European Journal of Human Genetics

Volume 22 Supplement 1 May 2014 www.nature.com/ejhg

European Human Genetics Conference 2014

May 31 - June 3, 2014
Milan, Italy

Abstracts

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PSYCHOSOCIAL ASPECTS OF GENETICS

European Human Genetics Conference

in conjunction with the

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May 31 - June 3, 2014
Milan, Italy

Abstracts

European Society of Human Genetics

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May 21 – 24, 2016

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PLENARY LECTURES

PL1.1
RASopathies. The other face of RAS signalling dysregulation

M. Tartaglia;
Istituto Superiore di Sanità, Rome, Italy.

RAS proteins are small monomeric GTPases that function as molecular switches controlling a major intracellular signaling network that, depending on the cellular context, guides diverse biological functions, including proliferation, cell fate determination, survival, migration, differentiation, and senescence. RAS signaling is switched on upon stimulation by numerous cytokines, hormones, and growth factors, and mediates the appropriate cell response to external stimuli through the regulation of transcription, cytoskeletal rearrangement, and metabolism. Within this network, signal flow through the RAF-MEK-ERK pathway, the first identified mitogen-associated protein kinase (MAPK) cascade, mediates early and late developmental processes, including determination of morphology, organogenesis, synaptic plasticity, and growth.
Signaling through the RAS-MAPK cascade is tightly controlled, and its enhanced activation has been known for decades to represent a major event in oncogenesis. Activating somatic RAS gene mutations occur in approximately 30% of human cancers, and upregulation of this signaling pathway can also result from enhanced function of upstream signal transducers or RAS effectors, and inefficient function of feedback mechanisms.
Unexpectedly, discoveries derived from a massive disease gene hunting effort have recently established a novel scenario in which the upregulation of this signaling cascade underlies a group of clinically related developmental disorders, the RASopathies, characterized by facial dysmorphism, cardiac defects, reduced postnatal growth, variable cognitive deficits, ectodermal and musculoskeletal anomalies, and increased risk for certain malignancies. These disorders are caused by mutations in genes encoding RAS proteins, regulators of RAS function, modulators of RAS interaction with effectors or downstream signal transducers. Based on the relatively high prevalence of some of these disorders (i.e., Noonan syndrome and neurofibromatosis type 1), the dysregulation of this signaling pathway represents one of the most common events affecting developmental processes. These discoveries have also provided us with unpredicted molecular mechanisms converging toward the dysregulation of this signaling network.

PL1.2
Evolution of the HD gene

E. Cattaneo;
Department of Bioscience, University of Milan, Milan, Italy.

The *Hdh* gene arose with no CAGs in *Dictyostelium discoideum* (*Dd*), around 800 million-year ago before the protostome-deuterostome divergence (Zuccato, Physiol Rev 2010). The CAG then has appeared in and is unique to the deuterostome branch. Two CAGs are found in *Hdh* in sea urchin (*Strongylocentrotus purpuratus, Sp*), the first specie to carry a primitive nervous system, and two CAGs are present in amphioxus (*Branchiostoma floridae, Bf*), the first specie to exhibit a rudimental hollow nerve tube and cephalization. Four CAG are found in *Hdh* from the more evolved fishes, amphibians, and birds. The CAG further expands in mammals and reaches its maximum length in human. A study of 278 normal subjects revealed an increase in grey matter with increasing length of the CAG repeat (Muhlau, PlosOne 2012), indicating that CAG size could influence normal brain structure. Our hypothesis is that the progressive increase in CAG length in the *Hdh* gene observed during evolution may be implicated in the evolutionary changes that have occurred in the developing and adult nervous system throughout vertebrate phylogeny, with a possible role for the CAG in newly emerging cognitive functions in the mammalian brain. We have now collected the *Hdh* gene from new species both in the protostome and deuterostome branch. In addition to further analyze the CAG tract during mammalian evolution we have collected genomic DNA and amplified the CAG tract from non-anthropomorphic and anthropomorphic primates. Our reconstruction of htt phylogeny supports further that the CAG tract expands during deuterostome evolution and seems to correlate with the appearance and/or evolution of progressively more complex nervous systems.

PL1.3
Genetic engineering of hematopoietic stem cells for the treatment of inherited diseases

L. Naldini;
Milan, Italy.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

PL2.1
Early-Onset Stroke and Vasculopathy Associated with Mutations in ADA2

I. Aksentijevich[1], *Q. Zhou*[1], *A. K. Ombrello*[1], *D. Yang*[2], *A. V. Zavialov*[3], *R. Sood*[1], *M. Boehm*[2], *D. L. Kastner*[1];
[1]*NHGRI/NIH, Bethesda, MD, United States,* [2]*NHLBI/NIH, Bethesda, MD, United States,* [3]*University of Turku, Turku, Finland.*

We initially observed 3 unrelated patients with fevers, livedo reticularis, vasculopathy, and early-onset recurrent ischemic strokes. We performed exome sequencing on affected patients and their unaffected parents that pointed to recessively inherited predicted-deleterious mutations in CECR1, encoding adenosine deaminase 2 (ADA2). All mutations are either novel or present at low frequency (<0.001) in several large databases, consistent with the recessive inheritance. Candidate gene screening was done in additional 6 patients. Nine patients shared 4/9 missense mutations in CECR1 along with conserved haplotypes. Computer modeling based on the crystal structure of the human ADA2 suggests that CECR1 mutations either disrupt protein stability or impair enzyme activity. All patients had at least a 10-fold reduction in serum and plasma concentrations of ADA2, and reduced ADA2-specific adenosine deaminase activity. ADA2 is expressed predominantly in myeloid cells and is a secreted protein. Animal models suggest that ADA2 is the prototype for a family of growth factors (ADGFs). Although there is no murine homolog of CECR1, there are 2 zebrafish homologs, Cecr1a and Cecr1b. Knockdown of a zebrafish ADA2 homolog caused intracranial hemorrhages and neutropenia, phenotypes that were rescued by wild type but not mutant human CECR1. Skin, liver, and brain biopsies from patients demonstrated vasculopathic changes characterized by compromised endothelial integrity, endothelial cellular activation, and inflammation although ADA2 is not expressed in the endothelial cells. Our data suggest that loss-of-function mutations in CECR1 are associated with a spectrum of vascular and inflammatory phenotypes ranging from early-onset recurrent stroke to systemic vasculopathy and/or vasculitis.

PL2.2
Disrupted auto-regulation of SNRPB causes cerebro-costo-mandibular syndrome

D. C. Lynch[1], *T. Revil*[2], *J. Schwartzentruber*[3], *E. J. Bhoj*[4], *A. M. Innes*[1], *R. E. Lamont*[1], *E. G. Lemire*[5], *B. N. Chodirker*[6], *J. P. Taylor*[7], *E. H. Zackai*[4], *D. R. McLeod*[1], *E. P. Kirk*[8], *J. Hoover-Fong*[9], *L. Fleming*[10], *R. Savarirayan*[11], .. *Care4Rare Canada*[12], *J. Majewski*[13], *A. Jerome-Majewska*[14], *J. S. Parboosingh*[1], *F. P. Bernier*[1];
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The discovery of highly conserved non-coding elements has unleashed a race to elucidate their functional significance. A class of intragenic highly conserved non-coding elements has previously been associated with regulating levels of core spliceosomal components, which affects the alternative splicing of downstream genes. These regulatory exons contain a premature termination codon, and trigger nonsense-mediated mRNA decay (NMD) when included in the associated transcript. In this study we identify mutations in one such exon in SNRPB as the cause of cerebro-costo-mandibular syndrome (CCMS). CCMS, one of the last unsolved classical Mendelian disorders, is a multiple malformation syndrome characterized by posterior rib gaps and micrognathia. The identified mutations fall within exonic splicing silencer sequences. We show by both qRT-PCR and a minigene reporter assay that these mutations cause increased inclusion of the alternative exon and decreased overall expression of SNRPB. This decrease is predicted to influence the splicing of a diverse but limited number of downstream target genes. Our data provide the first evidence for the functional importance of this class of conserved elements in the regulation of human development. We suggest that this exon, which is highly conserved across placental mammals but not other vertebrates, accords a subtle regulatory role to SNRPB which may have contributed to the complexity of mammalian development.

PL2.3
The First 100 patients diagnosed by whole-exome sequencing through FORGE Canada: Insights for Clinical Translation

S. L. Sawyer[1], *C. L. Beaulieu*[1], *T. Hartley*[1], *D. Bulman*[1], *J. Majewski*[2], *FORGE Canada Consortium, K. M. Boycott*[1];
[1]*Children's Hospital of Eastern Ontario Research Institute, Ottawa, ON, Canada,*

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An accurate genetic diagnosis is fundamental for pediatric patients with rare genetic disorders to improve disease management, access to resources, and recurrence risk counseling. A diagnosis also provides psychosocial benefits to families. The Finding of Rare Disease GEnes (FORGE) Canada project, which sought to identify novel rare disease genes by whole-exome sequencing (WES), shows that 36% of the solved disorders were secondary to mutations in known genes; 95 known disease genes were identified out of 264 total disorders studied. All patients had undergone standard of care genetic testing in Canada and no diagnosis was forthcoming. Thus, for 104 families, WES provided a clinical diagnosis; 24 of these were dominant (most de novo), 68 were autosomal recessive, four were X-linked mutations and one family had two disorders. Although many of the 104 families who received a clinical diagnosis were ascertained because of familial recurrence or consanguineous parents, 91 single affected patients without a family history were also included for WES. This latter subset of the above 264 disorders are representative of what geneticists often see in the clinic and had a diagnostic rate of 43%. Thus, WES would seem to be an efficient and cost-effective means of clinical diagnosis for many patients who are currently undiagnosed. Our findings suggest that patients with genetically heterogeneous disorders or sibling recurrence are the most likely to be diagnosed by WES. Canada is building on the success of these 104 diagnosed families to develop a national framework for clinical exome sequencing.

PL2.4
Transcriptomes of individual cells

C. Borel, *P. G. Ferreira, M. Garieri, F. A. Santoni, O. Delaneau, E. Falconnet, P. Ribaux, P. Makrythanasis, M. Guipponi, E. T. Dermitzakis, S. E. Antonarakis;*
University of Geneva Medical School, Geneva, Switzerland.

We sequenced hundreds of single-cell transcriptomes to decipher the cell-to-cell transcriptional variation. Starting from a homogeneous cell population of human female primary fibroblasts from one individual, we used the C1 Single-Cell-Auto-Prep-System to capture individual cells and to generate pre-amplified cDNA for next-generation sequencing. On average, 9322 genes per single-cell (RPKM >0.3) were detected, representing ~50% of the total genes detected by the bulk sample containing millions of cells. We noted a wide spectrum of transcriptional heterogeneity. Correlation analysis between single-cells showed an average coefficient of 62% (0.3-0.9). A large number of detected genes are cell-specific with gene mRNA levels variable between single-cells. Moreover, we identified cell-specific novel exons, multitude of alternative spliced isoforms and 3'UTR isoforms due to alternative polyadenylation. Hence, 1% of exons showed difference for exon inclusion between single-cells and 10% of 3'UTR contained alternative transcript termination sites.
To further assess the differential allelic expression at the single-cell level, WGS has been performed on this sample. Our data revealed that both alleles are actively transcribed for most of the detected genes. Interestingly, we observed a skewed monoallelic distribution in single-cells in a given snapshot of time. Indeed, for most of the genes, we detected one transcribed allele at the time in an individual cell. For a specific gene, rare are the individual cells expressing both alleles simultaneously. The results were validated in fibroblasts from a second individual. We will also provide a comprehensive survey of imprinted genes, X inactivation and escape.
S.E.A and E.T.D. laboratories contributed equally.

PL2.5
Chromosome X-wide association analysis discovers new loci for complex traits including a height locus not dosage compensated between men and women

T. Tukiainen[1,2,3], *M. Pirinen*[3], *A. Sarin*[3,4], *C. Ladenvall*[5], *J. Kettunen*[3,4], *T. Lehtimäki*[6], *M. Lokki*[7], *M. Perola*[3,4,8], *J. Sinisalo*[9], *E. Vlachopoulou*[7], *J. G. Eriksson*[4,10,9], *L. Groop*[11,3], *A. Jula*[12], *M. Järvelin*[13,14], *O. T. Raitakari*[15], *V. Salomaa*[4], *S. Ripatti*[3,16,17];
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The X chromosome (chrX) has often been overlooked in genetic association studies, thus representing one potential source for the "missing heritability" for complex phenotypes. Additionally, as up to 15% chrX genes escape from X inactivation (XCI), chrX may be enriched in sexually dimorphic associations, further highlighting that chrX warrants attention.
We investigated the contribution of >400,000 chrX SNPs to the levels of twelve common quantitative phenotypes in almost 25,000 individuals from Finland and Sweden. ChrX-wide association analysis pinpointed three new loci: two for height (FGF16/ATRX/MAGT1, P-value=2.71×10-9; ITM2A, P-value=3.03×10-10) and one for fasting insulin (Xq23, P-value=5.18×10-9), the first X-chromosomal ones for these traits in Europeans. Interestingly, the genetic effects for height near ITM2A, a gene implicated in chondrogenesis and know to escape from XCI, showed sex-heterogeneity in a manner consistent with no dosage compensation between men and women, observation further supported by statistical model comparison and female-bias in ITM2A expression. Given the converging evidence, this height locus likely represents the first link between an XCI-escaping gene and phenotypic variation in a population sample, and we estimate it explains approximately 1.5% of the height difference between men and women.
Together our findings underline the value of including chrX in large-scale genetic studies of complex diseases and traits. Our estimate that chrX accounts on average 2.6% of complex trait heritability, suggests there are tens of associated chrX loci to be discovered, with the intriguing possibility that some of the loci can also contribute to sexual dimorphisms.
Ref. Tukiainen et al. PLoS Genet. 2014

PL2.6
Genome sequencing identifies major causes of severe intellectual disability

C. Gilissen[1], *J. Y. Hehir-Kwa*[1], *D. T. Thung*[1], *M. Van de Vorst*[1], *B. W. M. van Bon*[1], *M. H. Willemsen*[1], *M. Kwint*[1], *I. Janssen*[1], *A. Hoischen*[1], *R. Leach*[2], *R. Klein*[2], *R. Tearle*[2], *T. Bo*[3], *R. Pfundt*[1], *H. G. Yntema*[1], *B. B. A. De Vries*[1], *T. Kleefstra*[1], *H. G. Brunner*[1,4], *L. E. L. M. Vissers*[1], *J. A. Veltman*[1,4];
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Severe intellectual disability (ID) occurs in 0.5% of newborns and is thought to be largely genetic in origin. The extensive genetic heterogeneity of the disorder requires a genome wide detection of all types of genetic variation. Microarray studies and more recently exome sequencing have demonstrated the importance of de novo copy number variations (CNVs) and single nucleotide variations (SNVs) in ID, but the majority of cases remains undiagnosed. Here we applied whole genome sequencing (WGS) to 50 patients with severe ID and their unaffected parents. All patients were negative after extensive genetic prescreening, including microarray-based CNV studies and exome sequencing. Notwithstanding this prescreening, de novo SNVs affecting the coding region provided a conclusive genetic cause in 13 patients and a possible cause for another 8 patients. In addition, we identified 7 clinically relevant de novo CNVs as well as one recessively inherited compound heterozygous CNV. These CNVs included single exon and intraexonic deletions of known ID genes as well as interchromosomal duplications. Local realignment of sequence reads allowed the mapping of most of these CNVs at single nucleotide resolution level and provided positional information for duplicated sequences. These results show that de novo mutations and CNVs affecting the coding region are the major cause of severe ID. Genome sequencing can be applied as a single genetic test to reliably identify and characterize the comprehensive spectrum of genetic variation, providing a genetic diagnosis in the majority of patients with severe ID.

PL3.1 - Summary
Incidental findings in clinical exome and genome sequencing

Diagnostic exome and genome sequencing data can be interrogated for clinically relevant variants other than those relevant for a diagnostic request. There are different opinions on the way to deal with these „incidental findings" in the clinic, on the potential benefits and risks to patients, on patient autonomy and on the obligation of laboratories to report these findings. These will be debated with representatives from both sides of the Atlantic.

PL3.1
Incidental findings in clinical exome and genome sequencing

R. Green;
Boston, MA, United States.

In 2013, the American College of Medical Genetics and Genomics (ACMG) issued Recommendations for incidental findings in clinical exome and genome sequencing. The Recommendations were written by a Working Group of 14 medical geneticists, laboratory geneticists, genetic counselors and ethicists who met regularly for 14 months to draft them. The draft Recommendations were presented for commentary in a public forum at the 2012 Annual Meeting of the ACMG, reviewed by 15 additional experts, and reviewed several times by the Board of the ACMG before being issued in March, 2013. The Recommendations

were recently amended in March, 2014.
The Recommendations were consensus-based, as empirical data on population penetrance and medical outcomes of identifying incidental variants are not available. The Recommendations called for molecular laboratories performing clinical sequencing for any indication on patients of any age to examine 56 genes associated with 24 actionable conditions for pathogenic variants. The Recommendations now suggest that patients be offered an option to decline these tests as a group at the time they are ordered.
Several clinical sequencing laboratories in the US have adopted the Recommendations and over 1000 reports of incidental findings have been issued. The incidence of the ACMG variants in unselected populations appears to be 2-4%. Additional data from our experimental laboratory of translational genomics on the issue of incidental findings will be presented.

PL3.3
Debate: Active search for clearly pathogenic variants that require direct clinical intervention; When related to late onset disorders, adults only.

M. Kriek;
Clinical Geneticist, Department of Clinical Genetics, Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

In 2013 the 'American College of Medical Genetics and Genomics' (ACMG) published a list of 56 genes that are proposed specific targets of a search for pathogenic variants in both children and adults (*Green et al.*). These genes are associated with diseases with an indication for immediate medical intervention of the patient or of a family member. The publication asserts that, in principle, the necessary patient data are available following a NGS procedure and therefore cannot be ignored. For purposes of clarity, the vast majority of these variants are removed during the various filtering stages of the normal trio analysis process and that identification of these variants will require additional actions.
During the American Society of Human Genetics meeting 2013, it was argued that consensus on a basic list of clearly pathogenic *variants* in the relevant genes is essential if this form of diagnosis is to be implemented in genetic diagnostics. A disadvantage of the approach as proposed by the ACMG (analysis of the entire gene, rather than focusing on known pathogenic gene variants) is that variants will be detected that have not been previously reported and/or are of undefined pathogenicity. This could result in considerable distress for the patient and family. To avoid this, only selected variants in the genes nominated by the ACMG will be analysed.
Another point of discussion is whether conditions that manifest later in life should be investigated in children. While the ACMG is in favour, *Ploem et al.* are of a different opinion: „When it comes to the unsolicited findings of NGS diagnostics in a young child, the interests of the child prevail over any wishes of the parents (not) to receive certain information." (NTVG 2014;158:A6757). This is consistent with ESHG recommendations (EJHG 2013;21:580-4)).
'In case of testing minors, guidelines need to be established as to what unsolicited information should be disclosed in order to balance the autonomy and interests of the child and the parental rights and needs (not) to receive information that may be in the interest of their (future) family'
A solution to these conflicting interests is as follows: where the index patient is a minor, a genetic predisposition for 'late onset' diseases for which immediate medical treatment is indicated will only be sought in parents. This is made possible by the setup of trio analysis in which variant lists are available for each parent.
Genetic variants that result in clinical manifestations that in require direct intervention during childhood will be analysed in the index patient, even when a minor.

PL3.4
Debate: Whole-genome sequencing in health care: proceed with caution and avoid incidental findings

M. C. Cornel;
VU University Medical Center, Amsterdam, Netherlands.

Whole-genome sequencing (WGS) of the human genome has a great potential to identify the genetic component of health problems, and probably, in the near future, at a lower cost than that of the current techniques. Proof of principle regarding the clinical utility of WGS followed by whole genome analysis (WGA) has been reported, especially for rare diseases. The Public and Professional Policy Committee of ESHG developed recommendations on whole genome sequencing for health care (EJHG 2013;21:580-4).
As a first element of the discussion, we have to consider all stakeholders involved: patients looking for a diagnosis, primary care physicians referring patients and following them for many years after the diagnosis, laboratory experts and clinical geneticists, legal and ethical experts. If a new technology is being implemented, a mutual learning process starts. Structures are needed for sharing experiences and establish testing guidelines at local, national and international levels.
When *in the clinical setting* either targeted sequencing or analysis of genome data is possible, it is preferable to use a targeted approach first in order to avoid unsolicited findings or findings that cannot be interpreted. The ACMG advocates (Genet Med 2013;15:565-74) that there is a potential for the recognition and reporting of incidental or secondary findings unrelated to the indication for ordering the sequencing but of medical value for patient care. The ESHG position is that whenever possible, such testing should be targeted to genome regions linked to the indication. Wider testing requires a justification in terms of necessity and proportionality. Adding screening targets to a diagnostic test violates the necessity criterion. Imposing this extra testing upon patients who need an answer to their clinical problem is at odds with respect for autonomy (Science 2013;341:958-9). Guidelines for the informed consent procedure in WGA must be developed (Hum Mutat. 2013;34:1322-8).
Also in a targeted analysis it is possible to detect an unsolicited genetic variant, indicative of serious health problems (either in the person tested or his or her close relatives) that allow for treatment or prevention. In principle, a healthcare professional should report such genetic variants (EJHG 2013;21:580-4).
To prepare the health care professionals for WGS in health care, a sustained effort at genetic education is required at various levels: in primary care to inform and refer people appropriately, and in specialized care to counsel or refer patients, and to discuss and interpret genetic test results adequately. Genetic experts should engage in discussing new developments in genetics, and explain the pros and cons of genetic testing and screening in clinical and commercial settings to inform the public and raise public awareness. Enhancing genetic literacy in patients and the lay public will help to involve wider society in this debate.

PL4.1
Gene Targeting into the 21st Century: Mouse Models of Human Diseases from Cancer to Neuropsychiatric Disorders

M. Capecchi;
Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City, UT, United States.

Gene targeting provides the means for modifying any gene in any desired manner in an intact, living animal, the mouse. This technology permits the evaluation of the function of genes in mammals. Because nearly all biological phenomena are mediated or influenced by the activity of genes, this methodology permits the analysis of the most complex biological processes such as development, learning, normal and aberrant behavior, cancer, immunology and a multitude of congenital human diseases. In the past, gene targeting has been used primarily to generate ‚knockout' mice, that is mice in which the chosen gene has been disrupted in the germline, such that every cell in the mouse carries the mutations. This methodology permits evaluation of the function of that gene in the intact mouse. However, many, if not most, genes have multiple functions. If one of those functions is critical to the survival of the mouse, then subsequent functions of that gene in the mouse cannot be evaluated by the above means. This problem can be circumvented by generating conditional mutations, that restrict the effects of the mutation temporally, spatially (to defined sets of cells or tissue), or both. In mice, conditional mutagenesis is achieved by combining gene targeting, which is achieved through homologous recombination, with a site-specific recombination system, such as Cre/loxP or Flp/FRT. Cre and Flp are recombinases that mediate recombination between specific, short DNA sequences, loxP and FRT, respectively. Gene targeting is used to flank your chosen gene with either loxP or FRT sites in the mouse germline. By restricting the production of the Cre or Flp recombinase to either a specific set of cells, a chosen temporal period, or both, within the developing or adult mouse, the gene is only excised from the genome of the mouse, in those selected cells, during that chosen temporal period, or both. I will describe a number of applications of gene targeting and conditional mutagenesis derived in our laboratory that address interesting biological questions from modeling human cancer to neuropsychiatric disorder in the mouse.

PL5.1
Signatures of Mutational Processes in Human Cancer

M. Stratton;
Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

All cancers are caused by somatic mutations. However, the processes underlying the genesis of somatic mutations in human cancer are remarkably poorly understood. Recent large-scale cancer genome sequencing initiatives have provided us with new insights into these mutational processes through the mutational signatures they leave on the cancer genome. In this talk I will review the mutational signatures found across cancer and consider the underlying mutational processes that have been operative.

SYMPOSIA

S01.1
From rare disease to management of common disorders
M. Summar;
Washington, DC, United States.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S01.2
Breast cancer genes: beyond BRCA1 and BRCA2
P. Pharoah;
Dept of Oncology, Cambridge, United Kingdom.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S01.3
Age-related Macular Degeneration
C. Klaver;
Rotterdam, Netherlands.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S02.1
Variation and genetic control of chromatin in humans
B. Deplancke;
Lausanne, Switzerland.

Understanding how a genome gives rise to complex phenotypes is one of the key unresolved questions in biology. In this talk, I will present results from our study aiming to examine the mechanistic relationship between genomic and molecular phenotypic variation. Specifically, we profiled three histone modifications (H3K4me1, H3K4me3, H3K27ac), two DNA binding factors (PU.1, RNA polymerase II), and gene expression in lymphoblastoid cell lines from 50 European individuals, reasoning that an integrated analysis of intermediate molecular phenotypes coupled with personal genome information might enable an in-depth characterization of non-coding variation. I will discuss how the resulting data allowed us to measure inter-individual variation in chromatin states, to predict sex-biased regulatory regions, and to map the regulatory architecture behind gene expression variation. As such, I will argue that we were able to provide a comprehensive view of chromatin state variation in a human population and to generate novel molecular insights into the propagation of genetic signals along the complex chain of molecular, regulatory events.

S02.2
Control of gene expression in disease
M. Georges;
Liège, Belgium.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S02.3
Computational challenges in single-cell transcriptomics
J. Marioni;
EMBL-EBI, Hinxton, United Kingdom.

Recent technical developments have enabled the transcriptomes of hundreds of cells to be assayed in an unbiased manner. These approaches have enabled heterogeneity in gene expression levels across populations of cells to be characterized as well as facilitating the identification of new, and potentially physiologically relevant, sub-populations of cells.
However, to fully exploit such data and to answer these questions, it is necessary to develop robust computational methods that take account of both technical noise and underlying, potentially confounding, variables such as the cell cycle.
In this presentation I will begin by briefly describing how we used spike-ins to quantify technical noise in single-cell RNA-seq data, thus facilitating identification of genes with more variation in expression levels across cells than expected by chance. Subsequently, I will discuss a computational approach that uses latent variable models to account for potentially confounding factors such as the cell cycle before applying it to study the differentiation of Th2 cells. I will show that accounting for cell-to-cell correlations due to the cell cycle allows identification of otherwise obscured sub-populations of cells that correspond to different stages along the path to fully differentiated Th2 cells.
To conclude, I will briefly discuss further applications of single-cell RNA-seq in the context of studying neuronal cell types, including olfactory neurons.

S03.1
Pontocerebellar hypoplasia
K. Kutsche;
Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Pontocerebellar hypoplasias (PCH) constitute a group of autosomal recessively inherited neurodegenerative disorders with prenatal onset. Classification based on clinical, neuroradiological and neuropathological characteristics divided PCH into eight subtypes; the underlying genetic cause has been identified in six PCH types (PCH1, PCH2, and PCH4-6 and 8). PCH2 represents the most frequent form of PCHs and typical clinical features are respiratory and feeding difficulties at birth, dyskinesia and chorea, severe impairment of cognitive and motor development, progressive microcephaly, and frequent death in late infancy or early childhood. MRI imaging in PCH2 shows brainstem and cerebellar hypoplasia, with the cerebellar hemispheres more affected than the vermis. In patients with PCH2, 4 and 5, biallelic mutations in genes encoding three subunits of the heterotetrameric transfer RNA (tRNA) splicing endonuclease complex, *TSEN54*, *TSEN2*, and *TSEN34* have been identified. PCH shows significant overlapping features with microcephaly with pontine and cerebellar hypoplasia (MICPCH), generally associated with loss-of-function *CASK* mutations. The classical MICPCH phenotype can be found in females who typically have moderate to severe intellectual disability and progressive microcephaly with pontine and cerebellar hypoplasia. Possible findings are ophthalmologic anomalies and sensorineural hearing loss. Males with a *CASK* mutation have also been identified. The phenotypic spectrum ranges from severe intellectual disability and MICPCH, or early-infantile epileptic encephalopathy to mild X-linked intellectual disability (XLID) with or without nystagmus. I will present an overview on clinical and genetic data of patients with PCH and MICPCH and how to discriminate the two conditions. My focus will be on the different *CASK* mutations in females and males and their associated phenotypes to help understanding genotype-phenotype relationships.

S03.2
The neurobiology of lissencephal
A. Wynshaw-Boris;
Cleveland, OH, United States.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S03.3
Neuronal migration defects associated with mutations in tubulins and MT-related proteins
L. Broix, K. Poirier, Y. Saillour, N. Bahi-Buisson, **J. Chelly**;
Cochin Institute, INSERM Unit 1016, CNRS –UMR 8104, Paris-Descartes University, Paris, France.

Neuronal migration disorders such as malformations of cortical development are frequent causes of epilepsy and intellectual disability. Disrupted biological and cellular processes such as neuronal progenitors proliferation, neuronal migration, and cortical organization, are traditionally used as a basis for the classification of MCD. Etiologies of these disorders are heterogeneous and genetic studies in humans and mice have identified a spectrum of mutations in genes involved in an array of crucial processes that often disrupt the development of the cerebral cortex. Aside from genes such as ARX, GPR56 and WDR62, the importance of genes encoding cytoskeletal proteins has become evident. For example, mutations in DCX and LIS1, both of which encode proteins involved in MT homeostasis, are associated with a large spectrum of neuronal migration disorders. Moreover, MCD associated with mutations in α- or β-tubulin-encoding genes such as TUBA1A, TUBB2B, TUBB3 and TUBB5, have been also described. These tubulin-related cortical dysgenesis are thought to involve a combination of abnormal neuronal proliferation, migration and differentiation. More recently, we reported the association between mutations in TUBG1, DYNC1H1, KIF2A and KIF5C, and diverse forms of malformations of cortical development with or without microcephaly. We further showed that the mutations in these MTs-related proteins: KIF5C, KIF2A and DYNC1H1 affect ATP hydrolysis, productive protein folding and microtubule binding, respectively. In addition, we showed by in utero electroporation that in vivo downexpression by shRNA of mouse, Tubb3 and Tubg1, as well as expression of Kif2a mutants, interferes with proper neuronal polarization and migration, morphogenesis and intermediate progenitor proliferation.
Altogether, these findings together with literature data support the hypothesis that proliferation and migration are genetically and functionally interdependent. Finally, they reinforce the importance of centrosomal and

MT-related proteins in cortical development and strongly suggest that MT-dependent mitotic and post-mitotic processes are major contributors to the pathogenesis of MCD.

S04.1
Disease, networks and epistasis
C. Webber;
Neurological Disease Genomics, MRC Functional Genomics Unit, Department of Physiology, Anatomy & Genetics, Oxford University, Oxford, United Kingdom.

I will give an overview of our recent work in identifying the pathways and processes underlying complex disorders, illustrating how different functional genomics resources can each provide novel biological insights into the same phenotype-influencing gene network. The topologies of the identified networks can identify pathway loading (both additive and epistatic) along with the direction in which the pathway is perturbed, thereby inviting drug repurposing. I will also illustrate some of the novel integrative approaches that we've been applying in GWA/exome studies and used to determine the significance of a functionally-clustered genome.

S04.2
Understanding molecular mechanisms of human disease mutations and coding variants through 3D protein networks
H. Yu;
Ithaca, NY, United States.

To better understand the molecular mechanisms and genetic basis of human disease, we combined the massive scale of network systems biology with the supreme resolution of traditional structural biology to generate the first comprehensive atomic-resolution 3D interactome-network comprising 3,398 interactions between 2,890 proteins with structurally-defined interface residues for each interaction. We found that disease mutations are significantly enriched both among interface residues and other non-interface ones within the same domains, contradicting the previous assumption that only a few interface residues are mutation hot spots for disease. We further classified 94,476 disease-associated mutations according to their inheritance modes and found that the widely-accepted "guilt-by-association" principle does not apply to dominant mutations. Furthermore, recessive truncating mutations on the same interface are much more likely to cause the same disease, even if they are close to the N-terminus of the protein, indicating that a significant fraction of truncating mutations can generate functional protein products.

S04.3
From protein networks to disease mechanisms
R. Sharan;
Tel Aviv University, Department of Computer Science, Tel Aviv, Israel.

In recent years, there is a tremendous growth in large scale data on human proteins, their interactions, and their relations to diseases. These allow for the first time a systems-level analysis of the molecular basis of disease. In my talk I will describe several recent works in this direction, aiming to uncover novel disease proteins and their underlying pathways with implications to diagnosis and therapy.

S05.1
Dynamic blastomere behaviour
R. Pera;
Stanford, CA, United States.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S05.2
24 chromosome copy number analysis for preimplantation genetic screening
A. H. Handyside;
Illumina, Cambridge, United Kingdom.

Chromosome aneuploidy in human gametes and embryos is a major cause of IVF failure and miscarriage and can result in affected live births. To avoid these outcomes and improve implantation and live birth rates, preimplantation genetic screening (PGS) aims to identify any euploid embryos prior to transfer but has been restricted to analysis of a limited number of chromosomes. Over the last 15 years, various technologies have been developed which allow copy number analysis of all 23 pairs of chromosomes, 22 autosomes and the sex chromosomes, or '24 chromosome' copy number analysis in single or small numbers of cells. The pros and cons of these technologies will be reviewed and evaluated for their potential as screening or diagnostic tests when used in combination with oocyte or embryo biopsy at different stages.

S05.3
Preimplantation genetic diagnosis
T. Voet;
Leuven, Belgium.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S06.1
Risk is More Than a Number: About Risks and Probabilities and People's Perceptions of Genetic Risks
D. R. M. Timmermans[1,2];
[1]Department of Public and Occupational Health, EMGO Institute, VU University Medical Center, Amsterdam, Netherlands, [2]National Institute for Public Health and the Environment, Bilthoven, Netherlands.

Risk communication is an essential component of genetic counseling . Genetic testing and providing information about genetic predisposition may enable early disease detection, targeted surveillance, and may lead to effective prevention strategies and behavioral change. However, the impact of genetic information on people's perceptions may be limited. Most people are unfamiliar with probabilistic thinking and find it hard to understand risks. Moreover, risks are experienced differently depending on the characteristics of the risk. A risk is more than a number. It is not only the probability, the quantification of the uncertainty, but also the nature of the risk, the severity of the consequences and the degree of control what matters. People process and evaluate information about potential risks in two different ways: analytical-rational if possible, but always intuitive-affective. The characteristics of the risks as well as the way risk information is processed impact people's understanding and perception of risks. This perception may be in discordance with the way experts' perceive genetic risks. In order to enable people to make informed decisions, information about (genetic) risks should not only be adequate but should also be in line with people's perceptions or mental model. Discrepancies with people's mental model of genetic risks as well the abstractness of the risk information may hamper people's informed decision making. In this presentation I will discuss the factors affecting the perception of health risks and genetic risks in particular, the way people understand the risks communicated to them and what this means for risk communication

S06.2
Risk perception: what could be at stake in multiple genetic testing?
C. M. Julian-Reynier;
Institut Paoli-Calmettes, UMR912 Inserm, Marseille, France.

First the concept of risk perception will be introduced, highlighting its interest for clinical practice or various research fields. We will focus on associated factors and in particular on the potential role of emotions in risk perception. Then the evidence for the relevance of previous findings will be reviewed in the context of genetic risk assessment and genetic test result disclosure such as investigated in clinical genetics/genetic counseling. Different application fields such as cancer genetics will be selected as illustrations of different situational contexts. Finally the way these previous experiences and body of knowledge could help to anticipate the potential consequences of multiple risk information disclosure and to document specific recommendations in the context of the process of multiple genetic testing or next generation sequencing will be discussed.

S06.3
Risk Communication Methods for Helping Patients Understand the Risks and Benefits of Genetic Testing
A. Fagerlin;
University of Michigan, Ann Arbor VA Center for Clinical Management Research, Ann Arbor, MI, United States.

Making decisions about whether to undergo genetic testing or how to use information from genetic tests is exceedingly complex. The complexity is due, in part, to the numeracy demands required of patients to understand the information being presented. In this talk, I will discuss methods for improving patients understanding of risk and benefit information. Furthermore, I will discuss how different risk communication methods can influence risk perceptions. Finally, I will discuss the role of family history and how that affects patients' (hypothetical) perception of risk and how patients weigh information about their family history and their risks to make screening decisions.

S07.1
Gene therapy of human genetic diseases with AAV vectors
A. Auricchio;
Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S07.2
Epithelial stem cell in cell and gene therapy
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Adult stem cells are cells with a high capacity for self-renewal that can produce terminally differentiated progeny. Stem cells generate an intermediate population of committed progenitors, often referred to as transit amplifying (TA) cells, that terminally differentiate after a limited number of cell divisions. Human keratinocyte stem cells are clonogenic and are known as holoclones. Human corneal stem cells are segregated in the limbus while limbal-derived TA cells form the corneal epithelium. Self-renewal, proliferation and differentiation of limbal stem cells are regulated by the ΔNp63 (α, β and γ), C/EBPδ and Bmi1 transcription factors. Cultivated limbal stem cells generate sheets of corneal epithelium suitable for clinical application. We report long-term clinical results obtained in an homogeneous group of 154 patients presenting with corneal opacification and visual loss due to chemical and thermal burn-dependent limbal stem cell deficiency. The corneal epithelium and the visual acuity of these patients have been restored by grafts of autologous cultured limbal keratinocytes. In post hoc analyses, success was associated with the percentage of p63-bright holoclone-forming stem cells in culture. Graft failure was also associated with the type of initial ocular damage and postoperative complications. Mutations in genes encoding the basement membrane component laminin 5 (LAM5) cause junctional epidermolysis bullosa (JEB), a devastating and often fatal skin adhesion disorder. Epidermal stem cells transduced with a retroviral vector expressing the β3 cDNA can generate genetically corrected cultured epidermal grafts able to permanently restore the skin of patients affected by LAM5-β3-deficient JEB. The implication of these results for the gene therapy of different genetic skin diseases will be discussed.

S07.3
Therapeutic targeting of Phosphatidylinositol-3-kinase/AKT/mTOR signalling in segmental overgrowth disorders
R. Semple;
University of Cambridge, Metabolic Research Laboratories, Addenbrooke's Hospital, Cambridge, United Kingdom.

The type 1A phosphatidylinositol-3-kinase (PI3K) enzyme complex serves as a signal transducer for a diverse range of hormone and growth factor receptors, and harbours somatic activating mutations in a large number of cancers. We and others have recently established that mosaicism for many of the same mutations underlies a spectrum of disorders of segmental overgrowth, ranging from isolated macrodactyly to catastrophic overgrowth affecting large parts of the body and several tissues, and commonly associated with complex vascular anomalies. Identification of the underlying signalling defect in affected tissues has immediately suggested that pharmacological targeting of the PI3K/AKT/mTOR pathway may offer the first rational, effective therapeutic approach for these disorders. The effect of mTORC or PI3K inhibition in dermal fibroblasts from affected patients will be discussed, as well as the early experience of sirolimus treatment in a severely affected proband.

S08.1
Demographic inference from identity by descent
I. Pe'er;
New York, NY, United States.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S08.2
Insights into European genetic history at fine geographic scales using haplotype-based approaches
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Wellcome Trust Centre for Human Genetics, Oxford University, United Kingdom

Modern genetic datasets are revealing features of our genetic history, and how this has shaped our genomes, in unprecedented detail. Across Europe, multiple invasions and migrations have taken place over the past several millennia, but the possible genetic effects of these and of subsequent regional isolation remain uncharacterised, partly due to the extremely subtle differences between the groups involved. We apply a set of approaches based on "painting" the genomes of people as haplotypic mosaics and describe insights into the existence and nature of extremely fine-scale differences among people from different regions of the UK, and Spain, as well as elsewhere in Europe. In the case of the UK, we show how these differences relate directly to specific historical migration events from other European countries, including invasions by Angles, Saxons and Jutes, and the Vikings. The groups involved in, genetic contributions, and dates, of these migrations are characterized based on genetic information alone.

S08.3
The role of population isolates in understanding genetic and complex diseases
P. Gasparini;
Trieste, Italy.

The use of isolated populations to reduce disease heterogeneity of complex disorders has already proven very useful in identifying DNA polymorphisms associated with complex diseases and quantitative traits. The study of complex traits in geographically and culturally isolated populations is particularly useful because the entire population can be analyzed, the relative weight of environmental variation can be controlled and genetic factors can be more easily identified. In these genetically and culturally homogeneous populations, a large proportion of individuals presenting a given trait is likely to share the same trait-predisposing gene inherited from a common ancestor. Furthermore, inbreeding, typical of small communities, reduces genetic heterogeneity and increases homozygosity, providing greater power for detection of susceptibility genes. We have created the Italian Network of Genetic Isolates (INGI) that collects the samples coming from several villages from 5 different Italian regions for a total of more than 6000 samples. Moreover, additional 1500 samples have been collected along the Silk Road. For all of them a great number of information regarding medical records, hematological parameters and lifestyle has been collected as well as DNA samples which have been genotyped with high density chip arrays. To evaluate the power to detect association in our cohorts we aimed at replicating several already published results and to verify if any new Italian specific loci were present. For example, GWAS were carried out on several hematological and serum lipids traits, blood glucose levels, blood pressure and anthropometric measures leading to the replication of 206 loci and to the discovery of some novel associations for BMI and weight. For 12 of these loci the top associated SNP was different from the one previously published highlighting the importance of having a population specific reference panel for personalized medicine. Moreover, specific genes/variants associated to phenotypes such as hearing, smell, taste and food preferences have been identified. More recently, new data have been obtained using whole genome sequencing data that allow refining the results previously obtained and will lead to the discovery of even more population specific genetic variants. Our results show that genetic isolates are a powerful resource for studying complex traits and thus to create genetic risk profiles which will be the bases for personalized medicine in Italy. Updated data will be presented and discussed.

S09.1
Twenty-five years of research in sarcomeric cardiomyopathies and therapeutic perspectives
H. Watkins;
University of Oxford, Oxford, United Kingdom.

Hypertrophic cardiomyopathy was one of the first monogenic cardiovascular disorders to be understood at the molecular level. Twenty years after the discovery of the first disease gene, HCM is still seen principally to be a disease of the sarcomere. At the biophysical level, the contractile protein mutations that cause HCM are seen to be activating in that they enhance Ca(2+) sensitivity, maximal force production, and ATPase activity. These defects then entrain secondary disturbances of energy deficiency and altered Ca(2+) handling that appear to be major common paths leading to the phenotype of hypertrophy and risk of sudden cardiac death. Importantly, these functional consequences of HCM mutations may lend themselves to specifically targeted approaches to disease modifying therapy for HCM, and phase I/II clinical trials have been completed or are underway.
In contrast, dilated cardiomyopathy is caused by mutations in genes encoding many different classes of proteins with very diverse roles in cardiomyocyte function, e.g. ranging from the nuclear envelope through to the contractile and force transduction apparatus. This indicates that the DCM phenotype is the end result of disparate pathways that lead to myocyte loss and fibrous replacement, suggesting that finding broadly applicable approaches to disease-modifying therapy may be difficult. In the subset of DCM caused by sarcomeric mutations the picture is clearer, and may lead to approaches to therapy, as DCM alleles result in functional changes that are the

opposite of the changes seen with HCM alleles: depressed motor function and reduced Ca(2+) sensitivity.

S09.2
Mendelian Randomization

M. V. Holmes;
University of Pennsylvania, Philadelphia, PA, United States.

Identifying causal biomarkers is important in the quest to prevent and treat cardiovascular disease. Mendelian randomization is a genetic epidemiological technique that can be used to make causal inference about biomarkers and environmental exposures.
I will talk about recent applications of Mendelian randomization with a focus on identifying potential therapeutic targets and understanding the role of physiological traits and environmental exposures in cardiovascular disease pathogenesis.

S09.3
Genetic testing in the clinical arena, current and future perspectives

P. Charron[1,2,3];
[1]Depart. of genetics, Pitié-Salpêtrière hospital, AP-HP, Paris, France, [2]INSERM UMR1166, Paris, France, [3]University UPMC Paris 6, Paris, France.

The recent development of molecular genetics in cardiovascular diseases has created a new understanding of their pathogenesis and natural history, and also new possibilities for the diagnosis of these genetic disorders through genetic testing. This has induced new expectations, and new demands, from both families and physicians regarding genetic counselling, DNA testing and application of this knowledge in clinical practice. The integrated use of genetic testing in routine practice developed rapidly in the context of monogenic cardiovascular disorders (such as cardiomyopathies, channelopathies, Marfan syndrome) with significant medical impacts on the management of patients or their families. This refers to diagnostic testing as well as predictive testing, prognostic evaluation and, rarely, prenatal or preimplantation diagnosis. Encouraging data were also identified in the context of pharmacogenetic interactions (such as Vitamin K antagonists, antiplatelet treatment) and multifactorial diseases (such as coronary artery diseases and heart failure).
The clinical utility of genetic testing was acknowledged through the production of international or national guidelines recommending routine genetic testing in monogenic disorders. However, its use in everyday clinical practice has been limited by the cost and complexity of conventional sequencing technologies. Advances in next generation sequencing technology (NGS) have the potential to solve this problem by analyzing substantially larger genomic regions at a lower cost than conventional capillary Sanger sequencing. But they may also pose new challenges. This was already obvious in preliminary studies that compared a number of variants found in cardiovascular diseases with exome or genome data from general population, and questioning the pathogenicity of variants previously considered as disease-causing. We will review the advantages, pitfalls, and clinical utility of NGS in the clinical setting of cardiovascular disorders, especially in the context of monogenic disorders.
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S10.1
Chromotrypsis

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De novo genomic rearrangements are a common cause of congenital abnormalities and mental retardation. However, in the majority of patients a molecular mechanism linking rearranged chromosomes to disease phenotype is lacking. This is particularly true for ultra-complex genomic rearrangements, such as those resulting from catastrophic chromosome shattering termed chromothripsis. We developed a family-based approach for dissecting the functional consequences of (complex) genomic rearrangements in patients with congenital disease. This approach combines gene-expression and chromatin profiling with functional studies in patient-derived cell lines and model organisms. We highlight two cases where family-based analysis and functional follow-up studies were instrumental to reveal the drivers of the disease phenotype. The described methodology is key for understanding disease in a large and broad category of patients with chromosomal aberrations of unclassified significance and thereby improves diagnosis and clinical decision-making.

S10.2
Kataegis: a mutation signature identified through whole-genome sequencing of human cancers

S. Nik-Zainal[1,2], *L. B. Alexandrov*[1], *B. J. Taylor*[3], *Y. Wu*[3], *D. Wedge*[1], *C. Rada*[3], *P. J. Campbell*[1], *M. Neuberger*[3], *M. R. Stratton*[1];
[1]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [2]East Anglian Medical Genetics Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom, [3]Laboratory of Molecular Biology, Cambridge, United Kingdom.

Cancer is the ultimate disorder of the genome, characterised by not one or two substitutions, indels or copy number aberrations, but hundreds to thousands of acquired mutations that have been accrued through the development of a tumour. The set of mutations observed in a cancer genome is not simply a random accumulation of variants. It is the aggregate outcome of several biological mutational processes comprising an underlying mechanism of DNA damage mitigated by the DNA repair pathways that exist in human cells. Each mutational process will leave its distinctive mark or mutational signature on the cancer genome.
Recently, we set out to extract the mutational signatures characterizing the mutational processes that have been operative in 21 whole-genome sequenced breast cancers. Multiple distinct known and novel substitution, insertion/deletion and rearrangement signatures were unearthed by these analyses. Here, I will describe one particularly intriguing signature of localized regions of dense somatic hypermutation, called kataegis, in which substitutions at C:G base pairs occurring within a distinctive sequence context were found associated with clusters of genomic rearrangements. Using an algorithm developed to allow efficient detection of kataegis, we investigate other whole-genome sequenced cancers to show that this phenomenon is not restricted to breast cancer. These studies harness the full scale of whole-genome sequencing. Furthermore, detailed and considered analyses of genomic data can provide biological insights that would otherwise remain buried.
The mechanism of kataegis however, is unknown. On the basis of similarities in mutation class and sequence context in experimental systems, members of the AID/APOBEC family of cytidine deaminases were implicated. Using whole-genome sequencing approaches in model organisms, the mechanism underlying kataegis is slowly being unraveled.

S10.3
Medulloblastoma links chromothripsis with TP53 mutations

J. O. Korbel;
Heidelberg, Germany.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S11.1
Copy number alterations in skin disorders

X. Zhang;
Beijing, China.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S11.2
Congenital heart disease

B. Keavney;
Manchester, United Kingdom.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S11.3
Copy number variants are a common cause of short stature

C. T. Thiel[1], *A. Reis*[1], *H. Dörr*[2], *A. Rauch*[3];
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Shortness of stature is one of the most common pediatric concerns and has an incidence of 3 % in the general population. In the majority of patients with idiopathic growth deficit the etiology remains elusive in the absence of morphological details. This unknown etiology prevents a sufficient medical care in most cases.
As it has been proposed that the growth fundamentally regulated by genetic factors, GWAS found significant evidence for both single nucleotide and copy number polymorphisms associated with height variation in the general population. However, these associations explain only a small fraction of the overall variability of human height.
Based on the early identification of SHOX gene deletions as a common cause

of idiopathic and syndromic (Leri-Weil syndrome) short stature as well as copy number variation (CNV) as a common cause of intellectual disability, the hypothesis of a "rare variant - frequent disease" hypothesis seemed to be feasible for short stature. To address this hypothesis we thoroughly build a study group of more than 400 families with idiopathic short stature and conducted SNP array analysis to demonstrate the presence of CNVs as a common underlying cause of short stature. Molecular karyotyping was performed and CNVs of a minimum size of 50kb scored and compared to healthy controls. Based on this technique we found a significant odds ratio for aberrations above 100 kb only. Due to the number of potential disease causing CNVs a gene-centric analysis comparing known CNVs, gene functions, tissue expression and murine knock-out phenotypes was neccessary. We confirmed that 10 % of the patients had de novo and inherited CNVs in agreement to the segregation of the short stature phenotype in the families. These CNV regions include known microdeletion/duplication loci expanding the phenotypical spectrum of these entities. The pathogenicity of novel loci was substantiated by comparison to available information, especially the overlap with loci of genome wide association for short stature. Our data showed a clear connection between the prenatal onset of short stature as well as the severity of the growth deficit with the likelihood of the identification of causal CNVs. Thus, we confirmed CNVs as a main cause of idiopathic short stature. Further improvement of the array technology as well as the application of CNV identification based on next generation sequencing will lead to a more elaborate and detailed view on even smaller CNVs. Application of these methods can help to illuminate the complex heterogeneity of short stature.

S12.1
The Epigenetic Basis of Common Human Disease

A. P. Feinberg;
Johns Hopkins University, Baltimore, MD, United States.

Although epigenetic changes in the cancer genome have been known for 3 decades, the role of epigenetics in common human disease generally and its relationship to genetic variation has only recently begun to be explored. We have been developing whole-genome approaches to epigenetic analysis of human disease. Surprising results have been the discovery of CpG island shores and large hypomethylated blocks corresponding to nuclear lamina / heterochromatin lysine-modification regions (LOCKs), and accounting for the vast majority of epigenetic changes in cancer. We have also been contributing to a new field of epigenetic epidemiology that integrates genetic, epigenetic, and environmental factors, and we are applying these approaches to the study of autoimmune and neuropsychiatric disease. One of the most exciting developments in this recent work is the idea that epigenetic plasticity under genetic control may itself confer a survival advantage in evolution, and may also be important in normal tissue differentiation and response to the environment. In support of this idea, we have identified variably methylated regions (VMRs) in normal development. These same VMRs appear to be targets for disrupted methylation in cancer, pointing to a unifying model of cancer in which epigenetic dysregulation allows rapid selection for tumor cell survival at the expense of the host. We have also identified genetic variants that increase epigenetic plasticity in patients with autoimmune disease. Thus, will need to integrate genetic and epigenetic analysis in common disease risk assessment, prevention, and treatment, and also consider the role of epigenetic plasticity itself in disease pathogenesis.

S12.2
Intergenerational epigenetic programming in a mouse model of undernutrition

A. C. Ferguson-Smith;
University of Cambridge, Department of Genetics, Cambridge, United Kingdom.

Environmental factors during early life are critical for the later metabolic health of the individual and of future progeny. In a mouse model of maternal caloric restriction during pregnancy the metabolic physiology of offspring over two generations is affected, including via paternal transmission to the second generation. Here we explore whether the paternal experience of in utero undernutrition, with an impact on the health of his offspring, is an epigenetically inherited memory transmitted via his sperm methylome.

S12.3
Cancer Genetics and Epigenetics: Two Sides of the Same Coin?

P. A. Jones;
Chief Scientific Officer, Van Andel Research Institute, Grand Rapids, MI, United States.

Genetic and epigenetic alterations used to be considered as providing two separate pathways leading to cancer. However, recent whole exome sequencing of a large number of human cancers has led to the realization that many previously unknown mutations occur in genes which regulate the epigenome. These mutations could potentially alter DNA methylation patterns, histone modifications and the positioning of nucleosomes to profoundly alter gene expression in cancer. Mutations in these genes can therefore contribute to cancer just as epigenetic processes can cause mutations in tumor suppressor genes and disable DNA repair enzymes such as MLH1 and MGMT. The cross talk between the genome and the epigenome is therefore a fascinating new area of research which gives us unprecedented opportunities to understand carcinogenesis. Because many of the genes which are mutated are enzymes, these findings may also lead to new avenues for drug discovery. Epigenetic therapies are already a reality and we can expect great progress to be made over the next few years as new targets are identified and tested for their potential new cancer targets.

S13.1
State of the Art of Non-Invasive Prenatal Testing

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Traditionally definitive prenatal diagnosis of genetic and chromosomal disorders has required analysis of fetal tissue obtained by invasive testing (usually chorionic villus sampling or amniocentesis) which carries a small but significant risk of miscarriage. The identification of cell free fetal DNA (cffDNA) in maternal plasma from four weeks gestation offered an alternative source of fetal genetic material for testing based on a simple maternal blood sample, thereby allowing earlier and safer prenatal diagnosis.
The majority of cell free DNA in maternal plasma emanates from the mother herself. This high background of maternal cfDNA meant that early applications were based on the detection of exclusion of alleles that were inherited from the father, e.g. SRY, or that arose de-novo, e.g. achondondroplasia. Fetal sex determination based on analysis of cfDNA is now widely available in Europe in pregnancies at risk of sex-linked disorders, where it is used to direct invasive testing. It is also available clinically in the UK for the definitive diagnosis of a number of conditions including some skeletal dypslasias and Apert syndrome, as well as for the exclusion of the paternal allele in families at risk of cystic fibrosis where parents carry different mutations. Elsewhere in Europe it has been used for the non-invasive diagnosis of other conditions such as Huntingdon Disease, but invasive testing has been required to confirm the fetal mutation status. The other major application is fetal Rhesus-D typing in RhD negative mothers where it is used both to inform pregnancy management in women with a history of haemolytic disease of the newborn, as well as to direct routine immunoprophylaxis with anti-D immunoglobulin to those RhD negative mothers carrying an RhD positive baby.
With the advent of next generation sequencing (NGS) it has become possible to estimate the proportion of sequence aligning to different chromosomes present in maternal blood and thereby detect fetal aneuploidy. This is now widely available in the private sector with detection rates for trisomies 13, 18 and 21 in excess of 99% with similarly high specificities. However discordant results are regularly reported and this test can only be considered a highly predictive screening test with invasive testing required to confirm positive results.
The potential for easy access to non-invasive prenatal diagnosis and testing has raised significant ethical concerns, largely with regard to maintaining informed parental choice, and the need for good health professional and public education. The huge commercial drive to implement NIPT for aneuploidy serves to heighten the need for early introduction of educational programmes and careful consideration of strategies required to facilitate appropriate implementation into public health services.

S13.2
Noninvasive prenatal testing creates an opportunity for antenatal treatment of Down syndrome

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Noninvasive prenatal testing (NIPT) for Down syndrome (DS) using massively parallel sequencing of maternal plasma DNA facilitates early detection of affected fetuses. If NIPT is performed at ~ 12 weeks of gestation it creates a potential 28-week window of opportunity in which to treat the fetus by orally administering small molecules to the mother. Our laboratory is using a 4 phase translational approach that involves human biomaterials (amniocytes and amniotic fluid), a mouse model of DS, and living human fetuses. In phase 1 we compared the transcriptome of fetuses with and without DS by analysis of cell-free RNA in amniotic fluid and showed that oxidative stress was a significant difference in the affected fetuses. In phase 2 we uploaded differentially-regulated genes into the Connectivity Map to identify candidate FDA-approved therapeutic molecules. In phase 3 drugs that had high efficacy in negating oxidative stress and showed low toxicity were selected for further studies in an animal model. We use the Ts1Cje mouse model of DS because affected males are fertile, yet

cognition is significantly impaired. We can therefore use wild type females for the treatment experiments, ensuring both a normal intrauterine environment and normal postnatal nurturing behavior. Treated and untreated affected pups and littermate controls are then evaluated using a variety of brain studies that include analyses of gene expression, histology, cellular proliferation and migration, and neurobehavior. In parallel, in phase 4 we are preparing for a human clinical trial by analyzing fetal brain growth using quantitative fetal magnetic resonance imaging (MRIs). To date, we have identified significant phenotypic differences in Ts1Cje embryos and neonates as endpoints to evaluate therapy. We have also shown encouraging, statistically-significant improvement in some neurobehavioral tests in adult mice. These results suggest that prenatal treatment in DS is an achievable goal.

S13.3
Clinical and social implications of NIPT

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Noninvasive prenatal testing (NIPT) became clinically available in late 2011. Since that time, hundreds of thousands of pregnant women have undergone NIPT. This talk will discuss the social and ethical concerns that preceeded the clinical use of NIPT, as well as the data that exists on patient, provider and stakeholder attitudes towards NIPT now that it is in use.

S14.1
Developments in rapid DNA sequencing technology

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No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S14.2
DNA sequencing in neonatal intensive care units

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No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S14.3
Impact of rapid DNA sequencing on diagnostic and public health microbiology

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Whole genome sequencing (WGS) promises to be transformative for the practice of clinical microbiology, and the rapidly falling cost and turnaround time mean that this will become a viable technology in diagnostic and reference laboratories in the near future. The objective of this talk is to provide an overview of a modern diagnostic microbiology laboratory in order to analyse at a very practical level where WGS might be cost-effective compared to current alternatives. I propose that molecular epidemiology performed for outbreak investigations and genotypic antimicrobial susceptibility testing for microbes that are difficult to grow represent the most immediate areas for application of WGS, and discuss the technical and infrastructure requirements for this to be implemented.

S15.1
Signaling networks in the auditory sensory cells unveiled by hereditary deafness

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The biochemical study of the components and associated molecular networks of the auditory sensory cells, the hair cells, are strongly impeded by the small number of these cells. In contrast, human and mouse genetic approaches have proven to be powerful to identify such components.
The presentation will focus on the hair bundle of the hair cells that operates the mechano-electrical transduction (MET). This sensory antenna is comprised of several rows of rigid microvilli, known as stereocilia of a few femtoliters in volume, that are organised in a staircase pattern. The MET process relies not only on the MET machinery but also on the proper architectural and biophysical properties of the hair bundle. In addition, any defect that affects the mechanical elements involved in sound-induced oscillation of the hair bundle or its ionic environment necessary to drive hair cell depolarization, also perturbs MET. For each of these contributors to the MET, several key components have been identified by studying inherited deafness forms; they are used as entry points to decipher the involved molecular networks.
The presentation will illustrate how the genetic approach led to the discovery of molecules controlling the development, maturation and maintenance of the correct structure of the hair bundle and the MET process. It will illustrate how it enlightens our understanding of the way these proteins form molecular complexes *in vivo* and how these complexes work and cooperate together. A special emphasis will be put on the networks composed of proteins encoded by the genes responsible for the Usher syndrome, the most frequent cause of hereditary sensorineural deafness associated with retinitis pigmentosa. Usher genes are critically involved in hair bundle development and the MET machinery as well as the functioning of the photoreceptor cells. Recent results based on mouse and human genetics, which provide evidence for a molecular maturation of the auditory MET machinery, will be discussed.

S15.2
Genes and cellular pathway of Fanconi's anemia

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Fanconi anemia (FA) is a rare genetic disease characterized by bone marrow failure, malformations, chromosome fragility, hypersensitivity to DNA interstrand cross-linking (ICL) chemotherapy and a high predisposition to cancer, including leukemia and solid tumors such as head-and-neck and gynecological carcinomas. The only cure of the hematological disease, which is the major cause of early death, is bone marrow transplantation using an HLA compatible donor. Those families with unavailable donor rely on preimplantation genetic diagnosis with selection of an HLA related embryo and in the future implementation of advanced gene and cell therapies. All these novel therapeutic applications to human health are further complicated by the fact that at least 16 genes, from FANCA to FANCQ, are involved in this disease and their products interact in a complex genome stability and tumor suppression network. Notably, 4 out of 16 FA genes (FANCD1/BRCA2, FANCN/PALB2, FANCJ/BRIP1 and FANCO/Rad51C) are breast cancer susceptibility genes in otherwise unaffected mutation carriers. Therefore, the discovery of novel Fanconi anemia genes is important not only for the affected patients but also for the general population. In this context, I will present data on the discovery of a novel Fanconi anemia gene by whole exome sequencing. Interestingly, this gene (ERCC4 o FANCQ) is involved not only in FA but also in xeroderma pigmentosum, a melanoma susceptibility disorder with defective nucleotide excision repair (NER), and in XFE-type progeria. Our genetic and biochemical results indicate that depending on the balance between ICL repair and NER, mutations in the same gene lead to clinically three distinct disorders.

S15.3
Analysis of signalling pathways in Tbx1 mutants identifies a novel mechanism in coronary artery Morphogenesis

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Much of the phenotype of 22q11 deletion syndrome (22q11DS) is secondary to haploinsufficiency of the transcription factor TBX1. Several studies have demonstrated distinct temporal and tissue-specific requirements for Tbx1 during development. Identification of candidate targets has led to new insights into cardiovascular morphogenesis. One such potential target is the signalling protein CXCL12 and its receptor CXCR4. This pathway is involved in collective cell migration, initiating contact inhibition of locomotion (CIL-"chase and run") between NCC and ectodermal placode. We predicted that disruption of this pathway in Tbx1 null mice would lead altered NCC patterning, and therefore conditionally mutated the receptor in cardiac neural crest cells. However, no defects were observed suggesting major species differences in the role of CXCL12-CXCR4 signalling. Rather, we showed that CXCR4 positive endothelial cells were the main target cell population, and that disruption of signalling led to VSDs, great vessel defects, and semilunar valve defects. In addition, lack of CXCL12-CXCR4 signalling led to a major failure in the elaboration of mature coronary arteries from the coronary endothelial plexus. This deficiency could be traced to a failure of invasion of the aortic root by endothelial cells and subsequent anastomosis of plexus vessels with the aorta. Reduction of Cxcl12 expression in the outflow tract of Tbx1 null embryos may therefore underlie the minor coronary artery dysmorphogenesis seen in these animals. Others have implicated the CXCL12-

CXCR4 pathway in some of the neurological deficits observed in chromosomally engineered mouse models of 22q11DS, suggesting multiple, independent roles for this pathway in 22q11DS.

S16.1
SINEUPs: a new functional class of antisense non-coding RNAs that activate translation

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No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S16.2
Molecular function of the repetitive (epi)genome in normal physiology and in disease

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Only about 1% of the genome encodes for the ~20000 human proteins, which are similar in number and largely orthologous to those found in organisms of significant lower complexity. On the contrary, the proportion of non protein-coding DNA has increased with developmental complexity reaching 98.5% in humans. Interestingly, up to two thirds of the human genome is composed of non protein-coding repetitive sequences. Furthermore, a significant portion of the epigenetic modifications is present in these regions and DNA repeats are dynamically transcribed in different cells and developmental stages producing a vast pool of non protein-coding RNA (ncRNA) molecules. Thus, ncRNAs produced by DNA repeats may hold the key to understanding the regulatory complexity inherent in advanced biological networks.
Long ncRNAs (lncRNAs) represent the most numerous and functionally diverse class of RNA produced by mammalian cells. Despite the growing interest on lncRNAs, they still remain poorly explored in terms of biological relevance, cellular function, mechanism of action and involvement in disease. We have recently contributed to this field through the identification of the first activating lncRNA involved in a human genetic disease: facioscapulohumeral muscular dystrophy (FSHD).
FSHD is one of the most important genetic diseases affecting the skeletal muscle. It is an autosomal dominant disorder with a strong epigenetic component. Unlike the majority of genetic diseases, FSHD is not caused by mutation in a protein-coding gene. Instead, the disease is associated with a reduced copy number of the D4Z4 macrosatellite repeat mapping to 4q35. Despite years of intensive research, the molecular pathogenesis of FSHD remains largely unknown. We recently identified DBE-T, a chromatin-associated lncRNA produced preferentially in FSHD patients. DBE-T mediates a Polycomb to Trithorax epigenetic switch at the FSHD locus, driving chromatin remodeling and transcription of FSHD candidate genes.
Here, I will discuss our recent results regarding the regulation of DBE-T expression, the mechanism responsible for DBE-T tethering to chromatin and how this lncRNA regulates the epigenetic status of the FSHD locus.

S16.3
The SMN complex: RNA processing and motor neuron disease

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At the post-transcriptional level, expression of protein-coding genes is controlled by a series of RNA regulatory events including nuclear processing of primary transcripts, transport of mature mRNAs to specific cellular compartments, translation and ultimately, turnover. These processes are orchestrated through the dynamic association of mRNAs with RNA binding proteins and ribonucleoprotein (RNP) complexes. Accurate formation of RNPs in vivo is fundamentally important to cellular development and function, and its impairment often leads to human disease. The survival motor neuron (SMN) protein is key to this biological paradigm: SMN is essential for the biogenesis of various RNPs that function in mRNA processing, and genetic mutations leading to ubiquitous SMN deficiency cause the neurodegenerative disease spinal muscular atrophy (SMA). I will discuss the expanding role of SMN in the regulation of gene expression through its multiple functions in RNP assembly and advances in our understanding of how disruption of SMN-dependent RNA processing pathways can cause motor neuron disease.

S17.1
Cancer genetic heterogeneity: implications for therapy responsiveness and acquisition of therapy resistance

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No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S17.2
Non-cell autonomous interactions promote sub-clonal heterogeneity

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Cancers arise and progress due to the underlying Darwinian somatic evolution. It has been commonly accepted that biological and clinical behaviors of individual tumors reflect properties of the most abundant cells that have achieved clonal dominance by virtue of carrying the most "advanced" complement of oncogenic driver mutations. However, the recent influx of data from tumor genome sequencing has revealed a remarkable degree of genetic divergence within tumors, including sub-clonal differences in mutational status of key driver genes. This clonal heterogeneity begs obvious questions: i) what are the mechanisms that enable co-existence of genetically distinct populations, and ii) what are the biological consequences of this co-existence? Microenvironmentally constrained tumors represent a particularly interesting scenario: overcoming the bottleneck requires changes in the tumor environment induced by secreted factors, but it is not clear a priori whether the expression of a secreted factor capable of driving tumor outgrowth can provide an autonomous fitness advantage. To address this scenario, we have developed a mouse xenograft model of microenvironmentally-constrained tumors. Using this model, we have interrogated the impact of sub-clonal expression of secreted factors in contexts of either competition against the parental clone or that of polyclonal tumors. We found that tumor progression can be driven non-cell autonomously by a sub-population of cells that is unable to achieve clonal dominance. This non-cell autonomous driving of tumor growth is predicted to stabilize sub-clonal heterogeneity within tumors throughout clinically relevant time frames. This co-existence of biologically distinct sub-populations enables inter-clonal interactions that can affect clinically important phenotypes

S17.3
Circulating tumor cells: Detection, biology and clinical implications

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Sensitive methods have been developed to capture circulating tumor cells (CTCs) in the peripheral blood at the single cell level (Pantel et al., Nat Rev Cancer 2008). CTCs are usually detected by immunostaining or RT-PCR assays, and more recently by the EPISPOT assay which measures the number of cells releasing/secreting tumor-associated marker proteins. Interestingly, detection of cell-free nucleic acids released by tumor cells into the blood might become an indirect way to detect micrometastatic disease (Schwarzenbach et al, Nat Rev Cancer 2011). At present, most CTC assays rely on epithelial markers and miss CTCs undergoing an epithelial-mesenchymal transition (EMT). New markers such as the actin bundling protein plastin-3 (Yokobori et al., Cancer Res. 2013) are not downregulated during EMT and not expressed in normal blood cells might overcome this important limitation and, therefore, increase the sensitivity of CTC assays. Recently, in vivo capture of CTCs with an antibody-coated wire placed into the peripheral arm vein has become feasible and allows now the "fishing" for CTCs from approx. 1.5 liters of blood within 30 minutes. CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy (Alix-Panabieres & Pantel, Clin. Chem. 2013; Pantel & Alix-Panabieres, Cancer Res., 2013). Interestingly, the subset of Ep-CAM^{low}, CD44^{high}, CD47^{+}, c-Met^{+} CTCs obtained from the peripheral blood of breast cancer patients might represent metastasis-initiator cells (Baccelli et al, Nature Biotech. 2013). Moreover, monitoring of CTCs before, during and after systemic therapy (e.g., chemotherapy, hormonal therapy, antibody therapy) might provide unique information for the future clinical management of the individual cancer patient. This information can be used as companion diagnostics to improve the stratification of therapies and to obtain insights into therapy-induced selection of cancer cells.

S18.1
Glyco-lipophobia: association with disorders of glycolipid and glycosylphosphatidylinositol anchor synthesis

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Thankfully, DNA and protein sequencing technologies revolutionized genetics and proteomics. Sadly, the two other macromolecules, carbohydrates and lipids, missed that technical revolution creating a malady called Glyco-lipophobia. Perhaps its development was predictable based on their molecular complexity, template-independent biosynthesis, and structural analysis without having defined functions. Fortunately, this condition is treatable, and given the discovery of over 100 genetic disorders in these pathways, treatment should not be delayed.
Glycosylation related genes comprise 1-2% of the human genome and those genes often fall into a series of distinct (sometimes overlapping) pathways. Each pathway generates sugar chains (glycans) for typical "clients". The best-known pathway is N-glycosylation found in secreted proteins, membrane-bound receptors, and signaling molecules. Some glycosylation disorders involve addition of glycans to lipids, not proteins. A few glycolipids are biosynthetic precursors of other pathways, but two define distinct pathways: glycosylphosphatidylinolsitol (GPI) anchors and glycosphingolipids (GSL). These molecules are often located in lipid rafts. Fifteen distinct disorders disrupt these pathways. All cells contain GPI and GSL, and affected patients display a range of mostly severe phenotypes that can include intellectual disability, seizures, ophthalmologic abnormalities, heart defects, and often plasma hyper- or hypo-phosphatasia. Simple biochemical indicators can help identify likely candidate genes from exome sequencing, and then also confirm those candidates. Cellular assays based on the established pathways can help design potential therapeutic screens.
Understanding the biosynthetic pathways not only suggests, and then confirms, candidate genes, but it creates avenues for therapy, based on the restoration of functional readouts. The powerful partnership of biochemistry and genetics can help eradicate or reduce Glyco-lipophobia. Patients and families have a vested interest in that victory: they need us to collaborate and cooperate, regardless of the target molecules and genes.
Supported by The Rocket Fund and R01DK55615

S18.2
Disorders of phospholipids, sphingolipids and fatty acids biosynthesis

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No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S18.3
Update on lipidomic approaches in disorders affecting complex lipids metabolism: the example of cardiolipin

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Lipids are important components of cellular membranes but also function as signal molecules in many cellular processes including apoptosis, autophagy and inflammation. In the last decade many new inborn errors of metabolism have been identified, many by next generation sequencing, that are caused by a deficiency of genes involved in phospholipid metabolism. A complementary technique that is frequently applied to investigate the functional defect in these disorders is lipidomics, which strives to measure and quantitate as many lipids as possible by (tandem) mass spectrometry. This technique will be introduced followed by different examples that show that combining next generation sequencing and lipidomics is a synergetic combination that yields novel biomarkers for inborn errors of phospholipid metabolism.

S19.1
Whole genome sequencing of 4000 individuals provides insight into genetic architecture of complex traits

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No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

S19.2
Using transcriptome sequencing to understand mechanisms of disease

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Detailed characterization of cellular effects of genetic variants is essential for understanding biological processes that underlie genetic associations to disease. This is a particularly pressing question in the interpretation of personal genomes that necessitates accurate prediction and measurement of genome function. One approach to address this challenge is to combine genomic data to a cellular phenotype, such as the transcriptome measured by RNA-sequencing. The early population-scale RNA-seq studies such as GEUVADIS are now being complemented by Genotype-Tissue Expression project (GTEx), where we are creating a comprehensive public atlas of genetic effects on the transcriptome across multiple human tissues. These data have been used to characterize regulatory and loss-of-function genetic variants as well as imprinting both at the population and individual level. Common expression quantitative trait loci that associate to gene expression levels provide a powerful tool to understand genetic architecture of regulatory variation and its role in GWAS associations. Furthermore, allele-specific expression driven by regulatory variants and imprinting can impact on penetrance of coding variants. Finally, we systematically analyzed transcriptome effects of protein-coding loss-of-function variants. Measuring nonsense-mediated decay (NMD) triggered by SNPs and indels allowed us to understand and better predict NMD trigger and escape. Importantly, effects of loss-of-function variants appear highly context-dependent with frequent tissue-specific effects and dosage compensation. Altogether, our results demonstrate the power of integrating genome and transcriptome data not only to improve our general understanding of genetic variants, but also as a practical approach in future medical and clinical applications of personal genomics.

S19.3
High resolution genetic analysis to detect variants associated with quantitative traits and diseases in the founder Sardinian population

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Sassari, Italy.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

EDUCATIONAL SESSIONS

ES1.1
Genetics of familial forms of thrombocytopenia

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Inherited thrombocytopenias (ITs) are a heterogeneous group of rare diseases characterized by bleeding risk sometimes associated with platelet dysfunction or other clinical features. More than twenty different forms have been characterized (Table 1). They account for almost 50% of the IT patients, suggesting that many forms are still unrecognizable.

The IT genes play a variety of roles in the complex process of megakaryopoisis and platelet production though their function is often unclear. In the rare forms of CAMT, CTRUS and TAR, thrombocytopenia is severe because of absence or reduction of megakaryocytes (MKs). Whereas in TAR, the platelet count tends to rise during life, in CAMT and CTRUS the disease progresses to bone marrow failure. Loss of function of MPL, the receptor of thrombopoietin, prevents production of Mks in the bone marrow of the CAMT patients. Less certain is instead the role of HOXA11 and RBM8A in CTRUS and TAR, respectively.

In another group of ITs there is a defect of MK maturation. In two of these forms, ANKRD26RD and PFD/AML, patients are also at risk of developing leukemias. Whereas the role of ANKRD26 remains obscure, RUNX1 is a master transcription factor regulating hematopoiesis. RUNX1 binds several other modulators, including FLI1, whose haploinsufficiency due to 11q23-ter deletions determines TCPT or JBS. In case of mutations of GATA1 or GFI1B, another two hematopoietic transcription factors, thrombocytopenia associates with red cells defects and reduction of alpha-granules. Absence of alpha-granules resulting typical gray appearance of platelets is a pathognomic feature of GPS. NBEAL2, the causative gene in this form, is likely to function in vesicle trafficking and generation of platelet granules.

The largest group of ITs could be related to defects of proplatelet formation, a process involving dynamic reorganization of the cytoskeleton, signaling pathways or apoptosis. Regarding the first aspect, mutations in genes encoding for components of the cytoskeleton (MYH9, ACTN1, TUBB1, WAS or FLNA) are likely to interfere with the correct proplatelet extension and platelet release. Of note, FLNA interacts with the C-terminus of GPIbalpha, one subunits of the von Willebrand factor receptor (GPIb/IX/V). Alterations of GPIb/IX/V cause BSS characterized by mild thrombocytopenia associated with reduction of platelet aggregation. Finally, defects of cytochrome c (CYCS) could be associated with increased apoptosis and premature release of platelets into bone marrow instead of blood stream.

This heterogeneity makes the molecular diagnostic testing a complex and time-consuming process. Next generation sequencing strategies will have a strong impact in the diagnosis of the known forms and cloning novel IT genes.

Table 1. Features of inherited thrombocytopenias classified according to possible defective processes of megakaryopoisis and platelet production.

Disease	*Abbreviation*	*OMIN entry*	*Inheritance*	*Gene (chromosome localization)*	*Platelet size*	*Other features*	*References*
Congenital amegakaryocytic thrombocytopenia	CAMT	604498	AR	*MPL* (1p34)	Normal	Reduced megakaryocytes, evolution into bone marrow aplasia	Ballmaier et al. Semin Thromb Hemost 37:673, 2011
Amegakaryocytic thrombocytopenia with radio-ulnar synostosis	CTRUS	605432	AD	*HOXA11* (7p15-14)	Normal	Reduced megakaryocytes. Possible evolution into aplastic anemia. Radio-ulnar synostosis +/-other defects	Balduini et al. Hum Genet 131:1821, 2012
Thrombocytopenia with absent radii	TAR	274000	AR	*RBM8A* (1q21.1)	Normal	Reduced megakaryocytes. Platelet count tends to normalize in adult life. Bilateral radial aplasia +/-other malformations	Albers et al. Nat Genet 44:435, 2012
Familial platelet disorder and predisposition to acute myelogenous leukemia	FPD/AML	601399	AD	*RUNX1* (21q22)	Normal	Increased risk (40%) of leukemia or MDS	Balduini et al. Hum Genet 131:1821, 2012
ANKRD26-related thrombocytopenia	*THC2*	188000	AD	*ANKRD26* (10p2)	Normal	Increased risk of leukemia or MDS	Noris et al. Blood 117:6673, 2011
Paris-Trousseau thrombocytopenia	TCPT	188025 600588	AD	Large deletion (11q23-ter)	Large	Cardiac and facial defects, developmental delay +/-other defects	Balduini et al. Hum Genet 131:1821, 2012
Jacobsen syndrome	JBS	147791					
Dyserythropoietic anaemia with thrombocytopenia	GATA1RD	300367	XL	*GATA1* (Xp11)	Large	Haemolytic anaemia, possible unbalanced globin chain synthesis, possible congenital erythropoietic porphyria	Balduini et al. Hum Genet 131:1821, 2012
X-linked thrombocytopenia with thalassemia		314050					
Gray platelet syndrome	GPS	139090	AR	*NBEAL2* (3p21.1)	Large	Pale platelets, evolutive myelofibrosis, splenomegaly, high serum vit B12	Bottega et al. Haematologica. 98:868, 2013
GFI1B thrombocytopenia	GFI1B	nd	AD	*GFI1B* (9q34.13)	Large	Red cell anisopoikilocytosis, platelet aggregation defects, α-granules reduction	Stevenson et al. J Thromb Haemost 11:2039, 2013
Thrombocytopenia associated with sitosterolaemia	STSL	210250	AR	*ABCG5*, *ABCG8* (2p21)	Large	Stomatocytosis, possible anaemia, tendon xanthomas, atherosclerosis. Defective MKs in a mouse model (Chase et al. Blood 115:1267, 2010)	Balduini et al. Hum Genet 131:1821, 2012
MYH9-related disease	MYH9RD	155100 153640, 153650, 605249	AD	*MYH9* (22q12-13)	Giant	leukocyte inclusions, cataracts, nephropathy and/or deafness	Balduini et al. Br J Haematol 154:161, 2011
ACTN1-related thrombocytopenia	ACTN1RD	615193	AD	ACTN1 (1q24.1)	Large	Anisocytosis	Kunishima et al. Am J Hum Genet 92:431, 2013
FLNA-related thrombocytopenia	FLNARD	nd	XL	*FLNA* (Xq28)	Small/giant	May be associated with periventricular nodular heterotopia (MIM 300049)	Berrou et al. Arterioscler Thromb Vasc Biol 33:e11-8, 2013
TUBB1-related macrothrombocytopenia	*TUBB1RD*	613112	AD	*TUBB1* (6p21.3)	Giant	None	Kunishima et al. Blood 113:458, 2009
Wiskott-Aldrich syndrome	WAS	301000	XL	*WAS* (Xp11)	Small	Severe immunodeficiency. Platelets have reduced life span	Mahlaoui et al. Blood 121:1510, 2013
X-linked thrombocytopenia	XLT	313900				No or mild immunodeficiency	Albert et al. Bood 115:3231, 2010
Bernard-Soulier syndrome Biallelic	BSS	231200	AR	*GP1BA* (17p13), *GPIBB* (22q11), *GP9* (3q21)	Giant	None	Savoia et al. Haematologica 96:417, 2011
Bernard-Soulier syndrome Monoallelic		nd	AD		Large		Noris et al. Haematologica 97:82, 2012
Platelet-type von Willebrand disease	VWDP	177820	AD	*GPIBA* (17p13)	Possiblly giant	Platelet count goes down under stress	Balduini et al. Hum Genet 131:1821, 2012
ITGA2/*ITGB3*-related thrombocytopenia	*nd*	187800	AD	ITGA2B (5q23-q31), ITGB3 (17q21.32)	Large	Platelet anisotropy, possible defects of platelet function	Balduini et al. Hum Genet 131:1821, 2012
CYCS-related thrombocytopenia	*THC4*	612004	AD	*CYCS* (7p15.3)	Normal	None	Morison et al. Nat Genet 40:387, 2008

ES1.2
Diagnosis and management of inherited thrombocytopenias

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Until the end of the last century, a few forms of inherited thrombocytopenia (IT) were known and these disorders were considered exceedingly rare. Spontaneous bleeding was considered as a feature common to all patients and as the main clinical consequence of these disorders. Recently, many new forms of ITs have been identified and our view of these disorders has considerably changed. We realized that ITs are less rare than previously thought and that most patients have mild or no spontaneous bleedings. In many cases thrombocytopenia is discovered incidentally during adult life and its genetic origin is missed, exposing patients to the risk of misdiagnosis with immune thrombocytopenia. To avoid this mistake, it is therefore essential that ITs are considered in the differential diagnosis of any thrombocytopenia of unknown origin both in children and adults. Family history, to search for other affected family members, and physical examination, to identify the defects that typically associate with thrombocytopenia in syndromic forms, are useful tools in this respect, although negative results do not exclude the genetic origin of platelet deficiency. Microscope evaluation of blood films is another key element for differential diagnosis, as morphological anomalies of platelets (increased or reduced size, defects of granules or vacuolization), leukocytes (Döhle-like bodies in neutrophils) or red cells (anisocytosis) are observable in many ITs.

Once an IT is suspected, a diagnostic algorithm can guide further investigation toward the specific patient's disease. It is likely that next generation sequencing techniques will simplify this process in the near future.

A definite diagnosis is essential for proper patient management. In some forms of IT, the major risk for affected subjects does not derive from bleedings, but from the increased propensity to develop additional disorders, as hematological malignancies or kidney failure. In syndromic ITs, associated extra-hematological defects often have a much higher impact on patients' quality of life than the reduced platelet count. The identification of genotype-phenotype correlations for same disorders made feasible to identify and quantify the risks affecting each patient and to design personalized follow-up protocols.

A definite diagnosis is also required to personalize treatment, as platelet transfusions are no longer the only remedy for ITs (Table 1).

Hematopoietic stem cell transplantation can cure ITs and this treatment is the best option for a few disorders that invariably lead to death during childhood. The thrombopoietin mimetic eltrombopag is effective in increasing platelet count in the IT induced by MYH9 mutations, the most frequent form of IT, and this drug has been successfully used instead of platelet transfusions to prepare patients for elective surgery. Splenectomy has no role in ITs, except for patients with WAS mutations, whose platelet counts increase after spleen removal. Finally, effective treatments are available also for some extra hematological defects of syndromic ITs.

Table 1. Treatments for inherited thrombocytopenias

	Indications	Comments
Platelet transfusions	All ITs. To stop bleedings when local measures failed or are not possible	Use HLA-matched donors to reduce the risk of alloimmunization
Hemopoietic Stem Cell Transplantation	- Wiskott–Aldrich syndrome - Congenital amegakaryocytic thrombocytopenia	Successfully used also in a few patients with biallelic Bernard Soulier syndrome and life threatening hemorrhages
Eltrombopag	- MYH9-related disease	Used instead of platelet transfusions for preparing patients to surgery. Efficacy in other conditions to be tested
Splenectomy	- Wiskott–Aldrich syndrome - X-linked thrombocytopenia	It increases platelet count but also the risk of infections
Desmopressin	- All mild forms of IT. To stop bleedings and to prepare patients to surgery	Experimental. A test dose is recommended to identify patients who might benefit from this treatment for future bleedings-surgery
Antifibrinolytic agents	All mild form of IT. To stop or prevent bleedings, and to prepare patients to surgery	Experimental (anecdotal evidence of efficacy)
Recombinant factor VIIa	All inherited thrombocytopenias. To stop bleedings when all other treatments failed	Experimental (anecdotal evidence of efficacy). Risk of thrombosis

ES2.1
Using prediction scores in cardiovascular medicine

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Genome-wide association (GWA) studies have provided hints and pointers towards causes of many common complex diseases and the current wave of large-scale sequencing efforts is likely to refine many of these signals. Using cardiovascular diseases (CVD) as an example, I will describe efforts to turn the association results into risk evaluations and efforts towards developing tools to help in preventive medicine.

Almost two thirds of the incident CVD cases are not identified as high-risk individuals using the currently widely used risk scores, such as the Framingham risk score. Almost 50 genetic loci have been indentified for coronary artery disease and even though the effect sizes of individual common variants identified by GWA studies are small, the large number of loci provide an opportunity to use the SNPs jointly in genetic risk scores (GRS). These scores have comparable risk profiles with quantitative risk factors like blood pressure or LDL cholesterol and have a potential to identify high risk individuals missed by the traditional risk factor screens.

Using results from prospective cohorts, I show how the genetic risk scores provide complementary information for prediction over the traditional risk factors and show how GRS help in identifying individuals with high risk. I discuss the potential benefits of targeted interventions using both traditional risk factor and genetic risk score information and show how already using the currently known risk loci provide an opportunity to prevent disease events. Finally, I discuss the need for developing tools to communicate the genetic risk to medical community and citizens, and the need to test the risk scores in clinical settings.

ES2.2
The benefits of using genetic information to design prevention trials

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No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

ES3.1
Novel sequencing approaches in genetic disease research

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Over the past decade the next generation sequencing has led to a revolution in human genetics and in disease gene identification in particular. This revolution was driven by new technologies that allowed new applications. But what's next?

In this educational session I will address the latest technological developments, for example: What to do with more and less expensive sequencing data? What do longer sequencing reads offer? Can we detect mosaicism reliably? I will also show new applications, for example the benefits of whole genome sequencing, new developments for exome sequencing and also new methods for highly multiplexed targeted re-sequencing.

ES3.2
Single cell genome and transcriptome sequencing

J. Lundeberg;
SciLifeLab, KTH- Royal Institute of Technology, Stockholm, Sweden.

Cells in a particular tissue are not identical. Instead, cells that are identical from a genomic point of view may have considerable variation in gene expression profile and protein levels, giving rise to a heterogeneous collection of cells with different behavior and appearance. Even the genomic DNA sequence may vary slightly between neighboring normal cells within a tissue albeit the differences are can be significant in adjacent tumor cells. In order to get information from single cells one need to isolate, study, and sometimes culture them, separately. This lecture will describe some of the advancements in genome and transcriptome analysis of single cells using massively parallel DNA sequencing and describe in more detail an approach to spatially identify gene expression in single cells in a tissue sections.

ES4.1
Protein replacement system: the case of polymerase-delta and MLH1 mutations in colon cancer.

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Next generation sequencing has revolutionized the search for disease-causing genetic alterations. Unfortunately, the task of distinguishing the handful of causative mutations from the multitude of harmless polymorphisms remains daunting. We have developed a system that permits the study of all types of mutations in any gene of choice. The system is based on the generation of stable human cell lines that simultaneously and inducibly express a cDNA encoding the protein carrying the mutation of interest and shRNA against the endogenous mRNA. In this way, the endogenous wild type protein can be replaced with a variant expressing any mutation and, if desired, also a tag for affinity purification or visualization. In this presentation, I shall focus on polymerase-delta and MLH1, which have been found to be mutated in familiar and sporadic colon cancers.

ES4.2
Aging and cancer: The impact of DNA damage

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Inherited defects in nucleotide excision repair (NER) removing helix-distorting DNA lesions are associated with cancer predisposition in xeroderma pigmentosum and neurodevelopmental deficits and segmental progeria in Cockayne syndrome and trichothiodystrophy (TTD). Mutations in single NER genes, such as XPD, are linked with all three disorders. Various single and double NER mouse mutants reveal that the severity of specific repair defects strictly correlates with the acceleration of selective premature aging features, whereas the type of DNA repair defect determines the kind of progeroid symptoms and/or cancer susceptibility. Microarray, functional and physiological studies revealed that persistent DNA damage, like caloric restriction, down-regulates the IGF1/GH-, lacto- and thyrotropic hormonal axes and upregulates anti-oxidant defenses, favoring maintenance at the expense of growth. This 'survival response' links accumulation of DNA damage and IGF1 control of life span. Micro- and mRNA expression profiling of normal, accelerated and delayed aging also revealed a clear parallel with the expression changes triggered by persistent transcription-blocking DNA lesions. These findings strongly support the DNA damage theory of aging. We will present phenotypes of conditional DNA repair models targeting aging to selected organs, parallels with Alzheimer's disease and the effect of nutritional interventions on the life span of progeroid repair mutants.

ES5.1
Genomic View of Mosaicism and Disease

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Mosaicism refers to the presence of two or more populations of cells with different genotypes within an organism, The timing of the events that lead to various mosaic genomic alterations can vary widely, leading to many different patterns of mosaicism. Mosaicism can result from abnormalities arising during meiosis, with correction during earliest development in some cells, or it may arise postzygotically, during early mitotic divisions. Mosaicism has been detected at surprisingly high frequency in the very early embryo, in miscarriages, in a wide variety of patients with clinical abnormalities and in normal individuals. In some cases, the clinical presentation might suggest mosaicism, as for example, in cases of ambiguous sex in an individual with XX/XY mosaicism, or in individuals with patchy pigmentation where lighter and darker skin may have different genotypes. However, in many cases mosaicism cannot be discerned at the clinical level. Mosaicism can be restricted to somatic tissues only, in which case it is unlikely to be inherited, or it can occur in both somatic and germline tissues, where transmission is possible, and in some cases it may be present in germ cells only, so that a normal individual is at risk for having multiple offspring with the mosaic finding. Mosaicism can be restricted to a single tissue, such as the brain or heart, causing tissue limited pathology, such as autism, schizophrenia or cardiac disease. In this session, we will trace the history of mosaicism from earliest examples of chromosomal mosaicism through recognition of mosaic DNA alterations and we will demonstrate how the utilization of SNP based chromosomal microarray analysis and next generation sequencing have vastly improved our ability to detect mosaicism, leading to markedly increased recognition of the role of mosaicism in human disease. We will discuss the clinical and molecular classes of mosaicism, their detection and the biological insights gained from these studies.

ES5.2
Revertant mosaicism in skin disease

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Revertant mosaicism (RM) refers to the co-existence of cells carrying disease-causing mutations with cells in which the inherited mutation is genetically corrected by a spontaneous post-zygotic event. RM in skin was first reported by us in 1997 in the genetic disorder epidermolysis bullosa (EB), which is characterized by lifelong fragile skin that easily forms blisters and erosions. In a patient with generalized atrophic benign EB, caused by compound heterozygosity at the COL17A1 locus, we found several patchy areas of healthy skin and provided molecular proof that the keratinocytes in the clinically unaffected skin were corrected by a gene conversion event, and consequently produced normal type XVII collagen. Mutations in as many as 18 genes can result in EB. Five of these genes have shown to revert: KRT14 encoding keratin 14 in EB simplex, LAMB3 encoding the β3 chain of laminin-332, and COL17A1 encoding type XVII collagen in junctional EB, COL7A1 encoding type VII collagen in dystrophic EB, and FERMT1 encoding kindlin-1 in Kindler syndrome. RM was also found in other heritable skin diseases: dyskeratosis congenita, and in ichthyosis in confetti (ichthyosis variegata) induced by increased homologous recombination of KRT10. Similar examples of "natural gene therapy" by RM have been described in Bloom syndrome, leukocyte adherence deficiency type 1, Wiskott-Aldrich syndrome, and RAG1-deficient severe combined immunodeficiency. This „natural gene therapy" phenomenon manifests as normal appearing skin areas surrounded by affected skin. Although initially thought to be rare, RM is now considered relatively common in genetic skin diseases. To address the issues relevant to RM, we will discuss the following questions: 1) What is the incidence of RM in heritable skin diseases? 2) What are the repair mechanisms in RM? 3) When do the revertant mutations occur? 4) How do you recognize revertant skin? 5) Do the areas of RM change in size? The answers to these questions allow us to acquire knowledge on these reverted cells, the mechanisms of RM, and utility of the reverted cells to the advantage of the patient. The revertant skin could potentially be used to treat the patient's own affected skin. Revertant skin cells can be used for transplantation by means of: 1) own skin biopsies, 2) cell suspension, 3) cultured epithelial cell sheet, 4) induced pluripotent stem cells reprogrammed as epithelial sheet for skin grafting or as haemopoietic stem cells for infusion. Transplantation of revertant skin biopsies has already succesfully been performed in a patient with EB.

ES6.1
Strategies for rare disease gene discovery in the era of next-generation sequencing

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Assigning medically-relevant roles to human genes is at the core of medical genetics as a field. In the area of Mendelian genetics, such assignment should be relatively straightforward since the phenotypic effect of Mendelian genes is usually large and measurable. The recent ability to interrogate the entire genome (or its coding portion) has provided an unprecedented opportunity to rapidly discover disease-causing genes by circumventing historical roadblocks that characterized previous approaches. However, even this cutting-edge tool has its own set of challenges. In this session, the two presenters will share with the audience the lessons they learned from their ongoing effort to unravel the "Mendeliome", one gene at a time. They draw from their combined experience not only in the successful identification of >120 Mendelian genes, but also, and as importantly, from the many unexpected results they encountered in their research programs. The first presenter will provide an overview of the recent successes and pace of novel gene discovery and discuss the implementation of next-generation sequencing tools alone, or in combination with autozygosity positional mapping, to identify autosomal recessive Mendelian genes. The second presenter will continue the same theme by sharing experience with autosomal dominant disorders and their own set of associated challenges. Both presenters will discuss in detail the pitfalls of the various approaches and suggest some helpful strategies. The immediate and critical need to exchange information about rare variants in genes, which is likely to grow in even more importance as we start to identify genes that contribute a decreasing percentage to the overall Mendelian mutation burden, will be emphasized by both presenters. At the end of the session, it is hoped that interested investigators will have acquired basic knowledge on optimal designs of projects aimed to discover novel disease genes.

ES7.1
From Mutations in the Few to Drugs for the Many

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Black swans have existed in the imaginations of philosophers for thousands of years as a metaphor of extreme outliers and unexpected rare events of large magnitude and consequence. Philosopher's argued that even though black swans were extremely rare, collectively they had a vastly larger impact than common, regular occurrences. In genetics and drug discovery, the exceptional black swans of rare genetic illnesses can lead to high-impact discoveries beyond the realm of normal expectations. Following „extreme genetics" and "opposite phenotype" strategies, we have made inroads in studying many devastating rare genetic illnesses in order to decipher the basis for common diseases. Investigating the "Opposite Phenotype" of pain in rare patients who are unable to perceive or understand pain, we have developed new drugs that may be able to treat pain in a profound way for the general population. The importance of the rare patient and clinical genetics is crucial in the identification and validation of novel drug targets.

ES7.2
Genetic, cell biological and clinical interrogation of disease-causing CFTR mutations informs strategies for future drug discovery

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Cystic Fibrosis (CF) is a common genetic disease caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene that lead to reduction of CFTR anion channel function on the surface of fluid transporting epithelial tissues. The clinical phenotype is variable but severely affected individuals typically suffer from airway obstruction with recurrent episodes of inflammation and infection as well as pancreatic insufficiency. The need to overcome this gap in knowledge is urgent, particularly given the recent success in developing drugs that target the basic defects caused by CFTR mutation. VX-770 (or Ivacaftor) has been approved as a drug for patients bearing the relatively rare mutation: p.Gly551Asp mutation. The most common mutation, p.Phe508del, has been studied extensively and found to impair CFTR protein folding during synthesis. Knowledge regarding the molecular defects caused by p.Phe508del has driven development of targeted, interventional compounds, such as VX-809. The development of these drugs highlights the therapeutic relevance of understanding the basic defects caused by CFTR mutation. Approximately 2000 different variants in the CFTR gene have been reported in the CF Mutation Database, yet, the molecular consequences of less than 10% of these variants are understood. In this presentation, our interrogation of a rare variant, c.3700 A>G, will be discussed. This mutation leads to aberrant splicing and the in-frame deletion of six amino acids (p.Ile1234_Arg1239del) in a conserved region of the CFTR protein. The deletion of these residues, caused protein misfolding as in the case of p.Phe508del. These insights led us to test the efficacy of an investigational compound in trials for p.Phe508del and found that, in cell culture, VX-809 partially ameliorated the folding defect of p.Ile1234_Arg1239del-CFTR. Hence, these studies support the rationale for defining the molecular consequences of rare CFTR mutations in guiding decisions regarding future drug discovery efforts.

ES8.1
New Proposals for the Regulation of in vitro Diagnostic Devices (IVDs)

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The 1998 IVD Directive regulates the market for diagnostic tests within the EU, setting out standards for the design and manufacture of in-vitro diagnostic devices (IVDs) and providing mechanisms for the oversight of these standards. The current system is inflexible, unresponsive and does not do a good job of protecting patients from harm. In September 2012, the European Commission launched a proposal for a complete overhaul of the IVD regulations. While welcoming these proposals as a move towards a flexible, transparent and proportionate system which would bring the EU closer to international best practice, EuroGentest and ESHG have also expressed concerns about a number of provisions which do not provide effective regulation of the burgeoning commercial market for genomic diagnostics, including direct-to-consumer genetic testing. We have made detailed submissions to the Commission, to MEPs and to regulatory bodies to improve the proposed regulation in these areas. Tests manufactured and used within health institution labs are exempt from the current IVD Directive - the so-called "health institution exemption". EuroGentest has proposed that the health institution exemption should, in future, be restricted to accredited laboratories and should not apply to commercial laboratories. We believe that this provides an appropriate balance of test availability and patient safety. This proposal has been included in the new proposed Regulation. The ENVI Committee at the European Parliament has proposed that the new Regulation should include far-reaching provisions that would govern and restrict the interaction between clinical geneticists and their patients. The ESHG has taken a strong stance against these proposals and has commissioned a legal opinion that demonstrates that they breach the principle of subsidiarity and are beyond the legal competence of the European Union. We will report on progress in these efforts and on our plans for further action.

ES8.2
Data protection regulation

D. Townend;
Maastricht, Netherlands.

No abstract received at production date. Check the online database at www.eshg.org/astracts2014.0.html for possible updates.

CONCURRENT SESSIONS

C01.1

Clinical implementation of non-invasive prenatal aneuploidy detection

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The presence of cell-free fetal DNA in the maternal circulation has allowed for the development of methods for non-invasive detection of fetal chromosomal aneuploidies. Non-invasive prenatal testing (NIPT) thus avoids miscarriages due to invasive sampling of fetal material. We developed an innovative, fast, cost efficient workflow and high throughput analysis pipeline. For validation, cell-free DNA of 297 maternal blood samples were collected from women between 9-15 weeks of gestational age who were also undergoing invasive sampling for increased risk of fetal aneuploidy. Whole genome sequencing was performed on cell-free DNA sequencing libraries on a HiSeq2500 using the fast mode 50bp single-ends. In addition to the Z-scores, traditionally the main measure for non-invasive aneuploidy detection, we defined different new parameters which allow for a higher accuracy aneuploidy detection. Moreover, the use of Z-scores across sliding bins enables the distinction to be made between maternal and fetal CNVs. This approach resulted in 100% specificity and sensitivity for trisomy 21 and 18 detection (trisomy 21, n=17; trisomy 18, n=7). This analysis pipeline has been clinically implemented and accredited (ISO15189). An overview of our clinical experience, currently standing at 500 samples, will be provided. In addition to the traditional trisomies, we will present data on other aneuploidies and clinical management thereof.

C01.2

Clinical Validation of Noninvasive Prenatal risk assessment for fetal sex chromosome aneuploidies in maternal plasma using Direct ANalysis of Selected Regions (DANSR™) assays

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Fetal cell-free DNA (cffDNA) in maternal plasma enables screening for fetal aneuploidy using next generation sequencing technologies. We have previously described using DANSR assays for biochemical analysis of chromosomes 13, 18, and 21, combined with the Fetal fraction Optimized Risk for Trisomy Evaluation (FORTE™) algorithm to compute the risk of trisomy with high sensitivity and specificity. We have developed additional DANSR assays for the X and Y chromosomes and have applied the FORTE algorithm on a two blinded sets of samples with and without sex chromosome aneuploidies (SCA). We report clinical validation results on HarmonyTM Prenatal Test's ability to detect fetal SCA.

609 samples comprised the two sets. All subjects provided informed consent. Matching fetal karyotype results from invasive testing were available for all subjects. Samples were processed as previously described with lab and analysis personnel blinded to fetal karyotype. FORTE models were built against monosomy X, XXX, XXY, XYY, and XXYY genotypes. Harmony results were compared against fetal karyotype.

All samples that passed standard Harmony QC metrics generated a sex chromosome result (100%; 95% CI: 99.4-100%). All were concordant with karyotyping for fetal sex (100%; 95% CI: 99.4-100%).

Directed analysis of cffDNA is accurate for risk assessment of non-mosaic fetal SCA. This is the largest fetal SCA validation study to-date. This demonstrates ability to expand Harmony to genetic conditions besides trisomies 13,18 and 21.

Harmony with XY Analysis performance

Karyotype	45, X	47,XXX	47, XXY	47, XYY
Sensitivity (95% CI)	93%(85.1-97.1%) 69 of 74	100%(60.1-100%) 6 of 6	100%(64.6-100%) 7 of 7	100%(43-100%) 3 of 3
Specificity (95% CI)	99.6%(98.6-99.9%)	99.4%(98.3-99.9%)	100%(99.3-100%)	100%(99.3-100%)

C01.3

mtDNA mutations variously impact mtDNA maintenance throughout the human embryofetal development

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Mitochondrial DNA (mtDNA) mutations cause serious disorders maternally inherited with high transmission risk, resulting in requests for preimplantation or prenatal diagnoses. These procedures are hampered by our poor knowledge on the pathophysiology of mtDNA mutations during human development. Specifically, how mtDNA mutations impact the mtDNA content? We collected oocytes, embryos, placentas and fetal tissues at various stages of development, from controls and carriers of m.3243A>G (*MTTL1*, MELAS), m.8344A>G (*MTTK*, MERRF) and m.8993T>G (*MTATP6*, NARP). We devised a test assessing simultaneously mtDNA copy number (CN) and mutant load in single cells.

MtDNA CN increased from the germinal vesicle to the blastocyst stage in m.3243A>G cells, suggestive of a mutation-dependent induction of mtDNA replication, that may compensate for the respiratory chain dysfunction.

Analyses of placentas showed that mtDNA CN significantly increased in m.3243A>G at 11-GW, becoming identical to controls at delivery, probably owing to a placental energy demand maximal at the end of the 1st trimester.

Analyses of fetal tissues showed that mtDNA CN was similar in m.3243A>G vs control tissues apart from the lung; lower in m.8993T>G muscle, heart, and liver vs control. Mutant loads were identical in all tissues from a given fetus, indicating an absence of mutant load/mtDNA content correlation.

These data highlight the complex relationships between mtDNA mutations and mtDNA content, depending on mutation types, mutant loads, cell types and development stages. Transcriptome and mtDNA replication studies should help us in unravelling the molecular bases of these observations, of particular relevance for therapeutic approaches in mtDNA disorders.

C01.4

Whole-genome single-cell haplotyping, a generic method for preimplantation genetic diagnosis

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Preimplantation genetic diagnosis (PGD) is the genetic testing of embryos prior to implantation to avoid the transmission of germline genetic disorders or of unbalanced chromosomal rearrangements when a parent is a balanced carrier. Current single-cell PCR or FISH PGD-assays require family-specific designs and labor-intensive workup. Array comparative genomic hybridization (aCGH)-based methods, which are mainly applied for preimplantation genetic screening (PGS) to discern diploid from aneuploid embryos, enable genome-wide aneuploidy detection but do not allow diagnosing single gene disorders. Here, we present a generic method that detects in single blastomeres not only the presence of Mendelian disorders genome wide, but also chromosomal rearrangements and aneuploidies, including their parental origin as well as the meiotic or mitotic nature of chromosomal trisomies. The method interrogates single nucleotide polymorphisms (SNPs) and uses a novel computational pipeline for single-cell genome-wide haplotyping and imputation of linked disease variants (siCHILD). Following stringent single-cell QC-metrics, a bimodal approach, based on discrete SNP-calls and continuous SNP B-allele fractions, respectively, reconstructs the parental haplotypes of the biopsied single cell. The approach proved accurate on 55 embryos from 12 couples carrying either autosomal dominant, recessive or X-linked Mendelian disorders, or simple or complex translocations. The method allowed diagnosing an embryo for multiple monogenic disorders at once, and, in contrast to current PGD for translocation cases, it enabled distinguishing embryos that inherited normal chromosomes from embryos that inherited a balanced configuration of the rearranged derivative chromosomes. The method facilitates genetic selection of embryos, and broadens the range of classic PGD.

C01.5

Scenarios for implementation of noninvasive prenatal testing (NIPT) for Down syndrome in a national health care system

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The implementation of non-invasive prenatal testing (NIPT) of Down syndrome (DS) in national health care systems requires decision-making about the possible restriction of NIPT to high-risk pregnancies, the timing of NIPT, and the combination with other tests. In this study, we combined ethical exploration with a decision-analytic model. We compared three implementation strategies: (1) restriction of NIPT to high-risk pregnancies on the basis of the combined test (CT) (risk for DS ≥ 1:200); (2) NIPT for all women at 13 weeks of gestation; (3) NIPT for all women at 10 weeks of gestation. Comparing strategy 1 to strategy 2, 95% fewer women underwent NIPT, and false positive 1st trimester screening results and invasive procedures were reduced by 95% and 37%, respectively. This was at the expense of a decrease in DS detection of 11% before 15 weeks of gestation and 4% throughout the entire pregnancy (3 DS cases/100,000 pregnancies). Comparing strategy 2 to strategy 3, NIPT was avoided in an additional 4% of pregnancies that would have resulted in miscarriage. Furthermore, delaying NIPT to 13 weeks allows for immediate confirmation by amniocentesis, and may be beneficial to women who may feel overloaded with information in 1st trimester. As NIPT currently does not detect all prenatal abnormalities, it should be offered subsequent to or alongside a nuchal translucency (NT) measurement. In all cases of fetal anomaly on ultrasound (including NT enlargement), broad genetic testing after invasive procedure should still be performed.

C01.6

Whole genome sequencing and analysis in prenatal screening: ethical reflection

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Non-invasive prenatal testing (NIPT) for fetal abnormalities currently focuses on Down's syndrome. It is to be expected, however, that it will become possible to broaden the scope of screening with NIPT. As proof of principle, it has been demonstrated that the complete fetal genome can be sequenced using fetal DNA from maternal plasma, potentially allowing prenatal whole genome analysis.
This scenario makes a proactive ethical reflection of utmost importance. Normative issues to be addressed include the following: First, the prerequisite of proportionality. Do the possible benefits of whole genome sequencing and analysis in prenatal screening outweigh the possible harms and disadvantages? Secondly, would it be possible to fulfill the requirement of informed consent, given the wide range of possible outcomes of whole genome prenatal screening? Could so-called *generic* consent, based on pre-test information about general categories of test outcomes, be accepted as a sound variant of informed consent ? And thirdly: if whole genome prenatal screening would result in the birth of children whose genotype has been elucidated prenatally, the child's right *not* to know may be violated, i.e. the right of the future, competent, person to decide about predictive testing for later onset diseases. How to handle, then, possible conflicts between future parents' right to know and future children's right not to know?
This presentation offers a systematic exploration of these issues, aimed at contributing to adequate ethical guidance for whole genome prenatal screening.

C02.1

A novel variant in the SLC9A9 gene influences disease activity in interferon-beta treated multiple sclerosis patients

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Aimed to identify genetic variants able to predict response to interferon beta (IFNβ), we performed a genome-wide association study in Italian relapsing-remitting multiple sclerosis (MS) patients treated with IFNβ. Rs9828519, achieved genome-wide significance (p=4.43x10^-8), and its effect was replicated in three independent cohorts of American, Italian and French patients (meta-analyzed p=7.78x10^-4). Rs9828519 is intronic of SLC9A9 gene, codifying for a sodium/hydrogen exchanger localised in the endosomes, not known to be involved in MS or in IFNβ pathways. With in vitro experiments, we observed an upregulation of SLC9A9 expression after IFNβ stimulation in PBMCs of 20 healthy controls (HC, p=4.01x10^-7) which was unrelated to the genotype status. Such upregulation was confirmed also in IFNβ-treated MS patients (GSE24427 and GSE26104 experiments: p=3.8x10^-3 and 0.030). No cis-effect of rs9828519 was observed in whole blood of MS patients, nor in PBMCs, CD14+ monocytes or CD4+ T lymphocytes collected from HC. For this reason we performed a trans eQTL analysis in 211 CD14+ monocytes isolated from HC followed by pathway analyses using three independent softwares. All of them enlighten an enrichment of IFNβ-related pathways of genes trans-regulated by rs9828519. We observed in the rs9828519GG samples an up-regulation of genes involved in IFNβ signalling, including STAT1, JAK1, STAT5B, but also an impairment of the final effects of IFNβ, with an upregulation of osteopontin and a downregulation of IL10. Here we report a new genetic marker that affects the response to IFNβ in MS patients supported by experimental evidences of the involvement in the IFNβ pathways.

C02.2

High throughput sequencing in sporadic forms of steroid-resistant nephrotic syndrome: heterogeneous genetic alterations can predict resistance to treatments

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Steroid-resistant nephrotic syndrome (SRNS) is a disorder that results in end-stage renal disease and can be potentially related to a genetic cause, especially when it occurs in children. However, because of genetic heterogeneity, the real burden of genetic alterations in sporadic cases is unknown. In this study, we explored the possibility that genetic alterations of podocyte's structure may explain lack of response to steroid treatment in children affected by sporadic childhood-onset nephrotic syndrome (NS), not only in a causative manner, but also as critical disease modifiers that influence response to steroid treatment and immunosuppressive agents.
We designed a custom sequencing array to target all exons and part of flanking sequences for known podocyte genes responsible for SRNS, that was applied through next generation sequencing to a selected cohort of 50 patients with sporadic NS and variable response to steroid treatment.
We identified a genetic cause in 40% of the 19 SRNS patients analysed. Modifier genes were observed in an additional 25% of patients exhibiting resistance to steroid, but nor disease causing neither disease modifier genes were observed in podocyte's genes of 30 additional children that were sensitive to steroids. Treatment with immunosuppressive agents was effective only among patients that were negative to the test, while none of the patients displaying genetic alterations responded to any immunosuppressive agent.
The results of this study suggest that in children affected by sporadic NS, resistance to steroid treatment may be related to genetic alterations in genes that control structure and function of the podocyte.

C02.3

Personalized thiopurine dosing based on TPMT genotyping reduces leucopenia occurrence and results in cost-savings in IBD patients; results from a randomized trial in the Netherlands

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More than 20% of inflammatory bowel disease (IBD) patients discontinue thiopurine therapy due to severe adverse drug reactions, among which leucopenia is one of the most serious. Thiopurine S-methyltransferase (*TPMT*) pharmacogenetic testing can be used to optimize thiopurine safety and efficacy. Nonetheless, in clinical practice it is only used on a limited scale. We performed a randomized controlled trial including 769 IBD patients starting on thiopurine treatment. Patients were randomly assigned to standard treatment (control) or pre-treatment screening (intervention) for three common *TPMT*-variants (*TPMT**2, *3A and *3C); patients heterozygous for a *TPMT*-variant received 50% of the standard thiopurine dose, and patients homozygous for the variants received 0-10%. Intervention and control groups were compared for leucopenia occurrence (leucocyte count $<3.0*10^9$/l) in the first 5 months after treatment initiation. Cost-effectiveness analysis included complete cases (patients with outcome (EQ-5D) and self-reported costs). Thirty-nine (9.8%) patients in the intervention and 35 pa-

tients (9.5%) in the control group carried a *TPMT*-variant. Among *TPMT*-variant carriers, leucopenia occurrence was significantly reduced in the intervention compared to the control group (2.6% versus 22.9%, relative risk 0.11, 95%CI=0.01-0.85).Overall, no difference in leucopenia occurrence was observed between the intervention and control group (7.3% versus 7.8%). Treatment efficacy (ΔEQ-5D) was similar for both study arms. Average costs (direct medical costs and indirect costs) were lower for patients in the intervention (n=238) compared to the controls (n=216): €4433 versus €6150, p=0.023. Prior-to-treatment TPMT screening reduces leucopenia risk in IBD patients treated with thiopurines without impeding treatment efficacy and results in substantial cost-savings.

C02.4

Genome-wide identification and phenotypic validation of loss of function mutations

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A typical human genome includes numerous apparent loss of function (LOF) variants, which may be attributed to sequencing or alignment errors, carrier states, hypomorphic alleles, gene redundancy, and true LOF alleles for haploinsufficiency-sensitive genes. It is critical for understanding gene function and for predictive medicine to assess the consequences of LOF variants. Prior efforts to understand the genotype-phenotype relationships were limited by ascertainment biases and the limitations of deidentified data. To approach this problem, we characterized the phenotypic consequences of LOF variation in an exome cohort of 950 individuals and iteratively validated the phenotypes. Exomes were generated by targeting and Illumina sequencing. Potential LOF variants were defined as nonsense, frameshift, and essential splice site variants. Analysis was restricted to HGMD genes. Variants with maf >0.005 in ClinSeq or dbSNP were excluded. We filtered for genes that cause disease in an autosomal dominant pattern by haploinsufficiency. Remaining variants were considered variants of interest, 87 were identified in 112 individuals. 85 of these individuals were evaluated for these disorders. We used stringent criteria for phenotyping and family history to identify variants that were causing disease. Of the evaluated patients, half (38/85) had evidence of disease. The negative cases illustrated issues with gene model errors, mechanisms of alternative translation starts, etc. The positive cases demonstrate the ability to perform predictive medicine and to use filtering methods to identify inapparent genetic disorders. These data also point to approaches that could unravel the functional consequences of genes not yet known to cause human phenotypes.

C02.5

The SickKids Genome Clinic: Developing and evaluating a pediatric model for individualized genomic medicine

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Genomic medicine is a clinical paradigm in which knowledge gained from analyzing an individual's genome is used to guide health care decisions throughout life, in order to anticipate, diagnose and manage disease. To pilot the implementation of genomic medicine in paediatrics we have developed the SickKids Genome Clinic, a multidisciplinary test bed that supports a wide range of research into the clinical uses of whole genome sequencing (WGS), from development of new bioinformatics pipelines and counselling models to health economics and bioethics studies. We are enrolling 150+ children/year who are undergoing diagnostic microarray analysis or multigene panel sequencing. Participants undergo WGS after their parents are counselled about WGS and have declared their preferences for learning their child's pharmacogenetic variants, adult-onset incidental/secondary medically-actionable variants (MAVs) and carrier status variants. Of the first 200 families approached, 117 agreed to participate, with ~75% of participants electing to learn about all available adult-onset secondary MAVs and carrier variants. Of the parents who decide to learn of their child's adult-onset secondary MAVs, approximately 60% also choose to learn if they carry the same variants. The most frequent concerns of parents are psychological burdens of identifying their child's secondary MAVs and fear of insurance discrimination. WGS data are analyzed using separate bioinformatics pipelines for primary disorder variants, structural variants and secondary variants. Initial WGS analyses have already yielded clinical diagnoses not revealed by conventional testing and support our hypothesis that WGS is superior to conventional multi-gene panels and clinical microarrays for identifying pathologic variants.

C02.6

Collaboration to integrate genomics into clinical care: a demonstration evaluation

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Genomic technologies are proving transformative, but ensuring full clinical and research benefit from their application in clinical settings requires planning and collaboration. Working to a five year vision, seven research and healthcare organisations are conducting a demonstration project to test an ambitious, unified approach to meeting their diverse needs. Demonstration projects examine the application of structural innovations such as technology and non-structural innovations like health programs. They provide new insights into the process and value of implementing innovation, thereby informing decision-making about future implementation. Our highly novel, prospective project evaluates the feasibility of whole exome/genome sequencing as a single common assay for germline and somatic conditions, replacing current tests, for use in clinical and predictive care. Mimicking usual clinical practice, patients with one of five diverse germline or somatic conditions are being offered whole exome sequencing in parallel to routine investigations. Sequence data is generated by multiple diagnostic laboratories, analysis is then targeted to genes known to be related to the clinical condition using a common analytic pipeline. Data is available for reanalysis and research. Patient and clinician engagement in this clinically-led project is paramount. The demonstration project is being evaluated in terms of feasibility (can the model be built?), requirements (what does it take to build?), and impact (can it make a difference?) across all processes and with feedback from patients, clinicians and scientists supplementing data collection. We will describe the 'real world' strengths, weaknesses and barriers identified during collaborative implementation of the demonstration project across the seven organisations.

C03.1

Dominant β-catenin mutations cause a recognizable syndrome with intellectual disability, and are associated with learning deficits and structural and functional brain abnormalities in mice

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Introduction: The recent identification of multiple dominant mutations in both humans and mice has enabled us to explore the molecular and cellular basis of beta-catenin function in cognitive impairment. Mutations in human beta-catenin have been identified as causative in a spectrum of neurodevelopmental disorders. Methods and Results: In identifying *de novo* beta-catenin mutations in patients with intellectual disability and careful characterization of their phenotype, we were able to define a recognizable intellectual disability syndrome. We have collected detailed clinical information of 7 patients with this novel syndrome. In parallel, the characterization of a chemically-mutagenized mouse line displaying features that are similar to these human mutations has enabled us to investigate the consequences of beta-catenin dysfunction through development and into adulthood. The mouse mutant, batface, carrying a Thr653Lys substitution in the C-terminal armadillo repeat in beta-catenin, displays a reduced affinity for membrane-associated cadherins. In association with this decreased cadherin interaction, we found that the mutation results in decreased intrahemispheric connections with deficits in dendritic branching, long-term potentiation and cognitive function. Conclusion: For the first time *in vivo* we showed how dominant mutations in beta-catenin underlie losses in its adhesion-related functions leading to severe consequences including intellectual disability, childhood hypotonia, progressive spasticity of lower limbs and abnormal craniofacial features in adults.

C03.2

De Novo loss of function mutations in *SETD5*, a novel methyltransferase gene within the 3p25 microdeletion syndrome critical region, cause intellectual disability

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To identify further Mendelian causes of intellectual disability (ID), we screened a cohort of 996 individuals with ID for variants in 565 known or candidate genes using a targeted next generation sequencing approach. Seven loss of function mutations (four nonsense variants- c.1195A>T, c.1333C>T, c.1866C>G, c.3001C>T and three frameshift variants- c.2175-76delCA, c.3770insG, c.3856delT) were identified in *SETD5*, a gene predicted to be a methyltransferase. All mutations were compatible with *de novo* dominant inheritance. The affected individuals had moderate to severe ID with additional variable features of brachycephaly; prominent high forehead with synophrys or striking eyebrows that are full and broad; a long, thin and tubular nose; long narrow upslanting palpebral fissures and large fleshy low set ears. Skeletal anomalies were a frequent finding including significant leg length discrepancy in two cases. Congenital heart defects, inguinal hernia or hypospadias were all reported. Behavioural problems were a prominent feature including an obsessive-compulsive disorder, hand flapping with ritualised behaviour and autism. *SETD5* lies within the critical interval for the 3p25 microdeletion syndrome. The *SETD5* mutation individuals have phenotypic similarity to those previously reported with a deletion in 3p25 and thus loss of *SETD5* may be sufficient to account for the clinical features observed in this condition. Our findings add to the growing evidence that mutations in methyltransferases that regulate histone modification are important causes of ID. This analysis provides sufficient evidence that loss of function of *SETD5* is a relatively frequent cause of ID (0.7%) and occurs as a rare *de novo* mutational event.

C03.3

Genetic heterogeneity in Hyperphosphatasia with Mental Retardation Syndrome due to mutations in PGAP3, a member of the GPI anchor synthesis pathway

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Different genes of the glycosylphosphatidylinositol anchor synthesis pathway, PIGV, PIGO, and PGAP2, have recently been implicated in hyperphosphatasia-mental retardation syndrome (HPMRS), also known as Mabry syndrome, a rare autosomal recessive form of intellectual disability with characteristic additional phenotypic features. We developed a diagnostic gene panel for targeting all known genes of the GPI-anchor synthesis pathway to screen patients matching these features, and detected compound heterozygous and homozygous mutations (c.439dupC, c.914A>G, c.314C>G) in PGAP3, a gene that is involved in the GPI-anchor maturation, in two unrelated patients with developmental delay, elevated serum alkaline phosphatase, seizures and particular facial anomalies. Our functional studies show that an impairment of the later GPI-anchor remodeling steps also causes HPMRS. In addition we analyzed the mutation spectrum of all four genes as well as the associated phenotypic spectrum in a large cohort of individuals diagnosed with HPMRS. Among this cohort biallelic PIGV mutations were identified in about 50 % of unrelated families whereas mutations of the other involved genes are less frequent. Our findings demonstrate that the severe end of the clinical spectrum presents as a multiple congenital malformation syndrome with a high frequency of Hirschsprung disease as well as vesicoureteral, renal, and anorectal malformations. At the other end of this spectrum HPMRS could present as apparently non-syndromic form of intellectual disability. Taken together with recent data, HPMRS displays a heterogeneous etiology caused by impairment of different steps of the GPI-anchor synthesis and is associated with a marked clinical variability regarding additional malformations and growth patterns.

C03.4

The significance of small copy number variants in neuro-developmental disorders

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Despite abundant evidence for pathogenicity of large CNVs in neuro-developmental disorders (NDDs), the individual significance of genome-wide rare CNVs <500 kb has not been well elucidated in a clinical context. By high resolution chromosomal microarray analysis, we investigated non-polymorphic exonic CNVs sizing 1-500 kb in a cohort of 714 patients with NDDs, of which 8.8% had an obvious large disease causing CNV. Excluding recurrent false positive sites, we detected 96 small CNVs, of which 58 (60.4%) could be confirmed by secondary testing.

Six of the confirmed de novo or likely de novo CNVs were clearly pathogenic affecting the recurrent microdeletion region in 17q21.31 or OMIM morbid genes (*CASK, CREBBP, PAFAH1B1, SATB2*). Two further de novo CNVs affecting single genes (*MED13L*, *CTNND2*) were instrumental in delineating novel recurrent conditions. In addition, two unreported homozygous deletions were found likely pathogenic: An intragenic deletion of *ACOT7* which likely defines a novel autosomal recessive disorder, and a deletion of the genes *PREPL* and *C2orf34* representing a new variant of the hypotonia-cystinuria syndrome. Four of the inherited CNVs affecting previously reported sites (16p11.2, *AUTS2*, *NRXN3*, *GRM8*) were also considered likely pathogenic. In total 14 (24.1%) of the small CNVs were categorized as pathogenic or likely pathogenic (median size 130 kb), nine (15.5%) as likely benign and the rest remained unclear. These results verifies the diagnostic relevance of genome-wide rare CNVs <500 kb (overall ~2%; 1.1% de novo, 0.3% homozygous, 0.6% inherited) and their inherent potential for discovery of new conditions enabling better characterization of NDDs.

C03.5

Rare large CNVs are associated with intellectual disability, education level, and female fertility in general population

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To investigate the burden of the rare large CNVs in the general population, we analyzed the Estonian Genome Centre cohort. It is a longitudinal, prospective, population biobank linked to comprehensive personal, educational, medical and daily life data of 5% of the Estonian adult population. Within a subset of 6819 individuals, we identified 60 carriers of known genomic disorder lesions, equivalent to a prevalence of 1% in the general adult population. Their phenotypes are reminiscent of those of carriers of identical rearrangements identified in disease cohorts. Importantly some of the associated traits appear to have been previously overlooked due to age-dependent penetrance.

We then generated the genome-wide map of rare autosomal CNVs and identified a total of 697 carriers of CNVs ≥ 250 kb with MAF ≤ 0.05%. While duplication carriers are equally distributed in both sexes, we observe a significant bias towards female carriers of deletion. This biased mutational burden supports the notion that females are somewhat "protected" from neurodevelopmental disorders. Cumulatively, these CNV carriers show a significant increase in the prevalence of intellectual disability and a decrease in the rate of higher-achieved education level, as well as a decrease of female carriers' fertility. In addition to CNV size, these effects are associated with the number and "quality" of encompassed genes and pronounced in carriers of deletions and duplications that encompass ≥3 and ≥10 genes, respectively. Our results suggest that large rare variants significantly impact life quality of carrier identified from non-clinical cohorts.

C03.6

Altered neuronal network in iPSC derived cortical neurons from patients with *MECP2* duplication syndrome

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We previously showed that increased dosage of methyl-CpG-binding protein-2 (MeCP2) leads to a severe neurodevelopmental disorder in males, designated as the *MECP2* duplication syndrome (MIM#300260). The increased dosage of MeCP2 is the result of a copy number gain at Xq28, including the *MECP2* gene and results in severe to profound neurodevelopmental delay with onset at birth, limited or absent speech, hypotonia, epilepsy, autistic behavior and motor dysfunction. We developed induced pluripotent stem cells from 3 patients with *MECP2* duplication syndrome carrying different duplication sizes, to study the impact of increased MePC2 dosage in human neurons. Differentiation of these iPSCs into neurons of cortical identity showed modulation in the expression of progenitor genes like *BLBP* and *FOXG1* and cortical genes like *RELN*, *CTIP2*, *TBR1* and *VGLUT*. Cortical neurons derived from Mecp2dup-iPSCs had more synapses, and altered network synchronization as well as dendritic complexity. Next, we tested a series of epigenetic drugs for the ability to rescue neuronal defects and validated two HDAC inhibitors as potential clinical candidates. Our model recapitulates early stages of the human *MECP2* duplication syndrome and represents a promising cellular tool to facilitate therapeutic drug screening for severe neurodevelopmental disorders.

C04.1

EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension

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Pulmonary veno-occlusive disease (PVOD) is a rare and devastating cause of pulmonary hypertension that is characterized histologically by widespread fibrous intimal proliferation of septal veins and preseptal venules and is frequently associated with pulmonary capillary dilatation and proliferation. PVOD presents either sporadically or as familial cases.
In the French referral centre for severe pulmonary hypertension, we have identified 13 PVOD families: 5 with a confirmed diagnosis based on histological studies and 8 with a highly likely diagnosis, based on clinical, functional, and radiological criteria. All PVOD families were characterized by the presence of at least two affected siblings and unaffected parents, suggesting that the disease segregates as a recessive trait.
We used a whole-exome sequencing approach and detected recessive mutations (homozygous or compound heterozygous) in the *EIF2AK4* gene that co-segregated with PVOD in all 5 families initially studied. We subsequently identified mutations in the 8 additional PVOD families. We also found biallelic *EIF2AK4* mutations in 5 of 20 histologically confirmed sporadic PVOD cases. All identified mutations disrupted the function of the gene. In conclusion, we identified the first gene responsible for PVOD. Biallelic mutations in *EIF2AK4* gene were found in 100% of familial cases and in 25% of sporadic cases of PVOD, making this new gene a major player linked to PVOD development. This discovery significantly contributes towards understanding the complex genetic architecture of pulmonary hypertension. Further studies are needed to decipher the molecular mechanisms underlying the responsibility of the EIF2AK4 gene deficiency in the development of PVOD.

C04.2

Rare variants in *NR2F2* cause congenital heart defects in humans

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Congenital heart defects are the most common birth defect worldwide and a leading cause of neonatal mortality. Left ventricular outflow tract obstruction (LVOTO) is an important subtype, with lesions on the most severe end of this spectrum being significantly life limiting. Although mutations in several genes have been associated with LVOTO in the majority of cases the cause is still unknown. In an individual with LVOTO and a balanced chromosome translocation, the highly conserved *NR2F2* was found to be interrupted. Whole mount studies in mice showed Nr2f2 to be expressed in the atria and in human fetal samples, expression was also confirmed in the heart including in atrial tissue. A further study conducted exome sequencing in 13 parent-offspring trios and 112 unrelated patients with nonsyndromic atrioventricular septal defects, a further important subtype of CHD for which the genetic architecture is poorly understood. Five rare missense variants (two of which arose *de novo*) were found in *NR2F2*, a very significant enrichment (P=7.7×10^{-7}) compared to 5,194 controls. A further two CHD families were identified with other variants in *NR2F2*; a *de novo* substitution disrupting a splice donor site and a 3bp insertion that co-segregated in a multiplex family. *NR2F2* encodes a pleiotropic developmental transcription factor, and decreased dosage of *NR2F2* in mice has been shown to result in abnormal heart development. Furthermore, using luciferase assays, it was shown that all six coding sequence variants observed in patients significantly alter the activity of *NR2F2* target promoters.
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C04.3

Loss of alpha1 beta1 soluble guanylate cyclase, the major nitric oxide receptor, leads to moyamoya and achalasia

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Moyamoya is a cerebrovascular condition of unknown mechanism characterized by a progressive stenosis of the terminal part of the internal carotid arteries (ICA) and the development of abnormal "moyamoya" vessels leading to stroke. We describe a novel autosomal recessive disease leading to severe moyamoya and early onset achalasia in 3 unrelated families. Using genetic linkage and exome sequencing we identified in all 3 families homozygous mutations of GUCY1A3, the gene encoding the alpha1 subunit of soluble guanylate cyclase (sGC), the major receptor for Nitric Oxide (NO). Platelet analysis showed a complete loss of the mutated protein and an unexpected stimulatory role of sGC within platelets.The NO/sGC/cGMP pathway is a major pathway controlling vascular smooth muscle relaxation, vascular tone and vascular remodeling. Our data suggest that alterations of this pathway may lead to an abnormal vascular remodeling process in sensitive vascular areas with low blood flow and shear stress,such as ICA bifurcations. These data provide treatment options for these patients. They also suggest that investigation of members of this NO/sGC/cGMP pathway is warranted in both non syndromic moyamoya and isolated early onset achalasia. Data accepted in the Am J Hum Genet.

C04.4

From Identification of Differing TIE2 Mutations with Distinct Cellular Characteristics in Four Types of Venous Anomalies towards a Murine Model and a Therapeutic Pilot Study

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Venous anomalies are composed of ectatic veins with irregular smooth muscle coverage. They are commonly cutaneous. They usually occur as a single lesion without family history (sporadic Venous Malformation, VM). Some sporadic patients have multifocal lesions (Multifocal Sporadic Venous Malformation, MSVM). In the sporadic Blue Rubber Bleb Nevus syndrome (BRBN), patients also have multifocal lesions; pathognomonic are rubbery palmoplantar lesions and those located in the GI-track. In rare cases, venous malformations are multifocal because of autosomal dominant inheritance (Mucocutaneous Venous Malformation, VMCM). VMs progressively expand causing deformity, pain and local intravascular coagulopathy. Despite sclerotherapy or excision, lesions often progress or recur.

We have identified activating mutations in the endothelial tyrosine kinase receptor TIE2 in all four forms. VMs are mostly due to a single somatic amino acid change L914F. MSVMs and BRBNs are due to double mutations in cis. The BRBN mutations are somatic, whereas MSVM mutations seem mosaic. Moreover, a distinct cis-mutation is seen in MSVM. The inherited VMCM is due to a germline mutation combined with a somatic second-hit. These clinico-genetic entities are reflected by phenotypic differences in cells overexpressing mutant receptors. Activation of AKT is yet a common phenomenon. The capacity to form lesions clearly resides in mutant endothelial cells, which when injected to immunodeficient mice generate lesions mimicking human VM. Interestingly, an mTOR inhibitor is able to deter lesion development. Finally, in our therapeutic pilot study comprising five patients with VMs refractory to standard-of-care, an mTOR inhibitor diminished pain, intravascular coagulopathy and improved quality of life.

C04.5

A high yield of variants with a putative role as modifiers in patients with hypertrophic cardiomyopathy

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Next Generation Sequencing enables simultaneous screening of multiple genes for multiple patients in a single run. We designed a panel of 111 genes known to be associated to CMs to study 94 unrelated patients (80 with Hypertrophic Cardiomyopathy, HCM; 18 with Dilatative Cardiomyopathy, DCM and 6 with Arrhythmogenic Cardiomyopathy, AC). Targeted resequencing was performed on Illumina platform (98,13% of the regions with a depth of coverage of 20X or more, mean coverage on target of 530X). A mean of 1016 variants were found for each patient. Rare (frequency <0.05), non-synonymous, loss- of- function and splice-site variants were defined as candidates. Pathogenic or likely-pathogenic variants were all confirmed by Sanger and cosegregation was tested when possible. Excluding titin missense variants, we identified 48 variants (27 novel) in sarcomeric or associated genes in 48/70 HCM patients (68%), with 14% of complex genotype. MYH7, MYBPC3 and TNNI3 resulted the high-yield genes; 19 additional candidate variants (13 novel) in desmosomal and ion-channel genes in 14 patients (20%) were identified in this group. We identified 10 candidate variants (7 novel) in 7/18 DCM patients (39%) and 5 candidate variants in 3/6 AC patients (50%). A targeted protocol allowed the identification of likely pathogenic variants in a large proportion of patients with CMs, irrespective of phenotype. The unexpected finding of rare non synonymous variants in desmosomal and ion-channel genes among HCM patients raises important issues regarding their role as previously unappreciated modifiers of the disease, potentially relevant to risk prediction and counseling.

C04.6

Causal relationship of body mass index with cardiometabolic traits and events: a Mendelian randomization analysis

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Elevated body mass index (BMI) associates with cardiometabolic traits on observational analysis, yet the underlying causal relationships remain unclear. We conducted Mendelian randomization analyses using 14 SNPs associated with BMI from a recent discovery analysis to investigate the causal role of BMI with cardiometabolic traits. We used eight population-based cohorts, including 34,538 individuals of European ancestry with 4,407 type 2 diabetes (T2D), 6,073 coronary heart disease (CHD) and 3,813 stroke cases. A genetically-elevated one kg/m^2 increase in BMI resulted in higher levels of fasting glucose, insulin, interleukin-6 and systolic blood pressure but reduced levels of HDL-C and LDL-C (values reported in Table). Apart from LDL-C, all causal estimates were directionally concordant to observational estimates. A genetically-elevated one kg/m^2 increase in BMI increased odds of T2D but did not affect risk of CHD or stroke. A meta-analysis incorporating published studies with 27,465 CHD events in 219,423 individuals yielded a pooled odds ratio of 1.04 (95%CI: 0.97, 1.12) per 1 kg/m^2 increase in BMI. In conclusion, we identified causal effects of BMI on several cardiometabolic traits, however whether BMI causally impacts on CHD risk requires further evidence.

Table. Causal estimates for the relationship of BMI with cardiometabolic traits and events.

Traits (units)	Studies (Individuals)	Regression coefficient (95%CI)
Metabolic		
Fasting glucose (mmol/l)	6 (20,677)	0.18 (0.12, 0.24)
Fasting insulin (% difference)	3 (12,758)	8.47 (5.94, 11.06)
Inflammation		
C-reactive protein (% difference)	7 (24,319)	12.00 (7.95, 16.19)
Interleukin-6 (% difference)	5 (9,885)	7.00 (4.01, 10.08)
Fibrinogen (% difference)	6 (19,041)	0.92 (0.25, 1.59)
Blood pressure		
Systolic blood pressure (mmHg)	6 (30,136)	0.70 (0.24, 1.16)
Diastolic blood pressure (mmHg)	6 (30,137)	0.28 (0.03, 0.52)
Lipids		
HDL-C (mmol/l)	6 (24,943)	-0.02 (-0.03, -0.01)
LDL-C (mmol/l)	6 (23,364)	-0.04 (-0.07, -0.01)
Triglycerides (% difference)	6 (24,761)	0.82 (-0.61, 2.27)
Surrogate marker of CHD		
Carotid intima medial thickness (% difference)	3 (6,260)	1.12 (-0.42, 2.68)
Events	**Studies (Cases/ Individuals)**	**Odds ratio (95%CI)**
Type 2 diabetes	7 (4,407/31,844)	1.27 (1.18, 1.36)
Coronary heart disease	7 (6,073/ 26,193)	1.01 (0.94, 1.08)
Stroke	6 (3,813/ 23,782)	1.03 (0.95, 1.12)

C05.1

Compound inheritance of a low-frequency promoter deletion and a null mutation in a new gene causes Burn-McKeown syndrome (BMKS)

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Burn-McKeown syndrome (BMKS) is characterized by cardiac defects, choanal atresia, sensorineural deafness and craniofacial dysmorphism and seems to be rare. X-linked or autosomal recessive inheritance was suggested.

We performed exome sequencing in five patients with BMKS and identified two heterozygous nonsense mutations in an autosomal gene (p.Glu117*; p.Glu13*) and one frameshift mutation (p.Val44Alafs*48). Screening for deletions in seven additional families revealed three large deletions (0.484, 1.164 and 4.7 MB), containing at least the entire candidate gene. Further three patients are currently being analysed. In two patients, no mutation was identified. All mutations and one deletion were inherited from a healthy parent, but in no case a second mutation was found in the coding region of the gene. Whole genome sequencing was performed in five patients, in all a 34 bp promoter deletion on the other allele of the same gene was identified. Segregation analyses for the promoter deletion revealed that all patients with a null mutation on one allele had the promoter deletion on the other allele. We screened 378 controls for the promoter deletion and found a minor

allele frequency of 1% for this population.
Preliminary results of primer extension analyses of RNA from peripheral blood suggest a negative effect of the 34 bp-deletion on the expression level of this allele. Further functional analyses are currently being performed.
In conclusion, our results indicate that BMKS is an autosomal recessive condition caused by an unusual mode of inheritance and highlight the importance of analyzing regulatory regions of causative genes.

C05.2

Genetic studies of mosaic birth defects affecting the skin by next-generation DNA sequencing

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A large group of sporadic developmental disorders involving the skin are or may be caused by postzygotic mutational events. Although mosaic birth defects traditionally rank among the most complex forms of monogenic diseases for gene discovery studies due to the challenges of detecting mutations present in only a fraction of cells, exome sequencing recently emerged as a powerful tool in the context of postzygotic mosaicism. In recent years, we and others have identified mutations of PIK3CA and other PI3K/AKT/MTOR-pathway genes in several mosaic overgrowth syndromes including megalencephaly-capillary malformation, hemimegalencephaly, fibroadipose hyperplasia, Proteus, CLOVES, and Klippel-Trenaunay syndromes. Somatic HRAS and KRAS mutations were reported in isolated and syndromic sebaceous nevi, and GNAQ mutations were associated with Sturge-Weber syndrome and port-wine stains. To further study the genetic basis of mosaic birth defects involving the skin, we ascertained and collected blood and skin specimens from > 100 unrelated individuals with patterns of skin lesions suggestive of cutaneous mosaicism. Systematic targeted deep sequencing of known causative genes on a MiSeq instrument (Illumina) in subjects with a clinical presentation consistent with the aforementioned syndromes resulted in a diagnostic yield over 50% and led to identification of variants with allelic fractions as low as 1%. Exome sequencing studies based on paired samples or trios in subjects with a clinical presentation of unknown genetic cause led to identification of two new genes underlying two mosaic skin pigmentation syndromes. These findings highlight the value of next-generation sequencing, both for research and genetic diagnosis of mosaic skin disorders.

C05.3

A point mutation in STIM1 (p.R304W) is associated with Stormorken syndrome

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Stormorken syndrome is a rare autosomal dominant disorder characterized by miosis, thrombocytopenia/thrombocytopathy, mild hypocalcaemia, muscle fatigue, asplenia, dyslexia and ichthyosis. Presence of tubular aggregates has also been reported in muscle biopsies of adolescent and adult patients with the disease. We diagnosed Stormorken syndrome in four patients from two unrelated families. Using targeted sequencing and whole exome sequencing we identified the c.910C>T transition in a *STIM1* allele (p.R304W) only in patients and not in their unaffected family members. Stromal interaction molecule 1 protein (STIM1) is a finely tuned endoplasmic reticulum (ER) Ca2+ sensor. The effect of the mutation on the structure of STIM1 was investigated by molecular modeling, and its effect on function was explored by calcium homeostasis experiments. We show that STIM1 p.R304W variant may affect the conformation of the inhibitory helix and unlock the inhibitory state of STIM1 molecule. Results obtained from calcium imaging experiments using transfected cells together with the fibroblasts from one affected patient were in agreement with impairment of calcium homeostasis. The p.R304W mutation probably causes a constant Ca2+ release activated Ca2+ channels (CRAC) opening leading to a permanent entry of calcium in many cell types. Our results are in agreement with already published models for STIM1 structure and activation. We conclude that p.R304W mutation in STIM1 may be the cause of the Stormorken syndrome.

C05.4

TashT is a novel mouse model that phenocopies both the variable penetrance and male sex-bias of Hirschsprung's disease

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Neural crest cells (NCC) are progenitors of diverse cell types such as peripheral neurons and glia as well as melanocytes. Via an insertional mutation screen for loci affecting NCC, we identified several mouse lines that combine defects in pigmentation and formation of the enteric nervous system. One of these lines, named TashT, displays an aganglionic megacolon phenotype in a subset of homozygotes and, most interestingly, almost exclusively in males. This is highly reminiscent of human Hirschsprung's disease, a neurocristopathy with an incidence of 1/5000 newborns and a currently unexplained 4:1 male-to-female bias.
We localized the TashT transgene insertion site in a gene desert containing multiple highly conserved elements on chromosome 10. Migration assays as well as time-lapse imaging showed that megacolon is due to defective NCC migration within the gut mesenchyme, a defect generally more severe in males than females. At the molecular level, RNAseq analysis of TashT enteric NCC notably revealed upregulation of many genes encoding secreted proteins and downregulation of several X-linked genes. This analysis also identified the novel gene Fam162b as a strong candidate for being the TashT causative gene. Fam162b is located near the transgene insertion site and reporter gene as well as 3C assays suggest that this TashT overexpressed gene is normally repressed in NCC via long range interactions with some of the highly conserved elements near the transgene insertion site.
Altogether, our results demonstrate that the TashT line represents a unique mouse model that will help understand the male sex bias of Hirschsprung's disease.

C05.5

WNT pathway downregulation and Cornelia de Lange Syndrome

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The cohesin complex is formed from a multi-subunit core and their associated regulatory proteins.
Genetic variants within components of the cohesin complex (NIPBL, SMC1A, SMC3, RAD21, PDS5, ESCO2, HDAC8) are believed to be responsible for a spectrum of human syndromes known as "cohesinopathies" that includes Cornelia de Lange Syndrome (CdLS), a multiple malformation syndrome affecting almost any organ and causing severe developmental delay. The cohesin complex has a canonical role in cell division and a non-canonical role in gene expression regulation. Cohesinopathies seem to be caused by dysregulation of specific developmental pathways downstream of mutations in cohesin components. However, it is still unclear how mutations in different components of the cohesin complex effect the output of gene regulation. In this study, zebrafish embryos and patient-derived fibroblasts were used to analyze abnormalities underlying CdLS, focusing on SMC1A, the second most commonly mutated gene responsible for the disease. We show that the knockdown of smc1a in zebrafish impairs neural development, increases apoptosis and specifically downregulates the canonical Wnt pathway that can be rescued by chemical activation suggesting a therapeutic potential for CdLS. The same downregulation of the WNT pathway is observed in SMC1A-mutated patient fibroblasts. Previously we have demonstrated that the haploinsufficiency of NIPBL, the cohesin gene mutated in Cornelia de Lange Syndrome, produces a similar phenotype in zebrafish and in CdLS patients. Finally, the double-knockdown of nipblb/smc1a in zebrafish shows a synergistic effect for the canonical role of cohesins in cell cycle and survival.

C05.6

Trio-based exome sequencing in ten unrelated cases of atypical CdLS

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Cornelia de Lange syndrome (CdLS) is a highly variable multisystem disorder with a broad phenotypic spectrum. Most typically-affected individuals carry de novo heterozygous loss-of-function mutations in NIPBL. Mutations in other components of the sister chromatid cohesion system, SMC1A, HDAC8, SMC3 and RAD21, result in phenotypes that overlap with CdLS but which can be highly atypical. We carried out trio-based exome sequencing in ten unrelated individuals with the diagnosis of atypical CdLS, who had previously been screened for intragenic mutations in the known CdLS genes and for genomic deletions or duplications. Using a likelihood-based approach, we identified nine de novo variants in eight cases, including de novo loss-of-function mutations in NIPBL (c.3150delA [p.Glu1050Aspfs*20]) and KMT2A (c.3649G>T [p.Glu1217*]), an essential splice-site mutation in PUF60 (c.604-2A>C) and a missense mutation in NAA10 (c.247C>T [p.Arg83Cys]). Furthermore, de novo, probably damaging missense mutations were identified in PIK3C3, PDCD6IP, UNC45A, NUP210 and CELF3. We then re-sequenced these genes in 85 of our mutation-negative CdLS and CdLS-like cases by AmpliSeq-Ion Torrent sequencing, which revealed three additional loss-of-function mutations in KMT2A. We also detected the aforementioned de novo mutation in NAA10 (c.247C>T [p.Arg83Cys]) in a similarly-affected, but unrelated individual. Molecular modelling suggests that the p.Arg83Cys conversion is likely to alter binding of NAA10 to acetyl CoA and would result in reduced acetylation activity of the protein. Our results highlight the genetic heterogeneity in atypical CdLS. They also demonstrate the phenotypic overlap between atypical CdLS and other dysmorphic conditions such as Wiedemann-Steiner syndrome as shown by molecular data.

C06.1

Resolving variants of unknown significance through reanalysis of 4,978 public RNA-seq samples

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In recent years, exome sequencing has emerged as a very effective strategy for genome diagnostics. However, the functional significance is unclear for many of the identified variants, hindering clinical interpretation. To improve upon this, we hypothesized that if a variant of unknown significance is affecting gene expression, it is more likely to be pathogenic (similar to what is known for common disease-associated SNPs, Westra et al, Nature Genetics 2013).

We therefore analysed publicly available RNA-seq data from 4,978 human samples from European Nucleotide Archive. We developed methodology to QC and harmonize the RNA-seq data and to account for differences in sequencing strategy, tissue differences and other (unknown) confounders. We subsequently called SNPs using GATK and imputed genotypes using BEAGLE. We assessed genotype quality using 462 samples for which both RNA-seq data and 1000G genotypes are available (Lappalainen et al, Nature 2013) and observed a 97% genotype concordance, indicating that RNA-seq is suitable for genotyping.

This enabled us not only to identify effects of common variants on the gene expression levels of 6,005 genes (cis-eQTLs), but also to identify the effects associations of rare variants and gene expression by assessing allele specific expression (ASE). We observed that many rare variants known to be pathogenic strongly associate with gene expression levels.

Since the amount of RNA-seq data that is available in public repositories is growing exponentially, we expect ASE analysis of rare variants will likely provide new tools to resolve many variants of unknown significance.

C06.2

The long non-coding RNA landscape of autoimmune diseases

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We have recently shown that the majority of predisposing autoimmune disease SNPs are intronic or intergenic and have the potential to be regulatory (Ricaño-Ponce & Wijmenga, 2013) by affecting expression of nearby genes (so-called eQTLs). It has become clear that non-coding RNAs are an important class of regulatory elements. Annotating autoimmune SNPs shows that close to 10% map to long non-coding RNA genes (lncRNA). Transcriptome analysis across 11 distinct immune cell types (granulocytes, monocytes, NK cells, B-cells, memory-T cells, naive CD4+ and CD8+ T-cells, and four CD4+ T-helper cell populations) revealed that these "autoimmune" lncRNAs are significantly enriched in immune cell types. We also correlated the autoimmune SNP genotypes with expression levels (eQTLs) of both coding genes and lncRNA genes and observed > 70% of the autoimmune SNPs to be eQTLs. Interestingly, ~16% of these eQTL SNPs also affect lncRNAs and as high as 6% of these eQTLs are specific to lncRNAs alone. To gain a first understanding on the biological processes in which these "autoimmune" lncRNAs are involved we performed pathway analysis based on co-expression profiles of lncRNAs and protein-coding genes using 5000 publicly available RNAseq samples. More than 90% of the autoimmune lncRNAs were significantly co-expressed with genes in cis (P = 0.009 to 4.64 x 10-92) that are at an average distance of 245 kb. Our results show that lncRNA-eQTLs represent a novel link between non-coding SNPs and the expression of genes, which can be exploited to understand the process of gene-regulation through lncRNAs in more detail.

C06.3

Population Scale Comprehensive Identification and Analysis of Complex Structural Variation Using Nanochannel Array

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Diseases are known to be associated with large (>1kb) genomic structural variation (SV). A variety of techniques such as karyotyping, FISH and array-CGH have been used for SV analysis. However, population scale comprehensive SV analysis remains impractical--too expensive or incomplete (exemplified by missing inversions and balanced translocations by aCGH methods). More recently, next generation sequencing (NGS) has been shown efficient for discovery of SNPs and small indels. However, complete and accurate SV discovery and analysis is complicated by the fact that variants often span tens to hundreds of kb or are rearranged throughout the genome, difficult to infer from short fragment sequencing. Thus, there is a blind spot in effectively detecting SVs within this range (1 kb ~ 1 Mb), referred to as the "dark matter" of the genome, overlooked in the past due to insufficient tools.

We demonstrate a new platform technology (Irys) to effectively linearize very long strands of gDNA (100 kb to Mbs) through nanochannels to directly visualize SVs and rearrangements preserved within intact and unamplified genomic DNA at single molecule level. De novo genome maps are assembled and hundreds to thousands of SV events are called. Data from individuals and trio families will demonstrate this highly comprehensive and cost effective approach, with results validated by multiple orthogonal methods.

For the first time, it is now feasible to do large population-based comprehensive structural variation studies using a single platform. This innovation will transform the diagnosis and treatment of diseases resulting from structural variants, particularly cancer.

C06.4

Chromatin loops and CNVs: the complex spatial organization of the 16p11.2 locus

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16p11.2 600 kb BP4-BP5 deletion and reciprocal duplication have mirror impact on body mass index (BMI), head circumference (HC) and autism spectrum disorder/schizophrenia (ASD/SCZ). The nearby distal 16p11.2 220 kb BP2-BP3 rearrangements are similarly associated with mirror phenotypes such as obesity/underweight and macro-/microcephaly, as well as neuropsychiatric disorders. We demonstrated chromatin interplays between these regions, likely via long-range acting regulatory elements, using high-resolution 4C-seq technology using the promoters of the SH2B1, MVP,

KCTD13, ALDOA, TBX6 and MAPK3 genes as "viewpoints". Between 172 and 354 Blocks of Regulators in Chromosomal Kontext are found per viewpoint. These 3-dimensionnally adjacent genomic regions encompass genes that encode proteins that interact together (P=1.69e-9) and have been associated with autism (P=0.008). A dramatic reorganization of these chromatin interacting networks is displayed in cells of carriers of 600 kb BP4-BP5 deletion or duplication. In parallel, we profiled the transcriptome of lymphoblastoid cell lines of 50 deletion, 32 reciprocal duplication and 29 control individuals and identified 2209 differentially expressed (DE) genes using a numerical variable to reflect a dosage effect. 566 DE genes show a concomitant significant change in chromatin interaction (enrichment P=0.007).
Our results show that relevant chromatin conformation changes may arise from copy number variants. They strongly suggest a link between the observed chromatin perturbations and changes in gene expression, with a possible contribution of the chromosome conformation to the disease phenotype.

C06.5
Informing rare disease mechanisms: informatics for the International Mouse Phenotyping Consortium

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The International Mouse Phenotyping Consortium (IMPC) is building the first truly comprehensive functional catalogue of a mammalian genome that will inform mechanisms of rare disease. The IMPC is coordinating efforts to generate a knockout mouse strain for every protein-coding gene. These mouse strains are characterized using a standardized, broad-based phenotyping pipeline and data is collected and archived centrally by the IMPC-Data Coordinating Centre. Dedicated 'data wranglers' are working with each phenotyping centre to ensure proper transfer and quality control of data. An automated statistical analysis pipeline identifies knockout strains with significant changes in phenotype parameters. Potential disease models are identified by orthologous gene and by orthologous phenotype features.
Over 3000 IMPC mouse strains have been produced, with emerging phenotype data available for hundreds of these strains. Users can freely access all data including new gene-phenotype via an intuitive web portal. Annotation with biomedical ontologies allows biologists and clinicians to easily find mouse strains with phenotypic traits relevant to their research. Users can register interest in genes so they may be informed as mouse models and new phenotype data become available. The community is invited to explore and provide feedback as we build this rich resource for rare disease research at:
www.mousephenotype.org

C06.6
Strategies for Exome Prioritization of Human Disease Genes

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Whole-exome sequencing has revolutionized rare disease research, with over 100 disease-gene identifications since the first published success in 2010. However, many cases remain unsolved due to the fact that ~100-1000 loss of function, candidate variants remain after removing those deemed as common, low quality or non-pathogenic. In some cases it may be possible to use multiple affected individuals, linkage data, identity-by-descent inference, trio analysis, or prior knowledge of affected pathways to narrow down to the causative variant. Where this is not possible or successful, one approach is to use model organism phenotype data to evaluate whether a variant is likely to result in the patient's clinical manifestations. We have developed an algorithmic approach (PHenotypic Interpretation of Variants in Exomes; PHIVE) to semantically compare clinical and mouse phenotypes. The output is combined with measures of variant candidacy such as pathogenicity and allele frequency and synergistically improves performance: the causative variant is identified as the top hit in up to 83% of exomes, with a 54-fold improvement over variant-based prioritization alone. A web implementation (Exomiser) is freely available.
Another complementary approach we have developed ranks exome candidates based on their proximity in protein-protein networks to genes shown, or are suspected, to be associated with the disease. Again, combining this with variant-based measures of candidacy leads to large improvements in performance with up to 47% of exomes having the causative variant as the top candidate.
We are currently testing our approach in large-scale projects such as the Undiagnosed Disease Program and CareForRare.

C07.1
LysoPlex: an efficient strategy to study the role of lysosomal-autophagic-endocytic pathway

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Lysosome is a control centre for cellular clearance and energy metabolism: it is responsible for the degradation of the foreign molecules and the endogenous macromolecules. The essential role of lysosomes in phagocytosis and autophagy puts these organelles at the crossroads of several cellular processes. To understand the role of the lysosomal-autophagic-endocytic pathway in health and disease, we have developed LysoPlex, a Next Generation Sequencing-based workflow to sequence at high coverage 12,786 human exons of 891 genes involved in lysosomal function, endocytosis and autophagy pathway. We designed the enrichment probes using a Haloplex custom platform targeting 99.48% of exons. To validate the methodology, we created a training set of 15 DNA samples belonging to patients affected by 14 different LSDs, whose the molecular diagnosis was already known. Using LysoPlex, we were able to detect all the known mutations. Moreover, we used our strategy to identify disease-causing mutations in 50 patients clinically diagnosed as affected by neuronal ceroid lipofuscinoses (NCL). About 50% of samples have causative mutations and a subset of additional variations in other genes not directly correlated with the disease was also identified in each sample. In conclusion, LysoPlex is a cheap and fast NGS targeted platform for the molecular diagnosis of Mendelian LSDs and it is a precious tool to have a complete view of sequence variants in the genes involved in the lysosomal-autophagic pathway. Moreover, it can also be used to identify causative or predisposing mutations in a variety of debilitating human conditions such as common neurodegenerative diseases.

C07.2
Comparing Clinical Exome Sequencing versus Whole Exome Sequencing for monogenic diseases and undiagnosed patients

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Since Whole Exome Sequencing (WES) demonstrated mutations in known disease genes in more than 30% of patients with severe non-specific ID (Rauch et al. 2012) and in 25% of mixed clinical patients (Yang et al. 2013) we investigated the diagnostic yield of NGS of large gene panels including all known disease genes (CES: "clinical exome sequencing") versus WES. So far we analyzed WES data of 99 unrelated patients with a variety of neurodevelopmental or congenital anomalies and CES data covering 2761 or 4813 known disease genes from 12 patients. WES revealed pathogenic mutations in known disease genes in a total of 23% of cases, which increased to almost 70% in cases with a precise clinical syndrome suspicion. Diagnostic yield of CES was 50% in total. Comparison of the average yield of ~135 private non-synonymous variants per case targeting the whole exome versus the ~65 for the 2761 and ~107 for the 4813 gene panel, indicates a more sensitive variant detection on the clinical disease panel. The increased sensitivity may be explained by the high average coverage of over 200x and a 10x coverage in almost 98% of the targeted region. Of 29 diagnosed cases, 41% represented autosomal dominant mutations, 52% were inherited in an autosomal recessive pattern and 7% had X-linked inheritance. In about 14% of WES cases in total, and in 31% of affected sib pairs we detected new possible disease causing candidate genes.
We conclude that CES is highly sensitive especially in cases with a syndromic clinical diagnosis.

C07.3
One generic automated workflow for both Sanger and ion semiconductor sequencing in routine DNA diagnostics

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Dideoxy-based chain termination sequencing developed by Frederick Sanger is the "gold standard" sequencing approach used by clinical genetic laboratories. Recently, new NGS technologies found their way into diagnostic

laboratories, enabling to sequence also large targeted gene panels or even complete exomes. These NGS approaches are normally used in parallel to conventional Sanger sequencing. The development of benchtop NGS machines now allows to analyze single genes or small gene panels using the new technologies, making these platforms increasingly competitive to Sanger sequencing.
Here we show that we have generated a generic automated ion semiconductor sequencing workflow which can be used in a clinical setting. Standard amplicon-based enrichment remains identical to the PCR for Sanger sequencing. A novel post-enrichment pooling strategy was developed, allowing to limit the number of library preparations and thereby reducing sequencing costs up to 60-70% compared to Sanger sequencing. A total of 1224 known pathogenic variants were used to perform a thorough validation, resulting in an analytical sensitivity of 99,92%,with a specificity of 99,99%. An additional study using a total of 100 patient-derived DNA samples was performed blinded and resulted in an analytical sensitivity of 99.60% and a specificity of 99.98%, comparable to Sanger sequencing. These data combined with the high scalability of our developed system makes it attractive to use ion semiconductor sequencing as our main mutation scanning technique, independent of the requested genes. We have therefore implemented this automated semiconductor sequencing workflow in our routine DNA diagnostics, and ISO15189 accreditation is in process.

C07.4
Setting sequencing thresholds for the use of next generation sequencing as a diagnostic tool

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Before replacing gene-by-gene Sanger sequencing with genome-wide NGS, it is essential that diagnostic laboratories investigate the relation between sequencing depth and test characteristics such as false-positive/negative rates, yield and coverage of relevant regions. In addition, capture bias needs to be evaluated when choosing between whole exome (WES) and whole genome sequencing (WGS).
To determine these factors we investigated 9 samples sequenced in diagnostic laboratories using both WES and WGS and 4 samples analysed by SNP-array and deep sequenced by WES. As intellectual disability (ID) is considered the primary indication for diagnostics using WES/WGS, we focused on a panel of ~400 ID genes. We observed no false-negatives in WES versus WGS for the ID gene panel region. In contrast, duplicated regions and regions with many variants in close proximity were problematic, suffering from increased false negative rates (> 1/100).
Subsequently we determined minimal sequencing criteria by applying GATK's experimental reference confidence score model on subsets of WES data (20-140*10^6 mapped reads). This revealed that (1) false positive rates are negligible, (2) false negative rates are below 1/1000, (3) the yield of identified variants is >95% with >30*10^6 mapped reads (~35X average depth), and (4) adequate depth can be reached for >95% of the bases of the ID gene panel with >60*10^6 mapped reads (~70X average depth) and >99% with > 140*10^6 mapped reads (~160X average depth).
We conclude that the applied methodology allows diagnostic laboratories to make informed decisions on sequencing criteria using few deeply sequenced WES datasets.

C07.5
EuroGentest guidelines for diagnostic next generation sequencing

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Next generation sequencing (NGS) is quickly being optimized for use in diagnostics. The technologies bring challenges at the technical level, in terms of data management and in the interpretation of results. Over the past 2 years, guidelines have been issued by the American, Australian, Dutch and British genetic professional societies. At EuroGentest, an expert group has been working on compiling, integrating and completing these guidelines. For instance, we believe that defining the 'diagnostic utility' of the NGS test is the laboratory's first duty when preparing to offer diagnostic NGS. Second, we introduce a scoring system for the different NGS assays depending on their quality and comprehensiveness. This is important for patients and clinicians to allow comparison of the diagnostic offer from the different laboratories. It could also be used by the health care system to evaluate and reimburse the tests. This scoring system is new, as it does not feature in any other guideline. As far as 'reportable range' is concerned, we propose the use of 3 specific percentages depending on the reference (technical target, coverage of transcript in a gene panel, coverage with reference to the genome) which will again allow to compare individual results within runs, between tests and between laboratories. The guidelines propose a template for reporting NGS results as well. Finally, the guidelines also deal with informed consent, unclassified variants and unsolicited findings, again from the laboratory standpoint. Similarly, they define the difference between research and diagnostics, with a practical solution to the 'duty to recontact'.

C07.6
Clinical exome sequence performance for reporting secondary genetic findings

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The American College of Medical Genetics and Genomics (ACMG) has recommended the reporting of clinically actionable incidental genetic findings in the course of clinical exome testing. Specifically, the pathogenic findings of 56 specific genes with known clinical importance should be reported. However, this assumes that exome sequencing returns data of sufficient quality using methods that were not validated for clinical use. To address this issue, we surveyed the potential false negative rate of mutations in the 56 ACMG genes. We retrospectively, analyzed forty-four exome datasets from four different exome capture kits and two-sequence platforms. The exome methods were examined for their ability to detect clinically relevant mutations in the 56 ACMG genes. A total of 17,774 pathogenic nucleotide variants are annotated in the Human Gene Mutation Database (HGMD) for the 56 genes, and data was examined for depth of coverage in the exome datasets. Overall, the four-exome methods had inadequate depth of coverage for accurate base calling ranging from 5.2% to 34.8% of the pathogenic variant positions. At least one gene in each exome method was missing >40% of the pathogenic variant positions. The worst performing method was predicted to miss >90% of clinically significant variant positions in the genes TMEM43, PCSK9, KCNQ1 and LMNA. The heterogeneous and occasional poor depth of coverage across this set of 56 genes illustrates the opportunity for further innovation in standardizing clinical NGS methods. Implementation of the ACMG incidental findings guideline requires recognition of the substantial possibility of reporting false negative results.

C08.1
Smc1a cohesin gene mutations in colorectal precancerous lesions

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Chromosome missegregation leading to chromosomal instability, is thought to play a pivotal role in cancer development. As major cohesin function is to assure correct chromosome segregation, increasing data suggests its involvement in tumorigenesis. Colorectal cancer (CRC) is a useful model for investigating the role of cohesin in carcinogenesis. CRC develops over the course of many years as a consequence of the accumulation of specific mutations in both oncogenes and tumor suppressor genes. These mutations arise in a characteristic sequence leading to early adenoma/dysplastic crypt, late adenoma and carcinoma. Two types of genomic instability have been identified in CRC: chromosomal instability (CIN), is present in around 85% of colorectal cancers, while the remaining 15% shows microsatellite instability (MSI). CIN was proposed as the major cause of cancer development more than 100 years ago, but its molecular mechanisms have not yet been completely defined. In this regard, the identification of gene(s) that gives rise to a CIN phenotype at an early stage of CRC development has been challenging. To this aim, we analyzed colorectal early adenomas and identified eleven mutations in SMC1A core cohesin subunit. Transfection of the SMC1A mutants identified in early adenomas and wild-type SMC1A gene silencing in normal human fibroblasts led to chromosomal instability. Since SMC1A is an X-linked gene, our finding suggests that a single allele mutation is enough to trigger chromosomal instability and tumorigenesis.
This work was supported by grants from Istituto Toscano Tumori and Associazione Italiana Ricerca sul Cancro to A.M.

C08.2

Comprehensive annotation of splice junctions supports pervasive alternative splicing at the BRCA1 locus: a report from the ENIGMA consortium

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Loss-of-function germ-line mutations in *BRCA1* (MIM #113705) confer markedly increased risk of breast and ovarian cancer. The full-length transcript codifies for a protein involved in DNA repair pathways and cell-cycle checkpoints. Several *BRCA1* splicing isoforms have been described in public domain databases, but the physiological role (if any) of *BRCA1* alternative splicing remains to be established. An accurate description of *naturally occurring* alternative splicing at this locus is a prerequisite to understand its biological significance. However, a systematic analysis of alternative splicing at the *BRCA1* locus is yet to be conducted.

Here, the Evidence-based Network for the Interpretation of Germ-line Mutant Alleles (ENIGMA) consortium combines RT-PCR, exon scanning, cloning, sequencing, and relative semi-quantification to describe naturally occurring *BRCA1* alternative splicing with unprecedented resolution. The study has been conducted in blood related RNA sources,commonly used for clinical splicing assays, as well as in one healthy breast tissue. We have characterized a total of 63 *BRCA1* alternative splicing events, including 35 novel findings. A minimum of 10 splicing events (Δ1Aq, Δ5, Δ5q, Δ8p, Δ9, Δ(9,10), Δ9_11, Δ11q, Δ13p, and Δ14p) represent a substantial fraction of the full-length expression level (ranging from 5% to 100%). Remarkably, our data indicates that *BRCA1* alternative splicing is similar in blood and breast, a finding supporting the clinical relevance of blood-based in vitro splicing assays.

Overall, our data suggests an alternative splicing model in which most non-mutually exclusive alternative splicing events are randomly combined into individual mRNA molecules to produce hundreds of different *BRCA1* isoforms.

C08.3

Germline mutations in MAP3K6 predispose to gastric cancer

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Mutations in the E-cadherin gene, *CDH1*, account for 40% of hereditary diffuse gastric cancer (HDGC) cases. The genes responsible for the remaining cases of HDGC, as well as other familial gastric cancers (FGC) are currently unknown. We examined a large family with FGC and no *CDH1* mutations using a combination of genetic mapping with high-density SNP arrays and exome sequencing and identified that the cancer is associated with a germline coding variant (p.P946L) in mitogen-activated protein kinase kinase kinase 6 (*MAP3K6*). A somatic second-hit (p.H506Y) was present in DNA obtained directly from a tumor specimen of an affected individual. Screening of 115 unrelated individuals with non-*CDH1* FGC identified the p.P946L variant, as well as four additional coding variants in *MAP3K6* (p.F849Sfs*142, p.P958T, p.D200Y and p.V207G). These probands were negative for mutations in another 50 genes with links to gastrointestinal cancers also being screened in this cohort. These findings, together with what is known from mouse models about the role *MAP3K6* plays in oncogenesis, and previous studies showing the presence of somatic mutations in *MAP3K6* in non-hereditary gastric cancers, indicate that *MAP3K6* variants are a predisposing factor for FGC.

C08.4

Germline mutations in SUFU cause Gorlin syndrome and redefine the risk associated with childhood medulloblastoma.

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Background Mutations in PTCH1 have been identified in approximately 70% of individuals with Gorlin syndrome. Methods Gorlin families were screened for PTCH1 mutations by DNA and RNA sequencing and MLPA. Selected cases without an identified mutation with more than one affected member underwent exome sequencing. Results PTCH1 mutations were identified in 42/70 (60%) of families fulfilling Gorlin syndrome criteria. Exome sequencing of four PTCH1 negative patients revealed a heterozygous germline SUFU mutation, c.550C>T (p.Gln184*), in one individual. Sanger sequencing of 23 further PTCH1 negative families identified a novel heterozygous SUFU missense mutation, c.544G>T (p.Asp182Tyr). MLPA screening of SUFU defined a deletion in one family, starting between exons 3 and 5 and removing the 3' end of the gene. All affected SUFU positive families contained a single case of medulloblastoma. Of 171 individuals meeting Gorlin syndrome criteria 115 (67%) have a PTCH1 mutation and 10 a SUFU mutation. Only two of 115 (1.8%) with a PTCH1 mutation have developed pathology proven medulloblastoma. Three of ten individuals from three families with SUFU-related Gorlin syndrome developed a medulloblastoma. Three cranial tumours occurred in follow up of two SUFU mutation cases, two meningiomas and a pilocytic astrocytoma. Conclusions The risk of medulloblastoma in Gorlin syndrome is reported as 5%. Our study redefines the risk of medulloblastoma in PTCH1 related Gorlin syndrome as ~2%, with a risk around 15 times higher in SUFU related Gorlin syndrome. We also show convincingly for the first time that SUFU mutations cause classical Gorlin syndrome.

C08.5

Evaluation of anti-cancer chemotherapy genotoxicity using a new p53 functional assay in human lymphocytes

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Most of the conventional anti-cancer chemotherapies inhibit proliferation and survival of cancer cells by inducing DNA damages, are potentially mutagenic and therefore might promote development of secondary tumours, in particular in patients with inherited cancer risk. The p53 pathway is activated in response to any genotoxic stress. Our team has recently developed a new functional assay of p53 in human lymphocytes in order to classify the different germline *TP53* mutations detected in Li-Fraumeni syndrome. We adapted this assay to develop an universal genotoxicity test based on specific detection of p53 pathway induction. This simple test is based on the exposure of human wild-type *TP53* lymphocytes to chemical or physical agents, then on the measurement by RT-QMPSF of p53 target gene expression. The specificity of the p53 pathway induction is demonstrated by the same experiment carried out in lymphocytes harbouring a heterozygous *TP53* mutation, which compromises the response to genotoxic stress. Using this test, we evaluated the genotoxicity of the conventional anti-cancer drugs commonly used. This analysis revealed that all classes of anti-cancer drugs are genotoxic, with the exception of the mitotic spindle poisons, such as paclitaxel or derivatives of vinca alkaloids. These results suggest that the remarkable high rate of multiple primary tumours observed in patients with Li-Fraumeni syndrome may be due in part to the mutagenic effect of chemotherapies, and that the use of microtubules poisons may be of particular interest in these patients. This new functional assay should facilitate the identification of new non-genotoxic anti-cancer molecules.

C08.6

Functional analysis of mismatch repair gene variants of uncertain significance and their possible contribution to Lynch syndrome

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Objective: Germline pathogenic mutations in DNA mismatch repair (MMR) genes and especially in MSH2 and MLH1 predispose to Lynch syndrome (LS). However, many of the found variants are of uncertain significance (VUS), which complicates their risk assessment and calls for functional analyses before predictive gene testing and genetic counseling are offered to a family. **Methods:** 5 MLH1 and 4 MSH2 variants were identified in a set of Italian families with suspected LS. The probands from these families were investigated by sequencing of the coding regions of the genes and by microsatellite instability (MSI) and immunohistochemical (IHC) analyses of MMR proteins on tumor samples. Functional significance of the 9 putative LS predisposing VUS was analyzed in an in vitro MMR assay and expression/stability of the mutated variants evaluated by Western blot analysis (WB). **Results:** As a result, 2 MLH1 and 3 MSH2 variants showed no repair in the assay and were assessed as pathogenic, whereas 3 MLH1 and 1 MSH2 variant repaired as the wild type protein and were assessed as proficient. Lowered protein expression patterns in WB as well as in silico analysis together with MSI and IHC data further supported their pathogenicity. **Conclusions:** The functional analysis together with clinical, tumor pathological and in silico analysis helped to confirm pathogenicity in 5 out of 9 putative Lynch syndrome variants.

C09.1

Functional analysis of SHANK2 mutations identified in schizophrenia patients

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Genetic variants in the *SHANK2* gene have been previously reported in patients with intellectual disability and autism spectrum disorder. Our study focused on the analysis of *SHANK2* variants associated with schizophrenia. We analyzed all exons and exon-intron boundaries of the ProSAP1A_AB208026 isoform of *SHANK2* by Sanger sequencing in a cohort of 481 schizophrenia patients (177 trios and 304 singleton patients) and 374 unaffected individuals. We detected ten missense variants that affect protein structure which are only present in the patient group.
We used mutation prediction tools that are based on evolutionary protein conservation and chemical properties of amino acids to select the four most promising variants. *In silico* investigation is helpful to estimate a functional relevance of mutations, but cannot substitute the functional analysis itself. To analyze the functional impact of the four selected mutations, we conducted overexpression and knockdown-rescue experiments in primary hippocampal neurons from rat with a major focus on morphological changes of the neurons. Another major point of our study was to investigate the effect of different *SHANK2* isoforms and schizophrenia mutations on the actin structures. We used COS-7 cells as a model system and live cell TIRF (Total Internal Reflection Fluorescence) microscopy for imaging of actin structures. Additionally, we also performed actin polymerization assays to measure *in vivo* F-actin/G-actin ratio in our mutants compared to SHANK2 wild type. With these functional tests we were able to show for the first time a functional effect of SHANK2 variants which were identified in schizophrenia patients.

C09.2

Exome sequencing of familial parkinsonism in Scandinavia

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Parkinson's disease (PD) is a progressive neurodegenerative disease, and the second most prevalent neurologic disorder of the elderly. Although ~14% of patients have a family history of parkinsonism in only a small proportion of such pedigrees have pathogenic mutations been identified to underlie disease susceptibility. The majority of causal and disease-modifying variants remain unknown. We report longitudinal clinical, genealogic and comparative sequence (exome) analyses of affected multi-generational families. Genome alignment, variant annotation and comparative analyses were used to identify shared coding mutations. Initially we have examined concordant/discordant variant sharing within multi-incident pedigrees (n=10), using Mendelian models and maximum parsimony. We subsequently inspected variant segregation with disease and extended these findings to unrelated patients (n=1500) and control subjects (n=1500) of Scandinavian descent. In two families missense mutations in NOVA2 and RPE65 were found to segregate with parkinsonism and were not observed in control subjects of Scandinavian origin. Both variants were subsequently genotyped in a multi-ethnic case-control series submitted by the GEOPD Consortium (n=5000). Interestingly, two additional patients with familial parkinsonism were identified as RPE65 carriers and no controls. Moreover, sequencing of entire coding region of RPE65 and NOVA2, in multi-ethnic probands with familial parkinsonism (n=100), led us to identify more missense mutations. Our data provides evidence for two novel genes in the etiology of PD.

C09.3

Genome-wide analysis of microRNA coding genes in bipolar disorder

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Bipolar disorder is a severe and highly heritable disorder of mood with a lifetime prevalence of about 1%. Molecular genetic studies have identified a number of susceptibility genes, with the relevant pathways, however, being still largely unknown.
microRNAs are a class of small non-coding RNAs. Accumulating evidence suggests that microRNAs contribute to basic mechanisms underlying brain development and plasticity thus suggesting their possible involvement in the pathogenesis of several psychiatric disorders, including bipolar disorder.
The aim of the present study was to systematically investigate whether common variants at all known microRNA loci (miRBase release 13.0) contribute to the development of bipolar disorder. We performed gene-based analyses for all microRNAs and +/- 20kb flanking sequences using VEGAS on the largest existing GWAS dataset of bipolar disorder comprising of 9,747 patients and 14,278 controls (Mühleisen et al., Nature Commun 2014). In this dataset we combined our data obtained from four European countries, Canada, and Australia with the GWAS results of the multinational Psychiatric Genomics Consortium.
Our analysis revealed that 98 of the 609 microRNAs showed nominally significant p values, indicating that bipolar disorder-associated microRNAs are enriched within the known microRNA loci (p=0.006). After correction for multiple testing, nine microRNAs (let-7g, miR-135a, miR-499, miR-581, miR-611, miR-640, miR-644, miR-708, miR-1908) showed a significant association with bipolar disorder. These included microRNAs known to be involved in neural development and synaptic plasticity.
Results from the investigation of the affected target genes and underlying regulatory networks supports these disease mechanisms and also suggests new mechanisms.

C09.4

Imbalance between excitation and inhibition in Neurons derived from *MECP2*, *CDKL5* and *FOXG1* iPSCs

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Rett syndrome is due to de novo mutations in *MECP2*, *CDKL5* or *FOXG1* genes. MeCP2 and FoxG1 are transcriptional regulators; *CDKL5* encodes for a

kinase protein involved in multiple cellular processes. In spite of their involvement in the same disease, a functional interaction between the three genes has not been proven and disease mechanism remain elusive. It has been suggested that an excitation/inhibition imbalance might play an important role in Rett pathogenesis. We have used a mouse model and a human model based on patients-specific iPSCs-derived neurons to test the hypothesis that in maturing neurons an initial excess of excitatory synapses might result in excitotoxicity finally leading to loss of excitatory synapses. We have established 3 iPSC lines for each gene. Quantitative RT-PCR on iPSCs-derived neurons demonstrated that *VGLUT1* is over-expressed while *GAD1* is down-regulated. Similarly, down-regulation of another inhibitory marker (*VGAT*) is observed in the mouse model. However, in this model a reduction of excitatory synapses (*VGLUT1*, *GluA1*, *GluA2*, *NR1*) is observed opposite to iPSCs-derived neurons.

Moreover, RNA and protein analysis shows an over-expression of *GRID1*, a member of the delta family of ionotropic glutamate receptors which induces preferentially inhibitory presynaptic differentiation of cortical neurons. Our data provide evidence in favour of excitotoxicity as an important contributing factor in Rett pathogenesis due to *MECP2* mutations and suggest a similar mechanism for *CDKL5*- and *FOXG1*-mutated patients. These results confirm the presence of an imbalance between excitation and inhibition thus providing novel insights into Rett pathophysiology.

C09.5

Left/right asymmetry genes are associated with handedness and appear relevant for neurodevelopmental disorders

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Humans display structural and functional asymmetries in brain organization, strikingly manifested through language and handedness. While we understand the biology of body asymmetries, the molecular basis of brain laterality is still unknown. We report a genome-wide association study (GWAS) for a quantitative measure of handedness and dexterity (pegboard) in individuals with dyslexia (n = 728). The most strongly associated variant, rs7182874 (P = 8.68×10^{-9}), is located in *PCSK6*, a gene known to activate NODAL, which is required to regulate left/right body axis determination. A novel approach for GWAS pathway analysis, based on gene-set enrichment strategies, showed that left/right asymmetry pathways are associated with handedness in both the dyslexia and a general population (n = 2666) cohorts. In particular, genes involved in corpus callosum development were enriched among the GWAS top hits. Furthermore, different markers at the PCSK6 locus were found to be associated with a measure of handiness in a completely independent study. The dyslexia-specific marker-trait association could be the result of an epistatic effect. Recent findings show that dyslexia candidate genes play a role in ciliogenesis, an important developmental process at the basis of left/right structural asymmetries determination. We propose that handedness is a polygenic trait controlled in part by the molecular mechanisms which establish left/right body asymmetry early in development, which in turn influence brain midline development and might be implicated in neurodevelopmental disorders. We are now investigating the molecular mechanisms underlying this association using neuronal stem cell and zebrafish models.

C09.6

Exome sequencing to disclose potential new pathogenetic variants in Rett patients without mutations in the known Rett genes

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Mutations in the X-linked gene MECP2 are the main cause of classical Rett syndrome (RTT), while 28% of patients with early onset seizures and a small percentage of those with congenital variant forms carry mutations in CDKL5 and FoxG1 genes, respectively. About 5% of classical and 40-50% of atypical RTT patient remain without a molecular diagnosis. Aiming at disclosing novel candidate genes , a cohort of 31 Rett patients with both classic and atypical phenotype and unascertained genetic defect was processed .on a HiScan Illumina platform by True Seq Exome Enrichment. As most mutations in the known RTT causative genes discovered up to day have a de novo origin, we adopted this model and filtered heterozygous variants, prioritizing those predicted to introduce a frameshift or stop codon or splice or missense changes. Prioritization of the interesting genes took into account their expression in Central Nervous System and/or neuronal function, previous reported involvement in a known genetic disease and the interaction to genes/protein responsible for a resembling phenotype. By this approach we have validated by Sanger sequencing 57 variants corresponding to 38 genes. In 8 cases parents analysis has proved the de novo origin of the SNVs, which occur in GABA receptors, Sodium and Potassium neuronal channels, synaptic vesicular releasing factor and a transcriptional regulator. Our data confirm exome targeted NGS as a promising tool to disclose novel pathogenetic mechanisms leading to RTT.

C10.1

PLS3 mutations in X-linked osteoporosis and fractures: unraveling a new bone regulatory pathway

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Osteoporosis with its consequences, i.e., fractures, is major health problem in ageing societies. As osteoporosis is a prevalent disorder, understanding its etiological factors is very important. We recently identified novel pathogenic variants in PLS3 (encoding Plastin 3 (PLS3), a filamentous-actin bundling protein) as a cause of X-linked osteoporosis and osteoporotic fractures in five Dutch families (van Dijk et al. NEJM 2013:369(16):1529-36). These loss-of-function variants cause decreased bone mineral density and increased risk of fracture in hemizygous young men whereas the clinical picture in heterozygous women ranged from asymptomatic to early-onset osteoporosis. It was highly unexpected that mutations in this gene would cause osteoporosis and fractures as it had never been described as a candidate gene for osteoporosis nor was it known to play a role in bone formation. However, results of in vivo analyses in zebrafish strongly supported a role for PLS3 as a bone regulatory protein. Furthermore, a rare variant (rs140121121) in PLS3 was found to be associated with a twofold increased fracture risk in elderly female carriers in the normal population indicating genetic variation in PLS3 as a novel etiological factor involved in common, multifactorial osteoporosis. However, the exact mechanism by which PLS3 mutations cause osteoporosis and fractures is unknown and currently subject of further investigations. Unravelling this new bone regulatory pathway is of great importance for understanding the aetiology of osteoporosis, increasing also the possibilities for prevention, diagnosis and treatment aimed at bone formation.

C10.2

Mutations in plastin 3 cause osteoporosis with fractures. Overexpression of PLS3 and other F-actin bundling proteins influence skeletal development in zebrafish and mice

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Osteoporosis affects a large proportion of the human population, particularly women after menopause. Recently we reported that pathogenic loss-of-function variants in the plastin 3 (PLS3) gene, localized on Xq23 are causative of familial osteoporosis with fractures. Furthermore, a rare variant in PLS3 (rs140121121) associated with a 2-fold increased risk for fractures among elderly heterozygous women in two large cohorts from Rotterdam (Van Dijk et al, NEJM, Oct 2013). PLS3 is an ubiquitously expressed actin-

bundling protein that largely influences the dynamics of the actin cytoskeleton. We demonstrated that PLS3 mRNA co-injection dose dependently rescued malformations of the craniofacial muscular-skeletal system, body axis and tail phenotype induced upon pls3 morpholino injection in 3 and 5 dpf col1a1::eGFP transgenic zebrafish. Remarkably, affected patients with PLS3 loss of function mutations only presented a bone phenotype. The absence of systemic manifestations has led us to hypothesise that other F-actin bundling proteins may compensate for the loss of PLS3 in other tissues. Interestingly, α-Actinin (ACTN) was found to be overexpressed in patients' fibroblasts, possibly preventing more severe disease manifestations. Indeed co-injection of ACTN1 or ACTN4 mRNA, rescued the muscular-skeletal phenotype induced by pls3 knock-down in fish. Moreover, analysis of femora by micro computed tomography in 3-month-old transgenic mice overexpressing human PLS3 showed significant differences in cortical and trabecular bone structures when compared to control mice. These results strongly indicate that PLS3 is a novel etiologic factor for monogenic and mutifactorial osteoporosis and PLS3 and other F-actin bundling proteins are important regulators of bone development and maintenance.

C10.3

XYLT1 mutations in Desbuquois dysplasia type 2

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Desbuquois dysplasia (DBQD) is a severe condition characterized by short stature, joint laxity and advanced carpal ossification. Based on the presence of additional hand anomalies, we have previously distinguished DBQD type 1 and identified CANT1 (calcium activated nucleotidase 1) mutations as responsible for DBQD type 1. We report here the identification of 5 distinct homozygous xylosyltransferase 1 (XYLT1) mutations in 7 DBQD type 2 cases from 6 consanguineous families. Among the 5 mutations, 4 were expected to result in loss of function and a drastic reduction of XYLT1 cDNA level was demonstrated in 2 cultured individual fibroblasts. No significant clinical or radiological differences could be found with the remaining 14 DBQD type 2 cases with unknown molecular bases. However, long term follow up of XYLT1 mutated individuals emphasizes the severity of the short stature (< -6 SD) contrasting with obesity, lower limb and foot deformities requiring often repeated surgeries and intellectual disability (5/7). Interestingly, respiratory distress was present at birth in 4/7 cases and spontaneously resolved in the first years of life but thorax narrowness persisted in the eldest children. Since xylosyltransferase 1 (XT-I) catalyzes the very first step in proteoglycan (PG) biosynthesis, we further demonstrated in the two individual fibroblasts a significant reduction of cellular PG content. Our findings of XYLT1 mutations in DBQD type 2 further support a common physiological basis involving PG synthesis in the multiple dislocation group of disorders. This observation sheds light on the key role of the XT-I during the ossification process.

C10.4

Mutations in endothelin 1 cause auriculocondylar syndrome and isolated question mark ears

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Auriculocondylar syndrome (ACS) is a rare craniofacial disorder involving micrognathia, condyle hypoplasia and question mark ear (QME). QME, which involves a defect in the fusion of the lobe and helix, can occur as an isolated anomaly. Recently, mutations in PLCB4 and GNAI3 were identified in ACS. Both genes are predicted to function in the endothelin 1 (EDN1)-endothelin receptor type A (EDNRA) signalling pathway during the development of the pharyngeal arches. Following exclusion of PLCB4 and GNAI3 in a series of ACS and QME patients, we performed exome sequencing in three unsolved families, and identified a mutation in EDN1 in each case. A fourth EDN1 mutation was identified by direct sequencing. Two of the four cases involved patients affected with ACS, born to consanguineous, healthy parents; these patients harboured homozygous missense mutations in EDN1, each predicted to interfere with enzymatic cleavage of the EDN1 pro-protein. The other two cases involved patients with dominantly-inherited isolated QME; they harboured heterozygous EDN1 mutations - a premature stop in one case and a missense mutation affecting a highly conserved residue of the mature EDN1 peptide in the other. The nature of the mutations and the different modes of inheritance suggest that heterozygous loss of function mutations in EDN1 cause isolated QME and that homozygous hypomorphic mutations cause ACS. These are the first reported mutations of EDN1 in humans and suggest that ACS and QME are a phenotypic continuum resulting from impaired EDN1-EDNRA signaling.

C10.5

Defects in TAPT1, involved in Axial Skeletal Patterning, Cause a Complex Lethal Recessive Disorder of Skeletal Development

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TAPT1 encodes Transmembrane Anterior Posterior Transformation-1 protein. TAPT1 is evolutionarily conserved, with similar orthologs from yeast to vertebrae. ENU mutagenesis of TAPT1 resulted in embryonic lethality of murine homozygotes, with posterior to anterior transformations of thoracic and lumbar vertebrae. The mechanism by which this ubiquitously expressed protein causes a specific patterning defect is unknown. We combined homozygosity mapping with exome sequencing for a Moroccan family with three lethal fetuses affected with fractures of ribs and long bones, undermineralized skull and axial skeleton, hydramnios with ascites and dilated ventricles. We identified a homozygous c.1108-1G>C mutation in TAPT1, causing in-frame skipping of exon 10. A second TAPT1 mutation was identified by direct sequencing in a Syrian pedigree with three lethal fetuses with fractures and multiple congenital anomalies of brain, face, heart and lungs. These probands were homozygous for a missense mutation in TAPT1 exon 9 (c.1058A>T, p.Asp353Val). In both families, a diagnosis of recessive osteogenesis imperfecta (OI) was initially proposed. Both variants affect the second luminal loop of TAPT1. Proband fibroblast type I collagen has broadened electrophoretic mobility of alpha-chains in the cell layer, suggesting abnormal post-translational modification of collagen and a relationship with OI forms. Immunocytochemical staining of dermal fibroblasts revealed co-localization of TAPT1 with the centrosomal protein gamma-tubulin at the basal body of the cilium, suggesting TAPT1 might have a ciliary function. The novel autosomal recessive skeletal defects reported here underscore the importance of the cilium in embryonic bone formation. Functional studies with siRNA, zebrafish and expression studies are ongoing.

C10.6

ZIC1 mutations cause coronal craniosynostosis and learning disability

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Craniosynostosis, the premature fusion of the cranial sutures, is a serious disorder affecting approximately 1 in 2,500 children. Presentation and underlying cause is very heterogeneous and approximately 25% of cases have a single genetic basis. Using trio-based whole genome sequencing we identified a de novo nonsense mutation (p.S388*) of ZIC1 in a patient with bicoronal synostosis and severe learning disability. Two further de novo nonsense mutations were identified by resequencing in our craniosynostosis panel; both cases had similar phenotypes to the original patient. A fourth ZIC1 mutation encoding a missense substitution, was identified in a 3-generation family with bicoronal synostosis and normal to mildly impaired intellectual function.
ZIC1 encodes a transcription factor which, when heterozygously deleted with its contiguous neighbour ZIC4, is associated with Dandy-Walker malformation rather than craniosynostosis. This suggests that the ZIC1 mutations identified in our study might act through a gain-of-function mechanism

to cause suture fusion. Interestingly, the 4 identified ZIC1 variants all cluster within 42 nucleotides of the terminal exon; analysis of fibroblast RNA from the first case showed that the mutant transcript escapes nonsense-mediated decay. To gain further insight into the molecular mechanism we are characterising the in vitro activity of the mutant proteins. Furthermore we show that in mouse embryos Zic1 is expressed in the supraorbital region at E11.5-E12.5, consistent with a role for ZIC1 at a very early stage of cranial suture biogenesis. Overall these findings confirm ZIC1 as a new disease gene in coronal craniosynostosis, particularly when accompanied by unexplained learning disability.

C11.1

Polygenic risk for ADHD is associated with impaired educational achievement and lower IQ in the general population

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Introduction
High levels of ADHD symptoms during childhood carry risk of worse academic performance and can impact on employment and earnings in adulthood. Polygenic score analysis was used to show that common risk alleles for clinical ADHD contribute to the risk of having higher ADHD symptoms in the general population (Martin et al. *in press*). We have used polygenic score analysis to investigate the contribution of common risk variants for clinical ADHD on educational performance and IQ in the general population.

Methods
Academic performance was assessed using results from Key Stage 3 national tests and externally marked GCSE examinations in 6,385 children from the Avon Longitudinal Study of Parents and Children (ALSPAC). Polygenic risk scores were calculated for ALSPAC children and their mothers based on the results of an ADHD GWAS (Stergiakouli et al. 2012).

Results
ADHD polygenic scores on the children were associated with worst educational outcomes as represented by both time points and also with lower IQ scores at age 15.5 (see Table). Moreover, ADHD polygenic scores on the mothers were associated with lower IQ in the mothers and worst educational outcomes in the children (see Table).

Discussion
Our results suggest that the same genetic variants that are relevant for an ADHD diagnosis are also implicated in impaired academic performance in the general population and lower IQ score in both children and adults.

Outcome	N	Beta coefficient (95% CIs)	p value
Score achieved at key stage 3	6385	-1.41(-2-(-0.81))	$4.2x10^{-6}$
Capped GCSE points	6298	-3.84(-5.96-(-1.72))	$4x10^{-4}$
IQ at age 15.5	3858	-0.77(-1.19-(-0.36))	$2.4x10^{-4}$
Mothers IQ	2313	-0.64(-1.2-(-0.08)	0.025

C11.2

Polygenic risk score analysis shows shared genetic aetiology between AN and five other psychiatric disorders

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Anorexia nervosa (AN) is marked by extremely low body weight and intense fear of gaining weight. We evaluated shared genetic determinants of AN and commonly comorbid psychiatric disorders (PsyD) by testing whether polygenic risk scores derived from genome-wide data of other PsyD can predict AN status. We obtained allele risk scores for major depressive disorder (MDD), bipolar disorder (BPD), autism (AUT), attention deficit hyperactivity disorder (ADHD) and schizophrenia (SCZ) from the Psychiatric Genomics Consortium (PGC). We divided each of these sets into 10 Pt significance level thresholds. The test set comprised AN cases and controls from a published Wellcome Trust Case Control Consortium 3 AN GWAS study. For every test set sample, we produced a polygenic risk score as a weighted sum of risk allele scores. Logistic regression was used to assess whether each PsyD polygenic score predicted AN case-control status. We computed pseudo R^2 values, and compared these to the values obtained when randomly permuting case-control status to measure the proportion of variance in AN explained by each PsyD risk score. Our pseudo R^2 values were ~0.5-1%, comparable to pseudo R^2 values in a recent PGC polygenic score analysis of five PsyD. We computed an empirical p-value for every significance threshold and found significant pseudo R^2 values at the lowest Pt threshold (Pt <0.001) for AN vs AUT (p=0.0009), MDD (p=0.009), and SCZ (0.0008), and at Pt <0.01 for BPD (p=0.0004). We demonstrated for the first time a shared genetic aetiology between AN and other PsyD using genome-wide data.

C11.3

Efficient estimation of pairwise genetic correlations between hundreds of quantitative traits from population samples of thousands of individuals

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Several modern technologies, such as nuclear magnetic resonance and mass spectrometry platforms in metabolomics, produce high-dimensional phenotype data on individuals. A first step towards utilising high-dimensional phenotypes in genetic studies is to understand how their genetic components are related.
Recent algorithmic advances in multivariate linear mixed models have enabled variance component estimation for pairs of traits using population samples of individuals and genome-wide panels of SNPs. However, current methods have not been tailored for situations where hundreds of traits are available on the same set of individuals. For such settings, we introduce an algorithm that efficiently decomposes pairwise phenotypic correlations into genetic and environmental components.
We illustrate our approach with an application to 105 pairs of metabolic and anthropometric traits measured on up to 14,000 Finnish individuals. For example, we estimate that the observed phenotypic correlation (-0.41) between triglyserides (TG) and HDL cholesterol decomposes into an additive genetic correlation (-0.59, s.e. 0.06) and an environmental correlation (-0.36 s.e. 0.02).
We discuss the interpretation of genetic correlations as correlations between locus-wise genetic effects and characterise settings where prior information about genetic correlation increases statistical power to identify pleiotropic loci, i.e. loci that contribute to multiple traits.

C11.4

The influence of genotype and phenotype data quality control on SNP based heritability estimates within and across studies.

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Currently, many GWA studies are accompanied by additional analyses to get an estimate of the amount of heritability explained by the GWAS SNPs. Such analyses in unrelated individuals critically depend on the construction of a Genetic Relatedness Matrix (GRM) as implemented for example in the software package GCTA [1]. What sometimes seems a bit surprising is the large variation in the obtained point estimates between studies. Although heritability is a population specific characteristic, these GRM-based heritabilities seem more variable than e.g. heritabilities based on twin data. Using genotype data and a wide range of phenotypes from two large population based studies in the Netherlands (NESDA: Netherlands Study of Depression and Anxiety and NTR: Netherlands Twin Register), in addition to a set of simulated phenotypes, we examined the effects SNP selection based on GWAS QC, relational filters and phenotype transformations. For continuous traits, the results show that the GRM method is sensitive to SNP selection criteria, with - or without imputations, as well as deviation of phenotypes from the assumed normal distribution. For dichotomous traits, additional issues including non-random missingness of genotypes, play a role. Thus, a substantial part of the variation in GCTA heritability estimates may depend on data quality control and the selection of study individuals prior to the analysis.
1. GCTA: a tool for genome-wide complex trait analysis. Yang J, Lee SH, Goddard ME, Visscher PM. Am J Hum Genet. 2011 Jan 7;88(1):76-82. PMID: 21167468.

C11.5

Co-regulated transcripts associated to cooperating eSNPs define bi-fan motifs in human gene networks

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Associations between the level of single transcripts and single corresponding genetic variants, eSNPs, have been extensively studied and reported. However, most expression traits are complex, involving the cooperative action of multiple SNPs at different loci affecting multiple genes. Finding these cooperating eSNPs by exhaustive search has proven to be statistically challenging. In this paper we utilized availability of sequencing data with transcriptional profiles in the same cohort to identify two kinds of usual suspects: eSNPs that alter coding sequence or eSNPs within the span of transcription factors (TFs). We devised a computational framework for examining pairs of such cooperating source eSNPs that are both associated with the same pair of target transcripts. We characterize such quartets through their genomic, topological and functional properties. We establish that this regulatory structure of cooperating quartets is frequent in real data, but is rarely observed in permutations. eSNP sources are mostly located on different chromosomes and away from their targets. In the majority of quartets, SNPs affect the expression of the two gene targets independently of one another, suggesting a mutually independent rather than a directionally dependent effect. Furthermore, the directions in which the minor allele count of the SNP affects gene expression within quartets are consistent. Same-effect eSNPs are observed more often than expected by chance. Cooperating quartets reported here in a human system might correspond to bi-fans, a known four nodes network motif previously described in model organisms. Overall, our analysis offers insights concerning the fine motif structure of human regulatory networks.

C11.6

Inferring the human embryonic selection via genomic data

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Selection constantly filters out genomes with an excess of slightly deleterious variants (SDVs) maintaining the fitness of human population. In case of consanguineous marriages high level of homozygosity, observed in offspring, uncovers the damaging effect of many SDVs variants making the purifying selection more effective. We hypothesize that healthy offspring of consanguineous marriages carry a deficit of these variants as a result of the embryonic selection acting against the high genetic load. Using 50 genotyped pedigrees with consanguineous marriages we revealed a signature of purifying selection. First, by means of modified transmission disequilibrium test we observed a genome-wide deficit of rare derived alleles, transmitted from consanguineous parents to their healthy offspring. Because rare derived alleles are enriched in deleterious variants we assume, that the deficit of rare derived alleles could be a signature of embryonic purifying selection, which filters out low-fitted genomes with high load of mutations. Second, we expect that haplotypes, which are rarely seen in long Runs Of Homozygosity (ROH) harbor loci with high load of recessive mutations. Comparing the density of slightly deleterious variants inside and outside ROH we present an approach and the first results describing transmission of haplotypes identical by descent in consanguineous marriages. Further investigation of the genetic load of long ROH and the pattern of their inheritance can help to allocate mutations causing recessive autosomal diseases.

C12.1

Next generation sequencing as a reliable and efficient technique to identify mutations in patients with retinal dystrophies

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Retinal dystrophies (RD) constitute a group of blinding diseases that show clinical variability and genetic heterogeneity. More than 20 different clinical diagnoses with over 200 genes have been associated with RD. This enormous heterogeneity is an obstacle to identify disease-associated mutations for any diagnostic- and research-based approach.
Next generation sequencing represents the most efficient technology to identify mutations in RD patients and families. In this study, we performed both, panel-based sequencing of 105 RD-associated genes for diagnostic purposes and exome sequencing for research cases.
We developed a diagnostic NGS pipeline to identify mutations in 170 genetically and clinically unselected RD patients. Underrepresented regions were examined by Sanger sequencing. We found mutations in 50 to 60% of retinitis pigmentosa and approximately 80% of syndromic (Bardet-Biedl or Usher syndrome) cases. Seventy-one novel mutations in 40 genes were identified, among which the genes USH2A, EYS, ABCA4, and RHO were more frequently affected by pathogenic sequence alterations. Occasionally, cases carried mutations in more than one RD-associated gene suggesting modifier effects of the additional variants. We further found possible dominant de-novo mutations in sporadic RD cases imposing difficulties for counseling of patients and families. Exome sequencing showed similar detection rates in 11 additional research-based RD cases confirming that both approaches are efficient to identify mutations in known disease genes. In contrast to panel sequencing, exome analysis provided the bases to search for gene candidates for RD.
In conclusion, NGS-based mutation analyses provide reliable and cost-efficient approaches to investigate genetically heterogeneous diseases like RD.

C12.2

New Hereditary hearing loss (HHL) genes/mutations identified by High throughput sequencing and genotyping in the Italian and Qatari populations.

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To overcome the remarkable genetic heterogeneity of HHL an extremely powerful 3 steps "gene-identification strategy" was designed. STEP1 consists in a screening of 96 HHL genes by targeted re-sequencing (TS) on Ion Torrent PGM™ (3487 amplicons). Positive cases contribute to define an accurate molecular epidemiology picture while negative ones undergo a combination of STEP2 (linkage studies) and STEP3 (whole exome sequencing) or directly STEP3 (depending on pedigree size).
Illumina protocols were used for STEP2 and STEP3. Sequencing variants were annotated/filtered according to standard pipelines. This strategy was applied to a first series of 28 Italian and Qatari families. STEP1 characterized 43% of all families and led to the identification of several novel alleles in known HHL genes (Table1a). STEP2 identified four new HHL loci (Table1b), while STEP3 had already led to the discovery of 2 new genes (BDP1 and TBLY1) and it is in progress in the remaining 14 families. Briefly, p*2625Gluext*11 mutation resulting in an elongation of 11 residues of the BDP1 protein was identified in a recessive family. Bdp1 inner ear expression was confirmed by immunohistochemistry. In a pedigree showing a Y-linked pattern, the predicted pathogenic D69V mutation was identified in *TBLY1* gene. Functional studies are in progress to confirm its pathogenicity. This strategy proved to be very successful by explaining several unsolved cases. Updated data will be presented and discussed

Table 1

a	Origin	inheritance	cDNA change	Amino acid change	Gene
2nd mutation described worldwide	Italy	dominant	c.1057G>C	pG353R	*P2RX2 (Faletra et al. 2013)*
Novel mutation	Italy	dominant	c.775G>C	p.G259R	*TECTA*
Novel mutation	Italy	recessive	c.1019C>G/c.1291C>T	p.T340R/p.P431S	*TMPRSS3*
Novel mutation	Qatar	recessive	c.1588G>T	p.E530X	*LOXHD1*
Novel mutation	Qatar	recessive	c.453_455delCGAinsTGGACGCCTGGTCGGGCAGTGG	p.E152GfsX81	*MYO15A*
Novel mutation	Italy	recessive	c.G989A	p.R330K	*POU3F4*
Novel mutation	Qatar	recessive	c.G1334A	p.R445H	*TMC1*
Novel mutation	Qatar	recessive	c.T1808A	p.L603H	*TMC1*
Novel mutation	Qatar	recessive	c.G634A	p.G212S	*TRIOBP*
Novel mutation	Qatar	recessive	c.C2170T	p.R724C	*OTOF*
Novel mutation	Qatar	recessive	c.A1837G/c.T2A	p.M613V/p.M1K	*LOXHD1*
Novel mutation	Qatar	recessive	c.C1294G	p.L432V	*WFS1*
b	**Markers**	**Chromosome**	**LOD SCORE**		
New locus	RS7388463 to RS12678154	chr8	3.327		

New locus	RS3128516 to RS893918	chr9	4.1413		
New locus	RS17093352 to RS10137845	chr14	4.2		
New locus	RS6583338 to RS2191041	chr7	2.1		

C12.3

Disclosure of false disease genes - an underestimated potential of targeted and genomewide NGS: The example of MYO1A and deafness type DFNA48

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MYO1A is considered the gene underlying autosomal dominant non-syndromic hearing loss type *DFNA48*, based on six missense variants, one small *in-frame* insertion and one nonsense alteration. By next-generation sequencing (NGS) targeting 66 deafness genes in 109 hearing-impaired patients, we identified three families (including a consanguineous one) that provide strong evidence against a causative role of *MYO1A* in inherited deafness: Two novel nonsense mutations (p.Tyr740* and p.Arg262*) and a previously described missense mutation were identified not only in the index patients in heterozygous state, but also in unaffected relatives. The hearing deficit in these families was clearly due to mutations in other deafness genes, *MYO7A*, *EYA1* and *CIB2*, respectively. All but two of the altogether ten *MYO1A* mutations have been annotated in dbSNP, and population frequencies (dbSNP, 1000 Genomes, Exome Sequencing Project) above 0,1% contradict pathogenicity assuming a dominant model. Moreover, one healthy individual from the consanguineous family was homozygous for the nonsense mutation p.Arg262*, compatible with a previously reported homozygous *Myo1a* knockout mice lacking any overt pathology. We conclude that *MYO1A* is dispensable for normal hearing and may even represent a non-essential gene, adding to the list of „erroneous disease genes" that is growing rapidly with increasing availability of data from large-scale sequencing projects. Data from individuals born to consanguineous parents are particularly valuable because they are enriched for homozygous loss-of-function variants, with the potential to unmask „false disease genes". Their identification is urgently needed to improve mutation databases and avoid pitfalls in diagnostics and genetic counseling.

C12.4

AON intravitreal injections to manipulate splicing in retinal cells

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Purpose: Leber congenital amaurosis (LCA) is the leading cause of hereditary blindness in children. *CEP290* encodes a ciliary protein important to photoreceptor connecting cilium assembly and function. The *CEP290* intronic c.2991+1655A>G change is the most common LCA-causing mutation (10%). It introduces a cryptic exon in the mRNA encoding a premature termination codon. Recently, we made the proof-of-concept of antisense oligonucleotides (AON)-mediated exon skipping to correct the splicing in patient fibroblasts which recovered ability to ciliate. The purpose of this study was to make the proof-of-concept of exon-skipping *in vivo* using intravitreal injections of AONs. **Methods:** AONs were designed to skip mouse *Cep290* exon 36. Variable concentrations of 6-FAM-AONs were injected into the vitreous of C57BL/6J mice. Retinal sections and mRNA were prepared during 30 days post-injections (dpi) to follow *i)* the distribution of oligonucleotides across cellular layers and *ii)* exon skipping efficiency. **Results:** The most efficient AONs identified by *in vitro* analyses were injected in the vitreous of animals. Excellent correlation between the efficiency of exon skipping and AON injected dose has been demonstrated. Histological analyses revealed a wide distribution of AON in all retinal cell layers at least until 30 dpi. A linear amount decrease of mRNA lacking exon 36 was measured but still detectable at 30 dpi. **Conclusion:** Here we report that single intravitreal injection of AON allows efficient and persistent exon skipping in retinal cells. Hence, this strategy may be regarded as an attractive alternative to gene replacement therapy for 10 % of patients affected with LCA.

C12.5

Mutations in the tricarboxylic acid cycle enzyme, Aconitase 2,cause either isolated or syndromic optic neuropathy with encephalopathy and cerebellar atrophy

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Inherited optic neuropathy has been ascribed to mutations in mitochondrial fusion/fission dynamic genes, nuclear and mitochondrial DNA-encoded respiratory enzyme genes or nuclear genes of poorly known mitochondrial function. On the other hand, enzymopathies of the tricarboxylic acid cycle (TCA) have been reported to cause severe encephalopathies or isolated retinitis pigmentosa, but no TCA-cycle enzyme deficiency has been hitherto reported in isolated optic neuropathy. Studying a series of five patients with optic atrophy, we found homozygous or compound heterozygous missense and frameshift mutations in the gene encoding mitochondrial aconitase (ACO2), a TCA-cycle enzyme, catalyzing interconversion of citrate into isocitrate. Retrospective studies using patient-derived cultured skin fibroblasts revealed various degrees of deficiency in ACO2 activity but also in ACO1 cytosolic activity. Our study shows that autosomal recessive ACO2 mutations can cause either isolated or syndromic optic neuropathy. This observation supports the view that optic atrophy is a hallmark of defective mitochondrial energy supply and that extra-ocular involvement is not related to severity of the enzyme deficiency.

C12.6

Isolated foveal hypoplasia with secondary nystagmus and low vision is associated with a homozygous SLC38A8 mutation

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Foveal hypoplasia, always accompanied by nystagmus, is found as part of the clinical spectrum of various eye disorders such as aniridia, albinism and achromatopsia. However, the molecular basis of isolated autosomal recessive foveal hypoplasia is yet unknown. Individuals of apparently unrelated non consanguineous Israeli families of Jewish Indian (Mumbai) ancestry presented with isolated foveal hypoplasia associated with congenital nystagmus and reduced visual acuity. Genome-wide homozygosity mapping followed by fine mapping defined a 830 Kb disease-associated locus (LOD score 3.5). Whole-exome sequencing identified a single missense mutation in the homozygosity region: c.95T>G, p.(Ile32Ser), in a conserved amino acid within the first predicted transmembrane domain of SLC38A8. The mutation fully segregated with the disease-associated phenotype, demonstrating an ~10% carrier rate in Mumbai Jews. SLC38A8 encodes a putative sodium-dependent amino-acid/proton antiporter, which we showed to be expressed solely in the eye. Thus, a homozygous SLC38A8 mutation likely underlies isolated foveal hypoplasia. Intracellular localization of SLC38A8 using confocal microscopy indicated that SLC38A8 resides within the nuclear envelope and in the Golgi apparatus as well. The sub-localization of SLC38A8 is different than that of other SLC38 proteins, as all other members of this family are cytoplasmic transmembrane proteins. This may suggest that SLC38A8 has a different function altogether, and may not be an amino acid transporter as previously predicted. The precise role of SLC38A8 in normal eye development and function, and the molecular mechanisms through which its mutation causes foveal hypoplasia are yet to be elucidated.

C13.1

Stratified cancer screening in Europe using genomic information: conclusions and recommendations from the COGS project

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As part of the Collaborative Oncological Gene-environment Study (COGS), we investigated using genomic and other information to estimate individuals' risk of developing cancer and offering different screening and other preventive interventions according to the results. We concluded that genetic testing may soon be used in risk-stratified screening for breast and prostate cancer and that policy-makers should prepare for this.
Stratified screening could produce high rates of diagnosis and early treat-

ment, while sparing lower risk, disease-free people the risks and inconvenience of screening, and reducing costs. Achieving this with genomic information is attractive and increasingly feasible.
We will describe how this approach can be implemented, including how the screening offer would be made, how risk would be estimated, the age at which this could occur and the potential use of genetic data for other purposes. We will describe how management might differ depending on individuals' risk, communication of results and follow-up arrangements, and the different issues raised by modification of an existing screening programme (breast cancer) and the establishment of a new one (prostate cancer).
These issues will need careful handling to ensure outcomes are optimal and harms minimised.
Although we do not think the evidence is yet adequate to support risk-stratified screening for breast and prostate cancer, we believe that point will soon be reached. Further research is needed into impact, utility, cost-effectiveness, acceptability and ethical, legal and social implications. A critical factor may be whether targeting resources according to risk is seen as acceptable by the entire screening population.

C13.2

Expanding access to genetic counseling for hereditary breast and ovarian cancer with telephone delivery: A cluster randomized noninferiority trial

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Geographic barriers limit access to in-person cancer genetic services. We conducted a cluster randomized trial with high-risk women to test the equivalency and noninferiority of *BRCA1/2* telephone counseling to remote in-person counseling. 988 women, 25-74 years of age, with a personal or family history of breast and/or ovarian cancer enrolled. Participants were randomized by family unit to either telephone or in-person counseling. Assessments were done 1 week following pre-test and post-test counseling, and at 6 months. Cluster bootstrap methods were used to estimate the 95% confidence interval (CI) for the difference between test uptake proportions. Between-group intervention differences in psychosocial outcomes were estimated using linear models together with one-sided 97.5% CIs. Telephone *BRCA1/2* genetic counseling fulfilled the criteria for noninferiority to in-person remote counseling with regard to anxiety, cancer-specific psychological distress, and quality of life measures, as well as assessments of the informativeness, interpersonal sensitivity and partnership building of genetic counselors for both urban and rural dwellers. *BRCA1/2* testing uptake was lower following telephone (21.8%) than in-person pre-test counseling (31.8%) (CI=3.9%-16.3%). However, in-person counseling had higher average cost per participant counseled than telephone counseling ($654 vs. $278). The higher uptake of testing in the rural (35.1%) compared to the urban (25.2%) subgroups suggests that the genetic screening interests of rural populations may be underserved by existing health care systems. *BRCA1/2* telephone counseling appears to be safe and as effective as in-person counseling with regard to minimizing adverse psychological reactions and delivering patient-centered communication for both rural and urban dwellers.

C13.3

New approaches to bridge the gap between genetics research and primary health care in Ireland

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The translation of research findings on rare disorders from the laboratory into hospital practice can be challenging. Translation into primary healthcare is even more problematic due to lack of funding, infrastructure and communication with primary care staff. Through funding specific for dissemination, we have been able to engage with healthcare professionals (HCPs) using a multi-faceted approach. We developed a microsite (http://www.ucd.ie/medicine/rarediseases/), animation videos, guidelines handbook, Bioinformatics course and four nation-wide educational seminars.
The animation videos were designed for HCPs to help explain inheritance patterns and to educate about consanguinity. These graphical educational tools are being used by HCPs to overcome poor literacy in some families. We liaised with HCPs to develop a guidelines handbook to help with the management of common genetic disorders at a local level. We produced a series of common clinical scenarios that HCPs might encounter to help with advising on relative risk and genetic testing procedures. The two-day Clinical Bioinformatics course aimed to update our laboratory staff to enable translation of our research findings into our local diagnostic laboratory.
Our educational seminars covered topics such as carrier testing, intellectual disability, consanguinity, rare disease research and the role of advocacy. Discussions with seminar attendees suggest that there is a significant knowledge deficit when it comes to genetics in healthcare. However, encouragingly, HCPs voiced an overwhelming interest in gaining more knowledge about genetics and its application to their practice. We plan to continue our efforts and develop eLearning tools to support the integration of genetics into mainstream medicine.

C13.4

Unanticipated results in whole exome study: we've still a lot to learn

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Mutations in SCN5A are associated with Brugada syndrome, LQTS, atrial fibrillation and other conditions with risk of sudden cardiac death. Evidence-based guidelines exist to significantly improve or avert the mortality and morbidity associated with these conditions. As such the ACMG included SCN5A on its list of incidental findings which it recommends should be returned as part of all whole-exome/genome sequencing tests. We outline three challenges faced in the return of unanticipated SCN5A variants: (1) Defining pathogenicity in the presence of reduced penetrance and a highly heterogeneous grouping of conditions; (2) Balancing potential psychosocial harm to patient/family with result disclosure vs. possible medicolegal ramifications of not reporting this type of results; and (3) Psychosocial impact of a risk for sudden cardiac death in the absence of a personal or family history context. Through the NCGENES whole exome sequencing study, these challenges are being explored in a cohort of ~500 patients. Incidental, medically actionable findings are reported and this is emphasized during the pre-test counseling. We present here our experience with incidental finding of SCN5A mutations detected in 8 of 145 (5.5%) participants. Each variant, previously reported as pathogenic, was independently reviewed and discussed with members of the molecular analysis team to reach consensus regarding interpretation of its pathogenicity. Upon review we determined there was convincing evidence for only one to be considered pathogenic and reported. The remaining were not reported, though future information may change the assessment of these interpretations.

C13.5

The stepping stone approach towards the Genetics Clinic of the Future

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Routine application of genomics in the clinic faces many cross-cutting, controversial challenges. To map them, we have introduced the concept of the Genetics Clinic Of the Future (GCOF). Moreover we have developed a stepping stone approach to meet these challenges, comprising of field laboratories (the 'stepping stones') that address controversial, multi-dimensional

and interdependent challenges. Here we present three field laboratories, each of which are rooted in one of the main areas of the GCOF. Initially, we considered the effect of new phenomena like genome-first approaches and health data management on the organisation of the 'doctor's office'. In the second, we developed a broadly supported map of conditions and views on knowledge and data exchange, which is rooted in the 'server room'. Finally, we explored the possibilities for involvement of patients and non-patients in the 'living room' of the GCOF. Each field laboratory establishes focused, constructive interdisciplinary collaborations around 'radically interdisciplinary' topics. Based on the 'stepping stones' initiatives we set the route towards the GCOF: researchers from the natural and social sciences, medical professionals, healthcare managers, industry representatives, policy makers, patients and citizens collaboratively identify the design principles of genome data infrastructures as genomic technologies mature and become embedded in routine diagnostic procedures and health management systems.

C13.6

Teaching Genomic Medicine to Physicians - this is our responsibility as medical geneticists

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Background: Due to new discoveries in genomic medicine, and the transition of genetic knowledge from research laboratories into clinical practice, an increasing number of medical societies incorporate recommendations regarding genomic tests and therapeutics into routine clinical guidelines. Primary care practitioners have inadequate knowledge and skills in medical genetics and many are unaware of the technical, ethical, legal and psychosocial implications of genetic testing. **Methods**: We initiated a „genomic education" program for the purpose of providing physicians from different medical fields advanced knowledge in genomic medicine . We emphasized the main take-home messages for physicians, defined as: risk calculation for various genetic diseases, recognition of the mode of inheritance from the pedigree, guidelines for decision-making on which molecular tests to use, interpretation of test results and their clinical implications. **Results:** To date, 82 physicians have participated in 6 courses of our „genomic education" program, which included lectures, workshops and guided tours in genetic laboratories. In the „pre-course" examination the average score was 56% (range 20%-80%), whereas in the „post-course" examination it was 79% (range 40%-100%). The average improvement in score as a result of the course was 21% (range 0%-80%). The physicians who participated in our program reported a very high level of satisfaction from the theoretical and practical knowledge they aqired as well as the concept of a „one-week update course". **Conclusion:** A one-week „genomic education" program is an effective strategy to update primary care physicians regarding the advances in Genomic Medicine in order to improve their care of patients.

C14.1

Insights into the genetic architecture of anthropometric traits using whole genome sequence data

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Body weight and fat distribution measures are associated with increased risk of cardiometabolic disease. As part of the UK10K study, we have investigated the genetic architecture of anthropometric traits in 3,538 individuals with 6.5x whole genome sequence (WGS) data from the ALSPAC and TwinsUK cohorts. Variants discovered through WGS, along with those from the 1000 Genomes Project (1KGP), were imputed into additional individuals from the ALSPAC and TwinsUK cohorts with GWAS data (total sample size 9,979). We investigated association between anthropometric traits and 8.6 million low frequency and common variants (MAF>0.01). We are in the process of obtaining *in silico* replication of prioritised signals. In interim replication analysis across ~15,000 samples, 43 out of 66 novel signals for BMI have the same direction of effect in the replication cohorts (p-value=0.0093). We examined the concordance of the direction of effect at established loci for each trait. Out of the 31 established independent loci for BMI that were present in our data, 28 have the same direction of effect (p-value=2.3e-06). For weight, 10 out of 11 known loci (p-value=0.006), and for height 151 out of 172 loci (p-value<2.2e-16) have the same direction of effect, respectively. We estimated the improvement in genome-wide signal captured relative to those present in HapMap 2, HapMap 3 or 1KGP. We find no appreciable increase in variance explained as density increases, suggesting that the contribution of variants with MAF>0.01 are likely to be well-captured by existing GWAS implementation. Larger sample sizes will be required to refine these estimates.

C14.2

Genome of the Netherlands imputation identifies seven new loci for quantitative ECG traits in meta-analysis of 30,000 samples

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Genome-wide association studies (GWAS) of quantitative electrocardiographic (ECG) traits in large consortia identified over 50 loci associated with QRS, RR, QT and PR intervals, with even more loci discovered in ongoing HapMap-based meta-analyses in larger sample sizes. We hypothesized that imputation from sequencing-based reference panels may help in identifying new loci as the contribution of lower-frequency variation has not yet been extensively characterized. In the current study, we meta-analyzed GWAS results from 30,000 samples on the QRS, RR, QT and PR intervals after imputing 19 million SNPs from the Genome of the Netherlands (GoNL) reference panel (998 unique haplotypes). This approach proved successful; in addition to many known loci, we identified seven novel locus-trait associations. Of the seven novel loci, three were for PR, three were for QT and one was for QRS. Two were novel trait/locus combinations for loci previously observed for other ECG traits. Several others are involved in ion handling crucial to cardiac electrophysiology. The dense coverage of the genome (~seven times more SNPs than HapMap-based studies) also enabled us to study known loci in much finer detail. In conclusion, we show that larger reference panels with more accurate haplotype estimates and denser SNP data enable us to find new loci and fine-map previously known ones.

C14.3

Genome-wide association analysis identifies a new gene involved in salt perception and liking

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Salt perception and genetic variation in taste receptors may be important determinants of individual differences in salt intake, which in turn represents a risk factor for the development of hypertension and cardiovascular diseases. Genetic variations in taste perception are well known for bitter, sweet and umami taste, while little is known on the genetic bases of human salt perception. To investigate this area, salt taste responses were collected on ~ 900 healthy adults coming from 6 different small villages from North-Eastern Italy. NaCl taste intensity was assessed with the labelled magnitude scale (LMS) using a concentration of 1M and the log10 of the intensity ratings were used for the analysis. Genotyping data were imputed to the 1000G SNP set and used to perform a GWAS of salt perception including sex and age as covariates. A significant association with rs547916 SNP (p-value=5.6x10-08), closely located to the KCNA5 gene, was detected. A replication analysis was carried out on an American cohort confirming the association with salt liking phenotype (p-value=0.002). KCNA5 encodes a member of potassium channel voltage-gated, shaker-related subfamily and belongs to the delayed rectifier K+ (DRK) channels that in the mammalian taste system play a central role in specific taste transduction pathways. A study has also shown that KCNA5 is the major functional DRK channel expressed in the in rat taste buds. Further experiments are needed to better clarify KCNA5 role in salt perception and liking and possible implications for cardiovascular diseases.

C14.4

ImmunoSeq: Discovery of novel rare variants implicated in autoimmune and inflammatory diseases by targeting regulatory regions in immune cells

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Comprehensive DNase I hypersensitive site (DHS) mapping by the ENCODE project identified all classes of cis-regulatory elements. Recent studies showed enrichment of complex trait GWAS noncoding hits in DHS. However, GWAS hits only partially explain complex trait heritability. The discovery of rare variants located in regulatory regions specific to immune cells should explain part of this heritability. Our approach combines selective DNA capture of relevant regulatory regions coupled to next-generation sequencing. Genome-wide DHS mapping data from the ENCODE and NIH Roadmap Epigenomics Projects were used to select regulatory regions of immune cells. We designed a custom DNA capture panel (Roche SeqCap EZ developer) to target these selected regions, as well as exonic and HLA regions. Captured DNA (163Mb) is indexed (5-fold per lane) for sequencing on Illumina HiSeq2000 system, yielding average coverage of ~28x. We first applied the "Immunoseq." assay to 30 healthy individuals from Sweden (Uppsala BioResource), where we have existing transcriptome, methylome and ChIP-seq data from three primary immune cells (monocytes, B-cells and T-cells). The capture panel was also applied to 150 trios from the Saguenay–Lac-Saint-Jean asthma familial collection (complex trait with immune and inflammatory components). We observe an enrichment of variants in the vicinity of allelicly differentially expressed genes (p<1e-10) as well as potential impact of rare conserved variants on allelic differential expression of genes (p<0.05). This highlights the 1) potential impact of rare variations on functional mechanism in immune cells and 2) power of our targeted capture approach to identify novel rare variants in regulatory regions.

C14.5

Exome array analysis in >30,000 Europeans establishes a functional role for G6PC2 and identifies novel coding variants influencing glycaemic traits

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To identify coding variants associated with fasting glucose (FG) and fasting insulin (FI) levels, we analysed exome array data in up to 33,407 non-diabetic individuals of European ancestry. We identified multiple glucose-lowering coding variants in *G6PC2*, which resides in an established FG genome-wide association study (GWAS) locus. Conditional single-variant association analysis established the presence of two coding variants at exome-wide significance (P<5x10^{-7}) in this gene, one common (P_{COND}=7.1x10^{-10}, 48.1% MAF, p.V219L) and one rare (P_{COND}=1.3x10^{-11}, 0.8% MAF, p.H177Y), both were independent of the GWAS SNP at this locus and of each other ($P_{V219L\ COND\ on\ H177Y}$=4.0x10^{-7} and $P_{H177Y\ COND\ on\ V219L}$=3.7x10^{-10}). Gene-based analysis (P_{SKAT}=8x10^{-10}) highlighted another coding variant p.Y207S (MAF=0.5%) with evidence of association with FG. *In vitro* studies of these *G6PC2* variants showed a reduction in protein expression by 98% (p.H177Y, p<0.001), 100% (p.Y207S, p<0.001) and 43% (p.V219L, p<0.01) compared to wild-type in HEK293 cells, with similar results in INS1E cells. Protein expression was rescued with a proteasome inhibitor implying degradation of unstable G6PC2 proteins via the ubiquitin-proteasome pathway. Expressed variant proteins localized as expected to the endoplasmic reticulum. We also identified two genes not mapping to previously reported GWAS loci in which coding variants were associated with FG (*GLP1R*: P=4.4x10^{-7}, 1.6% MAF, p.A316T) or FI (*URB2*: P=3.8x10^{-7}, 0.1% MAF, p.E594V). These data establish a functional role for *G6PC2* in FG homeostasis and identify novel glycaemic trait loci. They do not support a role for low-frequency or rare coding variants in explaining previously reported association signals at GWAS loci.

C14.6

Transethnic association study of IBD identifies novel risk loci and shows pervasive sharing of genetic risk factors across populations

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There are now over 163 loci associated with inflammatory bowel disease (IBD) susceptibility in European populations, though little is known about their contribution to disease risk in non-European populations. Using the custom Immunochip array, we genotyped 9,846 individuals of East Asian (Japan, South Korea, Hong Kong, China and UK individuals of East Asian descent), Indian and Indo-European (Iranian) descent. Combining these with the existing cohort of 81,248 individuals of European descent (Jostins et al. Nature 2012), we identified 14 novel IBD susceptibility loci at genome-wide significance (P<5×10-8). The majority of IBD risk loci were shared between both Europeans and non-Europeans, and differences in the amount of variance explained in disease liability at each locus were driven by a combination of differences in allele frequencies and effect sizes. For instance, the variance in Crohn's disease (CD) liability explained by four independent risk variants at TNFSF15 was 19 times greater in East Asians than Europeans, a reflection of the risk-increasing alleles being more common and having larger odds ratios in East Asians. Aggregation of all SNPs assayed on the Immunochip revealed significant genetic correlation (0.4-0.85) for IBD between European and non-European populations. Together, this study increases the total number of IBD susceptibility loci to 177 and demonstrates the pervasive sharing of genetic risk factors across populations, though there exists heterogeneity in the variance explained per locus.

C15.1

BCAP31 mutations cause a new X-linked syndrome with deafness, dystonia, central hypomyelination and disorganization of the Golgi apparatus

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BAP31 is one of the most abundant proteins of the membrane of the endoplasmic reticulum (ER). It is a chaperone protein involved in several pathways, including ER-associated protein degradation, export of proteins from the ER to the Golgi, and programmed cell death. BAP31 is encoded by the BCAP31 gene located in Xq28. It is highly expressed in neurons. We identified loss of function mutations in BCAP31 in seven individuals from three different families and one missense mutation in another fourth family. These people suffer from intellectual and motor disability, dystonia and sensorineural hearing loss with abnormal white matter. The association of these clinical signs define a new X-linked syndrome. In primary fibroblasts from patients, we found that the absence of BCAP31 changed the morphology of the ER and disorganized the Golgi apparatus in a significant proportion of cells. We demonstrated that the constitutive deficiency of BCAP31 did not activate the response to unfolded proteins (UPR) nor triggered cell death. Rather, our data suggest that the absence of BAP31 affects ER and Golgi apparatus metabolism and that BAP31 plays an important role in ER-Golgi exchanges. These results provide a molecular basis for a new Mendelian syndrome and connect intracellular protein trafficking with a severe congenital neurological disorder.

C15.2

Mutations in KPTN Cause Macrocephaly, Neurodevelopmental Delay, and Seizures

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The proper development of neuronal circuits during neuromorphogenesis and neuronal-network formation is critically dependent on a coordinated and intricate series of molecular and cellular cues and responses. Although the cortical actin cytoskeleton is known to play a key role in neuromorphogenesis, relatively little is known about the specific molecules important for this process. We identified nine affected individuals from four Ohio Amish families affected by an autosomal recessive, variable form of neurodevelopmental delay. The most consistent features were global developmental delay, macrocephaly, anxiety, and some features suggestive of a pervasive developmental disorder. In addition, a primary seizure disorder was described in three cases. Using a combination of autozygosity mapping, linkage

analysis and whole-exome sequencing, we demonstrated that two founder mutations in KPTN, encoding kaptin, can result in this phenotype. Our immunofluorescence analyses in primary neuronal cell cultures showed that endogenous and GFP-tagged kaptin associates with dynamic actin cytoskeletal structures and that this association is lost upon introduction of the identified mutations. Taken together, our studies have identified kaptin alterations responsible for this disorder and have defined kaptin as a molecule crucial for normal human neuromorphogenesis. Finally, our identification of two KPTN mutations within this Anabaptist population parallels the situation seen for a number of other genes with multiple mutations that also commonly cause inherited diseases globally (e.g., GJB2 mutations in inherited hearing loss, and ATM mutations in ataxia telangiectasia), indicating that kaptin neurodevelopmental delay might be similarly widespread.

C15.3

REPS1 is a novel gene of Neurodegeneration with Brain Iron Accumulation

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Neurodegeneration with brain iron accumulation (NBIA) encompasses a group of rare neurogenerative disorders with different clinical, brain MRI and molecular features, underlined by progressive extrapyramidal dysfunction and iron accumulation in the brain. To date, mutations in PANK2, PLA2G6, FA2H, ATP13A2, C2orf37, CP, FTL and WDR45 have been associated with NBIA. Nevertheless a large number of individuals have "idiopathic NBIA", with unknown etiology. Exome sequencing of two sisters with NBIA identified two heterozygous mutations in the REPS1 gene. REPS1 is involved in endocytosis and the two mutations (p.Ala113Glu and p.Val78Leu) affect its EH1 domain that interacts with RAB11FIP2. Western Blot analysis detected a low level of REPS1 in patient's fibroblasts. The function of REPS1 in iron metabolism is unknown, but it was shown that RABB11-FIP2 functions in transferrin recycling. We investigated the iron metabolism and oxidative stress in patient's fibroblasts. Patient fibroblasts exhibited a dramatic iron overload, measured by a colorimetric ferrozine-based assay. Consistently steady-state levels of ferritin, iron responsive protein (IRP1) and SOD2 were increased whereas aconitase activity was decreased. This indicates that REPS1 mutations induce deregulation of iron metabolism. Over-expression of the wild-type REPS1 cDNA in patient's cells reduces the iron overload of these cells that display almost normal iron content. Our experiments demonstrate that REPS1 is a new gene of NBIA. Improvement in our understanding of the biochemistry and pathophysiology of this form of NBIA will help develop novel therapeutics for this neurological condition.

C15.4

Interferon type 1 response regulator *USP18* is mutated in severe pseudo-TORCH syndrome

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Pseudo-TORCH syndrome indicates a group of disorders putatively linked to autoimmunity and is characterized at birth by cerebral calcification, microcephaly, enlarged ventricles, cerebellar atrophy and the development of hyperbilirubinemia, thrombocytopenia and hepatomegaly resembling congenital infections. There is overlap with Aicardi-Goutières syndrome (AGS), caused by mutations in genes involved in RNA catabolism, indirectly leading to increased Interferon type I signalling. We report on patients from two families with pseudo-TORCH, cerebral haemorrhage and early demise.
The first consanguineous family included 3 affected sibs. Using linkage analysis, whole exome sequencing and sanger sequencing of the non-covered exons in the linkage area, we identified a homozygous truncating mutation in USP18, causing total absence of USP18 transcript in cultured fibroblasts, compared to normal expression in controls. In the second family, including two sibs, the same truncating mutation in heterozygous form was found. No transcript was amplified from cells of the two patients, suggesting that a second mutation is present in areas not covered by Sanger sequencing.
USP18 is an interferon-stimulated gene and itself a key regulator of interferon response both in native antibacterial and antiviral immunity and in autoimmune response. Usp18 knockout mice have cerebral hemorrhage and hydrocephalus with ependymal necrosis. Microscopy of patient brain tissues shows severe ependymal abnormalities, similar to the mouse.
In conclusion, USP18 mutations are a novel genetic cause of pseudo-TORCH syndrome and severe cerebral hemorrhage which provide for the first time a direct link between the IFN type I signalling pathway and the disease phenotype.

C15.5

Loss of CTNND2 is associated with borderline intellectual dysfunction in humans and neuronal migration defects in zebrafish

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Cytogenetically visible chromosomal translocations are a unique resource as they can pinpoint strong effect genes. Here we report a mother and daughter with borderline intelligence and learning problems within the dyslexia spectrum and two apparently balanced reciprocal translocations; t(1;8)(p22;q24),t(5;18)(p15;q11). By low coverage mate-pair whole genome sequencing we were able to pinpoint the genomic breaks to within a 2kb window and the DNA breakpoints were then verified by PCR and Sanger sequencing. The 5p breakpoint was located between exon 7 and 8 of CTNND2. By genome wide array comparative genomic hybridization we identified an additional individual with similar phenotypic presentation and a maternally inherited 163kb microdeletion exclusively involving CTNND2. The microdeletion at 5p11.2 is present as mosaicism in the mother and absent in the patient's healthy siblings.
Inappropriate migration of cortical neurons has been postulated as a potential mechanism for various neurological disorders including dyslexia, given the potential role of CTNND2 in neuron motility, we were particularly interested in assessing defects in neuronal migration in vivo. Knockdown of one of two zebrafish CTNND2 orthologues using antisense morpholino oligonucleotides showed mostly normal development of the neuronal clusters. However, analysis of a subpopulation of forebrain neurons expressing GFP under the islet promoter (isl:GFP) in knockdown embryos showed the presence of misplaced isl:GFP cells between the telencephalon and diencephalon indicative of a defects in migration. This suggests that defective migration of subpopulations of neuronal cells due to loss of CTNND2 could be part of the underlying mechanism behind the cognitive dysfunction in the patients.

C15.6

Novel (ovario)leukodystrophy related to *AARS2* mutations

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Purpose: This study was focused on patients with a leukoencephalopathy of unknown cause with the aim to define a novel, homogeneous phenotype suggestive of a common genetic defect, based on clinical and MRI findings, and to identify the causal genetic defect, shared by patients with this phenotype.
Methods: Independent exome sequencing studies were performed in two unrelated patients with a novel leukoencephalopathy. MRI findings in these patients were compared to MRIs in a database of unclassified leukoencephalopathies. Eleven additional patients with similar MRI abnormalities were selected. Clinical and MRI findings were investigated.

Results: Exome sequencing revealed compound heterozygous mutations in *AARS2* encoding mitochondrial alanine-tRNA synthetase in both patients. Functional studies in yeast confirmed the pathogenicity of the mutations in one patient. Sanger sequencing was performed in the eleven additional patients and revealed *AARS2* mutations in four patients. The in total six patients with *AARS2* mutations had childhood to adulthood onset signs of neurological deterioration consisting of ataxia, spasticity and cognitive decline with features of frontal lobe dysfunction. MRIs showed a leukoencephalopathy with involvement of left-right connections and ascending and descending tracts, and cerebellar atrophy. All female patients had ovarian failure. Signs of cardiomyopathy were not observed.
Conclusions: Mutations in *AARS2* have been found in a severe form of infantile cardiomyopathy in two families. We present six patients with a novel phenotype caused by *AARS2* mutations, characterized by a distinctive leukoencephalopathy and, in female patients, ovarian failure, indicating that the phenotypic spectrum associated with *AARS2* variants is wider than previously reported.

C16.1

A congenital disorder of glycosylation, with lymphopenia, neutropenia, and skeletal dysplasia, caused by mutations in the gene encoding phosphoglucomutase 3 (PGM3).

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Human phosphoglucomutase 3 (PGM3) catalyzes the conversion of GlNAc-6-P into GlcNAc-1-P, during the synthesis of UDP-GlcNAc, a sugar nucleotide critical to multiple glycosylation pathways. PGM3-defects have earlier been associated with hematopoietic deficiency in a mouse model. We identified three unrelated patients with recurrent infections, congenital leukopenia including neutropenia, B and T cell lymphopenia, and progression to bone marrow failure. Whole exome sequencing revealed novel, deleterious mutations in the PGM3 gene in all three subjects, delineating a new type of congenital disorder of glycosylation (CDG). Functional studies of the human immunodeficiency-associated PGM3 gene variants in E.coli cells demonstrated reduced PGM3 enzyme activity with decreased phosphate group transfer from position GlcNAc-6-P to GlcNAc-1-P. Two of the 3 patients had skeletal anomalies with short stature, brachydactyly, and intellectual disability, however these additional features were absent in the third patient, showing the clinical variability of the disease. Two patients were transplanted using a cord blood hematopoietic stem cell and a matched-related donor bone marrow; both had successful engraftment and correction of neutropenia and lymphopenia. We define PGM3-CDG as a novel cause of treatable genetic immunodeficiency, document the power of whole exome sequencing in gene discoveries for rare disorders, and illustrate the utility of genomic analyses in studying combined and variable phenotypes.

C16.2

Lenz-Majewski syndrome: disturbed phosphatidylserine metabolism causes intellectual disability and a sclerosing bone dysplasia

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Lenz-Majewski syndrome (LMS) constitutes a unique rare disease characterised by the association of generalised sclerosing bone dysplasia, intellectual disability, cutis laxa and distinct craniofacial, dental, and distal limb anomalies. By using whole-exome sequencing in four patients and selecting variants according to the predicted dominant de novo etiology model, we identified causative heterozygous missense mutations in PTDSS1 gene, which encodes phosphatidylserine synthase 1 (PSS1). Subsequently, three additional patients were screened by Sanger sequencing and similar mutations were found. In total, five different missense mutations affecting four specific PSS1 amino acids were identified in the seven patients studied to date. PSS1 is one of two enzymes involved in production of phosphatidylserine. Phosphatidylserine synthesis was increased in patients' intact fibroblasts and end-product inhibition of PSS1 by phosphatidylserine was significantly reduced. Therefore, these mutations cause a gain-of-function effect associated with regulatory dysfunction of PSS1. In support of this model, craniofacial defects similar to those found in LMS, were observed following expression of mutant but not wild-type PTDSS1 in zebrafish. Interestingly, total phospholipid cellular content was similar between patient and control fibroblasts. Ongoing lipidomic studies suggest that the identified mutations cause abnormal fatty acid composition of individual subclasses of membrane phospholipids. In conclusion, we have identified LMS as the first human disease caused by disrupted phosphatidylserine metabolism and one of the few examples of gain-of-function mutations affecting an enzyme. Our results bring important insight to the understanding of phosphatidylserine metabolism and point to an unexplored link with bone development and homeostasis.

C16.3

Homozygous *FIBP* truncating mutation in a new multiple congenital anomalies syndrome with overgrowth, macrocephaly, Iris coloboma, and learning disabilities

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Fibroblast growth factors (FGFs) pathways determine crucial cellular roles for proliferation, migration, fate and survival, by various extracellular and intracellular modes of action. Acidic FGF extracellular action usually consists of binding multiple forms of cell surface receptors, leading to the activation of cytoplasmic tyrosine-kinase pathways but it also enters into the nucleus to stimulate DNA synthesis. The acidic fibroblast growth factor intracellular binding protein (FIBP) is a FGF1 binding protein widely expressed in human tissues, but its function remains unknown in FGF signaling. We performed exome sequencing in a 22-year-old man, born from healthy consanguineous parents, and presenting with an undefined phenotype associating overgrowth, marfanoid habitus, macrocephaly, facial dysmorphism, large thumbs, bilateral iris coloboma, ventricular septal defect, mitral valve prolapse, venous insufficiency, moderate scoliosis, and learning disabilities. A homozygous nonsense mutation (p.Gln218*) was identified and was absent in unaffected siblings. FIBP is known to be expressed in brain, eye, heart and kidneys, consistent with the patient's clinical features. Additional functional results argue for the pathogenicity of the mutation in the phenotype: 1) FIBP cDNA was undetectable in patient's fibroblasts; 2) the intracellular FGF pathway was deregulated with increased FGF-1 protein level; 3) The XTT test and Ki67 labelling exhibited a higher proliferation capacity in patient's

fibroblasts than controls. In
conclusion, we present the first implication of the *FIBP* gene in a novel autosomal recessive multiple congenital abnormalities syndrome and the results of pathophysiological studies.

C16.4

Hidden mutations in Cornelia de Lange Syndrome (CdLS) - Limitations of Sanger sequencing in molecular diagnostics

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CdLS is a clinically and genetically heterogeneous developmental disorder. Patients are characterized by distinct facial features, growth retardation and cognitive delay. Whereas half of the patients show mutations in the *NIPBL* gene, mutations in *SMC1A, SMC3, RAD21* or *HDAC8* account for additional 10%. Interestingly, recent data using DNA from buccal mucosa (BM) tissue could identify a high proportion of mosaic mutations in *NIPBL* which were not detected in DNA from blood samples. Here we report on two unrelated patients with characteristic CdLS phenotype, who were mutation negative by Sanger sequencing analyzing DNA from blood and BM samples.
Subsequent Ion Torrent panel sequencing on DNA of BM using a custom made Ion AmpliSeq enrichment that includes the five known CdLS genes beside eleven functionally-associated candidate genes, could detect a mosaic nonsense (17%) and a mosaic missense mutation (13%) in *NIPBL*. Interestingly SNaPshot fragment analyses were able to confirm both mutations in DNA from BM, fibroblasts and urine samples but did not detect these mutations in DNA from blood. Additional Sanger sequencing approaches using DNA from all four tissues could only detect both mutations in fibroblast DNA.
In summary, our data strongly support a high frequency of mosaic *NIPBL* mutations in CdLS-patients. More importantly, it also shows the limitations of current sequencing approaches in diagnostics, even when using DNA of BM as recently suggested. Thus we recommend the use of high coverage sequencing techniques on DNA of BM, especially when analyzing patients with characteristic CdLS-phenotypes and negative Sanger sequencing results.

C16.5

RNA Polymerase II activity is affected at the promoter regions in SMC1A-mutated Cornelia de Lange Syndrome cells

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Mutations in genes encoding cohesin subunits or regulators, namely NIPBL, SMC1A, SMC3, HDAC8 and RAD21, have been linked to Cornelia de Lange syndrome (CdLS). It has been hypothesized that the dysregulation of gene expression by chromatin remodeling likely represents the underlying pathogenesis of CdLS, however the exact mechanism by which this is effected is unknown. To gain a better understanding of this process we investigated whether the gene transcription machinery was somehow affected by SMC1A mutations. We used chromatin immunoprecipitation coupled with massively parallel DNA sequencing (ChIP-seq) to identify genomic regions co-occupied by cohesin, NIPBL and RNA pol II in normal human lymphoblastoid cells. Genes co-localizing cohesin, NIPBL and RNA pol II have been compared to gene expression data from CdLS cell lines. Finally, we investigated the recruitment of RNA pol II onto genes differentially expressed in CdLS mutated cells. Our results indicate that SMC1A mutations reduce the recruitment of Pol II at promoter regions and also affect the activity of the Pol II elongating form. These findings highlight the pivotal role of cohesin in transcriptional regulation and the effect on Pol II occupancy may explain the typical gene dysregulation observed in CdLS cell lines.

C16.6

In silico and functional characterization of KMT2D/MLL2 missense mutations as causative in Kabuki syndrome

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Kabuki syndrome (KS) is a multiple congenital malformation syndrome characterized by facial features, skeletal anomalies, dermatoglyphic abnormalities, mental retardation, and postnatal growth deficiency. Heterozygous mutations in the KMT2D gene are detected in 60% of Kabuki patients. KMT2D gene encodes a H3K4 histone methyltransferase that plays important role in the epigenetic control of active chromatin states modulating the expression of genes essential for embryogenesis and development. A subset of KS individuals was recently identified with mutations in the chromatin modifier KDM6A. We performed a mutational screening on 303 Kabuki patients by direct sequencing, MLPA, and qPCR, detecting 133 patients with KMT2D and four with KDM6A mutations. Among the KMT2D mutations we identified 46 missense variants across the entire length of the KMT2D gene, 16 were inherited from an apparently asymptomatic parent.
Aim of this study is to ascertain the pathogenicity of KMT2D missense mutations through an integrative analysis of bioinformatics tools and biochemical and cellular assays. We used an innovative in silico approach that combines comparative analysis and motif/domain search tools with threading/homology modeling protocols to predict functional/structural effect of KMT2D missense variants and identify and confirm potential pathogenic mutations. Due to the huge size of KMT2D gene, we devised a strategy where minigenes carrying KMT2D missense variants were generated. We evaluated their potential pathogenicity effects by measuring KMT2D enzymatic activity and expression level of KMT2D known target genes. Our strategy should offer a valuable support to estimate the real deleterious effect of KMT2D missense variants, an issue in diagnostic counseling.

C17.1

A dominant mutation in CHCHD10 causes neurodegenerative disorder with mitochondrial DNA instability

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Mutations in OPA1 and MFN2, two genes encoding membrane proteins involved in mitochondrial dynamics, are responsible for mitochondrial DNA (mtDNA) instability disorder with "Autosomal Dominant Optic Atrophy (ADOA) plus" phenotype. We report a large family with a late-onset complex phenotype including motor neuron disease, cerebellar ataxia, cognitive decline and myopathy. Muscle biopsy showed ragged-red and COX negative fibres with combined respiratory chain deficiency and abnormal assembly of complex V. The multiple mitochondrial DNA (mtDNA) deletions found in skeletal muscle revealed a mtDNA instability disorder. By whole-exome sequencing (WES), we identified a missense mutation (c.176C>T; p.Ser59Leu) in the CHCHD10 gene that encodes a coiled-coil helix coiled-coil helix protein, whose function was unknown. We show that CHCHD10 is a mitochondrial protein located in the intermembrane space and enriched at cristae junctions. Patient fibroblasts carrying the CHCHD10 mutation present with a respiratory chain deficiency and a fragmentation of the mitochondrial network. Furthermore, we show that overexpression of CHCHD10S59L triggers mitochondrial fragmentation in HeLa cells, thus confirming the deleterious effect of this mutant on mitochondrial morphology and network. DRP1-K38A, which is resistant to fission, did not modify the mitochondrial fragmentation observed in cells expressing CHCHD10S59L, suggesting that the CHCHD10 mutant leads to impaired fusion activity. This work, suggesting that CHCHD10 plays a role in mitochondrial fusion and/or in maintenance of cristae morphology, highlights the critical role of mitochondrial dynamics in terms of human disease and mitochondrial genome stability.

C17.2

Decoding Mitochondrial Disorders using Exome Sequencing

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Mitochondrial disorders are a heterogeneous group of diseases characterized by faulty oxidative phosphorylation. Despite good progress in the field, most disease causing mutations still have to be identified. We applied whole exome sequencing in 300 unrelated individuals with juvenile-onset mitochondrial disorder. In a quarter of patients, we detected mutations in known disease genes. In another quarter of patients, we identified mutations in ge-

nes previously not associated with mitochondrial disorders. Mutations in the majority of genes are rare and could be identified due to loss-of-function alleles in evolutionary conserved genes such as MGME1, the first exonuclease involved in mitochondrial replication (Kornblum et al., Nat. Genet. 2013). Mutations in other genes are more frequent, with ACAD9 being the most common finding with more than 15 cases, providing statistical evidence for the association with isolated respiratory chain complex deficiency. More difficult to identify are missense mutation in genes coding orphan proteins such as FBXL4, a protein with unknown function associated with reduced mitochondrial protein content. Additional diagnostic challenges are patients with recessive mutations in more than one gene resulting in a compound clinical phenotype.

Evolving topics are tRNA modifying enzymes (ELAC2, MTO1 and GTPBP3) and tRNA synthetases, both involved in the translation of mitochondrial proteins as well as cofactor metabolism defects. The later offers rational therapeutic options as for example riboflavin supplementation in the case of mutations in the riboflavin transporter SLC52A2.

In summary, the genetically heterogeneous group of mitochondrial disorders is an example par excellence for the application of genome wide sequencing.

C17.3

Lentivial vector based hematopoietic stem cell gene therapy mediates sustained expression of functional thymidine phosphorylase in mitochondrial neurogastrointestinal encephalopathy mouse model

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MNGIE is an autosomal recessive disease caused by deficiency of the enzyme thymidine phosphorylase (TP), resulting in systemic accumulation of nucleosides thymidine (Thd) and deoxyuridine (dUrd), and mtDNA deletions, depletion and dysfunction. We aimed to use *ex vivo* lentiviral vector (LV) hematopoietic stem cell (HSC) gene therapy to deliver human TP enzyme in $Tp^{-/-}Upp^{-/-}$ double knockout mice, a model for MNGIE disease. To that end, LV transduced *Tp-/-Upp-/-* HSCs containing the native cDNA sequence (TP) or codon optimized (TPco) driven by the phosphoglycerate kinase (PGK) or the spleen focus forming virus (SFFV) promoter were transplanted in sublethally irradiated *Tp-/-Upp-/-* mice. Wild type mice had detectable but very low levels of enzyme activity in blood cell fractions (0.07 ± 0.03nmoles/h/mg, N=4), 1 month post transplantation, enzyme activities increased at least 90-fold in LV recipient mice (LV-TP and LV-TPco = 150±4 and 96±4 nmoles/h/mg respectively, N=4), with a 400-fold increase observed in recipients of LV-SFFV-TPco (450±5, N=4), resulting in reduction of Thd and dUrd in plasma and urine. TP activity was detectable in brain of gene therapy-treated mice 14 months after treatment (average of 1.2-fold in LV-TP and LV-TPco compared to wild type and 36-fold for LV-SFFV-TPco), which significantly reduced the nucleoside levels.High molecular chimerism (76.5±8.2 % donor chimerism, N=12) with low LV vector copy numbers (1.01±1.1VCN/donor derived cell, N=12)were achieved.Overall, HSC gene therapy resulted in stable TP expression and long-term biochemical correction without geno-phenotoxicity in MNGIE mice to be further optimized to develop a clinical protocol to treat MNGIE patients.

C17.4

Deletion of a distant-acting enhancer near *C16ORF91* underlies recessive congenital diarrhea

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We studied eight patients from seven families of Jewish Iraqi origin, suffering from congenital diarrhea, historically defined as intractable diarrhea of infancy syndrome (IDIS). Next-generation sequencing and SNP arrays identified two structural genomic deletion alleles near *C16ORF91*, leading to a homozygous deletion of a 1500bp intergenic critical region (ΔV) shared by all patients. The ΔV region contained a segment with vertebrate evolutionary conservation and transcription factor binding clustering. ΔV was shown by mouse embryo transgenic assay to harbor a robust enhancer, active in specific sub-regions of stomach, duodenum and pancreas at early developmental stages (E11.5-14.5). In a mouse ΔV deletion strain, homozygous offspring had on average 36% smaller post-natal weight as compared to heterozygotes, significantly modified intestinal content reminiscent of diarrhea with a continued lethality of knockout from days 3 to 21 post-partum. RNA sequencing of gut and stomach biopsy in an adult patient showed major downregulation (X10 to X200) of several gastrointestinal peptide hormones transcripts compared to controls, suggesting enteroendocrine cell dysfunction. No gene in a ±1Mb interval around the enhancer showed mRNA disregulation, to be expected if enhancer action extends to adults. Intriguingly, we observed a 30 fold mRNA downregulation of *ARX*, a transcription factor previously implicated in syndromic diarrhea. Circularized chromosome conformation capture (4C) is currently employed at different mouse developmental stages, seeking further information on potential enhancer targets. Our results suggest that congenital diarrhea in our patients involves a rare case of enhancer-mediated phenotype, perhaps involving failure of enteroendocrine development or function.

C17.5

Genetic testing leads clinical care in neonatal diabetes: a new paradigm

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Before next-generation sequencing, genetic testing was based on selection of a gene (or small number of genes) in which mutations had been identified in patients with recognisable, discrete phenotypes. For neonatal diabetes this included a transient subtype and syndromes such as Wolcott-Rallison. In this study we investigated the impact of testing all known genetic causes of neonatal diabetes.

We studied 1020 patients from 79 countries with diabetes diagnosed <6 months of age. Mutations were identified by targeted next-generation sequencing of 21 genes, Sanger sequencing or 6q24 methylation analysis.

A pathogenic mutation was identified in 840 patients (82%). The most common cause was a potassium channel mutation (n=390); most of these patients achieve improved glycaemic control after transfer from insulin to sulfonylureas. The median age at referral decreased from 271 weeks in 2004 to 18 weeks in 2013. Patients with a genetic diagnosis of Wolcott-Rallison syndrome referred <12 months of age were therefore more likely to have isolated diabetes at referral (85% vs 33% >12 months) and develop additional features (e.g. liver failure) after testing. A genetic diagnosis also predicts diabetes remission: 88/94 patients (94%) referred <3 months received a genetic diagnosis of transient neonatal diabetes before remission.

Comprehensive genetic analysis identified causal mutations in >80% of cases. More patients are now referred at presentation with isolated diabetes and the genetic result predicts clinical course and development of related features. This represents a new paradigm for clinical care with genetic diagnosis preceding the development of clinical features and guiding clinical management.

C17.6

Safety and efficacy of pravastatin and zoledronate association in Hutchinson-Gilford Progeria: two-years treatment results of a phase II, open label, single arm clinical trial (ClinicalTrials.gov #NCT00731016)

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Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare developmental disorder affecting children with segmental features of aging, including thin skin, loss of subcutaneous fat, alopecia, osteopenia and accelerated cardiovascular arteriosclerotic disease, leading to death at the mean age of 13 years. The syndrome is due to a mutation in the gene encoding lamins A/C, leading to the production of an aberrant lamin A precursor called progerin. Intranuclear progerin accumulation exerts multiple toxic effects which ultimately lead to organismal premature aging. Preclinical data provided proof-of-principle that the combined use of zoledronate and pravastatin (ZoPra) could ameliorate several disease parameters, including

growth, bone density and survival. On these bases, we conducted in Marseille La Timone Hospital a phase II, open label, single arm trial including 12 European HGPS patients, aiming to test the safety and efficacy of two years of ZoPra treatment (10 to 20 mg/day oral pravastatin and 0.05 mg/kg intravenous zoledronate every 12 weeks). The adverse events observed in this study were consistent with those expected in the HGPS population and none warranted treatment discontinuation. ZoPra induced clinical beneficial effects, including an amelioration of rates of weight gain and lipid profiles, bone mass increases, reduced mesenteric fat together with increased leptin levels, reduced thrombocytosis and concurrent reductions of circulating endothelial microparticles and endothelial progenitor cells, suggesting reduced vascular injury. Our results are discussed in light of previous results of a phase II lonafarnib trial in HGPS.

C18.1

Molecular Inversion Probe based Resequencing Identifies Recurrently Mutated Genes in Intellectual Disability

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Exome sequencing studies of individuals with intellectual disability (ID) and related neurodevelopmental disorders have identified many de novo mutations but few recurrently mutated genes1,2. We therefore applied a molecular inversion probe (MIP) based resequencing method to screen 42 ID candidate genes - mainly from a previous study2 - in ~2,500 patients with the clinical diagnosis of ID and 1,000 healthy controls. This approach allowed targeted multiplex enrichment of 768 DNAs per week per fte, and barcoding allowed simultaneous sequencing of up to 3,072 samples for the 42 gene panel per HiSeq2000 sequencing run. This resulted in an average coverage of >300x coverage per sample, allowing highly accurate variants calling.
We discovered predicted loss-of-function (LoF) events for more than half of the candidate genes, including multiple recurrent de novo nonsense and frameshift mutations in several genes including CHD2, GATD2B and MYT1L. This together with ongoing 'reverse-phenotyping' of the affected individuals further supports the pathophysiological role of these genes in intellectual disability. We were also able to analyze copy number status for these candidate genes, and discovered de novo CNVs, deletions and duplications, that implicate new dosage sensitive genes. Despite the challenging task to prove causality, latest technological improvements allowed entering a golden age of 'neurodevelopmental-gene' discovery which promises to improve not only our understanding of disease but provide fundamental insight into the biology of human brain development.

1. Rauch, A. et al. Lancet 380, 1674-82 (2012).
2. De Ligt, J. et al. N. Engl. J. Med. 367, 1921-9 (2012).

C18.2

Efficient molecular diagnosis of Intellectual Disability: targeted High throughput exon sequencing of 217 ID genes detects causative mutations in at least 26 of 106 tested patients

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Intellectual disability is a major public health problem, characterized by an extreme genetic heterogeneity, with several hundred genes implicated in monogenic forms with or without autism, complicating molecular diagnosis. In patients without evocative syndromic forms the offer is limited to Fragile-X testing and array-CGH, leaving most cases undiagnosed. Trio-Exome sequencing was recently proposed as a diagnostic approach, but remains costly for general implementation. We thus tested targeted exon sequencing of 217 genes previously implicated in X-linked ID, or in autosomal dominant or recessive forms in which ID is the major clinical concern. We analyzed 106 patients (mostly males and sporadic cases) with molecularly undiagnosed ID. One third of them harbored autistic features.
We identified causative mutations in 26 patients: sixteen in X-linked genes (*ATRX*, *CUL4B, DMD*, *FMR1*, *HCFC1, IL1RAPL1*, *IQSEC2*, *KDM5C*, *MAOA*, *MECP2*, *SLC9A6*, *SLC16A2, PHF8),* 10 *de novo* in autosomal genes (*DYRK1A*, *GRIN1*, *MED13L*, *TCF4*, *RAI1*, *SHANK3, SLC2A1*, *SYNGAP1*). We detected likely-causative mutations requiring additional validation in 5 patients (in *NLGN3* for instance). Our findings confirm MED13L as an ID gene, but raise doubts on *SHROOM4* or *SRPX2*. We identified causative mutations in syndromic genes in patients deviating from the classic phenotype. Some genes were hit more than once suggesting they correspond to more frequent conditions. The identification of mutations in 25-29% of patients proves the diagnostic efficiency of this strategy, higher than CGH and comparable to trio-exome sequencing at lesser cost. We have now updated our captured gene list to 270 genes, and will report on its use.

C18.3

Comprehensive NGS based diagnostics in over 1000 patients with epileptic disorders

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Purpose: Epileptic disorders have a highly heterogeneous background and a strong genetic contribution. Knowing the underlying molecular defect can be very valuable for diagnosis, guiding treatment and estimating recurrence risks. For this purpose we have developed a comprehensive diagnostic panel. **Method:** 461 relevant genes were selected from the literature, and subdivided into subpanels according to their phenotypes. Following customized target enrichment, NGS was performed, followed by bioinformatic analysis. Variants with a global minor allele frequency <5% were selected for evaluation and identified mutations were validated using Sanger sequencing. **Results:** In our study, over 1000 patients with epileptic disorders were analyzed. 20% of patients had pathogenic mutation(s) and 29% were inconclusive, partly being predicted pathogenic but unknown variants, partly due to non-segregation of identified variants. 51% of the cases remained unsolved. We observed rare variants in 203 different genes, with *SCN1A*, *SCN2A, CACNA1A*, *MECP2* and *KCNT1* being mutated most frequently. Across the cohort, 78 genes were identified as causative only once, emphasizing the advantage of diagnostic panels for very rare conditions.
Conclusion: We have developed a highly reliable and cost-efficient diagnostic NGS panel to analyze the genetic basis of epilepsies. We detected mutations in patients with clear and unspecific epilepsies, as well as in patients suffering from very rare conditions. This enables better understanding of genotype-phenotype correlations, and gives new insights into complex modes of inheritance such as combinatorial effects of variants.

C18.4

Planar cell polarity gene mutations contribute to the etiology of human Neural Tube Defects

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Neural tube defects (NTDs) are congenital malformations affecting 1 infants per 1000 births. The most common forms of NTDs are anencephaly and myelomeningocele, which result from the failure of fusion in the cranial and spinal regions of the neural tube, respectively. Population and family studies indicate a complex etiology to NTDs involving environmental and genetic factors that remain undetermined. Animal models have strongly implicated the Wnt non canonical planar cell polarity (PCP) pathway in the pathogenesis of NTDs. PCP is the process of by which cells become polarized in the plane of an epithelium. PCP core members include the transmembrane protein Frizzled (Fz), the intracellular proteins Dishevelled (Dsh), Strabismus (Stbm), Flamingo (Fmn), Prickle (Pk) and Diego. Downstream effectors include the small GTPases of the RhoA family and JNK that upon activation lead to a variety of cellular responses including cytoskeletal rearrangements. Our research group has demonstrated a link between some of the PCP genes and human NTDs. We identified 52 rare mutations in a cohort of 629 NTDs patients in seven PCP genes (*VANGL1*, *VANGL2*, *PRICKLE1*, *CELSR1*, *FZD6*, *DVL2*, *FUZ*) that were absent in ethnically-matched controls. The 94% of the mutations were missense changes and a pathologic role was demonstrated for 46 of them by using bioinformatic prediction tools and *in vivo* and/or *in vitro* bioassays. Overall, the identified mutations realize of about 10% of NTD cases. We hypothesize that rare variants of PCP pathway must interact with other genes to modulate the incidence and severity of the disease.

C18.5

Clinical exome sequencing for cerebellar ataxia and spastic paraplegia reveals novel gene-disease associations and uncovers unanticipated rare disorders

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Clinical exome sequencing is an unbiased test that is used for straight-forward causative mutation detection, or for the discovery of novel (allelic) gene - disease associations. Often, it leads to broader disease spectra, or reverse phenotyping. Here, we describe the use of clinical exome sequencing for a group of 76 probands with ataxia or spastic paraplegia. Since these patients have had extensive testing (4.5 genes on average) before inclusion, mutations were not anticipated in the 'common' genes. In a two-tier analysis, variants in known disease genes were analysed first, followed by analysis of the 'full' exome data set if no causative mutations were identified. Subsequently, segregation analyses, enzyme tests or reverse phenotyping tests have confirmed or excluded the pathogenicity of most variants. In those 76 patients, we have thus detected causative mutations in 16, and likely-causative in another 9, all in the known disease genes. Some of the identified diseases are very rare or even the second case described. Furthermore, another 8 putatively-causative mutations were detected in genes outside the known disease genesl, and await confirmation (functional tests or other families with mutations in those gene). Some of the latter diseases may be allelic disorders, while others may be novel. In conclusion, exome sequencing for these neurological disorders has resulted in a molecular diagnosis in one-third of the patients, and has provided a candidate gene identification in approximately 1/10. More importantly, it has also provided some patients with a molecular diagnosis that was both unanticipated and otherwise not revealed.

C18.6

WES detects disease causing SNVs and CNVs in Primary immunodeficiencies

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Primary immunodeficiencies (PIDDs) constitute a heterogeneous group of genetic diseases affecting the immune system. Depending on the genetic etiology, symptoms range from mild to severe and life threatening. Knowledge of the molecular genetic cause and disease mechanism is important and can direct targeted and curative therapy. However, subtype classification is difficult as patients often have overlapping phenotypes. In addition, >250 PIDD genes have been reported, and few are offered for diagnostic genetic testing. We examined the utility of whole-exome sequencing (WES) to detect single nucleotide variants (SNVs) and copy number variations (CNVs) in the diagnosis of PIDDs. As of Feb 2014, 225 patients with extensive immunological and genetic testing from 200 families have been recruited from Texas Children Hospital (Houston, USA) and Oslo University Hospital (Norway). Strategies for genetic analysis were tailored based on clinical data, immunophenotyping and family history, but for most families only the proband was subjected to WES. Initially, WES data were systematically screened for variants in reported and potential PIDD genes. In addition, a computational CNV prediction pipeline was applied to enable identification of potential disease-causing CNVs from the WES data. Analysis of the first 110 families identified PIDD relevant variants in 60% of cases; half of these attaining a definitive molecular PIDD diagnosis. The other half had previously reported PIDD-causing variants, but with an unexpected or extended clinical phenotype, or heterozygote, potential deleterious variants in recessively inherited PIDD genes. Other interesting findings include PIDD-causing CNVs, somatic reversion causing mosaicism, and 13 potential novel disease genes.

C19.1

Constitutive Activation of PRKACA in Adrenal Cushing's Syndrome

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Endogenous hypercortisolism, referred to as Cushing's syndrome, is associated with excess morbidity and mortality. Corticotropin-independent Cushing's syndrome is caused by tumors or hyperplasia of the adrenal cortex.

We performed exome sequencing of ten cortisol-producing adenomas and matched control tissue to identify somatic mutations and evaluated recurrent mutations in candidate genes in adenomas of additional 171 patients. We further performed genome-wide copy number analysis in 35 patients with cortisol-secreting bilateral hyperplasias. We studied the effects of these genetic defects both clinically and in vitro.

Exome sequencing revealed somatic mutations in the PRKACA gene, which encodes the catalytic subunit of cyclic AMP-dependent protein kinase (PKA), in 8 of 10 adenomas. Overall, PRKACA somatic mutations were identified in a total of 22 of 59 adenomas (37%) from patients with overt Cushing's syndrome; these mutations were not detectable in patients with subclinical hypercortisolism (n=40) or in other adrenal tumors (n=82). Among 35 patients with bilateral cortisol-producing hyperplasias, 5 carried a germline copy number gain of the chromosome 19 region, including the PRKACA gene. In vitro studies demonstrated impaired inhibition of both PKA catalytic subunit mutants by the PKA regulatory subunit, while cells from patients with germline chromosomal gains showed increased protein levels of the PKA catalytic subunit; in both instances, basal PKA activity was increased.

This study links genetic alterations of the catalytic subunit of PKA to human disease. Germline duplications of this gene result in bilateral adrenal hyperplasias, whereas somatic PRKACA gain-of-function mutations lead to unilateral cortisol-producing adrenal adenomas.

C19.2

A mutation in SEC61A1 causes autosomal dominant interstitial kidney disorder associated with anemia and growth retardation

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Autosomal dominant tubulo-interstitial kidney disease refers to a group of disorders characterized by progressive loss of kidney function and the eventual need for dialysis and kidney transplantation. While mutations in the UMOD, MUC1, and REN genes have been identified as the primary causes of this disorder, there remain a number of families in whom the genetic cause has not been identified. Here we report a three-generation family with autosomal dominant progressive chronic kidney disease associated with congenital anemia and intrauterine growth retardation. Ultrasound examinations revealed small dysplastic kidneys without cysts, and a kidney biopsy revealed tubular atrophy with secondary glomerular sclerosis. We performed genome-wide linkage analysis and identified a candidate region with a maximum LOD-score of 2.7 on chromosome 3q. Combining whole exome sequencing data with our critical interval, we identified a c.553A>G variant causing a p.Thr185Ala change in SEC61A1. SEC61A1 encodes the alpha-subunit of the SEC61 complex, responsible for the translocation of proteins across the endoplasmic reticulum membrane. Suppression of se-

c61a1 in zebrafish embryos led to an absence of convolution of the pronephric tubules, whereas the pronephric ducts were unaffected, a phenotype similar to the tubular atrophy seen in our patients. This phenotype could be rescued by wild type SEC61A1 mRNA, but not with mRNA encoded from the p.Thr185Ala allele, suggesting that the variant is a loss of function. Taken together, our genetic findings and the functional studies support SEC61A1 as a causal gene for a novel, dominant syndromic form of progressive chronic kidney disease with tubular atrophy.

C19.3

TJP2 deficiency: a new cholestatic liver disease

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Progressive familial intrahepatic cholestasis (PFIC) is an autosomal recessive disorder manifesting as early-onset cholestatic liver disease. In 1998 mutations in three genes encoding membrane transporters were identified. However, 30% of patients remain without genetic diagnosis. Combined targeted resequencing (TRS) and whole-exome sequencing were used to analyse a cohort of 33 children, from 29 families, with chronic cholestatic liver disease and no mutations in known PFIC genes. Most were from consanguineous families. Homozygous mutations in *TJP2* were identified in 12 individuals of 8 families; all were predicted to abolish translation. *TJP2* encodes a cytosolic component of cell-cell junctional structures, linking integral membrane proteins such as claudins with actin filaments. Immunohistochemistry and western blotting of liver tissue from these patients showed a complete absence of TJP2 protein. Claudin-1 was not localised, but showed no alteration in protein level. Tight junctions were abnormal on transmission electron microscopy. A further 53 cholestatic patients were then examined by TRS. Mutations in *TJP2* were identified in 8 families. All mutations were protein-truncating mutations, except for three families with missense mutations. One missense mutation, p.His788Leu, was found in 2 unrelated families with severe early-onset cholestasis, which subsequently remitted. Previously a single, incompletely penetrant, missense mutation was identified in *TJP2* in Amish patients with isolated hypercholanaemia. In conclusion, a complete absence of TJP2 causes severe cholestatic liver disease, with destabilization of tight junction structures, whilst missense mutations in *TJP2* can lead to less severe phenotypes. These findings highlight the importance of TJP2 in the maintenance of liver integrity.

C19.4

Identification and functional characterization of ESR2, a new disease gene for 46,XY disorders of sex development (DSD)

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Disorders of sex development (DSDs) are congenital conditions with atypical development of chromosomal, gonadal or anatomical sex. The prevalence of these conditions is 1 in 4500, milder genital abnormalities are seen in 1 in 300 births. Today, the molecular cause is known in only 50% of cases.
Here, homozygosity mapping in a patient with syndromic 46,XY DSD revealed a potential functional candidate gene for DSD, namely *ESR2*, encoding the Estrogen Receptor beta. Sanger sequencing revealed a homozygous in-frame deletion in *ESR2* in this patient, c.541_543del (p.Asn181del). The deleted amino acid is located in the DNA-binding domain and is highly conserved. This deletion was absent in an ethnically matched control population. *ESR2* mutation screening in a 46,XY DSD cohort revealed an additional heterozygous mutation c.251G>T (p.Gly84Val). Prediction programs suggest an effect on protein function (SIFT, Polyhen, Mutation Taster). In addition the affected amino acid is conserved until fruitfly.
Immunohistochemistry in an 8-weeks old human male embryo showed ER-beta expression in the hindgut and the eyes, which might recapitulate the systemic manifestations of the index case.
Dose-response assays with DPN, ERE-luc and TK-3xEREluc constructs, and ER-beta wild type and mutant constructs in HEK293T cells showed a differential transcriptional activation of the ER-beta mutants.
In conclusion, our study sustains a role of *ESR2* as novel disease gene for syndromic 46,XY DSD. It is expected that further functional studies of these ER-beta mutants will provide insights into their molecular consequences and into the role of *ESR2* in the pathogenesis of 46,XY DSD.

C19.5

LRP5 variants associated with development of polycystic kidney and liver disease

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Autosomal dominant polycystic kidney disease (ADPKD) is a Mendelian disorder characterized by multiple renal cysts that progressively leads to renal disease. Polycystic livers are the most common extra-renal manifestation (83%). About 90% of ADPKD families harbor a *PKD1* or *PKD2* mutation, but others remain indistinct. Previous studies hypothesized involvement of a third locus. Recently, we identified mutations in a novel gene (*LRP5*) associated with isolated polycystic liver disease (PCLD).
We analyzed all exons of *LRP5* in a cohort of 29 patients with and 50 patients without prescreening of *PKD1/2* and *PKHD1*. We identified 2 novel and 2 rare variants in 4 unlinked ADPKD patients. One family demonstrated obvious segregation of the novel *LRP5* variant with the disease.
In silico analyses predicted both novel and 1 rare *LRP5* variant to be pathogenic, and the remaining variant as possibly damaging. All 4 variants were not found in exome data from 1,300 individuals of predominantly European ancestry sequenced in-house. The 2 novel *LRP5* variants were absent in several (inter)national databases and also excluded in a control set of 1,000 Dutch and 525 Moroccan DNAs of healthy, unrelated individuals.
For 3 mutations homology modeling predicted that ionic interactions disappear which diminishes the protein domain function. Luciferase activity assays were performed in eukaryotic cells transfected with wild-type and mutant *LRP5*. Wnt signal activation was significantly reduced in 2 novel and 1 rare *LRP5* variant.
In conclusion, these findings contribute to the pathofysiology of renal and hepatic cystogenesis with involvement of the canonical Wnt signaling pathway.

C19.6

Digenic model in Alport syndrome

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Alport syndrome (ATS) is a clinically and genetically hereditary nephropathy, that is often associated with deafness and ocular lesions. Monogenic inheritance models are well known, with semidominant X-linked inheritance, linked to *COL4A5* gene, or autosomal dominant or recessive inheritance, linked to *COL4A3* or *COL4A4* gene. The increased availability of high throughput sequencing permits identification of kindreds with pathogenic mutations in more than one disease-gene, also exploring digenic inheritance models. We have found 6 kindreds, in which the proband had 2 pathogenic mutations in different Alport associated genes. In 4 out of 6 cases it was shown that each

mutation was inherited from one of the parents. In most cases heterozygotes had less severe disease than the double heterozygotes. In two cases mutations involved the *COL4A5* and *COL4A4* genes, whereas in the remaining 4, mutations involved the *COL4A3* and *COL4A4* genes. Mutation pathogenicity was ascertained on the basis of the following criteria: non polymorphic missense mutations in key aminoacids, as Glycine in the collagen Gly-X-Y triple helical domain, or truncating mutations. In conclusion, results from new technologies showed that some cases of ATS may segregate according to a digenic inheritance model. This model has previously been shown in other diseases, such as retinopathies, cardiomyopathies or intellectual disability. Our results are of interest both from a scientific point of view and for genetic counselling. Clinical geneticists should be familiar with more complex models of inheritance, which could alter the recurrence risk.

C20.1

Single cell allele-specific expression (ASE) in Down syndrome and common aneuploidies

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Trisomy 21 is a model disorder of altered gene expression. Several studies have addressed the transcriptome differences between normal and affected individuals; however all of these studies suffer from the presence of 'noise' due to gene expression variation among different individuals. We have previously used a pair of monozygotic twins discordant for trisomy 21 in order to study the global dysregulation of gene expression (*Nature* in press 2014). The majority of previous studies focused on aneuploidies were conducted on cultured cell populations or tissues, but studies focusing on gene and allelic expression behavior at the single cell level are lacking. In this study we explore the allele specific expression in Trisomy 21 using transcriptome studies in single cells. We have used 40 normal cells and 48 trisomic cells from the fibroblasts of the monozygotic twins discordant for trisomy 21 and compared the ASE (allele specific expression), and their transcriptional metrics in these two cell groups. We observed a pattern of biased or monoallelic expression for the majority of the genes across single cells. These results will be presented and discussed. In addition a series of samples from mosaic trisomy 21, trisomy 13 and trisomy 18 are in different stages of investigation. These studies in single cells will provide a fundamental understanding of the gene expression dysregulation and allele specific expression in aneuploidies.

C20.2

Distinct properties of de novo mutations from whole genome sequencing of 50 patient-parent trios

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De novo germline mutations create the genetic variability that is the driving force of species evolution. However, they also can cause sporadic genetic diseases if they affect critical genomic regions. Whole Genome Sequencing (WGS) of parent-offspring trios allows us for the first time to study the result of mutational processes in a single generation in full detail. Here we report on the rate and pattern of de novo mutations based on WGS of 50 trios at high (80x median) coverage.
We identified an average of 58 de novo mutations (DNM) per trio (range 32-84), 2,883 DNMs in total. By using the segregation of informative SNPs we determined the parental origin for 678 DNMs, and found a paternal/maternal ratio of 4 to 1 (79%/21%), consistent with previous data. Using the parental origin of these DNMs we found a significant correlation between the paternal age and the number of paternal DNMs (Spearman's p=0.006) as well as a suggestive results for the correlation between maternal age and the number of maternal DNMs (p=0.08).
We find that DNMs do not occur completely random in the genome, but are spatially clustered within individuals (observed=28 (0.9%), expected=1.3 (0.004%), p-value<10-16) and have a sequence context that is enriched for CpGs (observed=482 (20.6%), expected=58 (2.6%), p-value<10-16). In conclusion, our study provides insight into the parental origin and distribution of de novo mutations throughout the human genome.

C20.3

Study of the regulatory landscape of *SHOX* in 503 LWD and ISS cases uncover a key role of the upstream *cis*-regulatory element CNE-3

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Leri-Weill Dyschondrosteosis (LWD) and idiopathic short stature (ISS) are caused by a variety of molecular defects of *SHOX* and the downstream PAR1 region. Interestingly, the first deletion and duplication of the upstream regulatory PAR1 region have recently been described in ISS, suggesting a new pathogenic mechanism for ISS. Here, we aimed to evaluate the contribution of copy number variations (CNVs) of upstream enhancers to the molecular pathogenesis of LWD and ISS, and to characterize the chromosomal architecture of the regulatory landscape of *SHOX*.
We performed CNV analysis of three previously characterized upstream enhancers (CNE-5, CNE-3 and CNE-2) using quantitative polymerase chain reaction (qPCR) analysis in 503 prescreened LWD and ISS patients with unknown molecular diagnosis. Three deletions and three enhancer duplications were found in five unrelated ISS patients respectively. Interestingly, their shortest region of overlap only contains CNE-3, pointing to a crucial role for this upstream enhancer. Using chromosome conformation capture assays (3C and 4C-seq), we demonstrated reproducible interactions of CNE-3 with the *SHOX* promoter. Finally, the 4C-seq study allowed us to map the complete regulatory landscape of *SHOX*, extending more than 1 Mb both upstream and downstream of the *SHOX* promoter.
In conclusion, our study substantiates a role of upstream CNVs in ISS patients. In addition, we provided evidence for a key role of CNE-3 as upstream enhancer. Finally, our study indicates an important role for the extended regulatory region of *SHOX* to be included in molecular diagnostics of *SHOX*-associated phenotypes.

C20.4

Pseudoautosomal region 1 length polymorphism in the human population

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The human sex chromosomes differ in sequence, except for the pseudoautosomal regions (PAR) at both the short and the long arm, denoted as PAR1 and PAR2. The boundary between PAR1 and the unique X and Y sequences was established during the divergence of the great apes. During a copy number variation screen, we noted a paternally inherited chromosome X duplication in 15 independent families. Subsequent genomic analysis demonstrated that an insertional translocation of X chromosomal sequence into the Y chromosome generates an extended PAR. The insertion is generated by non-allelic homologous recombination between a 548 bp LTR6B repeat with two paralogues located within PAR1 as well as at a distance of 105 kb from the PAR boundary on the X chromosome. The identification of the reciprocal deletion on the X chromosome in one family and the occurrence of the variant in different chromosome Y haplogroups, demonstrate this is a recurrent genomic rearrangement. In contrast to its perceived evolutionary stability, we demonstrate that a PAR1 length polymorphism exists in the human population. This finding represents a novel mechanism shaping sex chromosomal evolution.

C20.5

Comparative proteomic analysis of different fragile X syndrome cell lines

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Fragile X syndrome (FXS), the leading cause of inherited intellectual disability, is caused by absence of the FMRP protein due to expansion over 200 repeats of the CGG tract at the 5' UTR of the FMR1 gene and subsequent

DNA methylation. We have already characterized rare individuals of normal intelligence with CGG expansion over 200 repeats without DNA methylation (unmethylated full mutation, UFM), which have relatively normal transcription and translation, and represent the status of FXS cell lines prior to gene silencing. We have compared the three types of cell lines (normal control WT, FXS and UFM fibroblasts) with a proteomic approach in order to demonstrate possible differences that might clarify the mechanisms through which the rare UFM cells remain unmethylated and transcriptionally active. Protein extracts were compared by LC-ESI LTQ Orbitrap MS/MS analysis after mono-dimensional SDS-PAGE and trypsin digestion. Interrogation of the dataset for differential protein expression shows that some metabolic pathways are deregulated in UFM cells when compared to FXS cells. Among these pathways, mitochondrial metabolism (oxidative stress) is of particular interest considering its role in neurodegenerative diseases (like FXTAS) and particularly in epigenetic regulation. The deregulated proteins, specifically mitochondrial SOD (SOD2), were validated by Western blot. The interaction of target mRNAs with FMRP was assessed by RNA immunoprecipitation. Preliminary data suggest that mitochondrial metabolism is likely to have a role in DNA hypomethylation of UFM cell lines.

Supported by Telethon Onlus, FRAXA Foundation and Italian Association for fragile X syndrome.

C20.6

RNA-DNA Differences in Endoplasmic Reticulum Stress Response

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The endoplasmic reticulum (ER) is an organelle where proteins are synthesized and modified. ER stress occurs when there is an excess of misfolded proteins, and if the stress persists, then apoptosis is induced. Here we study RNA editing as a part of the ER stress response and how RNA editing modulates the cellular response to promote cell survival. We sequenced the RNA and DNA of B-cells from 10 individuals before and following 2 and 8 hours of tunicamycin treatment to induce ER stress. By comparing the RNA and DNA sequences, we identified 15,823 A-to-G editing sites in 1,523 genes as targets of RNA editing by ADAR (Adenosine Deaminase Acting on RNA). Among these sites, 314 showed significant changes in editing level following ER stress (p-value<0.01; ANOVA). These sites are found in genes known to be involved in ER stress response: RNA processing, protein transport and apoptosis. For example, sites found in *XIAP* (*X-linked Inhibitor of Apoptosis*) show a 3-fold increase in editing level. Furthermore, ADAR knock-down increases apoptosis following ER stress, suggesting that RNA editing is induced to promote cell survival. The effect of ER stress on editing level is not limited to canonical ADAR editing, as we also detected other types of differences between the RNA and DNA sequences whose levels change following tunicamycin treatment. In this presentation, we will describe RNA sequence modification as a critical step in ER stress response and cell survival.

C21.1

Mutations in POGLUT1, encoding protein O-glucosyltransferase 1, cause autosomal dominant Dowling-Degos disease

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Dowling-Degos disease (DDD) is an autosomal dominant genodermatosis which is characterized by progressive reticulate hyperpigmentation affecting the flexures, large skin folds, trunk, face and extremities. We previously identified loss-of-function mutations in KRT5 in fewer than half of the individuals of our DDD cohort.

We undertook an exome sequencing approach to identify additional genetic causes of DDD by focusing on five unrelated affected individuals without KRT5 mutations. Data analysis revealed three heterozygous mutations (c.11G>A (p.Trp4*), c.652C>T (p.Arg218*), c.798-2A>C) in these five individuals, all of which are in POGLUT1, encoding protein O-glucosyltransferase 1. By further screening of POGLUT1 in our unexplained cases of DDD, we identified six additional mutations. Immunohistochemistry of skin biopsies of affected individuals with POGLUT1 mutations showed significantly weaker POGLUT1 staining in the upper parts of the epidermis compared to healthy controls. Immunoblot analysis revealed that translation of the wild type POGLUT1 and a mutated form with an amino acid substitution led to the expected size of about 50 kDa, while a nonsense mutation led to a truncated protein of about 30 kDa. Immunofluorescence analysis identified a co-localization of the wild type protein with the endoplasmic reticulum and a notable aggregating pattern for the truncated protein. Protein modeling and transcript analysis supported the pathogenicity of the identified mutations. Recently, mutations in POFUT1, encoding protein O-fucosyltransferase 1, were also reported to be responsible for DDD. Both POGLUT1 and POFUT1 are essential regulators of Notch activity. Our results emphasize the important role of the Notch pathway in pigmentation and keratinocyte morphology.

C21.2

The phenotypic spectrum of SHOC2 c.4A>G (p.Ser2Gly)

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Noonan-like syndrome with loose anagen hair (Mazzanti syndrome) (MIM 607721) is caused by an invariant mutation in the *SHOC2* gene (c.4A>G, p.S2G). The disorder is characterized by facial features resembling Noonan syndrome, short stature, GH deficit, developmental delay, congenital cardiac defects, and a distinctive hair phenotype characterized by easily pluckable, slow growing hair in anagen phase.

We report on a large cohort of patients with molecularly confirmed diagnosis from an international research collaboration. In all patients, the c.4A>G change was invariably identified. Clinical data from a total of 89 patients (25 previously published) was available. The ages ranged from 0.08 - 39 years (median at 9.0 years). Short stature and an intellectual disability were present in most of the patients, and were more common than in patients with *PTPN11*, *SOS1* or *RAF1* mutations. Proven growth hormone deficiency was diagnosed in 42% of patients with known test results. *SHOC2* mutation-positive individuals also have a recognizable facial phenotype. Heart defects were common, but differently distributed compared to patients with mutations in other RASopathy genes. Multiple defects were documented in 51% of cases. Overall, Mazzanti syndrome represents a recognizable condition within the spectrum of RASopathies, with a single recurrent mutation, p.S2G, accounting for the vast majority of cases.

C21.3

Heterozygous germline mutations in *A2ML1* are associated with a disorder clinically related to Noonan syndrome

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Noonan syndrome (NS) is a developmental disorder characterized by short stature, facial dysmorphisms and congenital heart defects. To date, all mutations known to cause NS are dominant, activating mutations in signal transducers of the RAS/MAPK pathway. In 25% of cases, however, the genetic cause of NS remains elusive, suggesting that factors other than those involved in the canonical RAS/MAPK pathway may also play a role. Here, we used family-based whole exome sequencing of a case-parent trio and identified

a *de novo* mutation, p.(Arg802His), in *A2ML1* which encodes the secreted protease inhibitor Alpha-2-Macroglobulin-Like-1. Subsequent resequencing of *A2ML1* in 155 cases with a clinical diagnosis of NS led to the identification of additional mutations in two families, p.(Arg802Leu) and p.(Arg592Leu). Functional characterization of these human *A2ML1* mutations in zebrafish showed NS-like developmental defects, including a broad head, blunted face and cardiac malformations. Using the crystal structure of A2M, which is highly homologous to A2ML1, we identified the intramolecular interaction partner of Arg802. Mutation of this residue, Glu906, induced similar developmental defects in zebrafish, strengthening our conclusion that mutations in *A2ML1* cause a disorder clinically related to NS. This is the first report of the involvement of an extracellular factor in NS, and RASopathies in general, providing new leads for better understanding of the molecular basis of this family of developmental diseases.

C21.4

A mutation in PAK3 with a dual molecular effect deregulates the RAS/MAPK pathway and drives an X-linked syndromic phenotype

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Loss of function mutations in *PAK3* contribute to non-syndromic X-linked intellectual disability (NS-XLID) by affecting dendritic spine density and morphology. Linkage analysis in a three-generation family with affected males showing intellectual disability, agenesis of corpus callosum, cerebellar hypoplasia, microcephaly and ichthyosis, revealed a candidate disease locus in Xq21.33q24 encompassing over 280 genes. By sequencing all coding exons of the X chromosome, we identified a single novel variant within the linkage region, affecting a conserved codon of *PAK3*. Biochemical studies showed that, similar to NS-XLID-associated lesions, the predicted amino acid substitution (Lys389Asn) abolished the kinase activity of PAK3. In addition, it conferred a dominant negative function to the protein that drives the syndromic phenotype. The *in silico* tridimensional model of the mutated proteins and *in vitro* inhibition of protein neosynthesis by cycloheximide treatment revealed that NS-XLID mutations make the inactive kinase unstable and prone to degradation, while Lys389Asn confers stability to PAK3 protein, which escapes its physiologic degradation. Finally, *in vivo* studies in zebrafish embryos showed that PAK3^{N389} leads to a perturbation of the MAPK signaling and to alterations of cerebral and craniofacial structures, through an uncontrolled kinase-independent function. Our data expand the spectrum of phenotypes associated with PAK3 mutations, characterize a novel mechanism resulting in a dual molecular effect of the same mutation with a complex PAK3 functional deregulation, and provide evidence for a direct functional impact of aberrant PAK3 function on MAPK signaling, with the generation of a phenotype that could be included within the clinical spectrum of Rasopathies.

C21.5

Activating mutations in RRAS underlie a phenotype within the RASopathy spectrum and contribute to leukaemogenesis

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RASopathies, a family of disorders characterised by cardiac defects, defective growth, facial dysmorphism, variable cognitive deficits and predisposition to certain malignancies, are caused by constitutional dysregulation of RAS signalling predominantly through the RAF-MEK-ERK cascade. We report on two germline mutations in *RRAS*, a small monomeric GTPase controlling cell adhesion, spreading and migration, underlying a variable phenotype with features partially overlapping Noonan syndrome, the most common RASopathy. We also document that somatic *RRAS* mutations rarely occur in juvenile myelomonocytic leukaemia, a childhood myeloproliferative/myelodysplastic disease caused by upregulated RAS signalling, defining an atypical form of this haematological disorder rapidly progressing to acute myeloid leukaemia. Two of the three identified mutations affected known oncogenic hotspots of *RAS* genes, and conferred variably enhanced RRAS function and stimulus-dependent MAPK activation. Expression of a RRAS mutant homolog in *Caenorhabditis elegans* enhanced RAS signalling, and engendered protruding vulva, a phenotype previously linked to a RASopathy-causing SHOC2 mutant. These findings establish a functional link between RRAS and RAS signalling, and reveal an unpredicted role of enhanced RRAS function in human disease.

C21.6

A New Mouse Model for Costello Syndrome

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Costello Syndrome (CS) is a distinctive rare multisystem disorder comprising characteristic prenatally increased growth retardation, coarse facial features, redundant skin with deep palmar, plantar creases and papillomata of later onset. CS patients present also laxity of small joints, tight Achilles tendons, cardiac malformations, and developmental delay. The primary cause of CS was associated to the germ line activation of *H-Ras* oncogene, with a common missense mutation G12S in 80% of the patients. Here we describe the generation and the consequent phenotypic characterization of a genetically engineered mouse model of CS, by introducing the oncogenic G12S mutation by homologous recombination into the mouse Ras gene. The effect of the *H-Ras* G12S mutation was evaluated on behavioral, visual, metabolic, cardiac and histological traits in young adult animals. The behavioral analysis revealed that *H-Ras* G12S mutant males displayed reduced locomotor activity, accompanied by decreased muscle strength and altered motor coordination performance. In addition, the cardiac exploration revealed that *H-Ras* G12S mutants exhibit a hypertensive phenotype combined with tachycardia. In conclusion, the *H-Ras* G12S mutant mice showed a polysyndromic phenotype reproducing some of the CS features observed in patients. The future study of the here-described CS mouse model should have a significant impact of our understanding of CS disease. The use of *H-Ras* G12S mutant mice as a CS mouse model opens up new fields of investigation to better understand the pathophysiology of the disease and to evaluate drugs dedicated to the reduction of the disease associated symptoms.

C22.1

The impact of reporting exome and whole genome sequencing: Predicted frequencies of primary, secondary and incidental findings based on modelling

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The American College of Medical Genetics and Genomics (ACMG) has released practice guidelines recommending reporting of incidental findings (IFs) from exome and whole genome sequencing by Massively Parallel (Next Generation) Sequencing for multiple conditions. Policy statements from additional agencies are still being developed, with many attempting to take into consideration the predicted increase in workload of reporting IFs and secondary findings. We describe the effects on rates of various diagnostic findings of changing the sensitivity, the specificity, the implications of varying diagnostic criteria and a priori prevalence, and of increasing the number of included conditions.
Methods: We developed a simple mathematical model based on binomial

probability for predicting rates of diagnostic findings. We primed and validated the model using published variant frequencies and population carrier frequencies. Monte Carlo simulation was used to predict population carrier frequencies as a function of numbers of potentially deleterious genomic variants in sampled individuals.
Results: The model correctly calculates observed rates of IFs and genetic carriers. Changing the model's parameters shows that even minor changes in diagnostic criteria or sequencing accuracy causes large variation in rates of diagnostic findings.
Conclusion: Our model correctly explains observed rates of diagnostic findings. Key drivers of rates include diagnostic criteria, variant frequency, disease penetrance, and sequencing and bioinformatics accuracy. Rates of IFs are relatively insensitive to even large increases in the number of conditions included, but rates of genetic carriers are very sensitive to the number of conditions tested. These findings have great relevance to recommended practice and policy.

C22.2

Defending the child's right to an open future concerning genetic information

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There has been a discussion regarding the ethical acceptability of genetic testing of children for years, resulting in the majority view that minors should only be tested for early onset disorders where treatment or preventive options exist. Two principles underlie this consensus: first, the beneficence-based best interest standard that urges physicians to test for clinically relevant and actionable results. Second, the child's right to an open future principle that urges physicians not to test for adult onset disorders and carrier status, in order to preserve the child's future autonomy right to make its own decisions, also concerning the possible obtainment of genetic information.
The emergence of next generation sequencing (NGS) technology seems to challenge the previous consensus. There now is a growing list of commentators and examples from practice showing disagreement on whether conditions that do not have immediate consequences for the health of the child should be disclosed to parents. The American College of Medical Genetics and Genomics for example recently proposed to relinquish the current distinction between pediatric and adult genetic testing policy, thereby abandoning the child's right to an open future, while the American Academy of Pediatrics maintains the previous consensus.
In this paper we explain the normative rationale that underlies the current debate on a child- versus family centred genetic testing policy and argue that the right to an open future should remain a leading ethical principle in pediatric genomics.

C22.3

Implementation of a duty-to-recontact system in molecular and clinical genetics: perspectives from professionals and patients

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Advances in DNA-diagnostic techniques and NGS are leading to more diagnoses, but also to interpretation problems. Many findings cannot be interpreted yet, but may prove medically relevant in the future. Currently, there is no moral or legal obligation to recontact former patients. Many geneticists nevertheless consider it desirable to recontact patients when important new information arises. The UMCG (Groningen, NL) is investigating whether to develop a recontacting system in clinical genetics and how one could be implemented. We explored how professionals and patients from our university hospital feel on these two issues. We organised a focus group discussion with 12 professionals (clinicians, clinical geneticists and laboratory staff) and two group discussions with 3 and 5 patients, respectively, in which we discussed the desirability and requirements for successfully implementing recontacting. Both professionals and patients agreed that recontacting is desirable and implementation could be successful if the following requirements are met: (1) provide a guideline describing the responsibilities of molecular geneticists, genetic counsellors, and patients. This would also specify what information requires recontacting, which information should be given priority, and how recontacting should take place; (2) availability of a lab-based databank containing up-to-date genetic information and the possibility to automatically match new genetic information, e.g. on former variants of unspecified significance, with patients/groups for which the information is important; (3) patient's preferences regarding recontacting (e.g. what type of information and which recontacting medium to use); (4) development of e-health devices facilitating automatic recontacting.

C22.4

International views on sharing incidental findings from whole genome research

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Whilst genome-wide sequencing in a research setting may be used to explore the genetic basis of a phenotype it also offers the chance to opportunistically screen for additional results unrelated to the research project but relevant to the participants' future medical health (termed 'incidental findings', IFs).There is a wealth of medical and ethics literature supporting the feedback of IFs, yet there are limited empirical work offering a voice from both professional and public stakeholders directly affected by this.
A cross-sectional, web-based survey investigated the attitudes of 6944 individuals from across 91 countries towards searching for and sharing IFs. Participants included 4961 members of the public, 533 genetic health professionals, 843 non-genetic health professionals and 607 genomic researchers.
Eighty percent of participants believed that IFs from sequencing studies should be made available to research participants if they want them. Treatability and perceived usefulness of the data were important with 98% personally interested in learning about life-threatening conditions that were preventable. However, only 31% of participants thought genomic researchers should actively search for IFs that were not relevant to their research study. Genetic health professionals were the most likely to take this view (OR = 3.09, CI 2.23-4.28, $P < 0.0001$). This may be due to their appreciation of the complexities involved in translating genomic data in the clinic. Participants felt that genomic researchers should be able to focus on their research question without being forced to actively search for IFs, potentially at the expense of their study.

C22.5

Newborn screenings and whole genome sequencing: the real need of a genuine public involvement

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In the last decade newborn-screening programs and whole genome sequencing are making their way in clinical practice.
The question we focus on is whether the intrinsic persistence in time of genetic characters (monitored in newborn-screening programs), once combined both with the rules governing bio-banks which store those information and the natural lifelong evolution of personal individuality (from infancy to old age), creates an apparently inextricable set of problems.
In this paper we have the ambition simply to draw a list of them in order to open up a space for a public interdisciplinary discussion about new arising challenges that need to be framed and faced by public policies.
Some examples are:
- The complex and not yet unraveled relationship between the gradual evolution of the interested person and epigenetic developments.
- Regulations of parents' opting in/out screening programs and the chances of modifying their choices during time.
- The involvement of relatives and future siblings.
- The (im)possibility of a real opt out during the phase of reconsent.
- Difficulties arising from the organizational rules of biobanking in relation to the described long lasting process of consent.
- Privacy issues deriving both from the stable relationship between person and collected data (impossible anonymousness) and the possibility of control over informational flows.
We maintain it is necessary to foster a real engagement of community in genuine discussion on a) benefits of a universal system of genetic monitoring and b) ethical and legal implications for personal rights.

C22.6

Current Developments in the Regulation of Direct-to-Consumer Genetic Testing in Europe

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In recent years direct-to-consumer (DTC) genetic testing has provoked a lot of debates and lead to various statements and opinions of professional societies, medical associations and governmental bodies. In October 2013 the European Parliament voted a Regulation on In Vitro Diagnostic Medical Devices. This document is waiting for approval by the Council of the European Union which might still provide amendments to the text. When approved, this new Regulation is expected to introduce important regulatory changes in the field of medical devices, and especially in the field of genetic testing. In this presentation, we aim to present and evaluate the major impact of

this Regulation on the field of DTC genetic testing. First, adopting the current proposal will mean that genetic tests, including both health-related and lifestyle tests, will be subjected to a pre-market assessment by independent notified bodies. Second, according to the risk class they fall into, IVD devices will have to comply with revised requirements for clinical evidence. Third, the proposal requires appropriate genetic counseling for all genetic tests and classifies them as "prescription only". Finally, DTC advertising of devices classed as prescription only will be banned. Full implementation of these provisions would greatly impact DTC genetic testing companies as the present offer and advertising would no longer be tolerated. This work aims to clarify the proposed amendments and contribute to the ongoing discussion regarding the desirable degree of genetic testing regulation and the appropriate balance between promoting innovation and securing consumers' safety by using legal tools.

ESHG POSTERS

P01.002-M
Chromosome methylation and hydroxymethylation patterns are dynamically reprogrammed during human preimplantation development

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We analyzed the distribution of 5-hydroxymethylcytosine (5hmC) and 5-methylcytosine (5mC) in metaphase chromosomes from IVF-produced human triploid zygotes and morphologically abnormal cleavage stage embryos. To obtain metaphase chromosomes, zygotes and embryos were treated with 0.1% colchicines, 0.9% sodium citrate and fixed with freshly prepared 3:1 methanol:acetic acid. Then, QFH/AcD-banding technique was applied and cytosine modifications were detected by indirect immunofluorescence.
In zygotes, parental sets of chromosomes had reversed patterns of methylation and hydroxymethylation. Paternal sets, detected by the presence of chromosome Y, contained little 5mC, but were enriched in 5hmC, while maternal chromosomes were heavily methylated and contained little 5hmC. Thus, active demethylation in zygotes involves both parental genomes, but is more intensive in paternal genome. Metaphase chromosomes from zygotes had band-specific distribution of 5hmC: R-bands, but not G-bands and pericentromeric heterochromatin, were enriched with 5hmC. In contrast, 5mC did not demonstrate band-specific distribution. In 3-cell embryos, chromosomes with sister chromatids differed both in the levels of 5hmC and 5mC were identified. Number of these asymmetric chromosomes in blastomeres decreased with each cleavage division up to the blastocyst stage. At the blastocyst stage asymmetric chromosomes were rarely encountered. This advocates for the replication-dependant loss of 5hmC and 5mC.
Thus, maternal and paternal chromosomes demonstrate reverse patterns of 5hmC and 5mC at the zygote stage. The number of both methylated and hydroxymethylated chromosomes decrease during cleavage divisions, suggesting replication-dependant loss of modified cytosine.
Supported by RFBR, Administration of St. Petersburg, OPTEC grant and stipend from RF President.

P01.003-S
Single-tube multiplex-PCR panels of highly polymorphic STR markers for application to prenatal and pre-implantation genetic diagnosis of alpha- and beta-thalassemia and fragile X syndrome

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Alpha- and beta-thalassemia and fragile X syndrome (FXS) rank among the most common monogenic disorders globally. Prenatal diagnosis (PND) and pre-implantation genetic diagnosis (PGD) of these disorders are usually performed by direct mutation detection. Flanking polymorphic markers provide alternative indirect mutation detection through linkage analysis, or serve to corroborate direct mutation testing results. Currently, limited numbers of linked markers have been optimized and validated for PND/PGD of these diseases. We performed *in silico* mining to identify 24, 99 and 122 short tandem repeats (STRs) within 1 Mb on either end of the *HBA*, *HBB* and *FMR1* loci, respectively. Markers with low heterozygosity (HET) and/or polymorphism information content (PIC) after preliminary analysis on 16 anonymous DNAs were dropped, prior to large scale testing on 288 or 480 anonymous DNAs. The 9-plex *HBA* STR set (0.68≤PIC≤0.92; 0.71≤HET≤0.93; 10≤alleles≤34) can be further multiplexed with the Y1 box or other *HBA2* exonic fragments for simultaneous deletion/point mutation detection with linkage analysis, for either HbBart's or HbH disease. The 15-plex *HBB* STR set (0.70≤PIC≤0.89; 0.74≤HET≤0.90; 10≤alleles≤23) is further multiplexed with additional *HBB* exonic fragments to allow simultaneous thalassemia major mutation detection with linkage analysis. The 13-plex *FMR1* STR set (0.40≤PIC≤0.86; 0.49≤HET≤0.87; 6≤alleles≤17) can be analyzed in parallel with *FMR1* CGG repeat expansion mutation detection for FXS, using aliquots of whole genome amplified (WGA) product. All three single-tube multiplex-PCR STR assays have been optimized for use on genomic DNA, single cells, as well as WGA products of single cells.

P01.004-M
Two unusual unbalanced X chromosome rearrangements: a case report

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The human X chromosome is characterized by genomic instability and rearrangements, associated with various X-linked disorders such as moderate intellectual disability, muscular dystrophies or reduced fertility. In the Laboratory of Medical Genetics that operates as a department of IVF clinic, we are preferentially focusing on fertility connected scope. Here we show two women presenting amenorrhea associated with de-novo X chromosome aberration. First, we report on the case of a 23-year-old woman presenting phenotype close to isochromosome i(Xq) with short size and primary oligo-amenorrhea. Classical cytogenetic observation revealed a deletion of the chromosomal region Xp11-Xpter. With using FISH, CGH and mBAND methods, de novo duplication in the chromosomal region Xq25q28 was also discovered. As the second case, we describe 30-year-old woman who was referred to our IVF clinic because of infertility and amenorrhoea. At the age of 18 years, she was placed on oral contraceptive pills for cycle activation. Now she has been experiencing amenorrhea again and she has been unsuccessful to become pregnant. The cytogenetic analysis revealed de novo deletion in the Xq21.2-qter region. Further analysis by FISH, CGH and mBAND methods led to the revelation of Xp22.31-pter duplication. If we had contented ourselves only with the classical cytogenetic analysis, both cases could be underestimated as simple X chromosome deletions. However, our detailed observations led us to suspect that a complex aberration may be present. These two cases show that conscientious cytogenetic analysis at 550 ISCN bands level in combination with other molecular methods is essential for accurate diagnosis.

P01.005-S
Anti Müllerian Hormone (AMH) association analysis on about 1,000 caucasian women highlights 2 suggestive loci

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AMH is a dimeric glycoprotein member of the TGF-β superfamily expressed in the growing follicles of the ovary. AMH concentration in serum essentially reflects the ovarian follicular pool. AMH levels decreases during reproductive life, becoming undetectable after the menopause. Serum levels vary broadly by the age of decrease of female fertility (~ 41 years): the discovery of genetic variants responsible for the high variability could be useful to predict fertility and age of menopause.
AMH levels were measured in serum of 946 genotyped fertile women <=40 years old, 709 from the Italian Network of Genetic Isolates (INGI) and of 237 from the Obstetrics and Gynecology Unit of San Raffaele Hospital.
A meta-analysis for AMH adjusted for age was performed on genotypes imputed to the low variants enriched 1000G panel. Two suggestive loci were identified: a locus on chromosome 6 (MAF=0.017; p=8.05E-08) and a second locus on chromosome 3 (MAF=0.22; p=2.21E-07).
Meta-analysis will have to be enlarged by additional samples to increase the statistical power of the association analysis. Several hundred samples will be provided by different italian sources and 4,000 young women from Estonia will be available to replicate the results.

P01.006-M
Clinical and cytogenetic features of de novo Wolf-Hirschhorn (4p-) syndrome and partial trisomy 7p confirmed by array comparative genomic hybridization

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Wolf-Hirschhorn syndrome (WHS) is a well known genetic condition caused by a partial deletion of the short arm of chromosome 4. Although the critical region for determining the phenotype is at 4p16.3, the great variability in the extent of the 4p deletion and the possible contribution of additional genetic rearrangements lead to a wide spectrum of clinical manifestations. Here, we report perinatal diagnosis and molecular cytogenetic characterization of a newborn infant with WHS and partial trisomy of 7p. The patient was a female neonate with facial dysmorphia including ocular hypertelorism, micrognathia, both preauricular pit, low set ears, and short and stubby fingers. Fetal agenesis of the corpus callosum was suspected by cranial ultrasonography. Laboratory analysis showed that she had acute renal failure. Chromosomes analysis by G-banding revealed 46,XX,add(4)(p16.3) karyotype. To delineate the origin of additional genomic gain in chromosome 4, array comparative genomic hybridization (CGH) was performed. Array CGH sho-

wed a 1.34 Mb sized loss on chromosome 4p (4pter-->16.3) and a 32.48 Mb sized gain on chromosome 7p (7pter-->14.3). Therefore, the final karyotype of the patient was defined as 46,XX,der(4)t(4;7)(p16.3;p14.3). The parental karyotypes were normal. In this case, we describe for the first time, as far as we know, the clinical and cytogenetic analysis of a patient with concomitant occurrence of partial of Hirschhorn (4p-) syndrome and partial trisomy 7p. This report suggests that the array CGH would be a valuable diagnostic tool for identifying the origin of small additional genetic materials.

P01.007-S
Preimplantation genetic screening of copy number variations (CNVs) using oligonucleotide-based array comparative genomic hybridization

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Chromosome aneuploidy is the most prevalent genetic abnormality in human embryos and represents the leading genetic cause of miscarriages. In view of this fact, diagnosis of embryos for chromosome abnormalities using array-CGH, i.e. preimplantation genetic screening (PGS), is suitable way to improve clinical outcomes in patients undergoing in vitro fertilization (IVF). In our work we analyzed of the whole genome profiles from trophoectoderm cells in 23 patients and 9 healthy donors (DEM). Overall, we evaluated 118 embryos using oligonucleotide-based CytoSure Single Cell Aneuploidy Array 8x15K. The copy number abnormalities (CNAs) were found in 23.7% of all embryos. While incidence of CNAs in DEM embryos was 11.8% of samples, CNAs in patient´s embryos occurred in 28.6% of samples. Aneuploidies of chromosomes were observed in 26.7%, segmental imbalances were proved in 6.8% of embryos. In patient´s cohort, we found overall 35 different CNAs. The most common aneuploidies were trisomy 21 and 13 (both 8.3%), whereas the most frequent losses of genetic material were monosomy 8 (12.5%) and 18 (8.3%). Two patients had embryos with complex aneuploidy. Segmental CNAs were found in 5q, 8p, 8q, 14q and 16p. In DEM embryos, we observed two 2 structural CNAs (gain 13q; loss 7p) and 2 cases with aneuploidies (trisomy 19, monosomy 18). This study shows that oligonucleotide array is novel progressive tool for sensitive genome-wide analysis of chromosome instability and opens the route towards high-resolution preimplantation screening which improves selection of embryos and implantation rates. Supported by OP VK CZ.1.07/2.4.00/31.0155 and CZ.1.07/2.3.00/20.0183

P01.008-M
Copy Number Variation (CNV) in azoospermic males

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We tested 95 males from the biobank at Department of Medical Genetics, Oslo University Hospital for Copy Number Variations (CNV). All males were azoospermic and referred for infertility testing, but no futher information was known. They had normal karyotype, and no deletion in AzF region on chromosome Y nor mutations in the CFTR gene. As controls we used 94 males from our biobank, who had fathered a child and did not have the abberation found in their child on aCGH.
The samples were run on Agilent technology array comparative genome hybridization with 180K and 400K resolution according to the manufactures procedure. 23 patients were run on 180K aCGH against a single sample control, 35 against a multisample control, and 37 patients on a 400K array with multisample control. We found totally 1863 CNVs in the patient group (19.6/patient) and 973 (10.3/patient) in the control group. For patients and controls run with same resolution (180K) we found 9.2 and 10.3 CNVs per patients.
The CNVs in the control group were subtracted from the patient group. We also eliminated CNV's in regions with no known genes. The remaining 154 chromosome regions were checked for CNV's known for expression or function in testis in the Database of Genomic Variants (DGV). There were no major large chromosome regions common for all patients and no regions earlier described as candidate genes. This left us with 12 new interesting small CNV regions not described before, that might affect male infertility.

P01.010-M
Non invasive prenatal diagnosis of beta-Thalassemia: application of Ion Torrent sequencing and long-range PCR haplotyping in 18 cfDNA samples

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The discovery of fetal DNA in maternal plasma has provided a new source of fetal genetic material that can be safely obtained from maternal blood and successfully processed for non invasive genetic diagnosis (NIPD). We describe a protocol for non-invasive prenatal diagnosis of β thalassemia with a next generation sequencing approach and Ion Torrent technology. Eighteen experiments of NIPD of β-thalassemia have been carried in cfDNAs of women who underwent PD for β-thal. In total, we have amplified and sequenced 47 amplicons mapping in the beta-globinic gene cluster and further 3 amplicons (ZFX/ZFY, SRY and TSPY1) useful to detect fetal sex and fractional fetal DNA. In the validation phase we have included the parental DNAs and trophoblast DNA to infer parental haplotypes inherited from the fetus. In the meanwhile we have developed a protocol to infer parental haplotypes without fetal information. The method is based on haplotype selection by allele-specific long-range PCR (LR-PCR) with allele-specific primers with the 3' base complementary to the mutated or to the normal allele for the most common β-thalassemia mutations. These reactions were performed with an high fidelity polymerase able to amplify genomic targets extending both downstream and upstream the causative mutations. By this approach, the 69.4% of fetal haplotypes, mainly of paternal origin, has been correctly identified in the cfDNAs processed. Following these encouraging results, we are working in order to improve the performance of our platform by extending the analysis to a higher number of SNPs and by developing specific algorithms of analysis.

P01.011-S
Prenatal diagnosis of severe X-linked chondrodysplasia punctuata in 8 female fetuses

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Conradi-Hunermann-Happle (CDPX2) syndrome is a rare X-linked dominant skeletal dysplasia. It is usually lethal in males, while affected females show wide clinical heterogeneity. Mutations have been reported in EBP, which is involved in cholesterol biosynthesis. To date, severe prenatal occurrence has been reported in only 6 females.
In order to characterize this severe phenotype, we analyzed the 8 prenatally-diagnosed female cases of severe EBP mutations listed in France. The mean age at prenatal diagnosis was 22 weeks of gestation. The ultrasound features included mainly bone abnormalities: shortening (7/8) and bowing of the long bones (4/8), stippled epiphyseal cartilage (5/8) and irregular aspect of the rachis (5/8). The pregnancy was terminated in 6/8 cases. Fetal examination revealed ichthyosis in all cases and skeletal X-rays showed constant epiphyseal stippling, with frequent asymmetrically shortened long bones and bowing. All cases appeared de novo except for two fetuses with moderately affected mothers, who presented ichthyosis and short stature, and one case of germinal mosaicism. In order to explain the high intrafamilial clinical heterogeneity, the X-inactivation pattern was studied in one familial case. Skewed X-inactivation was found in the mother's lymphocytes (cDNA study showed that the wild allele was mainly expressed) and random X-inactivation was found in the different fetal tissues affected.
In conclusion, we report additional cases of severe prenatal CDPX2 presentations in female fetuses. Even though the majority of these were de novo cases, our familial cases argue for careful genetic counselling in this condition.

P01.012-M

Fetal Fraction estimate in twin pregnancies using directed cell-free DNA analysis

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Objective: To estimate fetal fraction (FF) in monozygotic and dizygotic twin pregnancies.
Methods: Maternal plasma samples were obtained from 35 monochorionic twin pregnancies with male fetuses (monozygotic) and 35 dichorionic pregnancies discordant for fetal sex (dizygotic) at 11-13 weeks' gestation. Cell-free DNA was extracted and chromosome-selective sequencing with digital analysis of selected regions (DANSR TM) was carried out. The fetal-fraction optimized risk of trisomy evaluation (FORTE TM) algorithm was used to estimate FF using polymorphic alleles. In dizygotic twins the FORTE algorithm was modified to estimate the smallest FF contribution of the 2 fetuses. In both types of twins, FF was also determined by analysis of Y-chromosome sequences. Results: In monozygotic twins, the median total FF was 14.0% (range 8.2-27.0%) and in dizygotic twins the median smallest FF was 7.9% (4.9-14.0%). There were significant associations in FF between the methods using polymorphic alleles and Y-chromosome sequences for both monozygotic ($r = 0.951$, $p < 0.0001$) and dizygotic ($r = 0.743$, $p < 0.0001$) twins.
Conclusions: The study demonstrates the feasibility of an approach for cfDNA testing in twin pregnancies. This involves estimation of total FF in monozygotic twins and estimation of the lower FF of the 2 fetuses in dizygotic twins.

P01.013-S

Prenatal Diagnosis of Aneuploidy by Cell Free Fetal DNA in Maternal Plasma

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This study examined the methylation difference in AIRE and RASSF1A between maternal and fetal DNA, and the implication of this difference in the identification of free fetal DNA in maternal plasma and in prenatal diagnosis of trisomy 21. Maternal plasma and amniotic fluid samples were collected from 30 singleton pregnancies. Methylation-sensitive restriction enzymes in digestion of differential maternal-fetal methylation followed by fluorescent quantitative PCR (MSRE + PCR) were employed to detect trisomy 21. Diagnosis of trisomy 21 was established according to the ratio of fetal-specific AIRE to RASSF1A that are hypermethylated in maternal plasma and are not digested with methylation sensitive restriction enzymes. All of the results were approved with karyotype results. Based on the data from 22 euploidy pregnancies, the 95% reference interval of the fetal AIRE/RASSF1A ratio in maternal plasma was 0.33-1.77, which was taken as the reference value for determining the numbers of fetal chromosome 21 in 30 pregnancies. Firstly, 18 from 22 euploidy pregnancies were detected euploid correctly and 4 cases incorrectly so the early sensitivity rate was 81.81% (18/22). But by repeating the test with better digestion, the four cases made correct results so the final sensitivity rate was 100% (22/22). All of the eight trisomy 21 pregnancies were diagnosed with this method correctly so the specificity of this method was 100%. Also with performing STR-Typing and checking paternal alleles in maternal plasma and comparison with maternal alleles in 16 loci, the protocol of cell free fetal DNA extraction from plasma were confirmed.

P01.014-M

Non-invasive EXamination of Trisomy (NEXT) Study: Directedcell-free DNA analysis versus 1st trimester combined screening for Trisomy 21 risk assessment in a large Routine pregnancy population

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Non-invasive prenatal testing (NIPT) with cell-free DNA (cfDNA) is highly accurate for fetal trisomy evaluation in high-risk pregnancies. Routine pregnancy population NIPT performance has not been evaluated in a large prospective study. Our objective was to compare NIPT with directed cfDNA analysis to first trimester combined screening (FTS) for trisomy 21 risk assessment in a general pregnancy population.
This prospective multi-center blinded cohort study compared HarmonyTM Prenatal Test, a directed cfDNA test, with FTS using first trimester PAPP-A, hCG and nuchal translucency measurement. Women with a singleton fetus presenting in the first trimester for routine prenatal screening for fetal aneuploidy were eligible. Participants had both FTS and Harmony. FTS results were provided as part of routine care. Participants and care providers were blinded to Harmony results, calculated as probability scores. Pregnancies were followed for newborn outcomes. Invasive test results or neonatal phenotype,with karyotype confirmation in cases of suspected aneuploidy, were used for trisomy 21 identification. Harmony, FTS results and outcomes were reported to an independent data coordinating center. Primary outcome was comparison of the area under the ROC curve for trisomy 21 test performance of the Harmony and FTS.
18,955 women were enrolled across 38 centers in USA, Canada and Europe from March 2012 to April 2013. The mean maternal age was 30.6 (18-52) years. The mean gestational age was 12.4 (10-14.3) weeks. Follow-up is complete.
Study results will be presented. Implications for use of NIPT for trisomy 21 risk assessment in the general pregnancy population will be discussed.

P01.015-S

A case report of a high level 46,XX/46XY true chimerism without clinical effect in a healthy female who gave birth to healthy twins after IVF

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Chimerismis a rare event in humans when two or more genetically distinct cell lines occur in an individual. It is mostly connected with ovotesticular disorder of sexual developpent. We now present a case of high level 46,XX/46,XY
chimerism in a healthy woman who was karyoptyped prior to IVF. The fenotype of the proband was that of a normal woman with an unambiguously female external and internal genitalia.The XX/XY cell lines were detected in peripheral lymphocytes, buccal mucosa, urine sediment and also in all other subsequently biopted tissues: skin, both ovaries,
peritoneum and endometrium.The male cell line was detected by FISH technique(CEP X/Y SateliteIII DNA probes) in all samples at a propotion of 20-50%. The presence of SRY was confirmed using PCR. The chimerism was confirmed by STR multipexes
on chromosomes X, 13, 18, 21 and by STR markers kit (AmpFlSTR Identifiler PCR Amplification Kit). The
result of the analysis proved tetragametic origin of the chimerism.Our proband underwent three cycles of IVF with own oocytes and ICSI. The third cycle was successful. ET of 2 embryos was performed and after normal preganacy healthy female twins were born.

P01.016-M

Cell culture conditions of chorionic villous samples do not modify the genomic imprinting pattern at locus 11p15.5

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Methylation of the CpG islands is a common epigenetic marker of gene repression. Monoallelic and parental-specific DNA methylation pattern at the Imprinting Control Region 1 (ICR1) and 2 (ICR2) regulates the expression of IGF2/H19 and KCNQ1/CDKN1C domains at the imprinted locus 11p15.5. Alterations of ICR1 and ICR2 methylation state are common in Beckwith-Wiedemann (BWS) and Silver-Russell (SRS) syndromes and a robust molecular investigation is crucial to support phenotypic evidences, particularly in prenatal diagnosis for BWS. Since it is known that cell culture conditions could per se modify the epigenetic signature of the cells, we aimed to compare ICR1 and ICR2 methylation profile in fresh chorionic villus samples (CVS) with the corresponding cell cultures (CVC) to verify whether methylation at ICRs is stable after cell culture. By pyrosequencing we analyzed 9 CVS and their relative CVC from healthy pregnancies that underwent prenatal

diagnosis for maternal age. The range of methylation levels of ICR1 and ICR2 in control CVS was previously reported by our group (ICR1: 38-48%; ICR2: 37-47%). Herein, we found that the mean methylation percentage of ICR1 and ICR2 remains stable after cell cultures: CVS= ICR1: 44% ± 1,7%; ICR2: 42% ± 1,8%, CVC= ICR1: 44% ± 2,3%; ICR2: 42% ± 3,1%. The high stability of such genomic imprinted regions makes them useful to be investigated in prenatal diagnosis.

P01.019-S
Prenatal diagnosis: chromosomal microarray in fetuses with increased nuchal translucency

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Chromosomal microarray has significant advantages over standard metaphase karyotyping for detecting large chromosomal imbalances and alterations smaller than 10 MB in size. The method has besome an important diagnostic instrument in the prenatal setting for pregnancies with abnormal ultrasound findings. In our clinical setting (The Central Region, Denmark), more than 90 % of pregnant women receive combined 1st trimester screening and 2nd trimester anomaly scan via the public health care system. Since January 2013 we have used a two-tiered approach for invasive diagnostics of all foetuses with nuchal translucency > 3.5 mm (99 percentile) in 1st trimester pregnancies. This consists of a fast prenatal analysis for common aneuplodies by QF-PCR followed by chromosomal microarray on samples with normal QF-PCR test results. The analysis methods used are the Elucigene QST*R kit (GenProbe) and comparative genomic hybridization-based microarrays (SurePrint G3 Human CGH microarray 180K, Agilent). DNA was extracted directly from chorionic villus samples using the Maxwell® system.
We demonstrate the usefulness of microarray testing in this clinical setting based on our laboratory experiences.

P01.020-M
Clubfoot as an indication to perform array-cgh on prenatal diagnosis even when isolated?

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We report on the first pregnancy of a 31 year old healthy female. At 13+2 weeks of gestation, a combined test was carried out, which showed a calculated risk for trisomy 21 of 1:38 (NT 1,8mm, free β-hCG 1,52MoM and PAPP-A 0,22MoM). The patient decided to undergo chorionic villus sampling which showed a normal foetal karyotype (46,XY). At 17+6 weeks of gestation an ultrasound showed isolated bilateral clubfoot, no further genetic testing was offered. At 19+6 weeks of gestation the ultrasound confirmed the isolated bilateral talipes equinovarus and at 23+5 weeks of gestation hypospadia and dilated hyperechogenic bowel was also noted: array-CGH testing on foetal DNA was offered and the patient accepted. The array-CGH analysis showed a de novo 3,4Mb deletion on 5q31.1 (131825068-135229731) encompassing, among many others, the PITX1 gene, whose heterozygous mutations have been associated with congenital clubfoot and other skeletal anomalies. Patients with partially overlapping deletions have been described in the literature and most share common features like developmental delay, short stature and dysmorphic features. The pregnancy was interrupted at 30 weeks of gestation. This case might suggest that clubfoot, which is generally considered a mainly isolated congenital defect and for which no further genetic testing is offered in prenatal diagnosis, might be the first sign of a more complex picture which could also include other congenital defects and, possibly, developmental delay. Should we consider clubfoot, even when isolated, an indication to perform array-CGH on foetal DNA?

P01.021-S
CNV and Aneuploidy Detection by Ion Semiconductor Sequencing

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Ion Torrent™ semiconductor sequencing, combined with Ion AmpliSeq™ technology, provides simultaneous identification of copy number variants (CNVs), single nucleotide variants (SNVs), and small insertions and deletions (indels) from a research sample by means of a single integrated workflow. 100% of assayed CNV regions (n=34) were detected using a reference set of 31 samples with known chromosomal aberrations. Low-pass whole-genome sequencing data, with approximately 0.01x read coverage, allowed the rapid ≤10 hour analysis of aneuploidies from research samples with extremely low initial input DNA amounts—even from a single cell. Using a control set of 10 samples with known chromosomal aberrations, 100% of the copy number changes were found, ranging from gains or losses of whole chromosomes to subchromosomal alterations tens of megabases (Mb) in size. The Ion PGM™ System minimizes the high cost and complexity of next-generation sequencing and, with Ion Reporter™ Software, facilitates user-defined CNV and aneuploidy detection, with three sensitivity options so that copy number analysis workflows can be tuned to achieve desired levels of sensitivity and specificity.

P01.022-M
Fetal intracerebral hemorrhage and cataract: think COL4A1

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The COL4A1 gene encodes the alpha1 chain of type IV collagen, a crucial component of nearly all basement membranes. Mutations in COL4A1 were first associated with cerebral microangiopathy and familial porencephaly and have later been implicated in a clinicopathologic broad-spectrum affecting the brain, eyes, kidneys and muscles. Recently, COL4A1 mutations have also been identified prenatally in fetuses with intracranial hemorrhage (ICH). We report two additional prenatal cases of COL4A1 mutations in fetuses with ICH and cataract.
Case 1: Fetal ultrasound examination (US) at 23 weeks' gestation (WG) showed left cataract, left ventriculomegaly and hyperechogenic lesion of basal ganglia. Fetal magnetic resonance imaging (MRI) at 32 WG confirmed the subependymal hemorrhage affecting the left hemisphere.
Case 2: Fetal US examination at 31 WG showed hyperechogenic lesion in left hemisphere with thalamic echogenicity and bilateral cataract. Fetal MRI at 32 WG showed a left-sided periventricular parenchymal hemorrhage and mild ventriculomegaly.
In both these cases, the involvement of COL4A1 was evoked because congenital cataracts had been previously reported in association with ICH in pediatric cases.
The sequencing of COL4A1, performed on fetal DNA after termination of pregnancy, evidenced two heterozygous novel missense mutations c.2317G>A (p.Gly773Arg) and c.3005G>A (p.Gly1002Asp) in fetuses 1 and 2 respectively.
The two cases reported here show that the COL4A1 mutation should be envisaged in fetuses with prenatal ICH especially in the presence of lens abnormalities at US examination. Molecular confirmation of a COL4A1 mutation may have important implications for the outcome of the pregnancy and for genetic counseling.

P01.023-S
Validation And Clinical Application Of A Next-Generation Sequencing (Ngs)-Based Protocol For 24-Chromosome Aneuploidy Screening Of Embryos

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The rapid development of next-generation sequencing (NGS) technologies has generated an increasing interest in determining whether NGS could be reliably used for preimplantation genetic screening (PGS), i.e. for comprehensive aneuploidy screening of human embryos produced from patients undergoing in-vitro fertilization treatments, with the purpose of identifying and selecting for transfer euploid embryos.
We performed a large validation study to determine the accuracy of a NGS-based 24-chromosomes aneuploidy screening protocol. NGS ability to accurately identify aneuploidy was assessed in three steps: 1) a blind evaluation of karyotypically-defined chromosomally abnormal single cells; 2) a retrospective blinded assessment of 244 embryos previously analyzed by array-comparative genomic hybridization (aCGH); 3) a prospective trial involving a parallel evaluation of 192 blastocysts, from 55 clinical PGS cycles, with both NGS and aCGH techniques.
The NGS method was robust, with 454/454(100%) samples yielding results. Aneuploidy diagnoses were fully concordant with those obtained using aCGH technique. NGS was also able to detect chromosomal mosaicism in 54/54(100%) of mosaic embryos assessed. Clinical application of the NGS

protocol revealed 76(39.6%) euploid blastocysts. Following transfer of 50 embryos, 30 women had a sustained pregnancy (63.8% clinical pregnancy rate/ET; 64.0% implantation rate).
This is the first study reporting extensive validation and clinical application of NGS for PGS purpose, allowing identification and transfer of euploid embryos resulting in healthy pregnancies. Evidence of accuracy demonstrates that NGS represents a reliable high-throughput methodology for comprehensive aneuploidy screening, capable of detecting whole chromosome aneuploidies and segmental changes in embryos, with the potential to revolutionize preimplantation diagnosis.

P01.025-S
A new case of Apert's syndrome detected prenatally in Romania

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Apert's syndrome (Acrocephalo-syndactyly) is a rare congenital condition characterized by primary craniosynostosis, mid face malformations and symmetrical syndactyly of the hand and feet. The incidence of Apert's syndrome is about 15/1.000.000 live birts and is inherited in an autosomal dominant fashion, but sporadic cases are also frequent. Case report: A 32-year-old, gravida 4, para 0 woman was referred for fetal evaluation at 22 weeks of gestation because of digital abnormalities in the fetus. A prenatal ultrasound at 22 weeks of gestation revealed frontal bossing, low set ears, depressed nasal bridge, digital fusion, and bilateral syndactyly of the hands and feet. Amniocentesis was performed and the cytogenetics and the molecular test was done. A DNA testing for the FGFR2 gene was immediately performed using uncultured amniocytes, which revealed a heterozygous (P253R) mutation in the FGFR2 gene. The karyotype was normal, 46,XY. The woman decided to discontinue the pregnancy, and a male baby was delivered with frontal bossing, midface hypoplasia and bilateral syndactyly of the hands (mittenhands) and feet. Conclusions: A molecular analysis of FGFR2 using uncultured amniocytes is useful for rapid confirmation of Apert syndrome at prenatal diagnosis. The baby was the first case with Apert's syndrome confirmed by genetic testing in Romania.

P01.026-M
Could a combination of QF-PCR and SNP-array methods replace standard karyotyping in CVS?

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In the 2010 - 2014 period 689 chorionic villus sampling **(**CVS) were successfully performed. QF-PCR and a complete chromosomal examination (karyotyping) was a standard examination schema for all samples. Abnormal results were found in 184 samples (26.9 %). Totally 165 (89.7 %) cases out of these 184 pathological results has been announced by QF-PCR (most frequently an autosomal aneuploidy) prior to karyotyping. This is in contrast to 19 abnormal findings detected only due to classic chromosomal examination (9 balanced aberrations, 9 mosaics of both autosomes and gonosomes, 1 unbalanced aberration). In the same period of time 102 CVS (with normal karyotype and QF-PCR results) were evaluated by SNP-array (Illumina). Well-defined pathogenic microdeletions or microduplications were detected in 7 CVS (6.8 %) indicated from following reasons: increased nuchal translucency (5 cases), heart defect (1 case) and anal and esophageal atresia (1 case).
In our study, an examination of CVS based on QF-PCR method only would let 19 abnormal findings (2.5%) undetected out of which only 10 (1.45 %) are defined as pathological (9 mosaics of both autosomes and gonosomes, 1 unbalanced aberration). In all 10 cases we suppose that these aberrations would be detected by SNP-array evaluation. In addition to it, re-examination of CVSs with normal karyotypes and QF-PCR results by SNP-array allowed us to detect another 7 submicroscopic pathological abnormities. Based on these data we consider the classic karyotype examination to be still an invaluable part of the CVS testing procedure.

P01.027-S
Invasive prenatal diagnosis in bizkaia: a 20-year experience

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Objective: To analyze trends in the number of invasive procedures and the incidence of chromosomal abnormalities over a 20-year single institution experience
Methods: Data of 16,894 invasive prenatal procedures performed between 1993 and 2013 in Bizkaia were retrospectively reviewed, with particular emphasis on indications
and number and type of abnormal results. Results: Traditionally, maternal age has been the main referral reason for prenatal testing (69.3%) but during the last 3 years, prenatal screening has become the
most important clinical indication, decreasing the number of invasive procedures performed. Related to karyotype analysis, chromosomal abnormalities were detected in 348 out of the 16,894 (2.55%) cytogenetic studies. Among chromosome aneuploidies (1.55%), the most frequent ones were classical autosomal aneuploidies (1.09%): Trisomy 21, 18 and 13 were diagnosed in 115 (0.94%), 23 (0.07%) and 9 (0.06%) cases respectively. Sex chromosome neuploidies were found in 59 cases (0.46%), being Klinefelter Syndrome the most common sex aneuploidy diagnosed (0.16%). Balanced rearrangements corresponded to 0.67% of the structural abnormalities while unbalanced rearrangements were found in 16 cases (0.15%). Conclusion: Nowadays, positive prenatal screening has progressively replaced advanced maternal age as the main referral reason for amniocentesis. Our study based in 16,894 amniocentesis contributes to establish a standard reference incidence of chromosomal abnormalities in pregnancies. Our data also confirm the karyotype as a reliable method for detecting complex chromosome abnormalities, providing an important basis for prenatal counseling and for prenatal screening policy in the national strategy.

P01.028-M
Observations following commercial implementation of a cell-free DNA (cfDNA) and single-nucleotide polymorphism (SNP)-based non-invasive prenatal aneuploidy test (NIPT)

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Objective: To describe the clinical observations following implementation of a SNP-based non-invasive prenatal aneuploidy test in more than 28,000 pregnant women.
Methods: 28,709 consecutive cases reported since March 2013 for NIPT. Isolated cfDNA was amplified via multiplex PCR targeting 19,488 SNPs covering chromosomes 13, 18, 21, X, and Y. Sequencing data was analyzed the NATUS algorithm. Reports were issued describing risk for trisomy 21, trisomy 18, trisomy 13, and Monosomy X, and in a subset of cases triploidy, sex chromosomes trisomies and fetal sex. All reports included fetal cfDNA fraction. Follow-up information on samples receiving a high-risk result for trisomy 21, trisomy 18, trisomy 13, or Monosomy X was collected from providers.
Results: 510 (1.8%) cases received a high-risk NIPT result for any of the four main indications (325 trisomy 21, 83 trisomy 18, 41 trisomy 13, 61 Monosomy X). Of the 28,709 cases, 49% were under 35 years of age. 17,529 low-risk results and 358 high-risk results were reported to centers participating in follow-up efforts. A response to requests for follow-up information was received for 317/358 (88.5%) of the high-risk patients; including 120 calls (33.5%) with karyotype. These requests identified 21 false positives (8 trisomy 21, 1 trisomy 18, 8 trisomy 13, 4 Monosomy X). Two (0.01%) trisomy 21 false negative results were voluntarily reported.
Conclusions: Performance of this SNP-based approach in a clinical setting, when adopted by a large and diverse population and distribution base, appears consistent with previously reported validation performance characteristics.

P01.029-S
Non-invasive prenatal testing of fetal aneuploidies and follow-up

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In the Netherlands the Minister of Health has licensed noninvasive prenatal trisomy testing (NIPT) as from April 1st 2014, for women with a risk above 1:200 based on the first trimester combined test . In anticipation, we validated the SOLiD WildFire on 154 blood samples obtained from pregnant women opting for invasive testing. We show a false negative result in a trisomy 18 case: a growth retarded fetus, small placenta, and maternal BMI of 29 may have resulted in a low fetal fraction. We also present a case of a super-

numerary derivative chromosome, partly consisting of chromosome 13 material: an example of unexpected findings with NIPT. In 2013, thousands of Dutch women had NIPT performed abroad, at their own expense. In one of these women, who had received normal NIPT results, fetal anomalies were seen at the 20 week ultrasound. Invasive testing showed a trisomy 18. NIPT was repeated in our centre prior to amniocentesis, and it clearly indicated a trisomy 18. Placental and fetal biopsies, investigated with FISH after termination of pregnancy, confirmed this finding. Data of the initial NIPT could not be shared with us, which prohibited tracing of the source of this false negative finding. False negative, false positive and unexpected results will be obtained with NIPT. For safeguarding and examining follow up samples, leading to a further understanding of possible underlying biological causes, close interaction between specialists involved is essential. This is also vital to ensure adequate care and counseling for couples confronted with such results.

P01.030-M
Dyscordant Chromosomal Finding in Monozygotic Twins

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We investigate a patient PL aged 34 after spontaneous pregnancy in 17 th weeks of her gestation with monozygotic twins having dyscordant chromosomes. Literature describes mosaicism only in over 20 cases if more foetal tissues were examined. Our case report refers to different twins chromosomal finding represented by lines 47, XXX/45, X. Amniocentesis because of patological NT (4,7mm) by fetus B proved fetus A (47, XXX/46,XX), and fetus B (45,X/46,XX). In our first genetic examination by QF PCR we demostrated trisomy of chromosome X in fetus A, and chromosome monosomy X in fetus B. Second FISH analysis of native amniocytes proved in fetus A chromosome X in 79% of its nuclei (21% with normal set of chromosome XX), in fetus B monosomy of chromosome X in 95% of nuclei (5% with normal set of chromosome XX). The 3 rd FISH exmination of cultured amniocytes confirmed in fetus A trisomy of chromosome X in 95% of its nuclei (5% with normal set of sex chromosome), in fetus B monosomy of chromosome X in 98% of nuclei (2% with normal set of sex chromosome). Pregnancy finished in 25 th week by spontaneous delivery (stillborn fetus B with monosomy X, 1120g/30cm, fetus A with trisomy X 675g/30 died 17 days after delivery owing to sepsis). Our case report is shrouded in question: at what stage of embryonic development occurred an error?

P01.031-S
Embryo quality and early pregnancy loss after IVF

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Genetic disorders are of the most common reasons of early pregnancy loss. Chromosome abnormalities can be found in up to 70% karyotypes of chorionic villi after miscarriages of both spontaneous and IVF pregnancies.
Here we find out if embryo quality is a sufficient criterion of successful pregnancy outcome after IVF, and identify the relationship between the embryo morphology and chromosomal pathology in spontaneous abortions or missed miscarriages.
We analyzed 45 samples from patients underwent dilation and curettage after IVF. Chromosome preparations were made using direct material processing, GTG-stained or FISH was performed on chromosomes 13,16,18,21,22,X,Y.
Embryo transfer (ET) was performed at the day 5 of embryo development. In the most (76%) cases 2 embryos per woman were transferred. All embryos were divided into groups based on their quality. Day 5 blastocysts AA were defined as 'excellent'; AB, BA, BB as 'good'; BC, CB, C as 'poor' quality. Chromosome abnormalities were found in 53% cases. In these cases 25% were ET of excellent embryos, 63% were ET of good, and 12% were ET of poor. In abortions with normal karyotype 33% of ETs were with excellent embryos, 66% with good. Control group was 53 cases of live birth after IVF at the same period of time. In these successful pregnancies 41% were ETs with excellent embryo quality, which is significantly higher than in pathology group. Otherwise, embryos of excellent and good quality also need preimplantation genetic diagnosis, because chromosomal anomalies in more than 70% cases could be identified using this technique.

P01.032-M
ESX1 gene expression is a predictive marker of residual spermatogenesis in azoospermic males

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ESX1 mRNA expression was previously investigated by our group in testicular biopsies of infertile men and correlated to the presence of residual spermatogenesis in Non Obstructive Azoospermic (NOA) patients. Here, we further deepen this issue investigating by real-time PCR ESX1 expression in both testicular fragments (TF) and seminal fluids (SF) of 78 NOA men. The aim was to verify whether a positive ESX1 expression in TF or even in SF was predictive of a successfully sperm recovery at TESE/microTESE. Concerning TF, ESX1 mRNA levels: 1) significantly decrease with the increasing of spermatogenesis defect, as classified by histology; 2) are higher in dilated tubules, likely containing spermatozoa, compared to thin ones. In addition, the presence of a positive or negative testicular expression of ESX1 strongly correlates ($p<0.0001$) with positive or negative sperm recovery at surgery. Regarding SF, ESX1 mRNA expression was detected in the ejaculate of 44/56 azoospermic men, at lower levels than normospermic men ($p<0.05$). No significant differences were found in ESX1 expression levels among samples with different degree of spermatogenic failure, based on histological classification. Regarding spermatozoa recovery and ESX1 expression in SF, the two variables were concordant in 33/56 (59%) of cases. The overall data reinforce a correlation between a positive ESX1 mRNA detection and residual spermatogenesis in testes of NOA patients, indicating a role of ESX1 as predictive spermatogenesis molecular marker. We can speculate that in SF discrepancies between ESX1 expression (+) and sperm retrieval (-) could be attributed to limitations in surgery techniques for sperm recovery.

P01.033-S
The importance of fœtal pathology. Regional assessment report for Montpellier for the years 2010 to 2012

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The Languedoc-Roussillon region in the South of France has a population of about 2 800 000. Over the period extending from 2010 to 2012 the number of births in this region was 91 715. Over the 3-year period 910 requests for termination of pregnancy (TOP) were authorized. 92% of these TOPs were done for serious congenital anomalies. 50% of the TOPs were performed for serious malformation syndromes without a precise genetic cause determined during the prenatal period. In order to characterise fœtal anomalies, maternities in the region are encouraged to send fœtuses to the Fœtal Pathology Unit for genetic investigations and an autopsy. Over the 3-year-period 985 fœtuses were received for expertise either following accidental fœtal demise or TOP (n=436). In 30% of cases fœtal pathology showed additional anomalies undetected on fœtal ultrasound examination and in 7% of cases the pathology results considerably modified the initial prenatal ultrasound diagnosis. In fœtuses addressed for diagnosis of a syndrome, genetic investigations and autopsy revealed 20% chromosome abnormalities and in 6% of cases put a name on various malformation syndromes (VATER association, CHARGE, Smith Lemli Opitz syndromes, osteochondrodysplasias, ⋯). The malformations most commonly implicated were central nervous system anomalies (33%), skeletal anomalies (30%), heart defects (18%) and urinary tract malformations (14%). Fœtal pathology clarifies fœtal anomalies, determines whether a particular genetic syndrome is involved and reveals chromosome anomalies undiagnosed before TOP. This information is important for families (genetic counselling) and for the physicians who managed the interrupted pregnancy or decided on the TOP.

P01.034-M
Analysis of FMR1 and FMR2 genes in women with primary ovarian insufficiency from the Basque Country

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Primary ovarian insufficiency (POI) is an ovarian dysfunction defined as irregular menses and elevated gonadotrophin levels before or at the age of 40 years. Several genes have been reported as having significance in POI but the FMR1 (intermediate and premutation alleles) is one of the most important genes associated with it. The FMR2 gene has also been related with the development of this condition. A group of 68 women with POI and 47 control women from the Basque Country has been analyzed. Considering the FMR1 gene, the number of women carrying at least one allele with >35 CGG

repeats (intermediate and premutation alleles) was statistically higher in patients (26.47% vs. 0%). The patient group was divided in three categories concerning their ovarian condition. Among patients with amenorrhea and elevated FSH levels, the frequency of alleles between 35 and 54 CGG was statistically higher than in controls (15% vs. 0%). This frequency is also statistically higher among women with irregular menses and elevated FSH levels (11.11% vs. 0%). Regarding the FMR2 gene, small alleles with fewer than 11 repeats have been associated with premature ovarian failure. The frequency of these alleles in this study is not statistically different (3.68% vs. 3.19%). The data suggest that carrying more than 35 CGG repeats in the FMR1 gene might be related with the development of POI. However, the FMR2 gene has not a clear association with this ovarian dysfunction.

P01.035-S

Follicle stimulating hormone receptor gene alterations and ovarian response to gonadotropins in Iranian infertile women

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Diminished Ovarian Reserve(DOR) and Ovarian Hyper Stimulation Syndrome(OHSS) are infertility disorders in which women's ovaries don't have proper response to gonadotropins. Follicle stimulating hormone(FSH) has a critical role in the maturation of the ovarian follicles from the antral to the graffian stage. FSH will start a signaling cascade in the granulosa cells after sitting on its receptor(FSHR). Alteration of this receptor may change follicle maturation and therefore result in improper response to gonadotropins. We investigated the association of FSH receptor gene alteration in DOR and OHSS patients. The presence of P.Ala307Thr, P.Ser680Ala, P. Ala665Thr and Mut.Val341Ala were analyzed in a case control study. 31 Iranian DOR and 34 Iranian OHSS patients were selected as the case group. 30 Iranian fertile women were enrolled as the control group. The patients DNA were extracted from their peripheral blood and amplified by relevant primers. For determining allelic variant status all PCR products were analyzed by Sequencing. The results were unexpected; the homozygous Ser680 and Ala307 variants seem to be significantly associated with OHSS. The FSHR P.Ala665Thr genotype frequency was similar in all patients and controls. The number of oocytes retrieved was comparable between patients with different FSHR genotype. Although data are accumulating with evidence suggesting that the ovarian response to gonadotropins is mediated by different genetic alterations, as in some previous studies homozygousity for Ser680 was significantly associated with DOR, the optimal biomarkers and the efficacy of the tests still remain to be evaluated.

P01.036-M

New molecular approaches for the detection of free fetal DNA

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One of the major effort in medical genetics is the development of non-invasive prenatal diagnosis, aimed to replace current invasive methodologies. It is well known that the circulating free fetal cells may be employed as a new alternative source of DNA for prenatal diagnosis, in order to avoid the risks associated to invasive diagnosis. The cell free fetal DNA (cffDNA) in maternal plasma or serum allows the development of non-invasive method for the detection of chromosomal and molecular diseases. The same approach can be even applied to determine fetal sex and the risk for X-linked genetic disorders.

To date, several techniques, such as PCR, Real Time PCR, Next Generation Sequencing, have been employed for cffDNA analysis. Here we propose a new molecular technology (Plexor® HY System), based on Real Time PCR for fetal sex determination. The Plexor® technology takes advantage of the specific interaction between two modified nucleotides and the reduction in fluorescence. The qPCR approach allows the simultaneous quantification and detection of fetal sex in pregnant women. Peripheral blood samples were obtained from 50 pregnant women at the 12th to 14th gestational week and the cffDNA was isolated from maternal plasma.

Determination of fetal sex of all samples demonstrated a concordance of 100% with the results obtained by traditional method (karyotyping). We also observed, as expected, that cffDNA increases together with gestational age.

The experimental data demonstrate that non-invasive and molecular methodologies are able to determine the fetal sex and quantify the cffDNA.

P01.037-S

FSHB -211 G/T polymorphism affects hormonal levels and sperm parameters

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FSHB gene transcription is the rate-limiting step for FSH production. The *FSHB* -211 G/T single-nucleotide polymorphism (SNP) is the only genetic variant which has a major effect on serum FSH concentrations in men. The aim of this study was to evaluate the effects of this *FSHB* SNP on male infertility. The SNP was analyzed in 83 men with oligoasthenozoospermia/azoospermia (group 1) and in 82 normozoospermic controls (group 2). Genotyping of SNP was performed by Real-time PCR with TaqMan Genotyping Assay. The *FSHB* genotypes frequencies were: 74.4% (GG), 22.7% (GT) and 2.9% (TT). The distribution frequency of heterozygous and homozygous T carriers was significantly different between the two groups: 65.8% of GT heterozygotes and 100% of TT homozygotes were found in group 1 (Z-test, p=0.0057). The T allele frequency was statistically different in the two groups: 18,6% and 7,9% in group 1 and 2, respectively (Z-test, p=0.007). Moreover, the FSH serum levels were differently distributed with a trend from the highest values in the GG genotype to the lowest levels in the TT genotype (Kruskall-Wallis test: p=0.037). The T allele was associated with significant declining levels of LH, testosterone (GG+GT vs. TT and GG vs. TT (p<0.05, ANOVA) and sperm concentration (p<0.05, Median test). These results corroborate the observation that the highly conserved promoter regions of the *FSHB* gene have a regulatory function on the transcription of this gene and demonstrate convincingly that the promoter variant may be involved to the modulation of testicular functions both in healthy and infertile men.

P01.038-M

Noninvasive prenatal diagnosis of Huntington disease in the Netherlands; a validation study

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Huntington disease (HD) is a progressive neurodegenerative disorder, that presents with motor symptoms, cognitive impairment and psychiatric disturbances. HD is caused by the expansion of an unstable polymorphic trinucleotide $(CAG)_n$ repeat in exon 1 of the *HTT* gene.

Our facility is the only laboratory for diagnostics of HD in the Netherlands. About one third of the requests for molecular prenatal diagnostics we receive are for HD. However, molecular testing is performed on fetal DNA derived from invasive procedures. Therefore, there is a request for alternative, less invasive methods. Noninvasive prenatal diagnosis (NIPD) using total cell-free DNA (cfDNA) from maternal plasma for the detection of paternally inherited mutations in the fetus is achievable for a range of genetic disorders. In this study, we have explored the use of NIPD for HD. We have compared our methods to previously described methods and subsequently optimized and validated them.

Since 2010, n=17 couples have been included in this validation study, resulting in maternal blood samples from n=22 pregnancies. Using a combination of PCR and fragment analysis, paternally inherited fetal CAG repeat length was determined using total cfDNA. All results were confirmed on genomic DNA derived from chorionic villi.

The full range of fetal CAG repeats tested in this cohort was 15-70 repeats. We show that paternally inherited repeats in the intermediate and affected range can be detected in a large background of maternal cfDNA. In addition, ins and outs of detecting trinucleotide repeats in fragmented cfDNA from maternal plasma will be discussed.

P01.039-S

Perinatal hypophosphatasia: A case of extreme intrafamilial variability

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Hypophosphatasia (HPP) is a clinically diverse condition characterized by defective bone and/or teeth mineralization in the presence of low activity of serum and bone alkaline phosphatase. Perinatal lethal and infantile forms of HPP have autosomal recessive mode of inheritance with prenatal ultrasound and postnatal x-rays showing extreme skeletal hypomineralization that often results in neonatal death. Intrafamilial variability for autosomal recessive conditions is rare and HPP is not an exception; however, occasionally unexplained variations have been noted among affected siblings. We report a family with extreme variability in the perinatal presentation of HPP. The couple had two pregnancies affected with HPP confirmed by DNA analysis showing compound heterozygous mutations in the ALPL gene. Fetal ultrasound in the first affected pregnancy showed short long bones, fractures and demineralization of the skull at 16 weeks gestation. The second affected pregnancy had normal skeleton and normal growth of long bones at both 16.5 and 19.5 weeks gestation ultrasound. Both pregnancies were terminated in the second trimester. Fetal autopsy on the first fetus confirmed the prenatal ultrasound findings while external examination and x-rays on the second fetus showed few significant findings of perinatal HPP. This case emphasizes that in HPP prenatal diagnosis should rely on molecular analysis rather than ultrasound and suggests that other genetic or prenatal environmental factors can significantly modify the phenotype even in known lethal skeletal dysplasias.

P01.040-M

Additional Genetic Testing in case of increased Nuchal Translucency and normal Karyotype

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In fetuses with increased nuchal translucency (NT ≥ 3 mm), after exclusion fetal chromosomal anomaly, more than 100 different developmental/genetic syndromes are reported, most of them are unique and difficult to diagnose before birth. At the same time the anxiety of prospective parents needs to be reduced and exclusion of at least some genetic disorders may be helpful. Our study group consisted of 51 fetuses with increased NT and normal karyotype (analyzed at Tartu University Hospital in 2012-2013). For genetic testing the arrayed primer extension system was used, allowing to examine different genetic diseases with same test: Noonan syndrome (NS) (PTPN11, SOS1, KRAS, RAF, MEK1); Smith-Lemli-Opitz syndrome (SLOS); congenital adrenal hyperplasia (CAH); and spinal muscular atrophy (SMA). Of 51 tested pregnancies we found four positive for NS with the following mutations: one with p.Asp61Asn in PTPN11, and three with p.Pro655Leu in SOS1; and one case of CAH (homozygous mutation p. Pro454Ser in CYP21A2). The mutation p.Pro655Leu in SOS1 was inherited from healthy parent at least in two cases (in one case parental testing is ongoing); and in literature the mutation is proposed to be as polymorphism. No SMA or SLOS cases or carriers were detected and there were only two CAH carriers in our group. In conclusion: only in two cases (4%) the test gave valuable information for family; in three cases this test gave concern and uncertainty about fetus's health. Therefore it is questionable whether this particular test would be the best choice for additional testing.

P01.041-S

Two familial cases with interstitial deletion of chromosome 13 due to maternal germline mosaicism

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De-novo structural chromosome aberrations are usually interpreted as a result of a random event during parental gametogenesis and the recurrence risk therefore is estimated to be very low.
We report on a case in which the same supposedly de-novo deletion recurred in a second pregnancy.
The patient was referred to our institution for fetal karyotyping in her first pregnancy due to a positive first-trimester screening test. Karyotype after amniocentesis at 17 weeks gestation showed an interstitial deletion of chromosome 13: 46,XX,del(13)(q22q32). To define the exact deleted region an array-CGH was performed, indicated one copy of 13q31.1q32.1, 15,9 Mb in range. The ultrasound examination at 23 weeks of gestation showed cerebellar hypoplasia, severe ventriculomegaly as well as associated facial features – hypertelorism, midface hypoplasia and micrognathia. The pregnancy was terminated. Both parents were found to have normal karyotypes.
In the second pregnancy, chromosomal analysis of the fetus showed an identical interstitial deletion of chromosome 13 as the one found in the first pregnancy.
Microsatellite analysis of genomic DNA extracted from tissues of both fetuses as well as from the blood of both parents revealed loss of heterozygosity for two markers located inside the deleted region. Missing alleles originated from the mother.
The results of molecular and cytogenetic studies strongly suggest that the maternal gonadal mosaicism could be the cause for the increased risk of recurrence in described case.
The possibility of germline mosaicism is of particular concern when counselling the parents of a child with a de-novo chromosomal abnormality.

P01.042-M

Gene Expression Patterns in a disturbed Karyotype: Keys to the Clinical Conundrum of Klinefelter Patients

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Klinefelter Syndrome (KS) is the most common chromosome disorder in men (47,XXY), exhibiting marked phenotypic variation, frequent hypogonadism and increased mortality. It is unclear to what extent the genetic impact of the supernumerary X-chromosome contributes to the pathology.
EXAKT (Epigenetics, X-chromosomal features and Clinical Applications in Klinefelter syndrome Trial) involves 132 KS men and their parents assessing a wide range of clinical parameters in comparison to male and female controls (n=50 each) in relation to genetic investigations. The objective was to elucidate gene expression patterns in KS and whether these would be related to inherent pathologies.
Gene-expression was substantially disturbed in KS vs. both control groups. The differential expression of 36 not only X-chromosomal genes puts these phenotypical males into a genetic framework located between men and women with normal karyotypes. A range of these genes has previously been attributed to gender-specific modulations of immune responses. The KS cohort exhibited increased insulin resistance/inflammatory status, a procoagulatory state, higher waist circumference, dyslipidemia and an altered cardiac rhythmogenic setting. The extent of clinical dyshomeostasis was associated with the expression of dysregulated genes. Paternal origin of the supernumerary X-chromosome was an additional confounder regarding insulin resistance and cardiac phenotype.
In KS patients, the supernumerary X-chromosome contributes to a number of pathologies by altering gene expression patterns: insulin resistance, dyslipidemia, enhanced inflammation markers as well as altered cardiac rhythmogenic setting are involved; this was observable independently from testosterone substitution treatment which may have attenuated responses in KS.
Funding IZKF Münster CRA03/09; DFG WI2723/4-1

P01.043-S

Klinefelter Syndrome testicular gene expression profile by a whole transcriptome approach

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Klinefelter Syndrome (KS) is the most common sexual chromosome abnormality (47,XXY) and represents the first genetic cause of male infertility. The mechanisms leading to KS testis degeneration are still unclear and no therapy is so far available for affected patients.
The present study is aimed to unravel information about molecules playing a key role in the disruption of the spermatogenesis.
Gene expression profiles analysis of KS azoospermic testis versus normal testis, could provide useful information about the molecular basis of the alteration of the spermatogenesis.
Transcriptome analysis was performed carrying out gene expression profile by a whole genome microarray approach on testis biopsies obtained from 6 azoospermic non-mosaic KS men and from 3 controls, for a total of 12 experiments. T-test and False Discovery Rate were used to evaluate differentially expressed genes. Identified transcripts were analysed by Ingenuity Pathways Analysis software to disclose genes biological functions.
Data analysis revealed the differentially up- and down-expression, in KS testis versus the control ones, of 656 and 247 genes related to Endocrine system development and function, Lipid metabolism, Reproductive disease, Free radical scavenging, and Cell death.

Take together these data show the presence of several genes involved in testis microenvironment deregulation leading to the spermatogenesis failure. This information, associated with an early diagnosis could help to unravel possible therapeutic targets for testis failure prevention and limitation.

P01.044-M
Immunoelectron microscopy reveals massive expression of laeverin in microvesicles in preeclamptic placentas

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Preeclampsia is a pregnancy-specific syndrome characterized by hypertension and proteinuria. It complicates 5-10% of pregnancies and is a major cause of maternal mortality world-wide. Laeverin (aminopeptidase-Q) is a plasmamembrane-bound aminopeptidase that is specifically expressed in the human placenta. We reported previously that the mRNA levels of laeverin gene are significantly up-regulated in preeclamptic compared to healthy placentas, indicating that laeverin might play a role in the pathophysiology of preeclampsia. Immunofluorescence studies have indicated that laeverin protein is highly expressed in the cytoplasm, instead of the plasmamembrane, of villous trophoblasts in preeclamptic placentas. In this study, we used immunoelectron microscopy to investigate the subcellular localisation of laeverin protein. Ultrathin sections of high pressure freezed (Tokyasu method) tissue samples of four placentas (two from healthy women and two from women with severe preeclampsia) were fixed and labeled with primary antibody raised against laeverin, followed by antibody conjugated with gold particles. Double labeling with markers for endoplasmatic reticulum (ER) and Golgi apparatus (GA) were also performed. We found laeverin in the ER, GA and massive expression in microvesicles within the cytoplasm of villous trophoblasts, extracellular space and in the fetal capillaries in preeclamptic placentas. Since the ER and GA in normal placentas did not show any evidence of accumulation of laeverin, we hypothesize that dysregulation of transport or folding of laeverin might have a role in the development of preeclampsia.

P01.045-S
Leri Weill syndrome findings in an infertile man with 45,X/46,X,derY, t(Y;Y)(p11.2;q11.21) and SHOXY deletion

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Leri-Weill dyschondrosteosis (LWD) or syndrome is a rare genetic disorder with skeletal dysplasia characterized by short stature, mesomelia, and Madelung deformity. We report a 29-year-old male with Leri Weil Syndrome phenotype. The patient was referred from Urology department because of azoospermia. Infertility was the first complaint that forced him request medical achievement. The patient had short stature with macrocephaly, mesomelia, Madelung deformity, and exaggerated flattened vertebral spine adressing Leri Weill syndrome. Simultaneously regular consultations, radiographic and other procedures and genetic analysis was planned. Conventional cytogenetic analysis and Y chromosomal microdeletion scan was performed. No microdeletions were present on the loci sY14(SRY), sY84, sY86 (AZFa), sY127, sY134 (AZFb), sY254, sY255 (AZFc), sY152, sY153 (AZFd) and RBM1. Chromosomal analysis revealed a mosaic finding of two series: 45,X and 46,X, with a der(Y), possibly t(Y;Y)(p11.2;q11.21). Additional metaphase FISH analysis with a commercial probe mix targeting DXZ1, LSI SRY supported the mosaic numerical finding with 15% of the metaphases only showing one DXZ1 signal without LSI SRY and 45 chromosomes counted on DAPI stain. Whole chromosome painting FISH studies demonstrated that the derived Y chromosome consisted of only Y chromosomal material. SHOX analysis by FISH technique revealed absence of SHOX signal but 2 DYZ signals on derived Y chromosome. Finally, chromosomal microarray findings (Affymetrix Cytoscan 750K SNP Array platform) confirmed a SHOX deletion with no additional significant findings above cut-off values.

P01.046-M
The prevalence of luteinizing hormone beta-subunit gene polymorphisms in Czech population and patients with ovarian hyperstimulation syndrome

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There are two common polymorphisms in luteinizing hormone beta-subunit (LHβ) gene, which form the variant LHβ (v-LHβ). These polymorphisms change the amino acid sequence (Trp8Arg and Ile15Thr) of the protein. The hormone with v-LHβ (Arg8/Thr15) was shown to have higher bioactivity (tests in vitro) but shorter lifetime in vivo. The presence of v-LHβ is associated with reduced woman´s fertility, menstrual disorders and abortions. Female IVF patients with at least one v-LHβ allele tend to be hypo-responders to controlled ovarian hyperstimulation. Thus, we propose that women with v-LHβ would be in a lower risk to develop an ovarian hyperstimulation syndrome (OHSS).
In our study, 102 fertile male-controls, 149 fertile female-controls and 58 patients affected by OHSS type III-V were analyzed. The genotype was determined by restriction fragment length polymorphism (RFLP). The prevalence of v-LHβ allele was 10,1% in female controls, 14,7% in male controls and 6,0% in OHSS patients.
There was no statistical difference between Czech control men and women in genotype or allelic frequencies (P=0,22; P=0,12, respectively). The Czech population frequency of v-LHβ is closest to the prevalence from Iceland, Italy and The Netherland.
There was no difference between female controls and OHSS patients in genotype or allelic frequencies (P=0,41; P=0,25, respectively). The protective effect of the v-LHβ from OHSS was not confirmed, but this needs to be verified in a larger cohort.
Supported by grants IGA NT13770-4/2012, project for conceptual development of research organization 00064203 and OPPK CZ.2.16/3.1.00/24022.

P01.047-S
Genetic basis of male infertility: Belorusian Centrum of Reproductive Medicine data.

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Genetic factors play an important role in male infertility etiology.
We present the data of spermatogenetic impairment infertile couples referred to ART. Results. 601 mentally normal men with azoospermia/severe oligozoospermia were examined using cytogenetic and moleqular methods. Klinefelter's syndrome (KS, 47,XXY) was found in 11 (1,8%) patients. Different AZF regions Yq microdeletions (DelAZF) were detected in 34 (5.6%) patients. AZFc deletion calculated for 29 cases (85,3%) with a chance of retrieving sperm by testicular biopsy (TESE) and performing ICSI: spermatozoa were received in 15 cases (51.7%) for following cryopreservation. Two AZFb and 3 AZFb+c deletion's patients showed sperm absence in testicular tissue. 2 men with complete AZFa+b+c region's deletion didn't undergo TESE/ICSI. 16 (2.6%) CFTR gene mutation's heterozygote carriers were identified: dF508del (10 cases), CFTR2,3del (3), 2184insA (2), 1677delTA (1). Counseling: Partners-carriers of CFTR gene mutations have 25% risk of affected outcome, DelAZFc male - 100% deletion's transmission risk for sons. We recommended donor sperm using or adoption for KS and DelAZFb; DelAZFb+c; DelAZFa+b+c's patients.ARTs: 12 DelAZFc patients underwent IVF+ICSI procedures. 5 couples (41.6%) got pregnancy: 3 pregnancies resulted in healthy girl delivery, two - ongoing (12 and 22 weeks gestation). Preimplantation testing was performed for 2 couples with CFTR gene mutations heterozygote's status for both partners. Two pregnancies were obtained: one is developing successfully, other resulted in miscarriage. Conclusion. KS/Yq microdeletions are specific for a severe spermatogenic failure. Genetic testing permits to establish the origin of azoospermia, prognoses, ART strategy's selection and to avoid unnecessary treatment.

P01.048-M
X chromosome-linked CNVs in male infertility: discovery of duplication load and recurrent deletions with potential clinical relevance

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The X-chromosome genetic content is predicted to be important in spermatogenesis but its role in male infertility remains unknown. We investigated whether X-linked copy number variations (CNVs) might have clinical significance in idiopathic infertile men. We previously detected by a-CGH a number of CNVs including 16 patient-specific gains and 3 recurrent deletions on Xq, exclusively (CNV67) and prevalently (CNV64; CNV69) found in infertile patients. Among the 14 gains, we selected 5 for further qPCR analyses on a larger study population including 276 idiopathic infertile patients and 327 normozoospermic controls. The difference in duplication load was statistically different (p=1.65x10-4). PAR1-linked DUP1A displayed the highest

frequency (1.44% of patients) and we hypothesize it may cause spermatogenic failure by disturbing meiotic X-Y pairing or by affecting the correct regulation of a gene potentially influencing spermatogenesis (PPP2R3B). Concerning the 3 deletions, 627 patients and 628 controls were tested for each deletion with PCR+/−. CNV64 and CNV69 were significantly more frequent in patients than controls (p>0.05). CNV67 was detected exclusively in patients (1.1%) and was maternally transmitted. The oligozoospermic phenotype of one carrier versus his normozoospermic non-carrier brother strongly indicates a pathogenic effect of the deletion on spermatogenesis. MAGEA9, an ampliconic gene reported as independently acquired on the human X chromosome with exclusive physiological expression in the testis, is likely to be involved in CNV67. Our investigation further indicates an association between X-linked CNV burden and spermatogenic impairment and identifies the first X chromosome-linked recurrent deletion and duplication with clinical significance.

P01.049-S
A novel m.9588G>A missense mutation in the mitochondrial COIII gene as a cause of low sperm motility in asthenozoospermic Tunisian infertile men

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Infertility affects 10-15% of the population, of which, approximately 40% is due to male etiology consisting primarily of low sperm count (oligozoospermia) and/or abnormal sperm motility (asthenozoospermia). Furthermore, it has been demonstrated that mtDNA base substitutions can greatly influence semen quality.
In this study we performed a systematic sequence analysis of the mitochondrial genes (cytochrome oxidase III (COIII) gene in 64 infertile men suffering from asthenozoospermia (n=31) in comparaison to normozoospermic infertile men (n=33) and fertile men (n=150) from Tunisian population. A novel m.9588G>A mutation was found in the sperm's mitochondrial DNA (mtDNA) in all asthenozoospermic patient and was absent in the normozoospermic and in fertile men. The m.9588G>A mutation substitutes a highly conserved Glutamate at position 128 to Lysine. In addition, PolyPhen-2 analysis predicted that this variant is ''probably damaging''. This novel missense mutation (m.9588G>A) detected in the mitochondrial COIII gene as a cause of low sperm motility in asthenozoospermic Tunisian infertile men

P01.050-M
New candidate genes for oligoasthenoteratospermia as a potential cause of human male infertility

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Oligoasthenoteratospermia (OAT) is a condition characterized by the presence of sperm with abnormal morphology. The causes of OAT are unknown in most cases. Routine genetic examination of patients with nonsyndromic male infertility (Y-chromosome microdeletions and *CFTR* mutations) does not cover this phenotype. Given the hundreds of genes identified as essential for male fertility in animal models it is probable that substantial fraction of cases with genetically caused OAT remains undetected. Our study aims for mutation screening of selected candidate genes which were linked to OAT in animal models.
We selected candidate genes whose mutation in model organism interfering with spermatogenesis and was demonstrated like OAT. We selected *CAPZA3, CDC42, CDC14B, CNTROB, CSNK2A2, GOPC, HOOK1, HRB, OAZ3, ODF1, RIMBP3* and *SPATA16.* We performed genealogy, karyotyping and mutation analysis of *CFTR* gene and Y-chromosome microdeletions. Control group are men with normospermia. PCR amplified exons of the selected genes were sequenced using GS Junior next generation sequencer.
To date we accumulated 131 cases and 88 controls. Sequencing is completed for 41 patients and 8 controls. We found 6 persons with chromosomal aberration and one man with Y-chromosome microdeletion. We detected 115 known sequence variants and 18 novel variants (in *CDC42*, *CNTROB, HOOK1* and *RIMBP3*). The clinical significance of the novel variants is uncertain, however, we identified two likely damaging frameshift variants (in *CNTROB* and *RIMBP3*).
The study is supported by Ministry of Health of the Czech Republic grant No. NT/12269-5

P01.051-S
Transcriptomic profiling of spermatozoa of infertile men with abnormal sperm morphology

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The pathogenesis of oligo/astheno/teratospermia is unknown in most patients. Especially, the proportion of genetic and environmental factors has been hard to tease apart. However, transcriptomic or proteomic approaches can identify common dysregulated genes or pathways in sperm, that are substantial for their function, regardless the ultimate etiological factor.
We analyzed twelve semen samples according to WHO criteria (2010). Four of the participants were fertile donors, clinically and andrologically normal, who had achieved pregnancy in a period less that twelve months and got a live born baby in the last year. Seven samples were from infertile patients examined in our clinic that presented with oligospermia and abnormal sperm head morphology, including decapitation. We performed genome-wide expression analysis using Affymetrix HuGene2.1ST microaarays.
The principal component analysis revealed substantial overlap between normal and abnormal samples. These results indicate that despite the common result of morphologically abnormal spermatozoa, the underlying causes of the infertility might be diverse.
Surprisingly four genes were markedly different between fertile and infertile sperm samples: an uncharacterized gene, a small nucleolar RNA gene, a microRNA gene and *RIPK4.* Protein kinase RIPK4 is known to phosphorylate the adaptor protein DVL2 (dishevelled 2) to activate the Wnt signaling pathway. In fact, the ortholog DVL1 has a role in regulating spermiogenesis via Wnt signaling.
The project was supported by University of Buenos Aires, Science and Technology, no. UBACYT-CB06, to G.M.; and Ministry of Health of the Czech Republic no. NT12269-5.

P01.052-M
Decrease of meiotic cohesins with maternal age in human oocytes

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Aneuploidy of fetal chromosomes is one of the causes of pregnancy loss or congenital birth defects. It is known that the frequency of oocyte aneuploidy increases with maternal age in humans. Recent data have highlighted the contribution of cohesin complex in the correct segregation of meiotic chromosomes. In mammalian oocytes, cohesion is established during the fetal stage and meiosis-specific cohesin subunits are not replenished after birth. This raises the possibility that the long meiotic arrest of oocytes facilitates the deterioration of cohesion leading to the age-related increase in aneuploidy. We here examined the cohesin levels in dictyate oocytes from different age groups. Samples were obtained from the ovarian tissues of 8 women (age range: 19-49 years) and also from 2- and 10-month-old female mice. Ovarian tissue sections were immunostained using cohesin antibodies and the cohesin levels were determined by immunofluorescence. The levels of the meiosis-specific cohesin subunits, REC8 and SMC1B, were found to be decreased in women aged 40 and over compared with those aged around 20. An age-related decrease in meiotic cohesins was also evident in mice. Interestingly, SMC1A, the mitotic counterpart of SMC1B, was readily detectable in human oocytes but only barely in mice. The mitotic cohesin levels of mice slightly increased with age. These results suggest that, mitotic and meiotic cohesins may act in a coordinate manner in humans to maintain the levels of this protein over a sustained period. The decreased meiotic cohesin subunit levels with age impairs sister chromatid cohesion leading to increased segregation errors.

P01.054-M
Maternal plasma miRNA analysis of fetuses with congenital heart defects

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Congenital heart defects (CHD) are common developmental malformations and often associated with chromosomal disorders. The major focus of current research is examining free fetal nucleic acids in maternal circulation aiming to create new noninvasive screening methods. The miRNAs are short, non-coding RNA molecules that play important part in regulation of eukaryotic gene expression. The aim of this study was to investigate the role of

miRNAs in heart development, measure miRNA concentration and expression in maternal blood.
Peripheral blood samples were collected from 53 women, 27 of them had healthy and 26 had fetuses with congenital heart defects. Blood samples were centrifuged and miRNA was extracted from plasma. MiRNA concentration was calculated by Nanodrop spectrophotometer. By using Gene Ontology and miRBase databases we searched for those miRNAs which can be detected in plasma and are associated with chromosomal and congenital heart defects. These criteria were fulfilled by *let-7c* miRNA of chromosome 21. qRT-PCR was carried out to validate the miRNA's expression.
There was no significant difference between the miRNA concentrations in the two groups (6.05 ng/µl vs 5.36 ng/ µl). We found significant differences in the *let-7c* concentrations between the control and patient group (1.12±3.19 ng/µl vs. 0.00047±0.00083 ng/µl; p<0.0001).
Fetal-derived miRNAs are part of the free nucleic acids found in maternal plasma, expression studies reveals new opportunities for congenital heart defect research and diagnosis. According to our study *let-7c* seems to be a potential biomarker for fetal CHD.

P01.055-S
Overexpression of mir-21 and mir-221 in preeclamptic placentas

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Preeclampsia is a multisystem disorder with partial genetic and immunological etiology; the apoptosis is thought to play a role in the development of preeclampsia. The microRNAs are small noncoding molecules involved in regulation of cellular processes including apoptosis. Here we designed a 96-well reaction plate for the analysis of 21 microRNAs in duplicates which are known to be involved in the apoptosis regulation and are expressed in placenta - pro- apoptotic miR-1, let-7c, let-7g, mir-200c, mir-143, mir-205, mir-122, mir-409-3p, mir-449, mir-708, mir-149, mir-204,mir-133, anti-apoptotic - mir-214, mir221, and mir 222, and miRNAs with both the anti-apoptotic and apoptotic targets mir29a and mir29c. The normalization was performed against RNU44. The microRNAs were extracted from placental tissues obtained after delivery from preeclamptic women and healthy controls. After stem-loop primer reverse transcription in one tube, the samples were analyzed on the plates and evaluated by deltadelta Ct algorithm. The aberrantly expressed miRNA were validated by TaqMan MicroRNA Assay by relative quantitation with the standard curves The analysis of 21microRNAs revealed overexpression of mir-122, mir-21, mir-221, mir- 29a, mir- 29c and mir-449 The validation was performed on 13 additional preeclamptic placental samples collected after delivery and 6 placental samples from normal delivery; a significant overexpression of mir-21 and mir-221 with p=0.0055 (CI95% 25.2 to 123.47) and p=0.0047 (CI95% 12.16 to 57.20) was observed, respectively. Although the validation on larger number of samples is necessary, the analysis of miRNA deregulation can help in the identification deregulated signaling in preeclamptic placentas for detection of new biomarkers.

P01.056-M
Analysis of micrornas expression profile in human placentas from pregnancies complicated by preeclampsia

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Preeclampsia is a main cause of maternal and neonatal mortality and morbidity However, the origin of this disease is rather obscure so far. MicroRNAs (miRNAs) as a key a regulators of gene expression in cell proliferation, apoptosis, and embryogenesis, whereas their abnormal expression has been suggested in various common disorders. The objective of this study was to compare expression profiles of miRNAs in placentas from preeclampsia patients with these ones from normal term pregnancies. The expression profiling of five patients was performed with Ion Torrent semiconductor sequencing. Fourteen miRNAs (miR-181a-5p, mir-143, miR-143-3p; miR-126-5p; mir-369, mir-136, miR-136-5p, mir-142, miR-142-3p; mir-516a-1, mir-516a-2, mir-519a-1, mir-518c and miR-518c-3p) were significantly overexpressed in preeclampsia placenta compared to these ones in the controls. The results are important prerequisite for further molecular studies of genetic and epigenetic contributors in preeclampsia.
The work was supported by grants of Russian Federation President № 16.120.11.5773-MC.

P01.057-S
miRNA as potential universal fetal marker in NIPT

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Introduction: Circulating miRNA molecules are currently studied as potential diagnostic markers in many physiological and pathophysiological conditions such as oncological diseases, heart diseases and pregnancy. The aim of our study was to determine if circulating miRNAs can be used as potential universal fetal marker in NIPT. **Methods**: Circulating miRNAs were isolated from plasma of pregnant women and miRNA samples were divided into two groups depending on fetal gender. Firstly, expression levels of 91 miRNAs were established in three female and three male samples by two-step RT-qPCR. Five miRNAs (miR-378, miR-122, miR-20b, miR-320a, miR-320b) whose expression levels significantly differ between two groups were chosen for further analyses. Subsequently, expresion levels of five selected miRNAs were evaluated in 14 female and 15 male samples by two-step RT-qPCR. Differences between groups were tested using Student t-test with p<0.05 was consider as statistically significant. **Results**: After the first part of this study where panel of 91 miRNAs were investigated, five miRNAs significantly differ in their expression levels between two sample groups. However, there were no significant differences in expression levels of five selected miRNAs between sample groups, when expression levels of these miRNAs were determined in a greater number of samples. **Conclusion**: Our study showed, that none of evaluated miRNA molecules in this work is not suitable as the universal fetal marker. However, since our work addressed only limited number of miRNA molecules, for determination if circulating miRNAs can be used as universal fetal marker, further studies are needed.

P01.058-M
Arthrogryposis multiplex congenita caused by compound heterozygosity to Ashkenazi founder mutation and a novel splice mutation in *NEB* gene

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Background: Nemaline myopathy (NM) is a heterogenous disorder. A homozygote 2502bp deletion in *NEB* - had been detected in 1:40,000 Ashkenazi Jews (AJ). Most cases have congenital hypotonicity that might progress, but arthrogryposis is relatively rare.
Patients: Three affected siblings with arthrogryposis and an increased nuchal fold / cystic hygroma in two, were detected at 11-15 weeks of gestation. Parents were AJ with non-contributory family history.
Methods and Results: Histopathology with electron microscopy of muscle samples suggested NM on two siblings. DNA sequencing confirmed compound heterozygosity to the known Ashkenazi mutation (maternal) and splice mutation c.9619-2A>G (paternal) predicted to disrupt the intron 66 splice acceptor site.
Discussion: The splice site mutation revealed in our patients, has been previously detected in two unrelated affected AJ with clinical stigmata of arthrogryposis (*Ludtke et al*. ICHG 2011; *Yonath et al.* Prenatal Diagnosis, 2012). The detection of this mutation in several AJ families suggests it is either hot spot site or a new AJ founder mutation. Further studies should be performed to validate its carrier frequency among this population. Also, the severe stigmata in affected patients harboring this splice mutation, suggests its critical role in disease pathogenesis. In line with this, when arthrogryposis detected prenatally, screening of the *NEB* should be considered.

P01.059-S
Familial chromosomal translocation t(3;5)(q26.2;p14): clinical characterization of affected members with neural tube defect as a common clinical feature

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A 26-years-old woman was referred for prenatal diagnostics because of aggravated obstetric anamnesis. Her first child (male) was born at 34 weeks of gestation via S/C due to polyhydramnios and hypotrophy. Hypotonia, dysmorphic facial features and multiple congenital malformations were observed, including hypertelorism, epicanthal folds, micrognathia, hypoplastic cerebellum, congenital heart defect, optic nerve dysplasia, micropenis, hypospadias, syndactyly, and suspected spina bifida occulta. The newborn died at age of 1 month. According genealogy, proband's mother had a miscarriage at the 14th week of gestation and stillborn at 26 weeks of gestation

with uncertain gender and myelomeningocele.
During this pregnancy at the 27th week of gestation ultrasound scan showed polyhydramnios and multiple congenital anomalies in the fetus. On the 31st week of gestation the fetus died in the uterus. Autopsy findings were pulmonary atresia, transposition of the great vessels, horseshoe kidney, cerebellum hypoplasia, hydrocephalus, clubfoot, and spina bifida. Subtelomeric FISH was performed to the proband and balanced translocation ish t(3;5)(qter-,pter+;pter-,qter+) was detected. Karyotype was 46,XX,t(3;5)(q26.2;p14).
The clinical phenotype of affected family members is similar and could result from the combination of dup(3q) and del(5p) syndromes. Only few cases of (3;5) translocations have been reported to date. Detailed clinical description of affected family members brings new data for the characterization of this rearrangement.

P01.060-M
The Effect of Radiofrequency Waves on Pregnant Mice in association with Genes involved in Neuronal Migration

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The advancements in technology have improved human life in numerous ways, and concerns have been raised regarding health and safety issues in parallel with the spread of those developments. One of those concerns has been focused on radiofrequency waves because of common usage in daily life, such as mobile phones. The aim of this study is to evaluate change in expression levels of 7 genes (Dcx,Tuba1a, Ywhae, Arx, Reln, Large, Flna), which are involved in neuronal migration following the exposure of radiofrequency waves. A total of 16 mice were included in the study. They were divided into two groups as study and control. Each group consisted of 6 female and 2 male mice. Study group was exposed to 0.725 W/kg SAR value for 12 hours per day continuously by establishing an exposure system throughout pregnancy. A total of 29 offspring in the study group and 12 in the control group were achieved. Gene expression analyses were performed by using real time RT-PCR. Significantly increased expression levels were found in 5 (Arx, Dcx, Large, Reln, Ywhae) out of 7 genes in the study group. In conclusion exposure to radiofrequency waves in pregnancy may cause significant changes in those gene expressions involved in neuronal migration, which may also be related with brain anomalies.

P01.061-S
Confined placental mosaicism in a case of false positive NIPT result for trisomy 18

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Non-invasive prenatal testing (NIPT) of cell-free fetal DNA (cffDNA) for fetal aneuploidy risk assessment has been shown to be both highly sensitive and highly specific. False positive rates can be as low as 0.1%. However, discrepancies between positive NIPT result and fetal karyotype on chorionic villus sampling or amniocentesis may occur. The source of aneuploidy may be due to maternal mosaicism, maternal malignancy, true fetal mosaicism, a demised co-twin, an anembryonic sac, confined placental mosaicism.
We present a case of a 38-year-old woman, pregnant in 16 gestational weeks, referred to our Unit for amniocentesis due to positive NIPT for trisomy18. The ultrasound scans were unremarkable.
DNA was extracted from uncultivated amniocytes, amplified with commercial QF-PCR kit Aneufast (Molgentix SL, Barcelona, Spain) and analyzed on ABI 3130xl. Karyotyping was performed on cultured amniocytes using standard protocol.
QF-PCR and cytogenetic analysis showed normal results, trisomy 18 was excluded.
The pregnancy was still ongoing, with no pathological findings on routine ultrasound scans. Cytogenetic and molecular-genetic analyses are to be done after delivery.
Since fetal cell-free DNA mostly originates from invading trophoblast cells, this false-positive result may be due to mosaicism confined to the placenta (CPM). CPM occurs in 2% of the viable pregnancies and before the introduction of NIPT was most often detected by direct analysis of chorionic villus sampling.
Our case illustrates that follow-up with diagnostic testing of chorionic villus sampling and/or amniotic fluid for abnormal NIPT results should be performed and that pre- and posttest counseling is important.

P01.062-M
Preliminary experience with the management of positive results of non invasive prenatal testing (NIPT) in a reference centre

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During the past year techniques enabling non-invasive prenatal testing for the most common trisomies have become available clinically. An increasing number of women have been offered the test in connection with their first trimester screening, regardless of their level of risk. Pregnancies with a positive test are often referred to our Centre for genetic counseling and invasive procedures as recommended. To this end, we have created a flow-chart to assist these patients who are not receiving enough information at time of sampling. Clinical aspects of the test, its limits and reasons for false positives are discussed and a guaranteed follow-up is scheduled to collect data at time of birth. Between July 2013 and January 2014 6 patients with positive NIPT (2 trisomy 21, 1 trisomy 18, 1 monosomy X, 1 47,XXY, 1 triple X) were referred. All patients underwent an amniotic fluid procedure and only one case of trisomy 21, which had fetal anomalies at ultrasound, was confirmed. Normal results were obtained in the other 5. Two term placentas have been studied, the others being ongoing pregnancies. Since false positive cases of chromosome aneuploides after NIPT may have different underlying biological causes ,(the majority, however, are due to placental mosaicism) we believe that the cytogenetic analysis of term placentas should be acquired in all such cases so as to increase our knowledge about the relationship between cffDNA and the fetal genome. Genetic counselling should always be carried out before and after testing to reduce misinterpretation of test validity.

P01.063-S
Non-invasive prenatal detection of a mosaic trisomy 16

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The presence of cell free fetal DNA in the maternal circulation has allowed for the development of methods, which allow for non-invasive detection of fetal chromosomal aneuploidies. Our laboratory offers non-invasive prenatal testing (NIPT) with an innovative pipeline that utilizes all of the information provided by shallow depth whole genome sequencing to identify possible aneuploidies of all chromosomes. We have encountered one case, referred because of high trisomy 21 risk (1/14), with unusually high Z-score (5.7) for the chromosome 16 indicating the presence of a trisomy 16. Follow-up with direct FISH and array analysis of amniotic fluid cells showed a low-level mosaicism (between 7 and 20%). The finding of low level mosaicism is contrasting with the high Z-score which suggests the presence of trisomy 16 in the majority of the cells. This discrepancy could be explained by confined placental mosaicism and, if so, should be taken into account in subsequent counseling. Whereas the gynecologist suggested interruption, the family decided to continue the pregnancy with expert ultrasound monitoring of the fetal development following clinical genetic counseling.

P01.064-M
Non-invasive detection of fetal deletions or duplications by low coverage massively parallel sequencing

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To report the performance of fetal chromosomal deletions/duplications detection by the massively parallel sequencing (MPS) of maternal plasma DNA, we recruited 1,324 participants and performed the MPS test in a double-blind study. The peripheral blood of each participant was obtained before invasive sampling at 10-28 weeks' gestation with a median of 21 weeks. Plasma DNA was extracted and sequencing. About 0.08 fold sequencing data per sample was generated and the bioinformatics analysis was performed using FCAPS algorithm. Deletions / duplications, ranged from 3.07 Mb to 26.98 Mb, were suspected in 17 of the 1,324 samples, of which 16 were consistent with the results of fetal karyotyping / aCGH. In one case the suspected abnormality was not confirmed by karyotyping, representing a false positive case. No false negative case was observed in the remaining 1,307 low-risk samples. The sensitivity and specificity for detection of fetal chromosomal deletions / duplications were100% and 99.92%, respectively. Our study demonstrated the MPS-based test is feasible and of high sensitivity and specificity in detecting fetal chromosomal deletions / duplications.

P01.065-S

Nonivasive prenatal KEL genotyping using TaqMan Real time PCR and by capillary electrophoresis minisequencing

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Introduction: There are two reasons for establishing a methodology for noninvasive determination of KEL genotypes in early pregnancy. To identify fetuses which are at risk of hemolytic disease of fetus and newborn by alloimmunized pregnant women and to prevent alloimmunization during pregnancy. Aim: KEL noninvasive determination of fetal genotype from cffDNA in plasma KEL negative pregnant women using TaqMan assay and minisequencing (SNaPshot). Project is supported by IGA MZ CR: NT12225. Material and methods: 1) TaqMan assay involving region of KEL (K/k) polymorphism and region of AMELY gene as an internal control was tested in DNA samples from leukocytes of k/k, K/K and k/K men. 2) SNaPshot: Determination of sensitivity threshold KEL calibration was performed using a dilution series. It was tested 141 samples of cffDNA from maternal plasma in the first trimester. Results: 1) TaqMan assay: KEL genotypes determined from leukocyte DNA were distinguishable, but there was fluorescence background. So there wasn´t able to exactly distinguish between false positive fluorescence background and fetal DNA admixture. 2) SNaPshot: On the basis dilution series it was possible to detect less than 0,78% admixture of K allele corresponding DNA concentration of 0,04 ng /µl. Seven fetuses with allele K were found in 113 k/k mothers, which corresponds to about 4-5 % of the population frequency. It wasn´t possible to determine KEL genotype at 8 fetal samples due to KEL maternal heterozygous genotype. Conclusion: SNaPshot assay is more suitable to distinguish fetal KEL genotyp, than TaqMan assay.

P01.066-M

Feasibility of utilization of semiconductor based low coverage genomic sequencing in noninvasive prenatal trisomy 21 detection

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Recent studies focused on utilization of next-generation sequencing (NGS) technology in non-invasive prenatal detection of trisomy of chromosome 21 (T21) showed high sensitivity and specificity if more than 10 milion of read per sample were used. For these analyses high-throughput NGS systems are needed. The aim of our study was to test feasibility of usage of NGS protocol with approx. 5 milion of reads per sample and to estimate sensitivity and specificity of such testing. In our study high-risk group samples of 42 pregnant women, represented by 37 samples without and 5 with trisomy 21 bearing fetus confirmed by previous invasive procedure were analyzed by low coverage genomic sequencing on IonTorrent PGM. For T21 detection Z-score calculation was used. In all 5 samples with T21 fetuses Z-score above 3 was recorded. In non-trisomic and trisomic samples on average 5 463 516 and 6 292 096 mapped reads per sample were used in Z-score calculation, respectively. In non-trisomic and trisomic samples Z-scores were -0.26 (SD = 1.15) and 7.65 (SD=3.73) on average, respectively. So in our small scale study 100% sensitivity and specificity were recorded. According to our results low coverage sequencing based on IonTorrent PGM sequencing is suitable for noninvasive prenatal trisomy testing, however, a further study based on data from larger sample cohort is needed for more precise calculation of sensitivity and specificity of our current protocol. The study continues on greater group of samples. This study was supported by grant ITMS 26240220067 funded by ERDF.

P01.067-S

Validating non-invasive prenatal testing (NIPT) using artificial and actual pregnancy plasmas: accurate detection of five microdeletions

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Introduction: Microdeletions, which may result in mental and physical handicaps, are challenging to detect using cell-free DNA-based NIPT due to their smaller size. Additionally, sample rarity and the later gestational ages at which samples are generally collected complicate test performance validation. We created artificial pregnancy plasma DNAs (plasmArts) to circumvent these issues, demonstrating accurate detection of five microdeletions: 22q11.2, Cri-du-Chat, 1p36, Prader-Willi, and Angelman.
Methods: DNA isolated from cell lines, affected or unaffected children, and their unaffected mothers, was treated to generate nucleosomal ladders, mimicking maternal/fetal cfDNA fragment lengths. These "cfDNAs" were mixed to mimic fetal fraction distributions in the target gestational age population, and were analyzed alongside pregnancy plasmas using the PanoramaTM NIPT, which reports copy number and associated confidence for each chromosome. 469 samples (111 artificial mixtures of mother/child DNA derived from 5 affected and 1 unaffected children and their unaffected mothers, 6 affected and 352 unaffected pregnancy plasmas) mimicking various fetal fractions were tested.
Results: NIPT correctly distinguished affected and unaffected "pregnancy" plasmArts, suggesting that mixtures behaved similarly to maternal plasma cfDNA. This SNP-based assay detected deletions to as low as 3.9% fetal fraction. The anticipated false positive rate based on the observed fetal fraction distributions and unaffected pregnancy plasma measurements is ≤1%.
Conclusions: PlasmArt samples generated appropriate NIPT results, allowing robust test performance validation for rare conditions. This approach accurately identified pregnancies with a fetus affected by various microdeletions. This will increase NIPT clinical coverage and allow for earlier prenatal screening for sub-chromosomal deletions.

P01.068-M

Discordant results between non-invasive prenatal testing by maternal plasma sequencing and fetal ultrasound examination

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Over the past year, noninvasive prenatal screening (NIPS) based on massively parallel sequencing to align and count DNA fragments floating in the plasma of pregnant women, has become clinically available into prenatal care as an advanced screening test. We report here a case of discordant results in fetal sex determination between NIPS and ultrasound examination. The patient was referred to our clinical genetic service at 23 week of gestation due to discrepancies between NIPS results performed in a different center and fetal ultrasound examination. In particular, a first NIPS report was of a normal female fetal karyotype but subsequent ultrasound examination showed a fetal male gender. The result of a second NIPS analysis on a new plasma sample offered and performed by the same center was of increased risk for Turner syndrome. After genetic counseling, amniocentesis was performed at 23 wog. FISH analysis with a probe targeting SRY gene showed a single signal for SRY onto an almost intact X chromosome, revealing an X-Y cryptic chromosomal translocation: 46,XX.ish der(X)t(X;Y)(p22.3;p?)(SRY+). Array-CGH showed a subtelomeric deletion of about 2.28 Mb in Xp22.33 and the presence of the short arm of Y chromosome. To exclude the possibility of phenotypic consequences due to haploinsufficiency of SHOX gene, MLPA subtelomeric analysis was carried out. These case support the recommendation that NIPT should be regarded as a screening test and offered in the context of genetic pre-test counseling as well as post-test counseling for screen positive individuals. Diagnostic testing is recommended to confirm positive NIPS results.

P01.069-S

Older mother's perspectives on non-invasive prenatal testing for fetal abnormalities and adult-onset conditions in the United Kingdom

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Since the 1970's there has been a steady increase in England and Wales in the number of women having a baby at the age of 35 or over. Birth rates for this group have risen from 6% in 1974 to almost 20% in 2012.
The use of new technologies such as non-invasive prenatal testing and genome or exome sequencing may result in the opportunity for women to request fetal testing for a range of conditions. While there is considerable support from prospective parents and health professionals for non-invasive prenatal testing for single gene disorders, ethical concerns have been identified. It has been suggested that prenatal testing discriminates against people with disability and there are fears it may become routine. Detecting multiple genetic conditions in the fetus may facilitate detection of adult-onset conditions. This has serious implications for parental decision making.

In this study we aimed to explore the attitudes of mothers aged 35 or over towards new diagnostic techniques for prenatal diagnosis of foetal abnormality. Women who had given birth in the UK within the last 12 months were recruited using a novel recruitment strategy via Twitter. Here we present the initial findings relating to NIPT and testing for adult-onset conditions. Mother's considered the reduced risk to the pregnancy and extra time to make a decision as being major advantages of NIPT whilst voicing concerns regarding health service readiness and test accuracy. Concerns about testing for adult-onset conditions included increased psychological burden on parents and respecting the child's autonomy.

P01.070-M
Prenatal diagnosis and outcome in Noonan Syndrome

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Noonan syndrome (NS) is an autosomal dominant inherited disorder caused by heterozygous mutations of several genes within the RAS/mitogen activated protein kinase pathway. NS is characterized by short stature, minor facial and cardiac anomalies. Frequent prenatal ultrasound findings are increased nuchal translucency in addition with distended jugular lymphatic sacs, cystic hygroma, hydrops, hydrothorax, ascites, polyhydramnios, cardiac and renal anomalies. There is high variability of clinical symptoms even within single families. We present a retrospective study of 23 prenatally diagnosed cases with mutations in PTPN11, SOS1, RAF1 genes from a cohort of 330 fetuses with ultrasound findings indicating NS and normal karyotype. Cases were seen in a single tertiary center between 2005 and 2014 by an expert team of experienced sonographers and human geneticists. Prenatal ultrasound findings, molecular genetic data and postnatal phenoptype will be reported. Some cases are exemplified with clinical, ultrasound and molecular genetic data. In our study we want to emphasize the importance of prenatal molecular testing of Noonan genes (panel) in cases with an increased nuchal translucency and at least one additional feature mentioned above. Prognosis depends on molecular genetic findings in conjunction with ultrasound abnormalities which can change dramatically in the course of pregnancy. The occurrence of severe progredient hydrops or hydrothorax in the second or third trimester is associated with a high risk for an unfavourable outcome. Genetic counseling remains demanding due to the high variability in the prenatal and postnatal spectrum of symptoms in NS and the difficulty to give a reliable prognosis.

P01.071-S
Follow up of 30 prenatally diagnosed cases of osteogenesis imperfecta

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Osteogenesis Imperfecta (OI) is a heritable connective tissue disorder, characterized by a wide clinical spectrum, ranging from few fractures in mild cases to perinatal lethality. During pregnancy, if severe forms are usually easily early recognizable, the mild and moderate forms may lead to notoriously difficulties in the evaluation of the postnatal prognosis. **Objective:** to evaluate the contribution of prenatal imaging, family history and molecular analysis in the postnatal prognosis in osteogenesis imperfecta. **Methods:** to analyze retrospectively the prenatal features (fetal ultrasound examination and 3D CT-scan), family history and postnatal outcomes (phenotype and genotype) of 30 cases diagnosed antenatally as OI in our hospital. **Results:** In 15 cases, the severe fetal presentation led to pregnancy termination. Among the 15 remaining cases that are still alive, 4 are considered as severe, 6 as moderate and 5 as mild. The family history appears to be the best prenatal predictor of postnatal severity. We emphasize the sensitivity of the 3D-CT scan. Molecular screening was not helpful in the prognosis given *a priori* to the parents. No correlation between the severity of skeletal phenotype and the genotype was demonstrated *a posteriori*. Importantly, in 3 cases with marked bowing of the long bones, a significant improvement of the bone deformities was observed after birth. **Conclusion:** OI is today easily diagnosed during pregnancy based on the improvement of the recent imaging tools; however, the postnatal prognosis is still often difficult to evaluate, highlighting the value of a family history.

P01.072-M
Prenatal diagnosis of de novo partial trisomy 4q3l→qter and partial monosomy 22q13.3→qter

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We report prenatal diagnosis of a fetus with partial trisomy of 4q31-qter and partial monosomy of 22q13.3-qter. A 35-year-old woman was referred for prenatal diagnosis at 28 weeks gestation because of fetal hydrothorax and polyhydramnios detected by ultrasound. Amniocentesis was performed and routine G-band analysis of cultured amniocytes showed additional material of unidentifiable origin at the long arm of one chromosome 22. Karyotype was 46,XX,der(22)add(22)(q13.3)?. Further molecular cytogenetic characterization of the additional genetic material was carried using FISH. Six commercial FISH were used: two centromeric chromosome probes 4(D4Z1) and 14/22(D14Z1/D22Z1); two whole chromosome paints 4 and 22; two locus specific probes 22q13.3(SHANK3) and 22q11.2(N25); two subtelomeric probes 4p(D4S3360) and 4q(D4S2283). The extra chromosomal material was found to be derived from chromosome 4 de novo and fetal karyotype was redesigned as 46,XX,der(22)t(4;22)(q31.3;q12)dn. A girl was delivered at 38 weeks of gestation with a birth weight of 3000g and AS 10/10. She has mild dysmorphic features including dolichocephaly, microretrognathia, small skin folds on ears, without other anomalies. At the age of one year she shows slight developmental delay especially in motor skills development. Reported children with distal partial trisomy 4q have the wide phenotypic variability which could be related to the location of the breakpoints and associated monosomies of the other chromosomal parts. Partial trisomies of 4q31 to 4qter in general were associated with only mild phenotypic anomalies like slight (mental) retardation and/or dysmorphism which is consistant with our case. Further follow up is necessary especially because of monosomy 22q13.3-qter.

P01.073-S
Prenatal diagnosis of UPD using genomic SNP array in a foetus with ultrasound abnormalities.

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We report the case of a 35-year-old gravida 1, para 0 referred at 18 weeks of gestation (wg) because of several fetal malformations. First trimester screening test showed an increased risk for trisomy 21 with a nuchal translucency of 4,1 mm; the fetal karyotype was 46, XY. Ultrasound scanning at 17 wg showed increased fetal biometry (>95°), small thorax, flaccid abdomen wall, ambiguous genitalia. At 19 wg the patient optioned for pregnancy termination and the fetal autopsy showed cleft palate, microglossia, edematous short neck with a prominent occipital region, low-set ears, ambiguous resembling male genitalia with hypospadias and short limbs with joint contractures. The fetal roentgenograms showed bell-shaped thorax with coat-hanger. SNP array analysis was performed on DNA from fetal tissue. Data analysis revealing two regions of runs of homozygosity in 14q: the first one is a subcentromeric region of about 10 Mb, the second is a subtelomeric region of about 9 Mb. Trio analysis performed by two computational tools (UPDtool and SNPtrio) for detection and classification of uniparental isodisomy (UPD) confirmed paternal uniparental iso/heterodisomy of chromosome 14. In particular, data revealed a region of isodisomy in 14q11.2-q12 from genomic position 20.2 Mb to 30.4 Mb, a region of heterodisomy in 14q12-q32.2 from 30.9 Mb to 97.9 Mb, and a terminal region of isodisomy from genomic position 98 Mb to telomere. Results was validated by the analysis of microsatellite markers across chromosome 14. This result may contribute to prenatal identification of similarly affected patients.

P01.075-S
Reporting the outcome of molecular PGDs from a single laboratory in Iran

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Reporting results of preimplantation genetic diagnosis (PGD) for single gene disorders, gender typing, HLA matching and chromosomal aneuploidies from 2009. We used mainly multiplex nested PCR containing several short tandem repeats (STR) and sometimes gene fragments for sequencing. PGD were mainly offered for families with common genetic disorder in Iran specially beta-thalassemia, HLA matching, PKU, sex typing, chromosomal aneuploidies and Hemophilia A. In total we have performed PGD for 29 cases (total of 158 blastomers). For ladies with advanced age only few (1-4) eggs could be retrieved. In total only 46 blastomers could be implanted.
Our results show that in total 36 blastomers were tested for F8 linked STRs, 5 for PKU (BH4, PTS gene), 8 for PKU (PAH gene), 8 for Deafness (GJB2 gene), 8 for Epidermolysis Bollusa and HLA matching, 20 for β-Thalassemia, 84 for aneuploidies and finally gender selection was done on 132 blastomers. Some of these figures belong to two or three types of simultaneous testing

(e.g. thalassemia and sex selection or thalassemia and HLA typing).
Implantation was succeeded to pregnancies and child births in 3 families and one is still pregnant. The birth outcome was a twin healthy boys for hemophilia A (born on May 2011), a triplet boys for gender and aneuploidies selections (born on December 2013), a carrier boy for β-thalassemia was born on February 2014 and another twin hemophilia A pregnant at 8 weeks.

P01.076-M
Pre-case haplotyping of the two monogenic diseases in one family in Gennet: Marfan syndrome and SMA

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Genetic pre-case haplotyping (PGH) is an important constituent of the preimplantation genetic diagnosis (PGD). In our center we have performed haplotyping studies for 86 different monogenetic diseases of all types - X-linked, autosomal recessive, autosomal dominant, translocation and small deletions. The list of diagnosis for which we can offer PGD is being continually extended. PGD is available for a large number of monogenic disorders. For most couples requiring PGD, common pre-case haplotyping analysis of disease-associated haplotype is derived from family anamnesis. We are presenting a case in which affected haplotype will be determined by standard PGH analysis . In this family 2 affected haplotypes were detected which are associated with AD disease - Marfan syndrome - OMIM # 154700 and AR disease- Spinal Muscular Athrophy(SMA) - OMIM # 253300. During the first PGD cycle in the year 2013 (11.2013): 11 cumulus-oocyte-complexes (COCs) were retrieved. 9 blastomeres were biopsied. All tested embryos were analysed .2 embryos were affected of SMA , 3 embryos were affected of Marfan syndrome .2 embryos were diagnosed as monosomy of chr.5 carrying high risk paternal haplotype SMN1 gene. 2 embryos did not show PCR-amplification. The embryos were not re- analysed because they didn´t show optimal development. In this first PGD cycle no embryos were transferred . While there was no transfer in this case during the first PGD cycle, PGD still increases the chances of families at risk of transmitting serious genetic disorders to plan a healthy offspring.

P01.077-S
Targeted capture and massively parallel sequencing for PGD of monogenic diseases

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PGD is an important option for known carriers of monogenic diseases to deliver healthy babies. Polymerase chain reaction (PCR) is the most widely used method in PGD for monogenic disorders. However, in order to establish a robust PCR based PGD protocol, extensive preclinical experiments are needed to ascertain the efficiency and reliability of the procedure, which is a labor-intensive, time-consuming and costly process.
Many clinical applications based on targeted Massively parallel sequencing (MPS) have been reported. In this study, we explored the applicability of targeted MPS for PGD of monogenic disease. Isolated single lymphocytes were used to evaluate the genotyping accuracy of target-MPS at single-cell level at first. Then two families carrying mutation of the HBB causing β-thalassemia were tested using targeted MPS.
Pedigree haplotype analysis was applied in the following analysis and double-blind design was used in the study.
Over hundred of informative SNPs flanking both sides of the disease causing mutation were detected for each embryonic haplotype. The average interval between informative SNPs and gene mutation was within 10 kb. The final result was 100% consistent with the diagnosis provided by the reference laboratory, and the whole test was finished within a week.
The new targeted MPS based method will make the PGD for monogenic disease more accurate, faster and more affordable, providing benefit to many patients requiring PGD for monogenic disease.

P01.078-M
Single cell segmental aneuploidy detection is compromised by S phase

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Carriers of balanced translocations are at high risk for unbalanced gametes which can result in recurrent miscarriages or birth defects. Preimplantation genetic diagnosis (PGD) is often offered to select balanced embryos. This selection is currently mainly performed by array comparative genomic hybridization (aCGH) on blastomeres. Current methodology does not take into account the phase of the cell cycle, despite the variable copy number status of different genomic regions in S phase. To evaluate the accuracy of single cell array CGH, different cell cycle phases from three cell lines derived from patients with different chromosomal imbalances were sorted by flow cytometry. Ten single cells were picked per cell line per cell cycle phase, whole genome amplified and analyzed by BAC arrays, the most commonly used platform for PGD purposes. In contrast to G phase, where the imbalances were efficiently identified, the log2 intensity ratios of less than half of the probes in the regions of interest were above the detection threshold in 17/26 analyzed S phase cells. The results demonstrate that the accuracy to detect segmental chromosomal imbalances is reduced in S-phase cells, which could be a source of misdiagnosis in PGD. Hence, the determination of the cell cycle phase of the analyzed cell is of great importance and should be taken into account during the analysis. This knowledge may guide future technological improvements.

P01.079-S
Placental growth factor (PlGF) levels for preeclampsia prediction

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The aim of the study was to verify the possibility to predict preeclampsia (PE) after the 20th week of gestation by reliable cut-off levels based on PlGF analysis at the time of pregnancy termination within weeks 24-41. Control PlGF levels for weeks 9-19 were derived from 800 cases; 100 PE sera comprised: 17 - PE weeks 24-29, 24 (30-33), 34 (34-37) and 25 (38-41). PlGF was examined by DelphiaXPress Tm platform (PerkinElmer). Reliable PlGF control levels (3, 5, 25, 50, 75 and 95 percentiles) were established for weeks 9-19. For 1st trimester PE risk determination commercial software was used, for weeks 14-19 levels < 3 or 5 pecentiles were applied. Inverse relationship of decreased PlGF levels to the onset of PE and its clinical impact was documented. PlGF median level increase during pregnancy is significantly retarded. Levels of PlGF for PE (weeks 24-29) correspond to medians of the 11th week, PE (weeks 30-33) to week 15 and intermediate/late PE to week 17. PlGF cut off levels for 100%/95% detection rate success for PE weeks 24-29,30-33, 34-37, 38-41 are 61.6/46.2, 267.8/19.5, 262.3/180.3, 168.3/146.0 (all pg/ml). Combination of 1 st trimester PE screening with software prediction of early intermediate and late PE with 2nd/3rd trimester PlGF examination could improve the complex care not only for PE, but also for other adverse risks of HELLP syndrome, SGA, preterm delivery and IUGR including increased efficacy of 1st trimester aneuploidy screening. Supported by FN Motol (00064203, Modern Therapy), IGA NT13770 and OPPK CZ.2.16/3.1.00/24022.

P01.080-M
Integrative transcriptome-based approach for association studies: identification of new genetic markers for preeclampsia

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Preeclampsia is a common pregnancy-specific disorder with unknown etiology diagnosed in 5-17% of pregnancies. It is the leading cause of maternal and perinatal morbidity and mortality. Our prior genome-wide transcriptional profiling of placental tissue led to a novel set of 63 preeclampsia candidate genes (differentially expressed genes, (Fold Change >1.5, FDR<0.1)). In this report, we present preliminary study on the role of variability in some of these genes in the genetic susceptibility to preeclampsia. We analyzed 51 tagging single nucleotide polymorphisms (tagSNPs) in 12 genes (ANKRD37, BCL6, BHLHE40, CCSAP, CORO2A, DGKG, GPT2, PLIN2, RDH13, SIGLEC6, SYDE1 and ZNF175) in 514 patients with preeclampsia and 627 women with uncomplicated pregnancies from Russian, Buryat and Yakut populations using MassArray iPLEX (Sequenom). We have detected significant associations for preeclampsia with tagSNPs in PLIN2, BHLHE40, DGKG, RDH13, SYDE1 genes in Russian and Buryat population. In Yakut population, only three genes (BHLHE40, CORO2A, GPT2) are associated with increased risk of preeclampsia. tagSNPs in ANKRD37 and ZNF175 genes were associated with preeclampsia in Buryat population only. Interestingly, we found an association with preeclampsia for twenty out of the fifty-one studied polymor-

phism. This results demonstrate the high informative value of the integrative approach in studies of the genetic components of preeclampsia and show that allelic variations of the differentially expressed genes in placental tissue are associated with preeclampsia in different ethnic groups. Nevertheless, the clinical significance of these findings remains to be determined. This work was supported by the Russian Foundation for Basic Research (grant №14-04-01467).

P01.081-S
Molecular understanding of the FMR1 premutation and Fragile X-associated Premature Ovarian Failure

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CGG repeats expansion (55-200 units, premutation range) in the 5'UTR of FMR1 gene (Xq27.3) is associated with Fragile-X Tremor/ Ataxia Syndrome and Premature Ovarian Failure (POF). FMR1 premutation represents the most significant single gene variant associated with POF, however no studies are available to evaluate its pathogenic effect in the ovary context. As for FXTAS, an RNA-mediated toxic gain of function could be hypothesized for FX-POF. Human granulosa cell line were transfected with plasmid containing 76CGG repeat elements in the 5'-UTR of EGFP reporter under the control of CMV immediate early promoter (CMV-76CGG-EGFP), or with CMV-EGFP as control. By RNA immunoprecipitation, the interaction of 76rCGG for rCGG-Repeat Binding Proteins (rCGG-RBPs), previously identified, was tested. 76rCGG solely immunoprecipitate with heat shock proteins, some hnRNPs and proteins involved in paraspeckles formation such as SFPQ and hnRNPM, suggesting that in vivo premutated FMR1 mRNA differs from the wild type in making RNA-protein complexes. In this regard in premutated granulosa cells a deregulation of structural components paraspeckles was observed suggesting an alteration of splicing. Furthermore an "heat shock like" response associated with a reduced cell viability in human ovary granulosa cells expressing expanded CGG mRNA was observed, indicating a toxic role of premutated mRNA itself in the ovaries. This work is supported by Telethon grant GGP009126.

P01.082-M
DNA copy number variations in women with premature ovarian insufficiency

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The cause of premature ovarian insufficiency (POI) remains unknown in many cases. In recent years many studies have focused on the genetic factors of POI. Conventional karyotyping analyses have identified several X chromosome regions that harbour genes critically required for normal functioning of the ovaries. Due to rise of DNA microarray technology, it has been shown that in addition to X chromosome rearrangements, POI is associated with aberrations in autosomal chromosomes. Single-nucleotide polymorphism (SNP) arrays are useful in identifying single genes and genome regions responsible for the onset of POI. The present study included 700 women with idiopathic POI with the cessation of ovarian function before 40 years of age. Women with a history of gynaecological surgery, cancer treatment and genetic syndromes were excluded. Genomic DNA samples were provided by Tartu University Hospital and Estonian Genome Centre and analysed for copy number variations (CNV) and runs of heterozygosity (ROH) using high-resolution SNP arrays. The majority of detected CNVs fall within the common polymorphic regions, while others have clinical significance and harbour previously reported and novel POI candidate regions and genes. Identified aberrations include a 24Mb Xp22.33-p21.3 hemizygous deletion and a novel microdeletion in 15q21.3. In addition to CNV, numerous ROH regions were detected, which may possibly contain homozygous mutations in POI associated genes. DNA microarrays are a suitable tool for evaluating genomic imbalances in POI patients when compared to the conventional cytogenetic methods. The present study provides novel data on associations between the genomic variants and aberrations and POI phenotype.

P01.083-S
Prenascan in Gennet, non-invasive prenatal test trisomy

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Prenascan project was created in the Czech Republic in cooperation with Health BGI Europe. Project introduced into clinical practise non-invasive prenatel test for trisomy of chromosomes 13, 18, 21 with the possibility of sex determination and determining defects in sex chromosomes X and Y. The method is based on the evaluation of fragment's relative abundance of cell-free DNA (cffDNA) in maternal plasma. The fetal fraction of the total free DNA is 5-25 %, according to the stage of pregnancy. Extraction of cffDNA exploits the fact that cffDNA is significantly smaller than the maternal DNA, with fragments approximately 200bp in size. cffDNA subsequently subjects to whole-genome sequencing using next-generation sequencing platform based on the semiconductor sequencing technology. Examination method Prenascan is perfomable between 10 and 21 weeks of pregnancy, only after genetic counseling. Diagnostic sensitivity of the method is about 96 % to detect the tested trisomy. The specificity of the test exceeds 99,7 %. Efficiency of the test is 99,9 % for the cases of trisomy 21, 98,8 % for trisomy 18 and 98,7 % for trisomy 13. The main limitation of the test is low concentration of cffDNA in maternal plasma. Test results can be distorted by foreign DNA if the mother received transfusion or transplantation of stem cells.
From October 2012 to December 2013 1263 patients were tested . Positivity was found in 22 cases, false pozitivity in 4 cases. False-positive patients were tested by amniocentesis or chorionic villus sampling with normal results.

P01.084-M
A unique ring chromosome 20 in a fetus with a complex heart malformation

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A 31-year old woman was referred at 20+6 weeks gestational age because of abnormalities at the standard anomaly scan. Advanced ultrasography showed a double outlet right ventricle with transposition of the great arteries and a hypoplastic left ventricle. Furthermore a mandibular retrognathia was noticed. Amniocentesis was performed for RAD and array analysis. The pregnancy was terminated at the parents request and external post-mortem examination revealed mild dysmorphic facial features including mild micrognatia and low set ears. SNP-array analysis showed a duplication deletion rearrangement in the long arm of chromosome 20. The interstitial duplication was 20,5 Mb and the adjacent deletion was 240 kb in size. Karyotyping revealed a ring chromosome 20 in all analysed cells. Fluorescence in situ hybridisation analysis demonstrated that the duplication was inverted. Parental chromosomes were normal indicating that this unbalanced rearrangement occurred de novo. Only a few cases with a ring chromosome with a telomere deletion and an additional inverted duplication have been described so far. These inv dup del chromosomes are the result of an asymmetric breakage of a dicentric chromosome that has been formed to stabilize a broken chromosome. The ring formation represents a new mechanism, in addition to telomere capture, through which inv dup del chromosomes can stabilize. In conclusion this study presents a prenatal case with a ring chromosome 20, which presented with ultrasonographic anomalies initially.

P01.085-S
A 3 years worldwide experience with Prenatal BACs-on-BeadsTM for prenatal diagnosis in over 9.500 pregnancies

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Chromosomal microarrays are the gold standard in prenatal cases with ultrasound abnormalities+normal karyotype. In low-risk pregnancies their use is still controversial because of their diagnostic yield in relation to that of variants of unclear significance. Consequently some laboratories, for low-risk pregnancies, adopted PNBoBs™ (in combination with karyotype) a transitional test investigating 9 critical-regions associated with dominant microdeletion syndromes with known genotype-phenotype correlations. We present the experience of 12 worldwide laboratories using PNBoBs™+karyotype. The purpose was to evaluate the usefulness of PNBoBs™ in pregnancies with low *a priori* risk of microdeletion/microduplication syndromes (advanced maternal age,AMA; maternal anxiety,MA; increased risk after maternal serum screening for Down syndrome,MSS-DS; soft marker/s). 9648 samples were analyzed: 17.4% AMA, 22.2% MSS-DS, 8.1% MA, 36.0% ultrasound abnormalities and 7.1% for unknown origin. Successful rate was 96.7%; false negative incidence 0.56% related to chro-

mosome abnormalities not covered by PNBoBs™ but detectable by karyotype. In 0.31% of cases PNBoBs™ was reflexed by FISH for further characterization. Overall abnormal results (PNBoBs™+karyotype) were observed in 9.1% of cases; a microdeletion/microduplication was retrieved in 0.7% of cases (n=69); the majority of them (68.1%) involved Di George syndrome critical-region (deletion=32; duplication=15). 23 cryptic imbalances were found in low risk pregnancy leading to an additional diagnostic yield of about 1/246 in the low-risk pregnancies; 41 were detected in high risk pregnancies providing a 1.8% of additional detection rate (1/54). We will present incidence of cryptic unbalances stratified by indication for prenatal diagnosis that was surprisingly higher than expected basing on theoretical incidence (1/1700).

P01.086-M
Detection of 7p14 deletion in a fetus with aortic coarctation: genetic and fetal morphology data

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We present the case of a fetus with aortic coarctation suspected by ultrasounds at 26th gw, in which an interstitial deletion 7p12.3p14.2 was identified on amniotic fluid. The deleted region, confirmed by FISH and aCGH analyses, spanned in the range chr7:36,671,630-46, 039.765bp, and involved several genes, as GLI3, ccm2, GCK, txndc3.
Decipher db shows at least 5 subjects with a microdeletion involving this region, all with delayed psychomotor development, dysmorphisms and abnormalities of the extremities. One of the subjects also presented coarctation of the aorta.
The literature also reports some cases of del(7)(p14) and Greig Cephalopolysyndactyly, an autosomal dominant disorder associated with GLI3 aploinsufficiency, characterized by a distinct combination of craniofacial, foot and hand malformations. Digilio et al reported a deletion 7p14 with cardiac anomalies (non-compacted myocardium, VSD, ASD and aortic valve dysplasia). Five other subjects, with cytogenetically visible deletions 7p14, had more severe findings as microcephaly, dysmorphism and mental retardation.
After several genetic counseling sessions with the equipe of the prenatal center, the parents decided for ToP.
Fetal autopsy identified cranio-facial dysmorphisms, a complex CHD with aortic coarctation, ASD, VSD and left ventricle hypertrophy, Meckel's diverticulum, accessory spleen and horseshoe kidney.
This case underlies that ultrasound, genetic testing (including aCGH) and genetic counselling should be included in the protocol of pregnancies with fetal malformations, once again reinforcing the necessity for the different specialists involved in prenatal diagnosis to cooperate in the identification of a complex fetal phenotype, to offer a prognosis that may help parents in their pregnancy options.

P01.087-S
Prenatal diagnosis and Preimplantation Genetic Diagnosis for inherited cardiac diseases in the Netherlands: an overview

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Inherited cardiac diseases are associated with an increased risk of sudden cardiac death (SCD). Common hallmark is the variable disease expression and incomplete penetrance. Therefore the phenotype varies widely between and within families. PND and PGD provide carriers of a mutation that causes an inherited cardiac disease the possibility of having a child without the familial gene mutation and reduce the risk of SCD in their children. The advice from the National Board for PGD indications is to be hesitant with PGD in inherited cardiac diseases due to reduced penetrance and variable expression of the phenotype.
In this study, we evaluated the number of PND and referrals for PGD for inherited cardiac diseases in the Netherlands in the past 15 years.
PND was performed for three different inherited cardiac diseases: Hypertrophic cardiomyopathy (HCM) (n=2), Dilated cardiomyopathy (DCM) (n=1) and Long QT syndrome (LQTS) (n=1). Couples came for PGD intake for HCM (n=9), Arrhythmogenic right ventricular cardiomyopathy (ARVC) (n=3), DCM (n=9), NCCM (n=1), LQTS (n=2), Brugadasyndrome (n=2), and idiopathic VF (n=3). After intensive counseling one couple (DCM) choose to continue the PGD procedure. Another couple (idiopathic VF) started the procedure, but withdrew after the genetic test was made, because of ethical considerations. The other couples choose to fulfill their child wish by other means. The families opting for PND or PGD for inherited cardiac diseases have experienced a severe phenotype or a severe family history of SCD. Nevertheless, the number of patients continuing with PND and PGD is low.

P01.088-M
High resolution chromosomal microarrays in prenatal diagnosis significantly increase diagnostic power

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Objective: The objective of this study was to determine for the first time the reliability and the diagnostic power of high-resolution microarray testing in routine prenatal diagnostics. Methods: We applied high-resolution chromosomal microarray testing in 464 cytogenetically normal prenatal samples with any indication for invasive testing.
Results: High-resolution testing revealed a diagnostic yield of 6.9% and 1.6% in cases of fetal ultrasound anomalies and cases of advance maternal age (AMA), respectively, which is similar to previous studies using low-resolution microarrays. In 3 (0.6%) additional cases with indication AMA an aberration in susceptibility risk loci was detected. Moreover, one case (0.2%) showed an X-linked aberration in a female fetus, a finding relevant for future family planning. We found the rate of cases, in which the parents had to be tested for interpretation of unreported copy number variants (3.7%), and the rate of remaining variants of unknown significance (0.4%) acceptably low. Of note, these findings did not cause termination of pregnancy after expert genetic counseling. The 0.4% rate of confined placental mosaicism was similar to that observed by conventional karyoytyping and notably involved a case of placental microdeletion.
Conclusion: We conclude that high-resolution prenatal microarray testing is a reliable technique that increases diagnostic yield by at least 17.3% when compared with conventional karyotyping, without an increase in the frequency of variants of uncertain significance.

P01.089-S
Noninvasive detection of a balanced fetal translocation from maternal plasma

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Noninvasive prenatal testing based on massively parallel sequencing (MPS) of circulating cell free DNA (ccfDNA) from pregnant plasma offers a powerful tool for detecting fetal chromosomal aneuploidies and other copy number variations; however, copy neutral structural rearrangements have proven challenging. We aimed to detect and characterize a balanced fetal specific translocation event by sequencing ccfDNA from maternal plasma. Simulations were used to develop an algorithm which leverages base incremental changes in mapping characteristics of ccfDNA to identify paired end reads potentially harboring structural rearrangements. We then applied this methodology on high coverage 100bp paired end data from ccfDNA isolated from a 38 year-old pregnant donor carrying a fetus with a balanced translocation. Our algorithm identified the known translocation (p=1.21e-8) and discounted the likelihood of others, enabling the base specific localization of the breakpoints. Furthermore, while no evidence of chromothripsis existed, we identified a 6bp deletion present within der(8) which is absent from the der(11) reciprocal rearrangement after de novo assembly of 76 chimeric reads. Overall, we have demonstrated here the first proof of concept study detecting and characterizing a balanced fetal specific translocation by sequencing ccfDNA from maternal plasma.

P01.090-M
Prenatal diagnosis and follow-up of a Patient with ASPM primary microcephaly MCPH

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Primary microcephaly is a heterogeneous disorder of brain development

where the brain is too small since birth but otherwise normally formed. It results from an insufficient production of mature neurons during neurogenesis. Many genetic defects have been identified in primary microcephaly, the common pathobiological endpoint being an alteration in the cell-cycle timing and fate determination of neural progenitors. *ASPM* is the most frequently involved gene in the Microcephaly, Primary Hereditary (MCPH), autosomal recessive phenotype. Detailed prenatal and postnatal phenotypic reports are currently lacking. We report on a Turkish family where the second child, a 36 months old boy, presented with typical MCPH. The parents were first cousins, and the mother was in the 9th week of her third pregnancy. Next generation sequencing of the proband's DNA showed a homozygous *ASPM* mutation, a 1bp insertion in exon 18 causing a frameshift and premature stop codon, c.6513dupA (p.Val2172SerfsX7). Both parents were heterozygous for the mutation. They opted for prenatal diagnosis, and amniocytes DNA showed homozygosity for the mutation. The pregnancy was continued, and the parents declined further examinations. This case illustrates the power of high throughput sequencing for rapid prenatal diagnosis of MPCH, where phenotypic correlations should eventually help understand how MCPH genes shape various parts of the cortex, central gray matter, and other parts of the encephalon.

P01.091-S

Identification of rare CNVs involving genes acting in oocyte maturation and differentiation in a cohort of patients affected by Primary Ovarian Insufficiency

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Primary Ovarian Insufficiency (POI) is a heterogeneous group of disorders with an incidence of 1:10,000 women by age 20, 1:1,000 by age 30, 1:100 by age 40. Despite the identification of numerous candidate genes in POI women, the genetic origin has been clarified only in about 20% of the patients. The patients showing the most severe phenotype, characterized by the absence of pubertal development and primary amenorrhea (PA) and 46,XX ovarian dysgenesis, are indeed rare but the search for genetic variations in this extreme phenotype may be more effective in identifying novel pathogenic mechanisms.
To unveil new POI causative genes we searched for rare high-penetrance CNVs involving genes essential for ovarian function in a cohort of 46,XX patients affected by PA. Forty-three patients were processed by high resolution array-CGH. Thirty-seven patients were found to bear 98 CNVs not reported to date in healthy subjects according to the Database of Genomic Variants (DGV), and 11 CNVs already reported in DGV but relevant to gene content, for a total of 109 CNVs. Several of these genomic alterations include genes implicated in: meiotic resumption, oogonia maintenance, first-polar-body extrusion, DNA repair, follicle adhesion/migration regulation, actin remodelling, cholesterol endocytosis, Ca2+ homeostasis.
Once characterized by other molecular and bioinformatics approaches, the results of this study are promising to expand the knowledge about the molecular pathways involved in POI pathogenesis and probably provide the basis for a more accurate genetic diagnosis of POI patients.

P01.092-M

Conflicting results QF-PCR and karyotyping due to structural aberration of the Y-chromosome

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Quantitative fluorescent polymerase chain reaction (QF-PCR) is an accurate and efficient technique for rapid prenatal diagnosis of trisomy 13, 18 and 21 and aneuploidies of the sex chromosomes. Discordant results between QF-PCR and karyotyping have occasionally been reported, mostly due to mosaicism.
A 20-year old female underwent chorionic villus sampling at 13 weeks' gestation because of an increased risk of a chromosomal aberration after first trimester screening. Ultrasound investigation demonstrated no abnormalities. The results from the QF-PCR analysis were interpreted as a possible mosaic 45,X/46,XY because of the low contribution of the Y chromosome. Conventional karyotyping of the LTC demonstrated a non-mosaic 45,X. Since no abnormal findings were detected on ultrasound, a confined placental mosaicism was suggested as a possible explanation for the discordant findings. A subsequent amniocentesis revealed a normal male genotype with QF-PCR. Karyotyping, on the contrary, demonstrated a mosaic pattern with 45,X and a structural rearranged Y chromosome, probably an i(Yp). Additional FISH confirmed the presence of an isochromosome of the short arm of the Y chromosome. The case presented here demonstrates that caution should be taken when conflicting results are observed in CVS - even if a normal profile is obtained with QF-PCR in a follow-up amniocentesis. This case further illustrate that mosaicism for an abnormal cell line can result in a normal QF-PCR profile.

P01.093-S

Persistent Elevated Population-based Rates of Congenital Malformations (CM) and Elevated Whole Body Counts (WBC) of 137Cs among Pregnant Women in the Polissia Region of the Rivne Province in Ukraine.

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Background: We established a population-based surveillance of CM in the Rivne (R) province of Ukraine. The northern half of R or Polissia (P) is polluted by Chornobyl ionizing radiation. Analyses of observations during 2000-2009 demonstrated elevated population-based rates of specific CM; that prenatal exposure to alcohol was an unlikely cause as well as WBC Bq counts of pregnant women residing in P that often were above the officially set safe limits (Wertelecki et al., in Congenital Anomalies, 2014 in press).
Objectives: determine the degree of persistence of population-based rates of CM, patterns of alcohol use during pregnancy and of WBC patterns among pregnant women.
Methods: adherence to procedures of the European CM monitoring consortium (EUROCAT); analysis of CM patterns among 180,559 births in R during 2000-2011; and analysis of WBC obtained from 9,169 pregnant women in R during 2008-2012.
Results: persistence of higher rates in P than in non-P of conjoined twins, teratomas, neural tube defects, microcephaly, and microphthalmia; less prevalent prenatal exposures to alcohol in P; and persistence of elevated WBC (above 3750 Bq) in 19% of pregnant women residing in P and nearly 0.2% among those residing in non-P.
Conclusions: Higher rates of specific CM, for the most part blastopathies, and elevated WBC of incorporated 137Cs by pregnant women are persistent in the P region of the Rivne province. The evidence is sufficiently compelling to call for a shift from descriptive epidemiology toward cause-effect investigations of IR impacts on CM in Rivne-Polissia.

P01.094-M

The VEGF+405 G/G Genotype may Influence Embryo Implantation in ART

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Repeated implantation failure (RIF) is the main problem after using assisted reproductive techniques (ART). The main causes of RIF as a multifactorial problem include decrease in endometrial receptivity, defects of embryo or combinational. Successful embryo implantation depends on trophoblast proliferation, migration and invasion to the endometrium, all associated with vascular endothelial growth factor (VEGF) as the major protein in stimulating of angiogenesis. This study aimed to determine the association betweenVEGF+405G/C polymorphism and RIF in infertile women. The patients group included 74 women with >3 RIF and the control group consisted of 149 healthy fertile women. Genotypes and allele frequencies of VEGF+405G/C polymorphism were determined by PCR-RFLP method and verified by Sanger sequencing. The frequencies of GG, GC and CC genotypes in patients group were 31.1%, 48.6% and 20.3%, respectively while those frequencies in controls were 2.0%, 47.0% and 51.0% respectively. The frequency of GG genotype was significantly higher in patients than controls ($p<0.001$). CC genotype frequency was higher in controls than patients ($p<0.001$). The frequency of GC genotype did not show any difference between groups. C as the wild allele was more frequent in controls while frequency of G as the mutant allele was higher in patients ($p<0.001$). The VEGF+405 G/G genotype may influence embryo implantation and lead to

RIF in ART candidates. Since this is the second report on association of this polymorphism with RIF, further studies in different ethnic populations require for determining this association.

P01.095-S

Gene-gene interactions and the risk of recurrent miscarriages in EG-VEGF and its receptor genes

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Endocrine gland-derived vascular endothelial growth factor (EG-VEGF) and its receptor genes (PKR1 and PKR2) play an important role in human early pregnancy. Our previous study showed that PKR1 and PKR2 polymorphisms are associated with recurrent miscarriages (RM). This study was conducted to find EG-VEGF, PKR1 and PKR2 variants in the coding regions of idiopathic RPL patients and further evaluate gene-gene interactions in 3 genes. Two hundred and ninety one blood samples from 142 RPL women and 149 controls were nucleotide sequenced in the coding regions of EG-VEG, PKR1 and PKR2. Gene-gene interaction was evaluated in 3 gene variants using multifactor dimensionality reduction (MDR) method. One each nonsynonymous variant of 3 genes were identified, and PKR1(I379V) and PKR2(V331M) were significantly associated with idiopathic RM (p=0.006 and p=0.002, respectively). Genetic interactions were founded not only between PKR1(I379V) and PKR2(V331M), but also among EG-VEGF (V67I), PKR1(I379V) and PKR2(V331M) (p=0.01 and p=0.01, respectively). Women carried low-risk genotypes reduced 77% risk of experiencing miscarriages compared with those carried high-risk genotypes. The present study corroborates the clinical relevance of the EG-VEGF system in human early pregnancy, and provides evidence for the gene-gene interactions of EG-VEGF and PKR variants.

P01.096-M

Study of chromosomal alterations and polymorphisms of MTHFR, Factor V and Prothrombin genes in patients with recurrent miscarriage referred to Royan Institute

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Introduction: Recurrent miscarriage(RM) is defined as two or more consecutive pregnancy losses before 20 weeks of gestation which is an important clinical problem, with an incidence of 1%-3% among couples wishing to have children. There are several factors in the etiology of RM. One of the main genetic causes involve in the pathogenesis of RM is balanced chromosomal rearrangements in one or both of partners. In 4%-8% of couples with RM, at least one of the partners has chromosomal abnormality. After chromosomal abnormalities, thrombophilia was identified as a major cause of RM, with a rate of up to 40%, especially in the first half of pregnancy.
Methods: The patients group included 1100 Iranian couples(2200 individuals) referred to Royan Institute between 2004 and 2013. Karyotyping was performed using standard cytogenetic techniques. Besides, thrombotic gene polymorphisms were studied in 128 women who had a normal karyotype .The results were compared with 70 healthy women as control group.
Results: Abnormal karyotypes were found in 124 people, 83 women (3.77%) and 41 men (1.86%). The frequencies of *FV Leiden*, *Prothrombin*-G20210A, *MTHFR*C677T and *MTHFR*A1298C mutations in patients were 10.93%,4.68%,43.75% and60.15%, respectively. These frequencies in control group were 2.85%,2.85%,34.28% and5.71% respectively.
Conclusion: Sex chromosome mosaicism is the most commonly detected chromosomal abnormality in couples with RM who are candidates for offering PGD.Patients with combined thrombophilic mutations are at higher risk for RM than women without these mutations.The most important issue with hereditary thrombophilia's is the prevention of maternal thrombosis.
Key words: Recurrent Miscarriage,Thrombophilia,Karyotype

P01.097-S

Polymorphisms in oxidative stress related genes and recurrent miscarriage

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Recurrent miscarriage (RM) is one of the important problems of modern reproductive medicine. RM affects approximately 1 – 5 % of all couples trying to conceive. Various factors have been identified that influence miscarriage, including parental chromosomal abnormalities, endocrine dysfunction, and others. However 50% of cases fail to reveal an identifiable cause and are therefore classified as idiopathic.
We investigated the association of polymorphisms in oxidative stress related genes with idiopathic RM. 331 idiopathic RM patients and 197 controls were genotyped for *ABCB1* rs1045642, *CYP1A1* rs1048943 and rs4646903, *COMT* rs4680, *CAT* rs17880664, *GCLC* rs17883901, *GPX4* rs713041, *NRF2* rs6721961, *SOD2* rs4880, and *OGG1* rs1052133. A protective effect of *COMT* rs4680-G allele on RM was shown in individual SNP analysis: P = 0.0016, OR = 0.47, 95% CI 0.29 - 0.75. The multi-factor dimensionality reduction (MDR) approach revealed gene-gene interactions for *ABCB1*, *COMT, GPX4*, and *OGG1* genes. Cumulative gene risk score analysis demonstrated that more than three risk alleles in the genes *ABCB1*, *COMT*, *GPX4*, and *OGG1* were associated with idiopathic recurrent miscarriage P = 1.2 x 10-3, OR =1.97, 95% CI 1.31- 2.97. In silico data interpreting by GeneMANIA analysis revealed genetic, physical, pathway and coexpression networks for these four genes.
The current study shows that cumulative effects of genetic variability in oxidative stress-related genes may play a role in the recurrent miscarriage with no known etiology.

P01.098-M

Results of conventional karyotyping and 5 thrombophilic gene mutations in a Turkish population with recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) is the spontaneous loss of clinically established intra-uterine pregnancy before the fetus has reached viability
Here we present the cytogenetic data of couples and prevalence of 5 thrombophilic gene mutations among 175 women referred with RPL to our center.
Chromosome analyses were performed in 179 couples with two or more consecutive miscarriages before 24 weeks' gestation between 19.10.2011-31.12.2013 using GTL banding. MTHFR c.677C>T and c.1298A>C, Factor II c.20210G>A, FactorV (Leiden) c.1691G>A and plasminogen activator inhibitor-1 (PAI-1) 4G/5G genotypes were determined using SNP primers designed by the manufacturer (NLM Diagnostics, Italy). Melting curve
analysis has been performed with labeled probes using Real-Time PCR method (Qiagen, Rotor Gene).
Table 1 shows the detail of the cytogenic findings of couples with RPL. The frequencies of studied 5 thrombophilic gene mutations in women referred with RPL were summarized in Table 2.
The prevalence of parental chromosomal aberrations was greater in our study (8.4%) than in most studies in the literature, which quote a 3% - 5% prevalence.
Comparing our results of 5 thrombophilic gene mutations with other studies we suggest considering the mutations in FV Leiden G1691A, MTHFR C677T and PAI-1 4G/5G genes in women with RPL.

Table 1: Details of cytogenic findings of couples with RPL

Cytogenic findings among women by conventional karyotyping	**n (%)**	**Cytogenic findings among men by conventional karyotyping**	**n (%)**
46,XX	156 (87,1%)	46,XY	152 (84.9%)
45,X [2]/46,XX [48]	2 (1,11%)	47,XXY[3]/46,XY[47]	1 (0,55%)
45,X[4]/46,XX[96]	1 (0,55%)	46,XY, t(4;10)(p14;p13)	1 (0,55%)
45,X[6]/46,XX[94]	1 (0,55%)	46,XY, t(2;3)(q35;q25.2)	1 (0,55%)
45,X[5]/46,XX[95]	1 (0,55%)	46,XY, t(1;8)(q25;q22)	1 (0,55%)
45,X[3]/47,XXX[2]/49,XXXXX[1] /46,XX[94]	1 (0,55%)	46,XY, t(6;14)(p23;q24)	1 (0,55%)
46,XX, inv(9)(p11q13)	2 (1,11%)	46,XY, inv(9)(p11;q13)	4 (2,23%)
46,XX, t(1;6)(p11;q11)	1 (0,55%)	46,XY, inv(9)(p12;q13)	1 (0,55%)
46,XX, t(11;18)(p13;q11.2)	1 (0,55%)	46,XY, inv(5)(p15;q31)	1 (0,55%)
45,XX, rob(13;14)(q10;q10)	1 (0,55%)	46,XYqh+	7 (3,91%)
46,XX, 16qh+	3 (1,67%)	46,XY, 9qh+	3 (1,67%)
46,XX, 21pss	2 (1,11%)	46,XY, 1qh+	1 (0,55%)
46,XX, 21ps+	1 (0,55%)	46,XY, 22pstk+ps+	1 (0,55%)
46,XX, 9qh+	2 (1,11%)	46,XY, 21pss	2 (1,11%)
46,XX, 1qh+	3 (1,67%)	46,XY, 9qh+, 16qh+	1 (0,55%)

Table 2: Frequencies of 5 thrombophilic gene mutations of women with RPL

	FII Prothrombin	FV Leiden	MTHFR 677	MTHFR1298	PAI-1
Homozygous Wild Type	168 (96%)	151 (86,3%)	81 (46,2%)	70 (40,2%)	Homozygous 5G 28 (27%)
Heterozygous Mutation	7 (4%)	23 (13,1%)	82 (46,8%)	79 (45,4%)	Heterozygous 4G/5G 40 (39%)
Homozygous Mutation	-	1 (0,6%)	12 (7%)	25 (14,4%)	Homozygous 4G 35 (34%)
	175	175	175	174	103

P01.099-S
The role of HLA-genes in complex immunogenetic preconditions for idiopathic recurrent pregnancy loss

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Influence of HLA-system on reproductive losses is considered from the standpoint of search for specific HLA genes, the similarity of spouses in HLA-antigens, or the study of modulating properties of HLA-system in the gene network complex. Aim. To analyze the distribution of allelic polymorphism of the HLA-DRB1, DQA1, DQB1 genes and HLA-G 14-bp insertion/deletion polymorphism in married couples with RPL. Results. Complex analysis of distribution and frequency of allelic variants of genes HLA-DRB1, HLA-DQA1 and HLA-DQB1 was conducted for 200 married couples with recurrent pregnancy loss of undefined genesis. It was determined that allele DRB1*0301 is an allele-aggressor in the group of women with RPL, and possessing this allele presents three-fold increased risk of idiopathic pregnancy loss for a woman (OR = 3.4;CI95%:1.0-11.1). The results demonstrated probable significant increase in frequency of genotype +14 bp/+14 bp of HLA-G 14-bp insertion/deletion polymorphism ($p < 0.05$) in women with RPL against the control group. The study demonstrated over two-fold increase of the risk of pregnancy loss for women-carriers of homozygous genotype by allele of insertion(+)14 bp 3' UTR region of HLA-G gene (OR = 2.65;CI 95%:1.06-6.68).
It was determined that the increase in total homology of spouses by 50% and more in allelic polymorphism in loci HLA-DRB1, HLA-DQA1 and HLA-DQB1 presents twelve-fold increased risk of idiopathic pregnancy loss for a woman (OR =12.8;CI95%:1.63-100.27).Conclusions. Changes in the major hystocompatibility complex genes can cause the failure of female reproductive function and lead to the early fetal loss.

P01.100-M
Association study of miR-196a2 rs11614913 polymorphism with risk of idiopathic recurrent pregnancy loss in Iranian women

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Introduction: Recurrent Pregnancy Loss (RPL) is defined as the occurrence of two or more consecutive pregnancy loss prior to 20th week of gestation. There are several leading causes of RPL including uterine anatomical defects, genetic factors, infectious, immunological, environmental and blood dyscariasis. However, despite in a large number of cases no cause has been identified and is classified as idiopathic. Recent studies have implicated miRNAs in endometriosis, preeclampsia, infertility and RPL. Therefore; the aim of the present study was to investigate the association of miR-196a2C>T (rs11614913) with RPL.
Methods: We conducted a case-control study of 185 Iranian women: 85 patients with at least two unexplained consecutive pregnancy losses and 100 healthy controls with at least one live birth and no history of pregnancy loss. Patients with recurrent pregnancy losses due to anatomic, hormonal, chromosomal, infectious, autoimmune, or thrombotic causes were excluded from the study group. Genotyping was performed using tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR).
Results: Significant difference in distribution of miR-196a2 rs11614913 genotypes was found in RPL patients in comparison to controls, with P-value of 0.04 and odds ratio equal to 2.69 (95% CI: 1.03-7.03).
Conclusion: We provide evidence for association between genetic variation in miR-196a2 and recurrent pregnancy loss. Further studies will be required to validate the significance of the studied genetic variation in diverse ethnic populations.

P01.101-S
Genetic variation in Circadian Rhythm Genes are associated with Recurrent Spontaneous Abortions

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Recurrent spontaneous abortion (RSA), the occurance of three or more consecutive pregnancy losses before the 24th week of pregnancy, occurs in approximately 0,5-3% of reproductive-aged women. Results from animal model studies demonstrate the importance of circadian rhytms in reproductive health. The aim of this study was to examine whether there is an association between genetic variability in the primary clock genes CLOCK and ARNTL and RSA in the Slovene population. The study group consisted of 152 unrelated women with RSA and control group of 170 age-matched women. Altogether, 8 SNPs were tested, 4 in CLOCK (rs6811520, rs6850524, rs11932595 and rs13124436) and 4 in ARNTL gene (rs3789327, rs1481892, rs4757144 and rs12363415). The significance of association for individual SNP was calculated to compare the allelic frequency and genotype distribution in patients and control subjects using the Chi-Square test ($\chi 2$). After using Bonferroni correction significant difference in distribution of CLOCK rs6850524 polymorphism genotypes were found in patients with RSA in comparison to controls, with P-value of $2.90 \cdot 10^{-6}$ and odds ratio equal to 2.65 (95% CI: 1.68 to 4.18). Other SNPs in CLOCK and ARNTL genes did not display any significant association with RSA susceptibility. We provide evidence for association between genetic variation in CLOCK gene and RSA. Further studies are required to validate the results obtained in the Slovenian population.

P01.102-M
Prenatal diagnosis for hereditary cancer in the Netherlands

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Background Since the 1980s the genetic cause of many hereditary tumor syndromes has been elucidated. As a consequence, carriers of a deleterious mutation in these genes can opt for prenatal diagnoses (PND). We investigated uptake of PND in the Netherlands for retinoblastoma (Rb) and compared this with use of PND for five other hereditary tumor syndromes, i.e. familial adenomatous polyposis (FAP), Von Hippel-Lindau's disease (VHL), hereditary breast ovarian cancer (HBOC), neurofibromatosis type 2 (NF2), and Li-Fraumeni syndrome (LFS). **Methods** A questionnaire was mailed to all DNA-diagnostic laboratories assessing: 1) Number of independent mutation positive families identified until January 2013 2) Number of PNDs performed for these syndromes until that date One-sided Fishers-exact test was used to compare uptake of PND for Rb with the other five tumor syndromes. **Results** 11.8% of mutation positive Rb families used PND, whereas uptake for the other syndromes was between never and 6.5%. For Rb PND was used both by couples with a 50% risk and by healthy couples with a child with a *de novo* mutation (2-3% risk). Overall uptake for PND was significantly higher for Rb than for FAP, HBOC and NF2. If just Rb couples with a 50% risk were taken into account, only a significant difference was found between Rb and FAP and HBOC. **Conclusion** Large differences in the use of PND between tumor predisposing syndromes were observed. Highest uptake was observed for Rb and other childhood hereditary tumor syndromes, which is of relevance for physicians caring for these families.

P01.103-S
Double Robertsonian translocation of chromosomes 13, 14 and 15: a case report

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Introduction. Robertsonian translocations (RT) are among the most common balanced structural rearrangements in humans that occurs in the acrocentric chromosome pairs (chromosomes 13-15, 21-22). Robertsonian translocations comprise complete centromere fusion of the long arms of two acrocentric chromosomes, and the two short arms are lost.
Materials and methods. Trisomy 13 with double RT was characterised by standart karyotype analysis and fluorescence in situ hybridization method (FISH) with probes for chromosomes 13, 18, 21, X and Y with critical regions (RB1, D18Z1, D21S259/D21S341/D21S342, DXZ1, DYZ3) from Abbott Molecular, Inc. Vysis AneuVysion DNA Probe Kit.
Case report. A pregnant 22-year old woman was referred to the prenatal genetic counseling after ultrasonography performed at 13 weeks of gestation revealed congenital fetal abnormalities, including polydactyly of both upper limbs and bilateral cleft lip and palate. First trimester biochemical screening showed decreased pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (HCG), calculated risk for trisomy 18

was 1:105. Triple marker screening showed decreased alpha-fetoprotein (AFP) and HCG, and normal free estriol levels. Risk recalculation with the FMF-2012 software (Fetal Medicine Foundation) adjusted risk for trisomy 13 showed 1:55. Diagnostic amniocentesis performed at 15/16 weeks of gestation revealed trisomy 13 by FISH and later 45,XX,+13,der(13;14)(q10;q10),der(14;15)(q10;q10) using standart karyotype analysis.
Conclusions. Currently the estimation of recurrence risk for future pregnancies is impossible because parents refused to investigate their karyotypes. There could be several possible explanations for double Robertsonian translocation.

P01.104-M
Sex chromosome classification by cell free DNA analysis of maternal plasma in a general obstetrical population

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Objective: To examine the performance of noninvasive prenatal testing (NIPT) by sequencing maternal plasma cell-free DNA for fetal sex chromosome classification in a general obstetrical population.**Study Design:** Blood samples were prospectively collected from pregnant women at 21 US sites in the Comparison of Aneuploidy Risk Evaluation (CARE) study (clinicaltrials.gov NCT01663350). Patients undergoing serum screening for fetal aneuploidy were followed to birth. Sex chromosome status was classified blindly by NIPT and compared to clinical outcome based on newborn physical examination, or karyotype if performed. Sensitivity, specificity and exact 95% confidence intervals based on the Clopper-Pearson method were calculated. **Results:** Sex chromosome classification by NIPT and outcomes were available for 1,948 subjects. 1,935 (99.3%) were classified as XX or XY by NIPT. Sensitivity and specificity for predicting female were 97.7% (CI:96.6-98.6) and 99.2% (CI:98.4-99.6), respectively, and 99.2% (CI:98.4-99.6) and 97.7% (CI:96.6-98.6) for predicting male. Two fetuses classified XY by NIPT had ambiguous genitalia at birth; one showed mosaic karyotype 45,X/46,XY. NIPT classified 13 (0.67%) samples with sex chromosome aneuploidy (SCA). Nine with Monosomy X (MX) - all bearing normal-appearing female infants; one had prenatal karyotype 46,XX. Three cases classified XXX by NIPT appeared female at birth and one classified XXY appeared male.
Conclusion: Results demonstrate that NIPT has excellent performance in the general obstetrical population for sex chromosome classification. Mosaicism, maternal contribution or co-twin demise may explain discordance. NIPT is useful in cases of ambiguous genitalia, pregnancies at-risk for sex-linked disorder, or when SCA is not readily detectable by ultrasound/newborn examination.

P01.105-S
The XY female: prenatal discrepancy between phenotype and genotype in bichorial diamniotic twin, what's the differential diagnosis?

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Sexual differentiation depends upon a series of complex events. A 32aa, healthy pregnant woman underwent a routine prenatal ultrasonographic examination at 12 weeks' gestation that showed a bichorial diamniotic twin pregnancy with female sex fetuses. Upon personal desire of the mother, amniocentesis was performed at 15+1 weeks of gestation. Ultrasonographic examination at the time of the procedure confirmed, in both fetuses, normal fetal anatomy with female external genitalia. However, amniotic fluid karyotype analysis was discordant with the female ultrasonographic sex, revealing a 46,XY male karyotype in both of them. Fetal re-exanimation was performed with 3D and 4D ultrasound for fetal sex, and a female genitalia was confirmed again. One hypothesis was the search for the presence of mutation on SRY gene on chromosome Yp11.3, causing 46,XY complete gonadal dysgenesis or XY sex reversal. the analysis has revealed no mutation in the analyzed gene. The other hypothesis was the analysis of androgen receptor (AR) gene. The molecular analysis showed a single nucleotide deletion in codon 766, that resulted in a frame-shift mutation in the steroid binding domain of the androgen receptor P766fsX. As expected, the carrier mother had both normal and mutant AR genes. After genetic counseling the couple decided upon voluntary termination of the pregnancy at the 20th week of gestation. The autoptic examination of both fetuses confirmed the external female genitalia and revealed the presence of sever hypoplasic uterus and abdominal testes that confirmed by histopathological examination.

P01.106-M
Prenatal detection of a case with Simpson- Golabi-Behmel syndrome as a consequence of GPS3 and GPS4 gene duplications

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We describe the case of Simpson-Golabi-Behmel syndrome (SGBS) discovered prenataly. Fetal scan in 23rd week of pregnancy identified male fetus with macrosomia, macrocephalia, dilatation of 3rd and 4th ventricle, hyperechogenic gut, agenesis of corpus callosum, cistical dilatated interhemispheric fissura, macrosomic kideny and liver, and kidney polyhydramnion.
Amniocentesis was performed. Molecular kariotyping using ISCA 4x180K arrays reveled two interstitial microduplications on Xq26.2 in size of 574 kb and 115kb. A larger microduplications encompassed four OMIM genes, including whole GPC4 gene. A smaller microduplications was located within the GPC3 gene and embraced exons 6 and 7 of the longest transcript of this gene. CNVs were inherited from the mother.
Persons with this SGBS are frequently affected by embrogenic tumors of kidneys. Also the mother had Wilms tumor in her childhood. The pregnancy was terminated because of ruptured amniotic membrane and amniotic leakage. An autopsy of the infant confirmed organomegaly seen on ultrasound. In addition, a ventricular septal defect, a complete agenesis of the corpus callosum, cerebellar hypoplasia were observed. Distinct facial features were also present, including hypertelorism, short nose with broad nasal bridge, macrostomia and macroglossia, nail hypoplasia, and an extra rib.
GPC3 and GPC4 are the two genes in which mutations are known to cause SGBS. There are only few reports on duplications of GPC3 gene and one report of duplication of CPG4 gene causing SGBS in the literature. Mostly deletions and mutations of GPC3 gene are described. In our presentation possible role of two genes involved in SGBS is discussed.

P01.107-S
The correlation between transcript expression levels of nuclear encoded and mitochondrial encoded genes in single human oocytes during oocyte maturation

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Introduction: Impairment of human oocyte maturation during oocyte maturation is a cause of infertility in infertile women. Therefore, Oocyte maturation is important in successful reproductive outcome of assisted reproduction technologies (ART). Mitochondria,which are the most organelle in the oocytes,have in important role during oocyte maturation. Little is known about mitochondrial genomes during oocyte maturation .This aim was to identify the correlation between transcript expression levels between mitochondrial and nuclear encoded genes included the cytochrome C oxidase1 (MT-CO1) gene and the nuclear respiratory factor 1(NRF1) as well as the mitochondrial transcription factor A (TFAM) genes , which using by single-cell real-time PCR during human oocyte maturation. 27 consenting women aged 21-35 years, with male factors were selected for ovarian stimulation and ICSI procedures.
Results:at the germinal vesicle (GV) stage oocytes ,no significance correlation between the relative expression levels ($P>0.05$),whereas there was significant correlation between the relative expression levels of nuclear (TFAM,NRF1) and mitochondrial (MT-CO1) encoded genes at the stages of metaphase I (MI)and metaphase II (MII)stages of oocytes ($P<0.05$).
Conclusion:human oocyte maturation is associated with the increased correlation between transcript expression levels of nuclear (TFAM , NRF1) and mitochondrial (MT-CO1)encoded genes. Thus, any defect correlation between transcript expression levels of nuclear mitochonderial genes leads to impaired developmental oocyte competence.

P01.108-M
Prenatal molecular diagnosis of skeletal dysplasias - a single center experience

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Skeletal dysplasias are a large, heterogeneous group of conditions involving the formation and growth of bone. Some skeletal dysplasias are associated with additional abnormalities in other organ systems.
Prenatal diagnosis relies primarily on fetal ultrasound findings, but molecular analysis is used to confirm the presumptive diagnosis and to determine the recurrence risk.

The diagnosis of a substantial number of the most frequent skeletal dysplasias can be confirmed in a short period of time by molecular genetic analysis of the involved genes (e.g. thanatophoric dysplasia, diastrophic dysplasia, campomelic dysplasia, Ellis-van Creveld syndrome or hypophosphatasia).
We present a retrospective analysis of 300 cases diagnosed prenatally by an expert team of experienced sonographers and human geneticists. The study was carried out at a single tertiary center during the last 20 years.
We demonstrate clinical findings and molecular genetic data and construct work-ups for the diagnosis of „difficult" cases (e.g. short rib-polydactyly syndromes, Filamin B associated skeletal dysplasias). Molecular genetic testing was conducted in 186 cases. We were able to establish a final diagnosis in 118 cases - which is equivalent to a detection rate of 65%. Some cases are exampflified with clinical, radiological, pathological and molecular genetic data.
We want to point out the importance of molecular genetic diagnosis for confirming the clinical diagnosis of skeletal dysplasias and providing exact information for genetic counselling.

P01.109-S
Susceptibility loci for neurodevelopmental disorders - genetic counseling and pregnancy management

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Objectives: SNP genomic array may detect susceptibility loci for neurodevelopmental disorders (SL), with possibly an increased but unquantified phenotypical risk. This study evaluates the effect of releasing the SL to pregnancy management. Psychological aspects will be reported separately.
Method: Every patient received *pre-test counseling* with "all pathogenic results will be communicated".
The *post-test genetic counseling* concentrated on the phenotype of the particular SL, its incidence in the normal and affected postnatal population and the difference between postnatal and prenatal ascertainment. Targeted parental array testing was offered. Extensive ultrasound (US) examination was offered when the SL (postnatally ascertained) was associated with physical abnormalities. Psychological help was available in all cases if needed.
Results: In 36 cases out of 2108 ongoing pregnancies a SL was found.
1. Two couples with an increased risk for aneuploidy and no US abnormalities opted for TOP. Both couples already had "a bad feeling" about the pregnancy before testing. They received psychological help. Pathological examination (N=1) revealed no structural abnormalities.
2. For parents with fetal US abnormalities the SL was usually considered as less important. The US abnormalities were the reason for TOP.
3.The inherited nature of SL seemed reassuring.
Conclusions: In 5,5 % (2/36) of the SL cases the pregnancy was terminated due to the presence of the SL. Further follow up of the families and their children is needed to evaluate the significance of prenatal diagnosed SL, to offer early intervention when neurodevelopmental disorders emerge and to evaluate the psychological impact of prenatally discovered SL.

P01.110-M
Origin of multilocus methylation defects of imprinted genes in first-trimester spontaneous abortions

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Genomic imprinting is one of the most important epigenetic mechanisms of fetal growth regulation. Our studies demonstrated that multilocus methylation defects (MLMD) at imprinted genes may be responsible for pathology of early embryonic development. The aim of the present research was analysis of origin of MLMD in first-trimester spontaneous abortions (SA). Chorionic villus samples (CVS) and extraembryonic mesoderm (EM) were collected from 217 SA from women who underwent abortion procedures. Induced abortion (IA) with normal karyotype (n = 60) were investigated as a control group. The DNA methylation patterns of 7 imprinted genes were analyzed in both tissues using MS-PCR, including analyses of 51 imprinted genes from 15 SA and 4 IA by GoldenGate Methylation Cancer Panel I microarray. Comparative analysis of epimutations distribution between studied tissues allows the identification its somatic or germinal origin. Presence of epimutations in both tissues indicates about mistake reprogramming in the primordial germ cells during gametogenesis. Whereas tissue-specific compartmentalisation of epimutations allows suggesting independent sporadic epigenetic events in various embryonic germ layers after its divergence. No epimutations were found in the 60 IA samples. Somatic MLMD in SA occurred more frequently than germinal epimutations (129 epimutations per 1808 alleles vs. 32/904; p<0.001). Multiple somatic epimutations were found more frequently in the EM (52/904; 31/904) than in the CVS (32/904; 14/904; p<0.05). Multiple hypomethylation in both tissues occurred more frequently than hypermethylation (96/1808; 33/1808; p<0.001). Thus, abnormalities of imprinting maintenance in the EM cells derived from epiblast progenitors are associated with fetal loss in first-trimester.

P01.111-S
Gonadal mosaicism for structural autosomal rearrangements with non-centromeric breaks: data from 76 carriers suggests male-specific selection against abnormal cell lines and association of clinical manifestation with a high proportion of abnormal cells

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Mosaicism for chromosomal structural rearrangements (Rea) is rare. There is much to learn about the timing and mechanisms of Rea formation and maintenance, and clinical manifestation. The question as to whether the proportion of abnormal cells in cultured blood is significant remains open. A recent study showed a strong female preponderance among carriers of mosaicism for Reas with pericentromeric breaks indicating female-specific instability in early embryos (Kovaleva NV. AJMG,136A:401-13). Objectives: (i) Comparative analysis of the male/female ratio in carriers of gonadal mosaicism (GM) for balanced and unbalanced Reas, (ii) comparative analysis of the proportion of cells with unbalanced Rea in blood cultures of asymptomatic and affected carriers. Method: Review of mosaicism for normal line/structural Rea cases of known sex identified from the literature. Results: (1) Among carriers of GM for balanced Rea (all asymptomatic) there was a typical male predominance, 18M/15F, unlike the strong female predominance among carriers of GM for unbalanced Rea (both asymptomatic and affected), 9M/34F, p<0.001. (2) Only one of eight male carriers with poor reproductive history was reported to have sterility, the others had partners with spontaneous/habitual abortion. (3) Seven of eight (88%) affected carriers of unbalanced Rea displayed a high proportion (≥50%) of abnormal cells compared to 2/27 (8%) in asymptomatic carriers, p<0.01. Conclusions: A strong female prevalence among carriers of GM for unbalanced Rea suggests male-specific selection against abnormal cells rather than impairment of male gametogenesis. A high proportion of abnormal cells detected in cultured T-lymphocytes is associated with clinical manifestation of chromosomal imbalance.

P01.112-M
Parental subfertility is not associated with an increased risk of a *de novo* mutation or microdeletion in the offspring

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Background Children born after *in vitro* fertilisation (IVF) with or without intracytoplasmic sperm injection (ICSI) are at increased risk for congenital anomalies. Recent publications suggest that the underlying parental subfertility is mainly responsible for this risk increase, rather than the IVF/ICSI procedures, but it is unclear how. Our study aimed to identify whether offspring of subfertile couples are at increased risk for a *de novo* mutation or microdeletion.

Materials and methods Data were used from the birth defects registry Eurocat Northern Netherlands. We included malformed foetuses and children born between 1997-2010 (N=5709). Of those, 5249 were born to fertile couples and 460 to subfertile couples (83 after IVF, 95 after ICSI, 282 conceived naturally after a time to pregnancy >12 months). We analysed whether parental subfertility was associated with *de novo* mutations or microdeletions.

Results From the 5709 malformed cases, 156 (2.7%) had a monogenic condition resulting from a *de novo* mutation and 61 (1.1%) had a *de novo* chromosomal microdeletion. Parental subfertility was not associated with *de novo* mutations (OR 0.88, 95%CI 0.47-1.63) or microdeletions (OR 1.04, 95%CI 0.41-2.60). Subgroup analyses showed that IVF or ICSI alone were not associated with a *de novo* event either. Adjustment for paternal and maternal age did not change the results.

Conclusion Parental subfertility, IVF and ICSI are not associated with *de novo* mutations or microdeletions in offspring. These results suggest that the previously established increased risk of congenital anomalies after IVF/ICSI is not explained by an increase in *de novo* events.

P01.113-S
Prenatal detection of TAR syndrome

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Thrombocytopenia-absent radius (TAR) syndrome is a rare genetic disorder that is characterized by the absence of the radius bone in each forearm and a markedly reduced platelet count that results in life-threatening bleeding episodes (thrombocytopenia).
Rare proximal microdeletions of 1q21.1 are found in the majority of patients but are also found in unaffected parents. Recently it was shown that TAR syndrome is caused by the compound inheritance of a low-frequency non-coding SNP and a rare null allele in *RBM8A.*
We present chromosomal, molecular, fetal ultrasound and pathological findings in a case of TAR syndrome diagnosed prenatally. In the first pregnancy (2010), ultrasound examination at 22 weeks of gestation revealed bilateral absence of the radii, pregnancy was terminated. A 1q21.1 microdeletion including *HFE2*, *PEX11B* and *CD160* genes was found using MLPA. Both parents were analyzed in order to determine the parental origin of the deletion, which was subsequently identified in the healthy mother. The second pregnancy (2012) ended in miscarriage. The third pregnancy (2013) with the same ultrasound findings, was terminated. Then the Sanger sequencing was used to analyze the DNA sequence of the region spanning the 5′UTR and the first intron of the *RBM8A* gene in the fetuses (2010, 2013) and parents. Genotyping of the sequence demonstrated the presence of compound heterozygote for the 5′UTR (rs139428292) and intronic (rs201779890) SNPs in the father. Both fetuses had compound inheritance of an *RBM8A* SNP (rs201779890 G>C) and a deletion in the 1q21.1 region. Now is the family in the PGD process.

P01.114-M
Tetrasomy 13q31.3q34 due to two marker chromosomes in a fetus with increased nuchal translucency

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Small supernumerary marker chromosomes (sSMC) are a group of structurally rearranged chromosomes that cannot be identified by conventional cytogenetic techniques. Prenatally sSMC are present in about 0.075% of the tests and postnatally in 0.044% of live born children. An overall risk of an abnormal phenotype is about 30%.
We report on a 31-year-old woman (first pregnancy, after IVF) referred for genetic counselling because of increased foetal nuchal translucency (5,5mm at CRL 55,9mm) and increased risk of trisomy 21 (1:11) and trisomy 18 (1:207) in first-trimester combined prenatal screening. Ultrasound scanning at 17th week of gestation revealed no heart defects but an increased nuchal fold, left-sided diaphragmatic hernia and hydronephrosis.
Cytogenetic studies of amniocytes showed a karyotype as follows: 48,XX,+mar1,+mar2. Parents' karyotypes were normal. To determine the origin of the markers, array comparative genomic hybridization (aCGH) was used (Agilent SurePrint G3 Human CGH Microarray Kit 4 x 180K platform). Tetrasomy of the region 13q31.3 to 13q34 - arr[hg19] 13q31.3q34(92507936-115092648) was showed.
The pregnancy was terminated at 18th week of gestation. Post mortem examination revealed dysmorphic facial features, head and body disproportion (head diameter: 8cm, CRL 12,5cm), wide neck, ambiguous genitalia. No other malformations of the internal organs, except listed above, were found.
It is known that a risk of an abnormal phenotype associated with de novo sSMC derived from chromosome 13 (or 14, 21, 22) is about 7%. Molecular characteristic of sSMC is important for genetic counselling and genotype/phenotype correlation.

P01.115-S
Additive effect of thromobhilic gene polymorphisims is associated with recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) is a heterogeneous condition affecting up to 5% of women of reproductive age. Thrombophilias (acquired and inherited) have been postulated as one of the causes of RPL. Here we examined the prevalence of nine thrombophilic gene polymorphisims among women with history of recurrent miscarriages and fertile controls. The study included 70 women with history of at least two early pregnancy losses (before 20th gestation week) and 30 fertile controls with no miscarriages. We investigated mutations in genes responsible for cloating and fibrinolysis, including FV Leiden, FV H1299R, FII G20210A, MTHFR C677T and A1298C, F XIII V34L, PAI -1 4G/5G and EPCR H1 and H3 haplotypes using reverse PCR Vienna lab CVD StrippAssays. Our results showed no statistically significant difference in prevalence of specific gene mutations between two tested groups. However, prevalence of heterozygous mutations as well as total number of mutations (homozygous and heterozygous) was significantly higher in RPL group than in control group (40% vs 24% and 26% vs 15% respectevly, p <0,01). Each woman with RPL had at least three mutations in examined genes, while the average number of mutations in control group was 1.75. Several studies have proposed that risk for RPL may be associated with additive effect of several mutations in genes involved with coagulation process rather than to some specific mutations. Our findings confirm these assumptions. Pregnancy itself represents a protrhrombotic state, which combined with multiple thrombophilic gene polymorphisms may contribute to adverse pregnancy outcome.

P01.116-M
Trisomy 16 Detected In Chorionic Villous Samples; Evaluation of 10 New Cases

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Since trisomy 16 (T16) is a lethal chromosomal abnormality, it is very rarely diagnosed in ongoing pregnancies, even in the first trimester.
We present ten new T16 cases detected in chorionic villous samples. Referral indication was increased first trimester-screening test (FTST) risk in eight cases, with additional pathological ultrasound (US) findings in two, pathological US in one, and positive triplet test results in the other. Pathological US findings included intrauterine growth retardation, generalized edema, cystic hygroma, omphalocele, oligohydramnios, echogenic intracardiac focus, choroid plexus cyst, and hyperechogenic bowels.
Combined risk of FTST in eight cases ranged between 1:2 to 1:62. PAPP-A levels were decreased in six cases (range 0,05-0,29 MoM). $_{\beta}$hCG levels were over 2,10 MoM in five cases.
T16 was detected by karyotyping using direct preparation and/or long term cell culture (LTCC) techniques in all cases, showing mosaic status in only one. Two cases (one unsuccessful DP, and one with normal DP results) were further investigated with I-FISH using centromeric chromosome 16 probe, and T16 mosaicism was demonstrated.
Eight cases were followed up by amniocentesis. Mosaic T16 was detected in only one of six by karyotyping, and I-FISH indicated mosaicism in four cases and normal results in two.
Our results are compatible with the literature, showing that the most sensitive screening test for T16 is the FTST, with the most reliable parameter being PAPP-A. I-FISH studies are helpful in the clarification of low-level mosaicism in AF cells, when T16 was found in chorionic villous samples.

P01.117-S
Non-invasive prenatal testing (NIPT): Laboratory experiences of PrenaTest®

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Non-invasive prenatal testing (NIPT) is an emerging new option in prenatal care. The commercially available PrenaTest® has been introduced in Europe in August 2012 and exhibits sensitivities and specificities over 99%. The laboratory experiences from routine application of NIPT are reported and test accuracy, limitations as well as patient profiles are discussed.
Within the past eighteen months about 8,000 successful PrenaTest® analyses have been reported, with 98.0% negative results. 1.7% were positive for trisomy 21, 0.4% positive for trisomy 18 and 0.1% positive for trisomy 13. According to ad hoc feedback from ordering specialists there were one false-negative result for trisomy 18 and 13 false-positive results (one T13, ten T18 and two T21) up to now. Further analysis revealed that one discordant positive T21 case was caused by a fetus papyraceus as determined by investigation of the placenta after birth, the other discordant T21 case was reported back by the responsible doctor as a known case of a vanishing twin. For one discordant positive T18 case, the fetus had a euploid karyotype after amniocentesis, but placental material exhibited 80% cells with trisomy 18 in FISH analysis.
It is intriguing that the majority of discordant results of NIPT and invasive diagnosis seem in fact to be consequences of fetal vs. extra-fetal cytogenetic discrepancies or due to undiscovered vanished twins. These findings suggest that biological reasons rather than methodical failures play the major role.

This confirms a strong collaboration between geneticists and gynecologists specialized in ultrasonography and further analysis of discordant results.

P01.118-M
Performance of in-house non-invasive aneuploidy test using benchtop next generation sequencing system

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Since the discovery of cell-free fetal DNA in 1997 by Dennis YM Lo, the main goal was formulated - a reliable method for non-invasive fetal aneuploidy detection. The arrival of next generation sequencing technologies finally gave scientists a proper tool to reach this goal. Today, so called large next generation sequencing instruments are used for the so called non-invasive prenatal aneuploidy testing. Few years ago, so called benchtop next generation sequencers were introduced, which allowed researchers worldwide to start adopting wide range of novel methods benefiting from next generation sequencing. However, they were generally considered not to be sufficiently parallel for non-invasive aneuploidy test.
In our study, we used one of benchtop next generation sequencers, the Mi-Seq by Illumina, to test its ability to detect aneuploidy. Plasma DNA from pregnant was isolated, fragment libraries were prepared and sequenced in multiplex setup. Data was analysed using in-house pipeline. A set of 20 samples (average read count 2 870 322 with SD 603 708) high risk group pregnancies with confirmed euploid fetus was used for training of our analysis pipeline. Analysis of testing set including 5 samples with T21 fetus resulted in 100% specificity and 100% sensitivity. Based on our results we believe that performance of benchtop next generation sequencers is sufficient for non-invasive prenatal aneuploidy testing. In addition, the inherently higher flexibility of benchtop systems could be of benefit for short turnaround and low sample throughput demands. This research was supported by ERDF grant with ITMS 26240220067.

P01.119-S
12 Mbp chromosomal gain on 7p detected prenatally without major dysmorphic features

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We describe a prenatally detected 12 Mbp chromosomal gain on 7p. Amniocentesis was performed due to risk for a chromosomal abnormality after first trimester screening.
Cytogenetic analysis revealed a duplication on chromosome 7p in the foetus. A SNP array was carried out showing a 12 Mbp gain - ISCN: arr[hg19]7p21.3p15.3(13,146,026-25,348,884)x3.
A literature review revealed one report from Miller et al. (Am J Med Genet 1979;4(4):323-32), describing a man with a duplication of the segment 7p21 to 7p25 with severe mental deficiency but normal growth.
In order to exclude a balanced rearrangement of the parents, conventional chromosome analysis was performed. The mother showed the same duplication as the foetus. A SNP array confirmed the cytogenetic result.
The mother presented without major dysmorphic signs and showed no intellectual disability. She had finished basic education.
Pregnancy was continued due to missing ultrasound abnormalities and the inheritance of the maternal duplication.
After caesarean section at 34+6th gestation week the premature infant presented with a weight of 2340g (P38), length 46.0cm, OFC 31.7cm and APGAR 9/10/10.
The slightly cyanosed girl showed mild facial dysmorphisms as deep set ears, mild retrognathia and mild hyperplasia of the clitoris. No further clinical abnormalities were reported. 19 days after birth the girl left the hospital in a good general state of health (weight 2486g). Two subsequent controls revealed no major clinical abnormalities.
The case report shows the importance of testing parents after a pathological prenatal result and that a 12 Mbp 7p duplication not necessarily causes a major malformation.

P01.120-M
Partial Trisomy 6q detected in Prenatal Diagnosis

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Partial distal trisomy 6q is a rare event and is characterized by a distinct phenotype which includes microcephaly, acrocephaly, joint contractures and profound psychomotor retardation. The authors present a case of a 30-year-old pregnant woman referred to prenatal diagnosis due to ultrasound anomalies. It was the first pregnancy of a non-consanguineous couple with no familial or personal story of anomalies. Parents karyotype was performed. Cytogenetic analysis revealed a chromosome 15 with an increase p arm similar to a variation in length of heterochromatic stalks on the short arm. Both parents presented a chromosome 15 with satellites but different from the one detect at the amniocytes. Subtelomeric FISH analysis revealed a trisomy of 6q27-qter present at p arm of chromosome 15 - it was a *de novo* rearrangement. The parents decided to terminate the pregnancy and foetal autopsy was required. Several polymorphic variants were described in human chromosome 15 including increased amounts of short arm hetrochromatin (ph+), interpreted as a normal polymorphism. In the majority of cases partial trisomy 6q results from a balanced chromosomal rearrangement in one of the parents, usually of maternal origin. There have also been rare cases in which partial trisomy 6q has appeared from spontaneous (*de novo*) errors very early in embryonic development. The authors compared the cytogenetic and the foetal autopsy findings with those described in the literature. Every new case of a rare chromosomal alteration should be reported in order to establish a genotype/ phenotype correlation, improving risk evaluation and genetic counseling.

P01.121-S
Assessment of chromosomal changes in trophectoderm cells in relation to maternal age

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Unbalanced chromosomal changes in embryos are one of the main causes of low human fecundity, with their incidence presumably increasing with maternal age. In the aim to assess the frequency of whole chromosomal and segmental changes in relation to maternal age, we evaluated 224 trophectoderm samples by aCGH (for details see table below). Our results showed a statistically significant increase in embryos with abnormal chromosomal results with advancing maternal age. Overall, 539 chromosomal changes involving similarly all chromosomes, with slightly higher frequency on chromosomes 15, 16, 21, 22, were described. The frequencies of losses and gains were not significantly different. The rising complexity of changes with higher maternal age was caused predominantly by increasing numbers of whole chromosomal abnormalities whereas frequency of segmental changes was shown to be independent on maternal age.

Group (according to maternal age)	No of abnormal embryos / No of analyzed embryos	No of segmental changes / No of chrom. changes	No of whole chromosomal changes / No of chrom. changes	Mean No of chromosomal changes per abnormal embryo	Mean No of segmental changes per abnormal embryo	Mean No of whole chrom. changes per abnormal embryo
(1) ≤ 32y	30/60 (50%)	45/83 (54%)	38/83 (46%)	2.8	1.5	1.3
(2) > 32y < 40y	63/94* (67%)	80/221 (36%)	141/221^{+} (64%)	3.5	1.3	2.2
(3) ≥ 40y	55/70** (79%)	49/235 (21%)	186/235^{++} (79%)	4.3	0.9	3.4

* (2) vs (1) P 0,04
$^{+}$ (2) vs (1) P 0,006
** (3) vs (1) P 0,00008
$^{++}$ (3) vs (1) P 0,00000005

P01.122-M
Our contribution to rescue line hypothesis for a case of Turner syndrome.

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Turner syndrome (45,X) is the only viable monosomy in humans, about 1% of recognized conceptus with this karyotype survive to livebirth. Ultrasound examination in 12th week of gestation disclosed hygroma coli cysticum with initial signs of hydrops. The NT was 6,2 mm. The QF-PCR analyses from na-

tive CVS was 46,XY. The long-term CVS cultivation revealed 45,X as well as QF-PCR and FISH. The same results were found in post mortal examination of muscule cells cultures. These findings suggest that in trophoectoderm initiated mosaicism with the 46, XY clone and loss of Y chromosome must occurred after fertilization. XY clone persisted in the embryo as a minor cell population and its prevalence increased in further chorion formation. It explains that in QF-PCR analysis of biopsied samples 46, XY represented at least 80% cells, while the second line 45,X was under detectable limit for this method (less than 20%). Our findings confirm recently published data, describing similar situation for 45,X genotype derived from 46,XX. This "rescue line" implies an origin of this disorder in mitotic loss. We also proved that syncytiotrophoblast is a strong candidate for the location of the rescue cell line. Most probably this may not be detected in extensive search for cryptic mosaicism. Our casuistic point out the importance of complex molecular genetics testing in native and long-term cultured fetal cells to provide the best reliable results allowing to make final conclusions properly. Supported by grants IGA NT13770-4/2012, project for conceptual development of reasearch organization 00064203 and OPPK CZ.2.16/3.1.00/24022.

P01.123-S
SNP-based non-invasive prenatal testing identifies vanishing-twin pregnancies

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Objective: To determine the ability of single nucleotide polymorphism (SNP)-based non-invasive prenatal testing (NIPT) to identify unrecognized vanishing twin pregnancies that could otherwise confound NIPT results.
Method: 30,795 consecutive reported commercial samples that were received for NIPT for fetal whole-chromosomal aneuploidies; known multiple gestations were excluded. Cell-free DNA was isolated from maternal blood samples, amplified, and sequenced. Sequencing results were analyzed using the NATUS algorithm to determine the fetal chromosomal copy number and presence of additional fetal haplotypes. Clinical follow-up for cases with additional fetal haplotypes is reported here.
Results: 130 (0.42%) cases with additional fetal haplotypes were identified, indicative of fetal triploidy or molar, vanishing twin, or undetected twin pregnancy. Clinical confirmation was available for 76 cases (58.5%); including 31 (40.8%) vanishing twin, 37 (48.7%) viable twin, 2 (2.6%) molar pregnancies, 3 (3.9%) triploid pregnancies, and 3 (3.9%) non-triploid pregnancies. Of the 5 vanishing twin losses with a known date of demise, 100% of losses occurred in the first trimester, and up to 8 weeks elapsed between the loss and detection by NIPT.
Conclusions: This SNP-based approach successfully identified previously unrecognized vanishing twin, viable twin, molar, and triploid pregnancies. Vanished twins have been identified in the literature as a significant cause of false positive results in NIPT. As vanished twins are more likely to be aneuploid, and residual cfDNA could bias NIPT results, the unique ability of this method to identify additional fetal haplotypes has the potential to decrease the false positive rate.

P01.124-M
Case-control analysis of VKORC1 gene polymorphisms (G1639A, C1173T) in women of reproductive losses from Republic of Moldova

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VKORC1 reactivates the reduced form of vitamin K- essential cofactor in the γ-carboxylation of blood coagulation factors. Decreased enzyme activity leads to blood clotting disorders that can cause reproductive problems. Aim of our research was to determine the prevalence of VKORC1 variant alleles G1639A (rs9926231) and C1173T (rs9934438) and correlate genotypes with recurrent pregnancy loss (RPL)
Materials and methods: Were tested 91 women with two or more (case) RPL, 69 women with two normal births (control) and 67 DNA samples of healthy Moldavian population. There was used PCR-RFLP assay to identify the VKORC1 rs 9926231 and rs9934438 with MspI and HinfI enzymes respectively. The distribution of genotypes was tested for deviation from Hardy-Weinberg equilibrium (HWE).
The results: All evaluated genotype frequencies were conformed to HWE expectation for VKORC1 polymorphisms. There was identify no significant difference in frequency in case and control groups for heterozygous state of rs9926231 (46.2%vs54.4%).The evidential differences of VKORC1 T1173T genotype frequency in case and control groups (19.8%vs7.4%). There was identified statistically significant that patients with T1173T genotype have increased risk of complications during pregnancy (OR 3.26; 95% CI 1.07, 9.91;p=0.032).
Comprising the allele frequencies of VKORC1 gene rs9926231 across different populations revealed that its are relatively close in Moldovan populations to those reported for Caucasians and Turkish. The allele frequencies of VKORC1 gene rs9934438 are statistically significant different from data available on NCBI-site, NCP, MASSource.
Conclusion: VKORC1gene rs9934438 is statistical associated with recurrent pregnancy loss and increasing the risk of complications during pregnancy

P01.126-M
The application of post-light semiconductor-based next-generation sequencing in clinical cases of preimplantation aneuploidy screening (PGS) with fresh embryo transfer

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Screening of all chromosomes is now a gold standard in PGD. Although NGS techniques are today best method of choice they require more than 24 hours to perform, consequently blastocyst vitrification is needed. 8 couples with the average maternal age of 34,4 was referred to PGS procedure from 08/2013. All together 28 blastomeres were biopsied. The short duration of the procedure allowed fresh embryo transfer without need of blastocyst vitrification. 7 out of 8 cases resulted in pregnancy in first cycle giving pregnancies rate of 87,5%. 3,5 blastomers on average per cycle were biopsied, resulted in 1,25 blastomers on average per cycle with no aneuploidy detected. 35,7% of embryos were euploid. We not only used cutting-edge technology in the field of PGD but we went further and designed and preformed in clinical IVF-PGD procedure innovative protocol adjusted to single blastomere biopsy and fresh transfer. The additional benefit is the cost 3 times lower in comparison to aCGH. Developing PLS-NGS technology require use of the latest chemistry and software updates to improve analysis quality. The highest standards and stringency in quality system of results is required to avoid diagnosis failure. New technology of PLS-NGS possess strong research potential allowing for generation of large amount of data in scale of hours. Our innovative single-plot short-time protocol require only single blastomere biopsy and is adjusted to fresh embryo transfer. We put efforts in increase of reproduction success rate by increase implantation and decrease miscarriages rates and this aspect need to be followed up.

P01.127-S
Analysis of the AZF region of Y chromosome in Slovak men with azoospermia

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Y chromosome microdeletions are the most common genetic cause of male infertility and screening for these microdeletions in azoospermic men is now standard practice. Analysis of the Y chromosome in men with azoospermia or severe oligozoospermia has resulted in the identification of three regions in the euchromatic part of the long arm of the human Y chromosome (Yq11) that are frequently deleted in men with otherwise unexplained spermatogenic failure. PCR analysis of microdeletions in the AZFa, AZFb and AZFc regions of the human Y chromosome is an important screening tool.
The aim of this study was to analyse type of microdeletions in men with fertility disorders in Slovakia. We evaluated 123 patients with azoospermia and with normal karyotype. All patient samples were analyzed cytogenetically. For PCR amplification of sequence-tagged sites (STS) of the AZFa, AZFb and AZFc regions of the Y chromosome was used Devyser AZF set. For all markers in one multiplex PCR reaction fluorescently labeled primers were used. For automated visualization and identification of the STS markers we used genetic analyzer 3500xL (Applied Biosystems). We reported 9 cases of deletions in the *AZF* region (7,32%). We have recorded particular types of deletions in each region *AZFa,b,c* but also a complete deletion of the whole *AZF* region. The most frequent was microdeletion(s) in the AZFc region. In Slovak azoospermic patients the percentage of microdeletions in the AZF region is low, but their detection is important for subsequent therapeutic procedures. This work was supported by grants APVV-0716-10 and ITMS 26220120041.

P01.128-M
Indisputable double paternity in twins caused by superfecondation

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Introduction: Paternity tests enable to establish the biological relationship between a child and his alleged father. Today, this test is based on DNA fingerprinting using mainly short tandem repeats (STRs) markers. Indeed, each

individual receives half of its genetic heritage from his biological mother and the other half from his biological father. Rare cases of twin pregnancy were induced by fertilization from two different parents. In this case, we speak about a "superfecondation". This situation is exceptionally confirmed.
Here, we report a case of genetically confirmed superfecondation in the context of paternity test by DNA fingerprinting.
Observation: In the context of paternity, we performed a genetic study of 4 members: a mother, her 2 twin infants, and a supposed father. This study involved the analysis of 15 STRs markers by „PowerPlex 16 System" kit. Results were analyzed by Genotyper® v 3.7.
Results: Genetic analyzes were performed under the same technical conditions and showed that one of the twins share the same alleles with the alleged father, while the other infant has different alleles in 11 of the 15 STRs studied. Therefore, despite that these two children are twins, their biological fathers are different.
Discussion and conclusion: The superfecondation is a rare and special obstetric situation. It is secondary to the fertilization of two eggs from the mother by two sperm each from a different father.
In this study, we have confirmed this situation by molecular genetics tools. However, their clinical and biological implications remain unknown.

P02.01-S
IL-8 is associated with age-related macular degeneration in Italian samples

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Age-related macular degeneration (AMD) is a macular degenerative disease, representing one of the main socio-economical health issues for the elderly population worldwide. The increasing prevalence of AMD is related to progressive aging of the population and affects more than one million Italian people. AMD presents a multifactorial etiology with several risk factors (age, cigarette smoking, diet).
Genes in the complement pathway (CFH) and a chromosome 10 region (ARMS2) showed to be the most involved loci in the disease. Several studies confirmed the crucial role of inflammation and angiogenesis in AMD pathogenesis and progression. Given these data a screening of IL8 gene has been proposed (4q12-q13) to evaluate the association of AMD with rs2227306 (C/T), that is an intronic SNP in the IL8 gene. The results demonstrated a strong association of T allele (p = 4.15*10-5, OR = 1.39, 95% CI = 1.19-1.62) in a cohort composed of 721 cases and 660 controls, suggesting the sequencing of the entire IL8 gene. Sequencing analysis revealed two haplotypes associated with AMD development (A-T-T-T, p = 2.08*10-9,OR = 1.68, 95% CI = 1.43-1.97 and T-C-C-A, p = 7.07*10-11, OR = 0.60, 95% CI = 0.51-0.70). It is notably that in human coronary atherosclerosis, IL-8 is an important mediator of angiogenesis and may contribute to plaque formation via its angiogenic properties. In this context IL-8 could represent a candidate gene to link the development and progression of AMD, the smoke habits and the occurrence of cardiovascular events in these patients.

P02.02-M
Molecular investigations in patients with oculocutaneous albinism.

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Oculocutaneous albinism is characterized by both ocular and skin and hair features. In addition to *TYR, OCA2, TYRP1, SLC45A2*, a locus in 4q25 (OCA5) and two new genes, *SLC24A5* (OCA6) and *C10ORF11* (OCA7) were recently discovered. Ocular (OA1) and syndromic albinism are also known. When studying 400 patients, we found 36 % OCA1, 25% OCA2, 2 % OCA3, 11% OCA4, 1.25% OCA6, 0% OCA7, 6% OA1 et 1% *HPS1*. Deletions represent 5.6% of the anomalies. In two patients we found a complex rearrangement of *OCA2* that comprised the deletion of exons 2 to 19, followed by the reinsertion, after reshuffling, of most of the deleted segment in intron 1 of the gene.
17.5% of patients remain without molecular diagnosis. A first hypothesis is that mutations are located in the introns or regulatory regions of the genes. We undertook the sequencing of the entirety of the OCA1-4 genes to search for alterations not only affecting the exons. A second hypothesis is that new OCA genes remain to be discovered. We have sequenced the exome of 11 patients.
In our diagnostic laboratory we have used NGS to analyze the 6 OCA, OA1 and HPS1 genes. This was efficient in terms of both finding mutations and rapidity. We are designing a larger panel including all syndromic albinism genes.
The extensive analysis of all albinism genes will allow us to better characterize gene interactions in this disease that may not be purely monogenic.

P02.03-S
Two novel (p.E930X and p.C2278X) mutations in ALMS1 leading to Alström Syndrome

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Alström syndrome (AS, OMIM#203800) is an extremely rare, autosomal recessive disorder with a significantly shorter life expectancy. It is mainly characterized by sensorineural hearing loss, early onset blindness due to retinal dystrophy, and obesity. Other reported features include recurrent pulmonary infections, progressive renal and hepatic dysfunction, cardiomyopathy, short stature, and endocrinological features. AS is caused by mutations in the ALMS1 gene, which encodes a protein of unknown function that localizes to the basal bodies of cilia, playing a role in intracellular trafficking. Here we report two AS cases born to unrelated healthy parents. The diagnosis of AS was performed accordingly to the criteria previously defined by Marshall et al. (2007). We ascertained two families with seemingly AS. Molecular analysis was performed by direct sequencing of the known hot spots in the ALMS1 gene (exons 8, 10 and 16) according to the mutational load described in AS. We identified a homozygous mutation (c.2788G>T, p.E930X) in patient 1 and a homozygous mutation (c.6834C>A, p.C2278X) in patient 2 that co-segregate with the disease phenotype in family 2. Both mutations cause a truncated protein and had not been previously described in literature. We show that direct sequencing of exons 8, 10 and 16 of the ALMS1 gene could represent a useful tool for molecular diagnosis of AS, whilst the mutations described here may contribute to extend the mutational spectrum in AS.

P02.04-M
Exome sequencing in 32 patients with anophthalmia/microphthalmia and other developmental eye defects

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Anophthalmia/microphthalmia (A/M) is a genetically heterogeneous birth defect for which the etiology remains unknown in more than 50% patients. A/M can occur without other structural eye defects (simplex A/M), or can be present with other ocular findings (complex A/M), such as anterior segment dysgenesis (ASD). We used exome sequencing (ACE ExomeTM, Personalis, Inc., 18 exomes; UCSF Genomics Core, 20 exomes; 6 were run twice) to sequence DNA from 32 children with A/M or other developmental eye defects for pathogenic mutations. In 19 patients with simplex A/M, we identified mutations in STRA6 (1 patient), RARB (1 patient; p.Arg387Cys), GDF6 (1 patient; p.Ala249Glu) and OTX2 (1 patient; p.Gln91His) and in 9 patients with complex A/M, we found mutations in STRA6 in 2 patients. Putative causative variants were identified in 3 out of 4 patients with other developmental eye defects - a homozygous mutation in GCNT2, p.Tyr347Cys, in a female with cataracts and microtia and a de novo mutation in COL4A1, p.Gly773Arg, in a female with cataracts and cardiomyopathy. In a male with a chorioretinal defect, microcephaly, seizures and sensorineural deafness, we identified a maternally inherited splice site mutation and a paternally inherited missense mutation, p.Ala507Ser, in PNPT1 which is critical for mRNA import into mitochondria and has not been associated with eye defects. In one child with ASD, no mutation was identified. Our overall detection rate for A/M was 6/28 (21%), illustrating high genetic heterogeneity and the need for further gene discovery.

P02.05-S
Molecular study of Brazilian patients with auditory neuropathy

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Auditory neuropathy (AN) is characterized by an absent or abnormal auditory brainstem responses and preserved otoacoustic emissions and/or cochlear microphonics. To date, four loci associated with nonsyndromic AN were mapped: DFNB9 (*OTOF* gene) and DFNB59 (*PJVK* gene), responsible

for autosomal recessive pattern; AUNA1 (*DIAPH3* gene) for autosomal dominant; and AUNX1 for X-linked. Connexin 26 mutations were also reported in subjects with AN. The main goal of the study was to investigate genetic mutations in patients with clinical diagnosis of AN, to verify its importance and involvement in the etiology of AN in Brazilian patients. Clinical information and genetic evaluation of 39 patients were analyzed. We investigated the most common causes of genetic hearing loss, including pathogenic variants in the *GJB2* gene, deletions in the *GJB6* gene and m.1555A>G mutation in the *MTRNR1* gene. Additionally, direct sequencing is performing for mutation screening of *OTOF* gene. The common Spanish p.Q829X mutation in the otoferlin gene was not detected in our cohort. The c.35delG mutation in the *GJB2* gene was found in two patients in homozygous genotypes. However, it is not established if pathogenic variants in connexin 26 could be involved with AN or if the otoacoustic emissions that were recorded from these subjects only represent the residual activity of few outer hair cells that still alive. Further investigation is needed to clarify the link between *GJB2* mutations and AN. The study of AN genetic basis is extremely important to improve the diagnosis, management, therapy and genetic counseling of the affected subjects.

P02.06-M
Targeted high-throughput sequencing for mutation detection in Bardet-Biedl Italian patients

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Bardet-Biedl syndrome (BBS) is characterized by truncal obesity, polydactyly, hypogonadism, developmental delay, learning disabilities, progressive retinopathy, renal disease and susceptibility to diabetes mellitus. Although BBS is mainly transmitted in an autosomal recessive manner, few families exhibit a tri-allelic mode of inheritance. To date, 16 different BBS genes (BBS1-BBS16) are known and BBS1 and BBS10 show the highest mutation frequency in BBS patients.
Six unrelated patients evaluated by standard ophthalmologic examination and with a clinical diagnosis of BBS were analyzed by targeted re-sequencing of 130 retinopathies-related genes on HiScanSQ Illumina platform (mean coverage 500X). Bioinformatic analysis identified a mean of 1100 sequence variants per sample. Filtering pipeline (exonic function, frequency, prediction and inheritance model) leads to distil a mean of 10 candidate variants per sample. The candidate variants were independently validated by Sanger sequencing and segregation analysis was performed.
We identified two known causative deletions in BBS9 (c.1877_1880del) and BBS7 (c.712_715del) genes and a novel frameshift deletion in BBS10 (c.804_805del). Morever, a patient is compound heterozygote for two novel variants in BBS10 (c.1677_1678insA and c.1220T>C); one patient present a homozygous variant in BBS2 gene (c.209A>G) and one novel heterozygous variant (c.538C>T) in BBS3 gene. The last patient has two variants in BBS12 gene both in homozygous status [c.1044del (novel) and c.61T>C (described)].
NGS approach has enabled us to detect variants in BBS genes. Although for described variants and ins/del frameshif variants the genotype-phenotype correlation is clear, in order to clarify tri-allelic variants' role in BBS-onset, further association studies are required.

P02.07-S
Neuro-developmental genes may underlie human congenital general anosmia

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The neuronal and cell-biological mechanisms underlying vertebrate olfaction have been studied in considerable detail, but there is a gap in our understanding of the molecular genetic basis of olfactory monogenic disorders. Congenital general anosmia (CGA) affects <0.1% of the general population, and appears in isolated or syndromic forms. We have recruited a large cohort of isolated CGA, with 66 affected families of Jewish origin. We applied whole-exome sequencing to 43 selected individuals from 8 multiply affected families. We identified in three members of one family with a rare X-linked mutation in the TENM1 (teneurin 1) gene, a neuronal signal transducer that functions in synaptic-partner-matching between olfactory sensory neuronal axons and target projection neurons in drosophila. The mutation affects a highly conserved Proline residue in one of the YD domains of TENM1. In another family we identified candidate variants in the genes FGFR1 (heterozygous mutation) and SEMA3A (heterozygous mutation). Affected individuals in this family obligatorily carry both mutations, suggesting a di-genic model of inheritance. Both FGFR1 and SEMA3A are implicated in Kallmann's syndrome, where GnRH neuronal migration developmental abnormalities typically lead to syndromic anosmia, associated with sterility. Our patients are unusual in having isolated anosmia. All mutations are currently under further scrutiny. The results suggest that decyphering CGA pathogenic variants might shed light on the embryonic development of the olfactory neuronal system.

P02.08-M
Prevalence of GJB2 (CX26) gene mutations in Turkish patients with autosomal recessive nonsyndromic sensorineural hearing loss

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Hearing impairment is the most common sensory impairment in humans, affecting 2:1,000 births. At least 50%of the cases are inherited. Non-syndromic hereditary hearing loss accounts for the 70% of the hereditary hearing loss. It is a genetically heterogeneous disorder.
The single-nucleotide guanine deletion (35delG) of the GJB2 gene coding for connexin 26 was shown to be the main genetic cause of autosomal recessive deafness among Europeans. The purpose of our study was to evaluate the prevalence of GJB2 mutations among affected individuals from Turkey. To provide appropriate genetic testing and counseling to families, we searched fifty patients presenting with autosomal recessive non-syndromic hearing loss from Turkey for variations in GJB2 gene by direct sequencing. Mutations were detected in 9 out of 28 people from 20 different families (9/28, 32 %). Four different mutations were identified; three of them were previously identified (35delG, V27I, E114G). The remaining one alleles, K221N was novel variants which determined 'uncertain significance' in different database. However, it is reported disease causing variant by 'Mutation Taster Database'. The most common mutations were 35delG followed by V27I with an allele frequency of 21 and 7 %, respectively. There is no correlation between mutation types and clinical findings of patients.
In conclusion, we determined GJB2 gene mutations responsible for 32 % of autosomal recessive non-syndromic hearing loss in Turkish people. This is higher than earlier studies which reported that the overall frequency of GJB2 mutations in comparable families in Turkey is 18.9%.

P02.09-S
A novel GJB6 mutation causes autosomal dominant palmo-plantar hyperkeratosis with hearing loss

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We report a boy affected by bilateral sensorineural deafness and palmoplantar hyperkeratosis. The pedigree suggests an autosomal dominant pattern of inheritance with variable expressivity. Molecular analysis of GJB2 was negative and GJB6 deletions were also excluded. GJB6 encodes connexin 30, which is highly expressed in the inner ear and in keratinocytes. Therefore, we sequenced the entire coding region of this gene in the proband and identified the c.175 G>A mutation, causing a substitution of glycine 59 with an arginine residue. The same missense mutation had been previously reported in a single patient affected by post-lingual deafness and palmoplantar hyperkeratosis (Nemoto-Hasebe et al, 2009). The G59R mutation affects a highly conserved amino acid, it was absent in 100 healthy ethnically matched controls and segregates with the phenotype in our family. The analysis by molecular modelling revealed that the residue is located at the top of the channel formed by the hemiconnexons. All these findings strongly indicate a pathogenetic role for the novel variant. To explore the functional consequence of the missense mutation, we expressed the mutated version of GJB6 in HeLa cells lacking connexins. We showed that GJB6 mutated protein is localized to the plasma membrane only when co-expressed with the wild type form. This model was employed to test the electrophysiological properties of the connexons formed by both the mutated and the wild-type connexin 30.

P02.10-M

Hearing and ageing: a complex genomic strategy leading to new genes/variants identification in European and Central Asian populations

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The lack of knowledge on the molecular basis of Normal Hearing Function (NHF) and Age-related Hearing Loss (ARHL), prompted us to develop a combined multiphase strategy using approx. 3000 individuals from isolated populations of Europe, Caucasus and Central Asia. Starting from our past experience on GWAS meta-analysis combined with expression studies (Girotto et al. 2014), the following strategy was designed: PHASE1: GWAS meta-analysis for common tag-SNPs and common functional variants, PHASE2: replication in independent cohorts, PHASE3: SKAT gene-based test for rare functional variants, PHASE4: Whole Exome Sequencing (WES) in a selected subgroup of 250 ARHL cases/controls. During PHASE1 the following strongly significant associations were identified: ITFG2 (p=6.92E-11), PCDH20 (p=4.71E-10), SLC28A3 (p=2.39E-09). The last two belong to gene-families already known as being involved in hearing-loss and expressed in the cochlea. During PHASE2 four SNPs within the PCDH20 gene were replicated at nominal level (p<0.05) in the 1958 British Birth Cohort. Applying PHASE3, the SKAT gene-based test for rare functional variants (0.001<MAF<0.05) revealed highly suggestive associations (ranging from 7.97E-06 to 1.90E-05) for the following genes: PTPRCAP, FN1, EAF1, METRNL, PDP2, ARRDC1, HDGF, TMOD1.

Finally, thanks to PHASE4 (carried out in collaboration with CRG, Spain), WES data are now available to identify population disease-specific rare variants. In vitro and in vivo functional studies will be carried out in the future to confirm the role of the whole list of genes/variants identified through the above-described phases. Up-to-date results will be presented and discussed.

P02.11-S

Targeted exome sequencing method identified Myo15A gene mutations as a frequent cause of non syndromic autosomal recessive hearing loss in Iran

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Human MYO15A is located on chromosome 17p11.2 and encodes unconventional myosin XV, which is critical for the formation of stereocilia. Mutations of MYO15A are associated with profound autosomal recessive nonsyndromic hearing loss (ARNSHL) in humans, and with deafness and circling behavior in Shaker-2 mice. To date, more than 50 mutations have been reported in MYO15Amost of which have been found by linkage analysis in consanguineous families from countries like Pakistan, Turkey, Iranand India.

In present study, we used targeted genomic enrichment and massively parallel sequencing to identify the cause of ARNSHL in probands from 94 families segregating recessive deafness. In eight probands, we identified MYO15A mutations as the cause of ARNSHL. Of the eight mutations, 6 were present in the homozygous state, 2 in compound heterozygous state and of them 7 were novel. This study implicates MYO15A as an extremely important cause of ARNSHL in Iran. With a mutation frequency of 8.5% in our cohort,MYO15A mutations are the most frequent cause of ARNSHL after GJB2 mutations.

P02.12-M

Whole exome sequencing identifies new causative mutations in Tunisian families with non-syndromic deafness

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Identification of the causative mutations in patients affected by autosomal recessive non syndromic deafness (DFNB forms), is demanding due to genetic heterogeneity. After the exclusion of GJB2 mutations and other mutations previously reported in Tunisian deaf patients, we performed whole exome sequencing in patients affected with severe to profound deafness, from four unrelated consanguineous Tunisian families. Four biallelic mutations were identified in three different genes, i.e. a nonsense mutation, c.208C>T (p.R70X), in LRTOMT, a missense mutation, c.5417T>C (p.L1806P), and splice site mutations, c.7395+3G>A, in MYO15A, c.2260+2T>A, in TMC1. We thereby provide evidence that whole exome sequencing is a powerful, cost-effective screening tool to identify mutations causing recessive deafness in consanguineous families.

P02.13-S

Diagnosing heterogeneous disorders such as hereditary hearing loss with targeted Next-Generation-Sequencing: a model for categorization and evaluation of the pathogenicity of sequencing variants

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DNA diagnostics of hereditary hearing loss (HL) is complicated considerably because of the genetic and phenotypic heterogeneity of the disease. Currently, only 10-20% of the patients receive a clear genetic diagnosis, because only two genes are analysed regularly. We developed a Next-Generation Sequencing (NGS) panel for HL, consisting of 79 known genes for all nonsyndromic sensorineural forms HL and Usher-, Stickler- and Jervell en Lange-Nielsen syndrome. 114 patients with presumed autosomal recessive nonsyndromic HL and ten patients with autosomal dominant nonsyndromic HL were analyzed using this panel. Target enrichment and sequencing was performed using the Illumina TruSeq Custom Enrichment and HiSeq2000. In total 307 variants were identified in the 124 different patients. In order to deal with the large amount of variants, a classification system was set up to assign the most plausible disease causing variant to each patient. Variants with a MAF below 0.003 were classified according to the variant type and predictions based on different prediction programs, in five groups ranging from definitely pathogenic (class 5) to not pathogenic/no clinical significance (class 1) at all. Approximately 3.7% of the patients had variants in two or more groups. In addition there was one patient with two variants in class four. The current diagnostic setting is likely to be less reliable than generally assumed. In addition, our study shows that a straightforward, reliable and a validated classification system is necessary to use targeted resequencing in the diagnostics of hearing loss in the future.

P02.14-M

Molecular diagnosis of Italian patients affected by hereditary Retinal Dystrophies, using targeted high-throughput sequencing

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Hereditary Retinal Dystrophies (RD) represent one of the most frequent genetic causes of blindness in the Western world. Because of their high clinical and genetic heterogeneity, an exhaustive classification of RD is difficult. The progressive forms may have an onset in early childhood, as Leber congenital amaurosis, or later in life, as Retinitis Pigmentosa (RP). There are also described many syndromic forms, as Usher syndrome (USH), and Bardet-Biedl syndrome (BBS).

RD can be inherited as autosomal recessive (ar) or autosomal dominant (ad), as well as X-linked (xl), or mitochondrial traits. Nevertheless, the majority of cases are sporadic.

In this study, we analyzed 115 retinopathies-related genes by targeted next-generation sequencing (NGS) in a cohort of 78 patient with sporadic RP, 10 patients with USH and 10 patients with BBS.

Sequence target enrichment was performed using SureSelect XT kit (Agilent). Sequencing of each library was carried out on HiScanSQ Illumina platform (mean coverage 500X).

We obtained a detection rate of 100% for BBS patients, 80% for USH patients, and 69% for RP patients. The candidate variants were independently validated by Sanger sequencing and segregation analysis was performed.

Were also identified 3 large deletions in the genes EYS, RPGR and USH2A, using method CONTRA and confirmed by MLPA.

This study confirms that NGS-based mutation analyses are reliable and cost-

efficient approaches in molecular diagnosis of genetically heterogeneous diseases like RD. NGS allows also a considerable reduction of costs and an early intervention for diseases for which there are available appropriate therapeutic strategies.

P02.15-S
Identification of three novel homozygous variants in the GJB2 gene in Mexican patients with hereditary sensorineural hearing loss

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Background: Hereditary sensorineural hearing impairment (HSHI) is a genetically heterogeneous disorder worldwide. Mutations in the GJB2 gene are a frequent cause of hereditary SNHL. Individuals that are homozygous for the GJB2 gene mutations manifest a wide spectrum of clinical data that ranges from moderate to profound SNHL; this suggests the participation of epigenetic and environmental factors in the phenotypic expression Objective: To describe three novel homozygous mutations in the GJB2 gene with HSHI. Materials and methods: Three subjects with prelingual HSHI were included in the study. Genomic DNA was extracted by conventional methods and all exons of GJB2 gene were analyzed through PCR and the DNA was sequenced on an ABI 3730XL automated sequencer. Results DNA sequencing analysis showed three novel homozygous mutation in the GJB2 gene that corresponded to p.V84M/p.F31I/p.W44X; /p.V84M/p.F31I/p.R32S and p.E47X/p.R32S/p.S19R. Parents were tested for this molecular defect and they present an heterozygous state that allows to confirm the recessive inheritance pattern. These mutations were searching in 100 normal controls to discard a possible polymorphism. Conclusion: We describe three novel varieties of homozygous mutations in patients with HSHI. All patients presented profound hypoacusia with no other anomalies. These data show that the genesis of HSHI is complex and that the genetic defects are greater than expected.

P02.16-M
Regional distribution of the GJB2/GJB6 gene mutations in Mexican population with hereditary hearing impairment

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Background: Hereditary sensorineural hearing loss (SNHL) is a genetically heterogeneous disorder worldwide. Mutations in the GJB2 gene are a frequent cause of hereditary SNHL. There is a prevalence of certain mutations in various populations which suggests that specific mutations may be influenced by ethnic background. Objective: To analyze the prevalence of GJB2, GJB6 mutations in several geographic areas of Mexico in patients with hereditary SNHL. Materials and methods: One hundred and forty Mexican unrelated propositi with prelingual SNHL were included in the study. All patients had three previous generations born in Mexico and belonged to no specific ethnic group. Analyses of the GJB2 and GJB6 genes and mt.A1555G were performed in all subjects. Results Twenty three homozygous mutations, 57 heterozygous mutations, 1 double heterozygous (GJB2/GJB6) and 59 wild-type genotypes in the GJB2 gene were observed. Three patients had the homozygous del35 mutation whereas 26 patients were heterozygous for this gene defect. Only one patient with the GJB6 gene deletion was present (it includes the double heterozygous GJB2/GJB6). The mt.A1555G mutation was not detected. Conclusion: We found a great variety of mutations depending on the analyzed region in patients with SNHL; 57.86% of patients had affection in one or two alleles in GJB2 or GJB6 genes whereas 42.14% were wild-type. In some cases, allele distribution depended on region. Molecular studies of more genes involved in hereditary non-syndromic SNHL are required to completely confirm the molecular basis of hearing loss in Mexican population.

P02.17-S
Panel base on next-generation sequencing for molecular diagnosis of inherited heterogeneous eye diseases

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Background: Inherited eye diseases are a leading cause of blindness in children and adults, a typical monogenic disease of which is retinitis pigmentosa (RP) that cause visual impairment. An accurate, econimical, and high throughput molecular diagnosis system can be very helpful in clinical diagnosis and essential in gene therapy in the future. **Methods:** We developed a panel based on next generation sequencing (NGS) that captures the exons of 283 inherited eye disease genes, which includes 58 known RP disease-causing genes. We applied 180 samples in this panel, 64 of these samples are previous tested by Sanger sequencing, and 99 of these samples are unselected patients with clinical diagnosis of retinitis pigmentosa. **Results:** We presented data on our first 99 probands with RP with systematic evaluation of our method and comprehensive molecular diagnosis. 96.85% of each targeted regions in 4,381 exons covered by at least 20 folds. In the 99 RP patients, 66.7% molecular diagnosis rate for RP disease was achieved, while in 5 patients, molecular diagnosis were not consistent with their initial clinical diagnosis. We revisited these 5 patients and reclassified the clinical diagnosis of them. In addition, altogether 3 patients were found to carry copy number variant (CNV). **Conclusions:** We have systemic evaluated and compared our method with Sanger sequencing, and have identified a large number of novel mutations. The results show that our method is sufficiently accurate for molecular diagnosis, and suggest the importance of molecular diagnosis in clinical diagnosis.

P02.18-M
An integrated next-generation sequencing approach identifies causative mutations in a family with syndromic and non-syndromic retinitis pigmentosa

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Routine molecular testing of Inherited Retinal Dystrophies (IRD) generally involves mutational screening of hundreds of clinically relevant IRD genes. Comprehensive analysis with available techniques has been challenging due to the extensive clinical and genetic heterogeneity present in this group of disorders. Here, we describe the clinical application of an integrated next-generation sequencing approach to determine the underlying genetic defects in a Spanish family with a provisional clinical diagnosis of autosomal recessive Retinitis Pigmentosa (arRP). Exome sequencing of the index patient and immediate family members resulted in the identification of the homozygous *BBS1* p.M390R mutation. Sanger sequencing confirmed the presence of this variant in this family branch but did not segregate in extended family members. Clinical reanalysis showed co-ocurrence of two different phenotypes in this family: Bardet-Biedl syndrome in the family harboring the *BBS1* mutation and non-syndromic arRP in the extended family members with unknown causal genetic defects. To identify possible causative mutations in the arRP family branch, we applied targeted resequencing on 26 IRD genes in one of the affected members using the 454 GS Junior system. The in-house custom panel was validated using 18 DNA samples known to harbor mutations in relevant genes and allowed us to reliably identify two novel heterozygous mutations in *RP1* (c.5962dupA; p.I1988Nfs*3 and c.4582_4585delATCA; p.I1528Vfs*10) as the most likely disease-causing variants in these arRP patients. Taken together, these results demonstrate the value of this combined next-generation sequencing-based approach as an efficient screening and diagnostic tool for complex genetic disorders.

P02.19-S
Partial *NMNAT1* deletions cause Leber Congenital Amaurosis

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In 2012, *NMNAT1* was identified as a novel disease gene for Leber Congenital Amaurosis. The mutation spectrum contains both coding and regulatory mutations. The starting point of this study was pseudohomozygosity of a known *NMNAT1* mutation (p.Arg237Cys, exon 5) in two unrelated Japanese families (F1 and F2), assuming hemizygosity. Here, we aimed to identify the putative *NMNAT1* deletions.

Copy number variation (CNV) screening was performed for all exons using qPCR. Subsequently, identified deletions were refined with additional qPCR amplicons located in the breakpoint regions. Finally, the junction product was amplified with long-range PCR and sequenced (Nextera XT, MiSeq, Illumina).

In F1, CNV analysis showed a heterozygous deletion of exon 4 and 5, which was subsequently refined to a region of 13.0-18.7 kb. In F2, the amplicon for exon 4 was deleted, whereas the copy number for exon 5, located downstream of the p.Arg237Cys mutation, was normal. Subsequent refinement at

the 5'end delineated the deletion to a region of 1.5-6.7 kb. Long-range PCR revealed a band of 2.4 kb and 2.1 kb in F1 and F2, pointing to two different deletions of approximately 16.6 kb and 4.8 kb, respectively. Sequencing analysis of the junction products is currently ongoing.
In conclusion, we report the first deletions in *NMNAT1*, expanding the mutation spectrum of this gene. Hence, we presume that the high number of patients in which only a single heterozygous mutation could be identified, can be explained by structural variations, requiring additional strategies apart from resequencing of the coding region.

P02.20-M
Prevalence and mutation analysis of *NMNAT1* gene in Leber Congenital Amaurosis in Spanish population

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Leber Congenital Amaurosis (LCA) is the most severe blinding retinal dystrophy that represents near 5% of cases. At the moment 20 genes have been associated to this disease, more of them expressed in the photoreceptors or in the retinal pigment epithelium, explaining around 70% of LCA cases. The ubiquitous gene encoding nicotinamide nucleotide adenylyltransferase 1 (*NMNAT1*) was recently associated to this pathology using exome sequencing. The aim of this work was to screen the *NMNAT1* gene in our Spanish cohort of uncharacterized LCA families. We were selected a total of 96 LCA families previously screened for known mutations to cause LCA using a commercial genotyping chip with negative results. Index cases of these families were analyzed by direct Sanger sequencing of the coding *NMNAT1* sequence. This screening allowed us to identify five novel mutations in the *NMNAT1* gene and to characterize 6 of the 96 total families (6.25%), carrying all of them compound heterozygous mutations. As described in other populations, the p.E257K variation which was not found in any of the 176 Spanish controls tested, is the most frequent variation. Five additional families were found to carry the p.E257K common variation in heterozygosity with no other apparently change in the second allele. In order to fully characterized these families other studies (RNA expression in peripheral total blood and lymphoblast cell line, intronic sequencing and others) should be performed.

P02.21-S
Mutation hotspot sequencing reveals RPE65 as the most frequently mutated LCA gene in Denmark

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Leber congenital amaurosis (LCA) represents the most severe and earliest onset form of inherited retinal dystrophies, and affects 1 per 50,000 individuals worldwide. Presently, mutations in twenty genes with diverse roles in the retina are known to be associated with LCA. The large number of LCA genes necessitates a systematic genotyping approach in a cost- and time-effective manner. Previous studies in LCA have described some recurrent mutations and mutational hotspots in particular exons of LCA-associated genes, i.e. an intronic variant in *CEP290* (c.2991+1655A>G), a nonsense mutation in *AIPL1* (p.W278*), a missense change in *GUCY2D* (p.R768W), and various mutations clustering in exons 7 and 9 of *CRB1*. As gene-specific therapies are emerging, identifying mutations in *RPE65* and *LRAT* can also be of tremendous importance for patients. Sanger sequencing of these mutation hotspots, and all protein-coding exons of *RPE65* and *LRAT* in 64 Danish LCA probands revealed one or two variants in 22 (34%) cases. Upon the identification of heterozygous variants, Sanger sequencing was performed in the relevant genes to identify the second allele, *RPE65* was shown to be the most frequently mutated gene in Denmark (20% vs. 8% in other Caucasian populations). This is important as 9-*cis*-retinoid supplementation and *RPE65* gene augmentation therapies have been developed.

P02.22-M
Propensity for localized provoked vulvodynia by *TRPV1* and *NGF* polymorphisms

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Localized provoked Vulvodynia (LPV) is a prevalent chronic pain condition. Familial occurrence (FO) suggests genetic susceptibility in its etiology. We studied possible associations between LPV in 70 affected women and seven Single Nucleotide Polymorphisms (SNPs) in the genes Transient Receptor Potential Vanilloid type-1 (*TRPV1*), Nerve Growth Factor (*NGF*) and *heparanase*, hypothesized to be involved in the pathophysiology of LPV. Prevalence of SNPs was compared between women with severe primary LPV and 132 healthy, ethnically matched controls. Women participating in the study have answered a detailed questionnaire, addressing possible FO of LPV and comorbid pain conditions.
SNaPshot™ genotyping revealed a novel statistically significant high prevalence of non-synonymous polymorphism rs222747 of *TRPV1* and rs11102930 located in the promoter region of *NGF,* , in women with LPV, especially those from Ashkenazi Jewish ancestry compared to the control group. Logistic regression model for rs222747 and rs11102930 frequent alleles indicates significant risk for LPV in all affected women and Ashkenazi Jewish group, respectively. Significant higher rate of FO of LPV, Temporo mandibular joint (TMJ) symptoms, recurrent vaginitis and irritable bowel syndrome was found in affected women compared to healthy controls.Ad hoc analysis compared pain conditions with frequent alleles among the 202 women studied. Interestingly, rs222747 minor allele of *TRPV1* was found in association with women presenting TMJ and women with recurrent vaginitis, suggesting a possible common genetic predisposition to pain comorbidities. These data open a new horizon for understanding the pathophysiology of LPV and might lead to the development of new personalized therapeutic modalities.

P02.23-S
Genetic Disorder with Diverse Modes of Inheritance Implicates Sporadic Form of Mild to Moderate Sensorineural Hearing Loss in a Pediatric Population

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arNSHL (autosomal recessive Nonsyndromic Hearing Loss), the most frequent mode of hereditary hearing impairment, is typically severe or profound. In contrast with severe or profound hearing loss of which genetic etiologies have been intensively investigated, relatively less attention into mild to moderate hearing loss has been paid. This study was designed to delineate genetic contributions, if any, to moderate hearing loss in a pediatric population using the next generation sequencing technologies. We performed whole exome sequencing (WES) for 12 unrelated children with moderate degree of hearing loss. Hearing loss from 11 of 12 probands was sporadic. We performed bioinformatic analyses of the WES data and selected the candidate variants compatible with either autosomal recessive inheritance pattern or *de novo* autosomal dominant pattern. Strong candidate variants were detected in 6 (50%) of 12 probands, suggesting a strong genetic contribution to moderate degree of hearing loss in a pediatric population. Diverse mode of inheritance pattern was observed in this disease population unlike in severe to profound hearing loss. Compound heterozygotes from deafness genes, *OTOGL,* and *SERPINB6* were detected. One proband segregated moderate degree of hearing loss as a digenic inheritance involving two deafness genes, *GPR98* and *PDZ7.* In addition, strong candidates from another three subjects were selected as a *de novo* mutation from *MYH14*, *MYO7*, and *P2RX2*, respectively. This study, for the first time in the literature reveals a strong genetic contribution to sporadic forms of moderate hearing loss in a pediatric population.

P02.24-M

Identification of genes and related mutations in 10 Iranian families with non-syndromic autosomal recessive hearing loss by whole exome sequencing

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With prevalence figures close to 0.2% at birth, hearing loss (HL) is the most frequent sensory impairment in childhood. In developed countries, genetic causes account for more than 60% of congenital HL, most often resulting in non-syndromic deafness, which is usually autosomal recessive.
Hereditary nonsyndromic hearing loss (NSHL) in Iran is highly heterogeneous, and more than 50% of patients with a presumed genetic etiology lack a specific molecular diagnosis with STR analysis. Whole-exome sequencing (WES) has recently opened a new page in Mendelian disease gene discovery - enabling to study autosomal recessive HL in a new way.
The aim of this study is to find more causative genes and their mutations for NSARHL in Iranian families by WES. After ruling out any association to prevalent genes for NSARHL in Iran, ten families will be subjected to WES.
Until now, WES has been performed with genomic DNA from affected individuals of two consanguineous families with profound deafness. Analysis of these data revealed a novel homozygous mutation in MYO7A gene in one family but co-segregation study failed to confirm this variant as the only cause of HL in this family. Additional clinical investigation revealed that an intra-familial phenotypic variation and existence of both syndromic and non-syndromic HL in this family is possible. Further studies to find the other variants which may be associated with HL in this family, the data analysis of the second family and also WES of remaining families are underway.

P02.25-S

Nonsyndromic hearing loss in Moravia: one fifth due to GJB2, no mutations in SERPINB6, TMIE, COCH, ACTG1, KCNQ4, GJB3

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GJB2 gene mutations are the most frequent cause of nonsyndromic hearing loss. There are many other genes less frequently causing this disorder. The aim of our study was to look for other genes mutated in Moravian population of patients with deafness. We have performed sequencing of *GJB2* coding region on ABI3130 and Δ(GJB6-D13S1830) detection using PCR and gel electrophoresis in 142 patients with nonsyndromic hearing loss. Biallelic pathogenic *GJB2* mutations were found in 31 patients (22%) thus explaining their hearing defect. In 9 patients (6%) only one pathogenic *GJB2* mutation was found. No patient carried Δ(GJB6-D13S1830). Sequencing of *SERPINB6, TMIE, COCH, ESPN, ACTG1, KCNQ4* and *GJB3 g*enes was performed on ABI3130 in 13, 13, 13, 30, 20, 14 and 30 patients without *GJB2* mutation, respectively. No pathogenic mutation was found in *SERPINB6, TMIE, COCH, ACTG1, KCNQ4* and *GJB3* genes. In *ESPN* gene, two variants with unknown pathogenicity were found in two unrelated patients: c.337C>T, p.Arg113Cys (Polyphen score 1.00) and c.1797_1808delCCCACCGCCGCC, p.Pro600_Pro603del. Both variants were inherited from parents without hearing loss. We cannot exclude big genomic deletion/duplication of *ESPN* gene on the other allele in the patients. There may also be bigenic mechanism of hearing loss pathogenesis. So far, however, we cannot conclude that these variants are causal in our patients. We are going to analyze *WHRN* gene encoding the whirlin protein, functionally associated with *ESPN* gene product espin.

P02.26-M

Comprehensive genetic diagnosis of non-syndromic hereditary hearing loss by targeted resequencing of 32 genes with use of Haloplex design and IonTorrent PGM sequencer

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Non-syndromic hearing loss (NSHL) is one of the most common hereditary diseases with incidence higher than 1:1000. It is characterized by high genetic heterogeneity as causal mutations in more than 70 genes were found to be associated with it. Altough mutations in single gene - GJB2, are the most prevalent worldwide in remaining cases screening for causal mutation will be expensive and time consuming by conventional gene by gene approach. Therefore simultaneous sequencing of multiple genes with utilization of next-generation sequencing (NGS) should be the right solution.
In our pilot study 5 samples with familial incidence of autosomal dominant or recessive non-syndromic hearing loss without causal mutations in GJB2 gene were analyzed. The NGS was performed on IonTorrent PGM with use of customized Haloplex assay covering coding sequence of 32 most frequently mutated NSHL associated genes.
In 3 of 5 cases candidate causal mutations were identified and its presence was verified with Sanger sequencing. The detected mutations were p.H455Q in KCNQ4, p.G662E in MYO1A and p.T1866M in TECTA.
According to our results the multiparallel resequencing of panel of NSHL candidate genes could significantly improve genetic diagnosis of this geneticaly heterogeneous hereditary disease. Nevertheless, the lack of information about functional consequencies of low frequency variants could complicate the variant validation process and lead to false positive results.
This study was supported by grant ITMS 26240220067 funded by ERDF.

P02.27-S

Evidence for genetic linkage of a North Carolina-like macular dystrophy phenotype in association with digit anomalies to chromosome 5p15.32 or 9p24.1

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Introduction: Developmental macular disorders are a rare cause of visual impairment in children, but identifying the causative genes will help understand the biological pathways involved in human macular development. One such disorder is North Carolina macular dystrophy (NCMD), an autosomal dominant fully penetrant developmental macular dystrophy. NCMD has been mapped to chromosome 6q16 (MCDR1) and NCMD-like disorder has been mapped to chromosome 5p13.1-5p15.33 (MCDR3). To date, the genes underlying NCMD are unknown. The case of a British family affected by a dominant macular dystrophy resembling NCMD in association with digit anomalies was presented by A. Sorsby in 1935. We present a second unrelated family of French origin affected by the same phenotype and report the results of genetic linkage studies in both families. Methods: Fifteen members of the two families, including 11 affected, were genotyped using the Affymetrix 250K Sty array (238,304 single nucleotide polymorphisms). Parametric linkage analysis under a dominant model with complete penetrance was performed. Results: Linkage analysis identified two loci at 5p15.32 and 9p24.1 with LOD score of 2.7. Given the involvement of other ADAMTS proteins in several different ocular phenotypes, all exons of gene ADAMTS16 (5p15.32) were sequenced in affected individuals and no mutations were found. Discussion: The linkage overlap with the MCDR3 locus on 5p might indicate allelism or more likely the presence of mutations in two different adjacent developmental genes. Exome sequencing is undergoing and expected to shed light on the genetic basis of this disorder.

P02.28-M

Identification of novel NSHL-causing mutations by whole exome sequencing

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Inherited nonsyndromic sensorineural hearing loss (NSHL) is characterized by a high level of genetic heterogeneity, making extremely challenging to obtain a molecular diagnosis with traditional screening methods. Whole exome sequencing (WES) has been recently introduced as an alternative approach to search for alleles underlying Mendelian disorders and has been successfully applied for gene/mutation discovery. In this study, we used WES to identify the pathogenic mutations responsible for NSHL in three families (NSHL6, 11, and 12), with a recessive inheritance pattern and at least two affected siblings. A total of 9 individuals were subjected to WES using the SeqCapEZ Exome capture kit (Roche) and the HiSeq 2000 sequencer (Illumina). In particular, the NSHL6 patients were compound heterozygous for two novel mutations within the TMPRSS3 gene (DFNB8/10 locus). Both variants segregate with post-lingual, bilateral, high-frequency NSHL and affect evolutionary conserved amino acids located within the TMPRSS3 catalytic domain. In the NSHL11 family, two novel missense variants were found in the heterozygous state in OTOGL, a gene that was only recently associated with NSHL (otogelin-like protein, DFNB84 locus). Finally, in the consanguineous

NSHL12 pedigree, WES coupled with homozygosity mapping analysis pointed out a known missense variant (rs74315438) in the CLDN14 (DFNB29 locus) gene, coding for the claudin 14 protein. In conclusion, we provide evidence of the usefulness of WES for the diagnosis of NSHL and increase the knowledge on the genetic defects underlying this disease. This study was supported by: Italian Telethon Foundation (grant#GGP11177) and Fondazione Cariplo, grant N°2013-0825.

P02.29-S
Phenotypic and genotypic variability of Pendred syndrome

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Pendred Syndrome (PDS) is an autosomal-recessive disorder characterized by progressive sensorineural hearing impairment and goiter. PDS is associated with temporal bone abnormalities ranging from isolated enlargement of the vestibular aqueduct (EVA) to Mondini dysplasia. Hearing loss is prelingual in the majority of the cases; a minority of patients has a progressive hearing loss later in life.
EVA and PDS are caused by mutations in the SLC26A4 gene. The SLC26A4 gene is expressed in the non-sensory epithelia of the inner ear. Additionally the genes FOXI1 and KCNJ10 may also be responsible for PDS and inner ear malformations. The FOXI1 gene is involved in the transcriptional control of the SLC26A4 gene. The KCNJ10 gene encodes for a K+-channel located in the stria vascularis. In this study we analysed 167 patients with PDS and EVA hearing loss to accomplish a phenotype genotype correlations for PDS related deafness.
Individual exon and intron transitions of the SLC26A4, FOXI1 and KCNJ10 genes of patients were sequenced. Audiometric thresholds were performed, and radiologically, the vestibular aqueduct midpoint and opercular width were measured.
In the analysed patients with PDS, a total of 34 different SCL26A4 mutations were detected, mutations could not be detected in 64 % of the cases. FOXI1 and KCNJ10 analyses reveal no mutation.
The present results obtained in patients with PDS indicate that this phenotype is likely to be complex. Moreover, analysis of FOXI1 and KCNJ10 genes in our cohort suggests a minor implication of these two genes in the phenotype of EVA and PDS.

P02.30-M
The 965insA in SLC26A4 gene is a founder mutation in Azeri families from western Iran

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Background: Mutations in the *SLC26A4* gene cause both Pendred syndrome [sensorineural hearing loss (SNHL), enlarged vestibular aqueduct (EVA), and goiter] and autosomal recessive nonsyndromic hearing loss (ARNSHL) at the DFNB4 locus. Pendred syndrome is the most common type of syndromic HL.
Materials/Patients and Methods: We performed comprehensive clinical and genetic evaluations of 15 patients from four unrelated families from the Ardebil Province in western Iran. After testing STR markers to confirm linkage to the *SLC26A4* locus, we sought to identify genetic mutations in *SLC26A4* by Sanger sequencing all 21 exons, exon-intron boundaries and the promoter region of this gene.
Results and Conclusions: A known frameshift mutation (965insA, p.N322Fs7X) in exon 8 was identified in all four families. Three families were homozygous for this mutation and one family was compound heterozygote (L597S/965insA). To date, this mutation has been reported only in the Iranian population, so we investigated the possibility of a founder effect. Haplotypes were constructed by genotyping seven single nucleotide polymorphisms throughout the *SLC26A4* gene, thus identifying a single haplotype that co-segregated with the 965insA mutation. We conclude that this mutation originates from a common founder in the west province of Iran.

P02.31-S
Identification by whole-exome sequencing of two novel *LARS2* mutations in an Italian family with Perrault syndrome

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Perrault syndrome (PRLTS) is a rare autosomal recessive disorder characterized by ovarian dysgenesis and premature ovarian failure (POF) in females, and by progressive hearing loss in both genders. Recently, mutations in four genes (i.e. HSD17B4, HARS2, CLPP, and LARS2) were found to be responsible for PRLTS, although they do not account for all cases of this genetically heterogeneous condition.
In this study, we used whole-exome sequencing (WES) to identify the pathogenic variants responsible for PRLTS in an Italian pedigree with two affected siblings (one female and one male). All five family members were subjected to WES using the SeqCapEZ Exome v2 kit (Roche) and the HiSeq2000 platform (Illumina). Data analysis highlighted compound heterozygosity, in both patients, for two novel missense variations, p.Thr300Met (c.899C>T) and p.Glu638Lys (c.1912G>A) within LARS2, encoding the mitochondrial leucyl-tRNA synthetase,. The segregation of the two mutations in the pedigree is compatible with the autosomal recessive inheritance of the disease. Both Thr300 and Glu638 residues are evolutionary conserved, and are respectively located within the editing domain and immediately before the KMSKS sequence, a unique signature of the catalytic domain of class 1 aminoacyl-tRNA synthetases. The identified mutations were confirmed to be absent in an in-house database of about 3500 ethnically-matched control exomes.
Apart from the original report in 2013, to our knowledge, this is the first study confirming the role of LARS2 mutations in PRLTS pathogenesis.
This study was supported by: Italian Telethon Foundation (grant#GGP11177) and Fondazione Cariplo, grant N°2013-0825.

P02.32-M
Whole exome sequencing in a multiplex family case of Plateau Iris Syndrome

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Plateau iris configuration (PIC) is a unique subtype of angle-closure glaucomas presenting in younger patients. PIC has been recognised as one of the anatomical mechanisms of angle closure development along with papillary block. The diagnosis is always based on gonioscopic and ultrasound biomicroscopy (UBM) findings. PIC-associated anterior chamber characteristics include a normal central anterior chamber depth (ACD), a flat iris plane, an iris root that angles sharply forward, and anteriorly positioned or large ciliary processes. Peripheral iridotomy is proposed as a treatment. Post-iridotomy complications, such as persistent angle narrowing or occlusion with intraocular pressure elevation, give rise to the plateau iris syndrome (PIS). An autosomal dominant pattern of inheritance with incomplete penetrance was proposed by Etter et al. in 2006 because of a higher prevalence of PIC in first-degree family members. To our knowledge, no familial case has been reported to date.
Here we described a multiplex family of seven affected members in two generations. Two members have PIC and five have PIS with early-onset and severe prognosis for some of them. Whole-exome sequencing was performed in five of the cases. Among the about 50 candidate variants left after filtering out by segregation, frequency and pathogenicity, the four more relevant ones -according to their function- were validated by Sanger sequencing in the two remaining affected members. Further experimental investigations and/or sequencing of additional families with a similar phenotype are needed to highlight the causative gene.

P02.33-S
Two Tumor Necrosis Factor promoter polymorphisms in Romanian patients with Primary Open Angle Glaucoma Results from a pilot-study

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Glaucoma is a complex group of optic neuropathies, characterized by the degeneration of the optic nerve, eventually leading to blindness. Primary Open Angle Glaucoma (POAG) is the most common form of disease, affecting 8 million people. The risk factors for POAG include genetic background. TNF-alpha polymorphisms were studied in different populations regarding their influence on susceptibility of POAG.
We aimed to investigate two TNF-α polymorphisms regarding susceptibility to POAG. To our knowledge, this is the first study of TNF polymorphisms

in Romanian POAG patients. We assessed 258 subjects (112 POAG patients and 146 healthy unrelated matched controls) for -857C/T (rs1799724) and -308 G/A (rs1800629) TNF-alpha polymorphisms. These were genotyped by Real Time PCR (Taqman SNP Genotyping Assays C_2215707_10 and C_7514879_10 respectively, Applied Biosystems, USA). Statistical analysis was performed using the SNPStats program for genetic association studies (http://bioinfo.iconcologia.net/SNPstats); p-values ≤0.05 were considered significant.
All studied groups were in HWE for both polymorphisms. The frequencies of minor alleles -857T and -308A were similar in POAG patients and controls (0.22/0.10 and 0.21/0.13 respectively). There was no significant difference in SNPs regarding the allele carriage or genotype frequency between POAG and control subjects. Three main haplotypes were constructed with similar frequencies in patients and controls -857C/-308G/, 857T/-308G and -857C/-308A: 0.683/0.661; 0.218/ 0.205 and 0.98 / 0.133 respectively. There was no significant association between the global haplotype and POAG (p-value = 0.5).
Conclusion: TNF-alpha polymorphisms (-857C/T and -308G/A) seem not to influence susceptibility of POAG. These results should be confirmed on larger patients' cohort.

P02.34-M
Genetic screening for disease-associated mutations in human retinal diseases using whole exome sequening (WES)

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Monogenic diseases of the retina and vitreous affect approximately 1 in 2000 individuals. They are characterized by tremendous genetic heterogeneity and clinical variability of symptoms involving more than 20 different clinical phenotypes and mutations in more than 200 genes. Clinical manifestations of retinal degenerations (RD) range from mild retinal dysfunctions to severe congenital forms. A detailed clinical diagnosis and identification of the underlying mutations are crucial for genetic counseling of affected patients and their families, for understanding genotype-phenotype correlations and developing therapeutic interventions.
We make use of WES and have established a reliable and efficient high-throughput analysis pipeline of next generation sequencing (NGS) data to identify disease-causing mutations in RD. Our data indicate that this approach enables us to genetically diagnose approximately 56% of the patients (N=28) with mutation(s) in known disease-associated genes. Thus, 44% of the cases, that do not carry mutation(s) in a known gene, are crucial for identification of novel candidate genes and biological pathways underlying the disease phenotype. Amongst identified mutations, 47% were previously described in the literature while 53% are novel. The types of mutations included missense mutations (47.4%), frameshift insertions or deletions (26.3%), stop gains (15.8%) and mutations predicted to interfere with splicing (10.5%). In conclusion, WES can rapidly identify mutations in various families affected with different forms of RD. Functional studies of the identified mutations are needed to understand the underlying disease mechanisms, which in future might aid in the development of therapeutic approaches.

P02.35-S
Large number of new mutations found in patients with inherited retinal dystrophies using Panel-based Next Generation Sequencing

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In inherited retinal dystrophies (RD) genetic heterogeneity is a well known feature. Therefore, we used next generation sequencing (NGS) technology as a tool to identify known as well as unknown mutations in RD.
More than 150 genes associated with RD were selected from literature or databases. A custom target-in-solution-enrichment was used, followed by sequencing on either the SOLiD 5500xl or the Illumina HiSeq 2500 platform. Variants were annotated using transcript, variant, medical as well as population frequency databases. Identified mutations were validated using Sanger sequencing.
We analyzed 300 patients with all forms of RD including syndromic forms such as Usher-syndrome and Bardet-Biedl syndrome. The detection rate of solved cases was 56.7%. Interestingly, in 54% of the solved cases we were able to detect mutations which were not previously described in literature at the date of the medical report. 70% of these cases show exclusively a new mutation in case of dominant or x-linked inheritance and two new mutations in case of recessive inheritance. 30% show one new and one previously described mutation.
Therefore, we conclude that NGS is the most promising tool to identify known as well as unknown mutations for this genetically highly heterogeneous disease entity. New mutations were found in 47 different genes. Genes most frequently affected by mutations were *USH2A* and *EYS* followed by *RPGR, PRPF31* and *CDHR1*.
The high number of novel mutations detected in our cohort shows the advantage of NGS over often used conventional chip-array analysis that assay only known mutations.

P02.36-M
Splice factor-based gene therapy to correct mislocalization of the ciliary protein RPGR in retinal degeneration

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Retinal degenerations (RD) and other genetic diseases are often caused by splice site mutations. This type of mutation accounts for approximately 20% of all pathogenic sequence variants associated with retinal degeneration.
Previously, we reported the identification of an X-linked RD patient that showed skipping of exon 10 in the RPGR mRNA. To treat this mutation-induced splice defects, we applied mutation-adapted splice factors. In case of the splice factor U1snRNA, the adaptation yielded an increase in its affinity towards the mutated splice site. This novel gene therapeutic approach was highly efficient in increasing the amount of correctly spliced RPGR transcripts in minigene splicing assays and patient-derived fibroblasts.
In this study, we aimed at evaluating the efficacy of the U1-based therapy on the protein level. We established an assay to locate the RPGR protein in patient-derived and control skin fibroblasts. In control fibroblasts, we found that the RPGR protein locates along the primary cilia including basal body, transition zone and axoneme. In contrast, patient-derived cells showed mislocalization of the RPGR protein restricted to the basal body and transition zone. Upon treatment with mutation-adapted U1, localization of RPGR along the axoneme was significantly increased compared to control treatments.
We conclude that the efficacy of the U1-based gene therapy is sufficient to correct not only misspliced transcripts but also their translated proteins. Thus, the adaptation of U1snRNA is a promising technique to treat patients who carry pathogenic splice defects.

P02.37-S
Homozygous deletion of glutamate receptor gene *GRID2* causes new human hotfoot mutant phenotype, characterized by early-onset cerebellar ataxia and retinal dystrophy

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It was our aim to identify the genetic cause of early-onset autosomal recessive cerebellar ataxia (ARCA) associated with retinal dystrophy in a consanguineous family.
Homozygosity mapping and copy number analysis revealed a homozygous deletion of exon 2 of *GRID2*, p.(Gly30_Glu81del), in the proband, compatible with mouse hotfoot mutant *ho15J*. *GRID2* encodes an ionotropic glutamate receptor known to be selectively expressed in cerebellar Purkinje cells. Here, we demonstrated *GRID2* mRNA expression in human adult retina and retinal pigment epithelium. In addition, *Grid2* expression was demonstrated in different stages of murine retinal development. GRID2 protein expression was observed in both murine and human retina, more specifically in photoreceptor inner segments, the outer plexiform layer and ganglion cell layer.
In order to rule out involvement of mutations in another gene, whole exome sequencing was conducted but did not reveal any other disease-causing mutations, supporting the phenotype observed here represents a single clinical entity.
We identified *GRID2* as underlying disease gene of early-onset ARCA with retinal dystrophy, expanding the clinical spectrum of *GRID2* deletion mutants, which thus far only involved cerebellar but no retinal phenotypes. We demonstrated, for the first time, GRID2 mRNA and protein expression

in human and murine retina, providing evidence for a novel functional role of GRID2 in the retina. To the best of our knowledge *GRID2* is the second glutamate receptor gene, apart from *GRM6*, leading to retinal disease when mutated. Finally, we provided further evidence for evolutionary conservation of a hotfoot fragile site between mouse and human.

P02.38-M
Homozygosity mapping and whole exome sequencing identified four novel candidate retinal dystrophy genes

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Inherited retinal dystrophies (IRD) are a remarkably genetically and phenotypically heterogeneous group of inherited eye diseases, with over 190 causative genes identified to date. In the highly consanguineous Saudi population, autosomal recessive forms of IRD are thought to account for the overwhelming majority of cases. Consanguinity is known to increase the frequency of recessive disorders since it increases the coefficient of inbreeding, which is a measure of the percentage of the genome that is identical by descent. Homozygosity mapping, targeted candidate gene analysis and whole exome sequencing were used to identify the causes of IRD in the Saudi population. Mutations in RP1 were found to be a common cause of recessive RP in the Saudi population. Novel and previously identified homozygous mutations in the KCNV2 gene were identified in a cohort of patients with a distinct recessive retinal disorder, 'cone dystrophy with supranormal rod response,' demonstrating phenotype/genotype correlation. In addition, a founder homozygous CABP4 mutation was identified . Causative homozygous mutations were also found in the IRD genes RBP3, RDH12, CRB1, BBS4, CNGA3, CNGB1, EYS, RLBP1, ABCA4 and PCDH12. Four novel candidate genes for retinal degeneration were identified in this study. Potentially pathogenic homozygous variants were identified in EMC1 (c.G430A, p.A144T), KIAA1549 (c.2399_2400insAA, p.T800fs809X), GPR125 (c.C2504G, p.S835C) and DHX29 (c.C2738T, p.A913V). In the majority of cases (31 families) the genetic cause of IRD was identified, demonstrating the power of homozygosity mapping and whole exome sequencing.

P02.39-S
Next-generation sequencing for retinal dystrophy: two years' clinical experience

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Retinal dystrophy (RD) is a genetically and phenotypically heterogeneous group of conditions; it can be syndromic or non-syndromic and follow dominant, recessive or X-linked inheritance. Since April 2012 we have been using next-generation sequencing (NGS) technology with a panel of 105 known retinal genes; genetic testing and the potential identification of causative mutations is now available for a much broader range of patients with RD than was previously possible.
We are currently conducting a notes-based review to evaluate our clinical delivery of this service and its impact on families; we will present data on a cohort of 40+ patients who have undergone NGS testing for retinal dystrophy. Analysis so far shows a wide age range (2-87 years) of patients, around 50% of whom have no family history.
A common reason for testing is to clarify the mode of inheritance for the sake of children or other relatives. Patients also underwent testing to clarify a diagnosis or prognosis, for example, whether the co-occurrence of hearing loss in the family was due to Usher syndrome.
We have reported a range of results: clearly pathogenic mutations (50-60% detection rate), negative test results, and unclear or ambiguous results requiring additional family samples and careful genetic counselling.
We will present data on the impact and value of NGS testing for families and clinicians, including incidental carrier findings and unexpected results such as altered inheritance pattern or change in management. Our experience suggests NGS testing for retinal dystrophy is relevant for a significant number of patients.

P02.40-M
Exome sequencing reveals a rare pathogenic mutation in C2ORF71 as the underlying genetic defect in a Retinitis Pigmentosa family.

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Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinal dystrophies ultimately characterized by the loss of photoreceptors leading to blindness. The molecular diagnosis of RP has long been hampered by the large genetic heterogeneity of this group of disorders. The clinical application of next generation sequencing (NGS) techniques may overcome, in part, these limitations. In this study, we conducted whole exome sequencing (WES) to uncover the genetic cause of arRP in a consanguineous Spanish family, in which genotyping and resequencing microarrays failed to identify the genetic defect. This strategy allowed the detection of one rare homozygous mutation located in exon 1 of C2ORF71 gene (c.1795T>C, p.Cys599Arg). Although this variant has been previously annotated (rs377190272), it has only been detected in heterozygosity in one control individual out of 6304 exomes from the Exome Variant Server database, being probably an asymptomatic carrier. Moreover, the variant co-segregated with the disease in the extended family and was absent in 400 matched chromosomes. Clinically, patient with mutation p.Cys599Arg shows signs of typical RP and resembles other patients with missense mutations in this gene. Taking into account the high prevalence of carriers of deleterious mutations in RP genes among the control population, and the growing number of experiments in which new variants identified by exome sequencing are reported, identifying real novel mutations is an increasingly difficult task. All together this data allow us to conclude that rs377190272 is the most likely causative mutation underlying the phenotype arRP in this family.

P02.42-M
Panel-based Next Generation Sequencing as an efficient technique to detect mutations in Italian patients with Retinitis Pigmentosa

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Inherited retinal disorders affect approximately 1 in 2000 individuals worldwide. Symptoms and associated phenotypes are really variable and, to date, more than 150 genes have been implicated. Retinitis pigmentosa (RP), a degenerative disease of the retina, accounts for approximately one-half of cases. Molecular diagnostics for RP patients by Sanger sequencing is hampered by genetic and clinical heterogeneity. Next generation sequencing (NGS) technology provides a new approach for RP genetic testing, allowing for the screening of other known retinal disease genes in addition to RP genes, particularly important due to their clinical and genetic overlap. To test NGS potential for RP molecular diagnosis, we analyzed a panel of 123 retinal disease genes by targeted NGS in a cohort of 95 Italian probands with non-syndromic RP. All identified genetic variants entered a systematic data analysis pipeline for their prioritization and prediction of pathogenicity, followed by direct sequencing validation. Here, we report preliminary results on 52 probands, for which data analysis and Sanger validation have been completed. Our screening resulted in a molecular diagnosis for 47 RP patients, comprising 18/20 recessive, 11/11 dominant, and 18/21 sporadic cases. A total of 92 likely causative variants in 45 different genes were identified; 46/92 are novel mutations. The genes *USH2A*, *ABCA4*, *EYS*, *NR2E3* and *RP1* were more frequently affected than others. In this group of 52 patients, NGS proved to be an efficient (diagnostic rate: 90%), faster and less time consuming method compared to conventional ones for molecular diagnosis of genetically heterogeneous diseases such as RP.

P02.43-S
Hypomorphic variants in the splice factor genes DHX38 and SNRNP200 are associated with autosomal recessive retinitis pigmentosa

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Purpose: To identify the underlying genetic defects in two consanguineous families with autosomal recessive retinitis pigmentosa (arRP).
Methods: Ophthalmologic analysis was performed in selected persons from both families, that included visual acuity assessment, fundoscopy and electroretinography. Genome-wide homozygosity mapping was performed for multiple affected and unaffected individuals using single nucleotide polymorphism arrays. After exclusion of arRP-associated genes residing in homozygous regions, the probands were analyzed using whole exome sequen-

cing. Pathogenicity of the candidate disease-causing variants in the homozygous regions was assessed by in-silico analysis.
Results: Ophthalmologic examination revealed typical features of RP in both families. In the first family a homozygous missense variant, c.3269G>A;p.(Arg1090Gln), was identified in SNRNP200, which previously was implicated in autosomal dominant RP (adRP) and shown to impair splicing. In the second family a missense mutation, c.995G>A;p.(Gly332Asp), was identified in DHX38, which encodes the pre-mRNA splicing factor PRP16 and previously has not been associated with arRP. The DHX38 variant shows a lod score of 3.25, which is highly suggestive of linkage. Segregation analysis indicated that both variants segregated with RP in respective families, in an autosomal recessive manner. In-silico analysis supported the causality of the p.(Arg1090Gln) and p.(Gly332Asp) variants.
Conclusions: Since the SNRNP200 variant p.(Arg1090Gln) appears only to be causative when present homozygously, we hypothesize that it is a hypomorphic mutation. So far mutations in pre-mRNA splicing factor genes have only been associated with adRP, thus this is the first report that implicates defects in the splicing machinery proteins DHX38 and SNRNP200 to be associated with arRP.

P02.44-M
RS1 gene exon 2 deletion in a large Pedigree with X-linked juvenile retinoschisis

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Juvenile retinoschisis (XLRS) is a vitreo-retinal disorder characterized by early-onset cystic macular dystrophy that can evolve in central atrophy, thus leading to blindness. More than 200 disease-causing mutations have been reported in the X-linked gene *RS1* (Xp22). The clinical diagnosis is based on instrumental ocular examination and can be confirmed by molecular genetic testing. In this report, we present the molecular characterization of an XLRS family with 4 affected males and a pedigree suggestive of an X-linked inheritance. **Methods:** *RS1* gene point mutations screening was performed by PCR e direct sequencing. *RS1* gene copy number variation was assessed by "home made" MLPA analysis and by SNP-array analysis using CytoScan HD Array (Affymetrix, Santa Clara, CA). **Results:** the exon 2 fragment of *RS1* gene failed to amplify in the proband; given the strong suspicion of a deletion a confirmation was made by MLPA and SNP-array: all affected males were positive for exon 2 deletion of *RS1* gene. Carrier females were also identified. SNP-array analysis showed a deletion of less than 7 kb including exon 2. **Conclusion:** this is the first report of a deep characterization of a whole exon deletion in the *RS1* gene, accounting in 10% of XLRS. SNP-array analysis mapped the deletion breakpoint in an intronic region rich of several repeated elements, thus prone to rearrangements. Female carriers can be easily detected by MLPA analysis that represents a useful test in terms of time and costs for copy number detection.

P02.45-S
Spectrum of SLC26A4 gene mutations in Slovak NSHL patients

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Nonsyndromic hearing loss is characterized by hearing impairment that is not associated with other signs and symptoms. This form of deafness accounts for 70% of all inherited hearing loss and it is caused mostly by malformations of structures in the inner ear. A large number of genes is associated with nonsyndromic hearing loss, including CLDN14, COL11A2, GJB2, GJB3, GJB6, KCNQ4, MYO1A, MYO15A, MYO6, MYO7A, POU3F4, SLC26A4, TECTA, TMPRSS3, and many others.
In the Slovak population, mutations in GJB2, GJB6 and mitochondrial genes were only screened to date. In our work we focused on the mutation screening of the SLC26A4 gene, which was found as another of the most prevalent disease genes in NSHL. The SLC26A4 gene encodes a protein called pendrin, which functions as an anion transporter of mostly chloride, iodide and bicarbonate ions across the membranes of cells of the thyroid, inner ear, and kidneys. Recessive mutations in this transporter are associated with hearing loss DFNB4, which is accompanied by an enlargement of vestibular aqueduct. Mutated pendrin affects the levels of fluid in the inner ear that leads to malformation and hearing loss.
In our work, we screened 324 patients for mutations in the SLC26A4 gene. We found several missense mutations, of which the most frequent were G6V (probably non-pathogenic), R185T, R409H, T416P, L445W, R470H, Y530S, L597S, and two splicing mutations c.919-2 A>G and c.2089+1G>A. In two patients we confirmed Pendred syndrome by identifying genotypes R409H/c.919-2 A>G and L445W/c.2089+1G>A.

P02.46-M
Prevalence of mutation c.11864G>A (p.Trp3955X) in the USH2A gene in patients with Usher II Syndrome from Volga-Ural Region of Russia

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Usher Syndrome (US) is an autosomal recessive condition characterized by a combination of congenital hearing impairment and retinitis pigmentosa. To date, ten genes have been associated with US, representing up to 90% of cases. Three types of US are known and differ by onset of the symptoms, severity and progressiveness of deafness and additional vestibular dysfunction. Patients with type II US have congenital bilateral sensorineural hearing loss that is mild to moderate in the low frequencies and severe to profound in the higher frequencies, intact vestibular responses, and bilateral retinitis pigmentosa.
40 unrelated Usher II type families (60 patients) from Volga-Ural Region of Russia were studied using genotyping microarray (Usher, Asper-Biotech) for screening 614 mutations in genes CDH23, MYO7A, PCDH15, USH1C, USH1G, USH2A, GPR98, CLRN1, DFNB31 and automatic sequencing of Usher's genes. Diagnosis was based on pedigree data, ophthalmologic, audiological and vestibular examination.
We revealed homozygous and heterozygous genotypes for the c.11864G>A (p.Trp3955X) mutation (USH2A) in six unrelated families among Russian, Tatar and Chuvash patients with Usher II syndrome. We found four pathogenic mutations in coding region of 8 patients (p.Glu4458fs, p.Trp3955X, p.Glu4078fs, and p.Gly1392X), confirming their clinical diagnosis. The most frequent USH2A gene mutation was c.11864G>A (9/80 alleles; 11,25%). Mutation c.11864G>A in heterozygous state was also found in one Russian subject out of 1066 examined individuals from 16 various populations of Eurasia: Bashkirs, Tatars, Chuvashes, Udmurts, Komi-Permyaks, and Mordvins, Russians, Belarusians, Ukrainians, Veps, and Karelians, Abkhazians, Kazakhs, Uzbeks, Yakuts, Altaians. Study was supported by grants (№12-04-00342_a, №12-04-98520_r_vostok_a, №14-04-97002_r_povolgie_a, №14-04-97007_r_povolgie_a,№14-04-01741_A).

P02.47-S
SOX2 whole gene deletion without ocular malformation but with isolated bilateral third degree microtia : a new manifestation of SOX2-related disorders?

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SOX2-related eye disorders are characterized by anophthalmia and/or microphthalmia, which is usually bilateral and severe. Molecular genetic testing of this gene, including sequence analysis and MLPA (for large gene deletions), identifies mutations in about 10-20% of individuals with such ocular involvement. Other common findings include brain malformations, oesophageal atresia and male genital abnormalities. Postnatal growth failure with pituitary insufficiency, seizures, sensorineural hearing loss, delayed motor development and learning difficulties are described. Recent studies have demonstrated a broader ocular phenotype in cases with missense mutations, consistent with a role for *SOX2* in both posterior and anterior segment development. Severe *SOX2* (OMIM 184429) mutations (whole gene deletion/nonsense mutation) with complete loss-of-function alleles, almost uniformly result in anophthalmia/microphthalmia.
We report the case of a newborn female, evaluated at birth for isolated bilateral third degree microtia. All investigations (cardiac, abdominal, cerebral, ophthalmologic) were normal.
Array-CGH analysis has shown a *de novo* 40 kb deletion of 3q26.33 (chr3:181407035-181442290) [hg19] deleting the entire *SOX2* gene.
More detailed ophthalmologic evaluations confirmed the absence of any ocular abnormality. The girl benefits from osseous conduction hearing aids. Is there a link between *SOX2* deletion and microtia, consistent with results

of a recent study showing under-expression of *SOX2* in human auricular chondrocytes from microtia samples ? Our case also confirms the reduced penetrance of the ocular phenotype, even in severe *SOX2* mutations, and raises the question of a further broadening of *SOX2*-related disorders….

P02.48-M
Exome sequencing identifies POU3F4 p.Ala116fs mutation among family with hearing loss

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One of the most common genetic diseases in human is hearing loss (HL). The majority of cases are nonsyndromic (70%) and 1-5% are nonsyndromic X-linked. Most cases are due to mutations in a single gene. Nevertheless, DNA diagnostics for hearing loss are challenging, since it is an extremely heterogeneous trait. Although more than 70 causative genes have been described for the nonsyndromic hearing loss alone, diagnostic application of the scientific progress has lagged behind. Some previous reports have shown that „next-generation DNA sequencing techniques" have the potential to offer a novel testing platform that could test all known genes in a sensitive, specific and cost-efficient manner. In this study, whole exome sequencing (WES) for direct genetic diagnosis in NSHL was used. Sequential filtering of variants obtained from WES, bioinformatic analyses, and Sanger sequencing validation identified premature termination p.Ala116fs mutation in POU3f4 gene as the candidate disease-causing mutation in the family. POU3F4 belongs to a subfamily of transcription factors, which are characterized by 2 conserved deoxyribonucleic acid-binding domains, a 75- amino acid POU- specific domain and a 60-amino acid homeodomain, both helix-turn-helix structural deoxyribonucleic acid-binding motifs. In this study clinical features and genetic analysis of a male child from a Polish family with congenital deafness and POU3F4 p.Ala116fs mutation is described. Usually clinical features of DFNX2 (DFN3) often include a mixed, progressive hearing loss, temporal bone anomalies, and stapes fixation.

P02.49-S
Common copy number variations on the origin of disease

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Introduction: It is well established the relation between CNV and intellectual disability and/or autism, as it is the existence of a large number of CNV not associated with disease. These polymorphisms although benign, could represent a risk of disease when occurring in homozigosity, or when combined, depending of its genetic content. Objective: We present a case of a girl with congenital deafness, and homozygous deletion of OTOA gene, resulting from the presence of a benign CNV in each chromosome. Method: PAMS, 8 yo was referred for genetic analysis because of congenital deafness. Conexin 26 and conexin 30 sequence analysis was done using Sanger sequencing, conexin 30 deletions was done using MLPA. Exclusion of additional genetic mutations was done using a proprietary mutation panel (312 mutations on 32 genes). Array CGH analysis was performed using Affymetrix Cytoscan 750K. Results: Conexin results were negative and CGC Mutation panel and direct sequencing results demonstrated absence of amplification on OTOA. Parents testing with array CGH revealed the presence of a CNV (heterozygous deletion) on 16p12.2 in each. Both CNV are registered on DGV as normal variants. However, each CNV encompasses the OTOA gene, thus leading to the conclusion that the co-existence of two overlapping (individually benign) CNVs was the mechanism leading to congenital deafness on the index case. Conclusions: This case reveals the utility of array CGH as a complement technique in the investigation of molecular mechanisms of genetic disease that allowed establishing the molecular diagnosis and proper genetic counseling to this family.

P03.01-S
In-house-designed synthetic probe set for MLPA identifies a novel chimeric CYP11B2/CYP11B1 gene in a patient with 11β-hydroxylase deficiency

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11ß-hydroxylase deficiency (11ß-OHD) represents the second most common cause of Congenital Adrenal Hyperplasia (CAH). It is caused by mutations in the *CYP11B1* gene localized in 8q21, 40 kb distant from its highly homologous *CYP11B2* gene codifing for aldosterone synthase. Pathological alleles derived from the asymmetric recombination of these two genes have been described. The formation of a chimeric *CYP11B2/CYP11B1* gene leads to 11ß-OHD; instead the chimeric *CYP11B1/ CYP11B2* gene causes the glucocorticoid-remediable aldosteronism (GRA). As the detection of these rearrangement is still laborious or indirect, our objective was to project a synthetic probe set for multiplex ligation-dependent probe amplification (MLPA) analysis in order to simplify the detection of these chimeric genes and other variation in the copy number of these genes. We disegned a set of 8 specific probes for both *CYP11B1* and *CYP11B2* genes to be used with the commercial control kit SALSA MLPA P300 Human DNA reference (MRC-Holland). The methods was tested on 15 control samples and then applied to 6 patients with the suspition of 11ß-OHD. The analysis with the CYP11 probe set has led to the identification of one copy number variation in an adult patient with adrenal rests misdiagnosed as 21-hydroxylase deficiency and not properly treated. He resulted compound heterozygous for a novel chimeric *CYP11B2/CYP11B1* gene, with the breakpoint region locolized within intron 2, and the known A306V mutation. In conclusion, the described MLPA kit represents an optimal complement to DNA sequence analysis in patients with 11ß-OHD, enabling detection of deletions, duplications or chimeric genes.

P03.02-M
A Novel mutation of FGD1 in four members of a Turkish Family with Aarskog-Scott Sydrome and growth hormone Therapy

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Aarskog-Scott syndrome (AAS), also known as facio-digito-genital dysplasia is a rare, clinically and genetically heterogeneous condition characterized by short stature, and facial, limb, and genital anomalies. The best characterized form of the syndrome which was caused by mutations in *FGD1* is inherited as an X-linked trait (MIM#305400). This gene located on the short arm of chromosome X (Xp11.21), includes 18 exons. The population prevalence of AAS is probably lower or equal to 1/25 000. The clinical singns may range from mild to severe. Herein, clinical features and intrafamilial heterogeneity of four affected individuals in a Turkish AAS family with a novel mutation were presented. Only one of these patients had received growth hormone therapy and this treatment provided height velocity gain in our patient.

P03.03-S
Deciphering variability of PKD1 and PKD2 genes in Italian patients affected by Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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ADPKD is the most common genetic nephron-pathology in humans, affecting about 1/1000 individuals. Aim of the present work was to define genetic variation of PKD1 and PKD2 in Italian patients affected by ADPKD. Analysis of PKD1 and PKD2 variation would allow to: confirm the diagnosis in clinically uncertain/atypical cases; offer genetic counseling in at risk families; exclude the presence of a mutation in related donors for kidney transplantation; define gene variability in Italian patients. 576 subjects has been analyzed with a semi-automated Sanger protocol: 298 unrelated patients belonging to families and 171 relatives; 72 patients with no familiarity and 35 cases with no data about familiarity. In 90% of the 405 probands, variants were present: PKD1 80%; PKD2 4%; PKD1+PKD2 6%. 84.5% of the identified variants have never been described. An average of 12 SNPs/patient in PKD1 and 2 SNPs/patient in PKD2,was observed. By combining results for truncating and known variants we classified variants as pathogenic in 65.4% (265/405) of patients. For unclassified variants, a prediction was attempted according to PKDB criteria. Concordance of the results obtained with SIFT, AGVGD and PolyPhen2 allowed calling of 18 likely-neutral and 12 highly-likely-pathogenic variants. In many cases interpretation of additional information becomes relevant (i.e. family segregation increases the score for pathogenicity). In patients with no mutations detected, MLPA analysis has been performed: we identified 2 patients with a huge deletion in PKD1 (ex2-

46); 1 patient with a deletion spanning exons 2-4 in PKD1; 1 patient with the deletion of the whole PKD2 gene.

P03.04-M
Scattered deletion of Pkd1 in mouse kidneys causes a cystic snowball effect and recapitulates human polycystic kidney disease

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Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) patients carry a germline mutation in *PKD1* or *PKD2*, leading to thousands of kidney cysts. It is poorly understood why a rapid progression of the disease is preceded by a lag-phase of several decades. Studies in which *Pkd1* is inactivated in large percentages of cells in animal models, led to the presumption that inactivation of the remaining allele initiates cystogenesis, and that the progression can be accelerated by renal injury. To mimic human ADPKD, we lowered this percentage and found important characteristics of cystogenesis that have not been described before. **Methods:** The percentage of *Pkd1*-deficient cells in Tamoxifen-inducible kidney-specific *Pkd1*-deletion mice was controlled by varying the tamoxifen dose, visualized with reporter mice and quantified by eMLPA. Several renal injuries were applied. Cyst progression was followed by MRI-analysis and PKD-related signaling was analyzed by Immuno-histochemistry. **Results:** Interestingly, no pathological changes occurred for six months after scattered-*Pkd1*-deletion and renal injury did not trigger rapid PKD. However, in the following 3-4 months, clustered cyst formation led to a severe human-like PKD phenotype. This shift was preceded by increased pSTAT3, pCREB, pERK1/2, LCN2 and Ki-67 expression near the initial cysts. **Conclusions:** Our data argue against the presumption that renal injury is the major trigger for cystogenesis but suggests that initial cysts themselves, by imposing persistent stress on surrounding tissue, triggers a ‚snow-ball' effect driving the formation of new cysts. In addition, our model mimics human ADPKD more precisely and can be used for pre-clinical testing.

P03.05-S
Identification and characterisation of six novel SERPINA1 null mutations

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Alpha-1 antitrypsin (AAT) is the most abundant circulating antiprotease and is a member of the serine protease inhibitor (SERPIN) superfamily. The gene encoding AAT is the highly polymorphic SERPINA1 gene, found at 14q32.1. Mutations in the SERPINA1 gene can lead to AAT deficiency (AATD) which is associated with a substantially increased risk of lung and liver disease. The most common pathogenic AAT variant is Z (Glu342Lys) which causes AAT to misfold and polymerise within hepatocytes and other AAT-producing cells. A group of rare mutations causing AATD, termed Null or Q0, are characterised by a complete absence of AAT in the plasma. While ultra rare, these mutations confer a particularly high risk of emphysema. We report here the identification and characterisation of 6 new SERPINA1 Null mutations.

P03.06-M
Chromosome Microarray and non-coding DNA copy number variants - a case of Alveolar Capillary Dysplasia at FOXF1 locus

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Chromosome microarray (CMA) analysis typically focuses on coding DNA (RefSeq and OMIM genes). Although non-coding intergenic and intronic variants may be critical in disease pathogenesis, copy number variants (CNV) in these regions are usually interpreted as variants of unknown clinical significance. We present a case of a lethal neonatal condition in which the pathogenic CNV lies in a distant, upstream non-coding region. It highlights the importance of relevant clinical information for CMA interpretation, and the importance of the analysis of flanking regions if an identified CNV does not initially appear pathogenic. A term female, with a prenatal diagnosis of AVSD, presented with severe neonatal respiratory distress out of keeping with her cardiac issues. The clinical picture and early lethality suggested congenital surfactant deficiency (CSD). Sequencing of a CSD-gene panel was normal. CMA analysis revealed a *de novo* 1.5 Mb deletion at 16q24.1. None of the 16 RefSeq genes mapping within the deleted region appeared causative. However, this deletion is located 157 kb upstream of *FOXF1*, a gene responsible for congenital alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV). The observed deletion encompasses a recently characterized distant regulator/enhancer of the *FOXF1* gene. The pathological diagnosis of ACDMPV was confirmed posthumously. As our knowledge of epigenetics and the genomic landscape improves, an increasing number of non-coding CNVs are poised to gain clinical relevance. We suggest that a database of well-characterized non-coding regulatory regions be developed and incorporated into CMA analysis.

P03.07-S
Novel mutations of PKD1 gene in autosomal dominant polycystic kidney disease (ADPKD)

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Background Polycystic kidney disease (*PKD*) can be inherited as an autosomal dominant (*ADPKD*) or an autosomal recessive trait (*ARPKD)*. *ADPKD* is one of the most common genetic diseases in humans affecting all ethnic groups worldwide with an incidence of 1 in 500 to 1 in 1,000. *ADPKD* is genetically heterogeneous and can arise from mutations in two genes, named *PKD1* and *PKD2*. Mutations of *PKD1* located on chromosome 16p13.3 are responsible for 85% of cases. **Aim** Although there is no hotspots reported in *PKD1*, most mutations are seen in the downstream exons of this gene. So, we developed a clinical assay for *PKD1* gene analysis using sequencing of 16 exons from 31 to 46 in 22 patients. **Material and Method** This study was carried out with a total of 22 patients. Genomic DNA was extracted from blood lymphocytes (5ml of whole blood) with standard methods and fragments were amplify using PCR. Screening of *PKD1* mutations was performed by direct Sequencing. **Result** Sequencing result of exons 44 and 45 showed one completely pathogenic mutation causing Gln4005Arg change. Two novel non-synonymous variation including Arg4091Glu and Val4035Ilu and five likely neutral SNP including rs10960, rs3087632, rs3087632, rs10960, rs3087632, rs3087632 are detected. There was no any variation in exons 35, 36 and 37. **Conclusion** It is expected that confirmation of pathogenic mutations can be useful for studies on disease molecular pathways. Early and prenatal diagnosis and personalized treatments recommended for family members after genetic consoling.

P03.08-M
Molecular diagnostics of autosomal recessive polycystic kidney disease by next-generation sequencing

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Autosomal recessive polycystic kidney disease (ARPKD) is a severe form of PKD with typical occurrence in neonates and infants. It is characterized by cystic dilations of the collecting ducts and congenital hepatic fibrosis. Perinatal mortality has been estimated to account 30-50% of ARPKD neonates and the majority of patients develop renal failure within 5 years, however presentation of ARPKD at later age and survival into adulthood have been also described. The aim of this work was to establish molecular diagnostics of ARPKD which could be used in "at-risk" families, as fetal ultrasonography has limited reliability in early pregnancy and some abnormalities typical for ARPKD become evident after 20 weeks of gestation. ARPKD is caused by mutations in PKHD1, whose longest transcript comprises 67 exons. Conventional mutational detection methods are therefore time-consuming and relatively expensive. For this reason next-generation sequencing has been chosen as a suitable method. After the setting up of NGS for PKHD1 gene, the analysis of 9 infants/children and 1 fetus from interrupted pregnancy, all with clinically suspected ARPKD, was carried out. Two mutations were found in 5 patients and one mutation in 3 patients. No mutation was identified in two patients, which could be caused by misdiagnosis. Interesting combination of p.G2705fsX and p.S2861G in cis appeared 3 times in our group of patients. Next-generation sequencing seems to be relatively fast, sensitive and cheaper in comparison to other methods and allows us molecular diagnostics of ARPKD. Supported by the grant project IGA MZCR NT 13090-4 and PRVOUK- P25/LF1/2.

P03.09-S

Dysregulation of cytoskeleton organization, carcinogenesis and immune response pathways involved in Balkan endemic nephropathy development

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Background: Balkan endemic nephropathy (BEN) is a chronic interstitial nephritis with endemic distribution, spreading over territories of several Balkan countries. The intricate interplay between genetic background and environmental factors is believed to be at the basis of this progressive disease leading to chronic kidney failure and associated with high incidence of upper urinary tract uroepithelial cancer.
DNA methylation is the most studied primary epigenetic mechanism that is involved in processes such as cancer, genomic imprinting, tissue differentiation etc. Epigenetic alterations could be a major contributing factor to BEN's elusive etiopatogenesis.
Materials and methods: We performed whole genome DNA methylation analysis on DNA blood samples from 159 affected individuals and 170 healthy controls assigned to 8 DNA-pools based on gender and ancestral origin (Bulgarian and Serbian). After determining the methylation status of ca.27000 CpG-islands throughout the whole genome (Agilent DNA methylation array 1x244k) we defined the differently methylated regions (DMRs) between patients groups and respective control groups. We then compared DMRs across different ancestral and gender groups.
Results: Analysis of the common DMRs between patient-control pairs revealed that genes involved in major biological processes appear to be affected in BEN - cell adhesion and cytoskeleton organization/regulation of cell cycle - 14.8% of DMRs in both Bulgarians(BG)/Serbians(SER), carcinogenesis and metastasis - 7.41%(BG)/8.8%(SER) and immune response - 14.8%(BG)/6.4%(SER) respectively.
Conclusions: Data obtained from our experiments suggest that dysregulation of multiple major pathways such as cytoskeleton organization; cell cycle regulation and immune response could contribute to BEN pathogenesis.
Acknowledgements: funded by BNSF grant DMY03/35

P03.10-M

NGS nominated CELA1, HSPG2 and KCNK5 as candidate-genes for predisposition to Balkan Endemic Nephropathy

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Balkan endemic nephropathy (BEN) is a familial chronic tubulointerstitial disease with insidious onset and slow progression leading to terminal renal failure. The results of molecular biological investigations propose that BEN is a multifactorial disease with genetic predisposition to environmental risk agents.
Exome sequencing of 22 000 genes with Illumina Nextera Exome Enrichmen kit was performed on 22 DNA samples (11 Bulgarian patients and 11 Serbian patients). Software analysis was performed via NextGene, Provean and PolyPhen. The frequency of all annotated genetic variants with deleterious/damaging effect was compared with those of European populations. Then we focused on non-annotated variants (with no data available about them and not found in healthy Bulgarian controls).
There is no statistically significant difference between annotated variants in BEN patients and European populations. From non-annotated variants with more than 40% frequency in both patients' groups, we nominated 3 genes with possible deleterious/damaging variants - CELA1, HSPG2 and KCNK5.
Mutant genes (CELA1, HSPG2 and KCNK5) in BEN patients encode proteins involved in basement membrane/extracellular matrix and vascular tone, tightly connected to process of angiogenesis. We suggest that abnormal process of angiogenesis plays a key role in the molecular pathogenesis of BEN.

P03.11-S

Whole exome sequencing reveals rare homozygous ARID1B and heterozygous MTOR missense variants in a patient with bilateral cystic-dysplastic kidneys and features of Coffin-Siris syndrome and tuberous sclerosis

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ARID1B variants have recently been described as a major genetic cause of Coffin-Siris syndrome, a developmental disorder associated with anomalies of the kidneys and urinary tract (CAKUT). Of 82 patients with Coffin-Siris syndrome carrying an ARID1B variant and examined for renal anomalies that have been described in the literature to date, 6 (7%) presented with a non-severe CAKUT phenotype. The causative ARID1B mutations detected in patients with Coffin-Siris syndrome so far were all heterozygous truncating variants. Here, we report a rare homozygous ARID1B missense variant detected by whole exome sequencing in a patient with bilateral cystic-dysplastic kidneys, bilateral vesicoureteral reflux grade IV, and a septal myocardial tumor. Stage 4 chronic kidney disease was diagnosed at 6 months of age, arterial hypertension developed, and pre-emptive kidney transplantation was performed at age 3.5 years. Additionally, the patient presented with a number of features frequently described in patients with Coffin-Siris syndrome carrying ARID1B mutations, such as thick eyebrows, synophris, thick eyelashes, strabism, anteverted nares, long philtrum, high arched palate, sandal gap, and behavioral anomalies. However, unlike all patients with heterozygous truncating ARID1B mutations, our patient had normal intelligence. Interestingly, whole exome sequencing also revealed a rare deleterious heterozygous MTOR missense variant inherited from a healthy mother in our patient. mTOR is regulated by TSC1/TSC2, encoded by genes that when mutated can cause tuberous sclerosis associated with renal cystic disease and myocardial tumors. Therefore, the MTOR and ARID1B variants may have acted together to cause the severe bilateral cystic-dysplastic kidneys present in our patient.

P03.12-M

New genetic abnormalities underlying chronic intestinal pseudo-obstruction (CIPO)

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Background and Objective: CIPO is a severe motility disorder affecting the entire gut. Most patients are sporadic, however some CIPO cases show a familial clustering suggesting genetic components. The aim of this study was to identify a CIPO cause in a consanguineous family with several affected members. Results: Whole exome sequencing in the affected individuals revealed a novel homozygous mutation, p.622 Ala>Thr in RAD21, cohesin complex subunit regulating cell replication and gene expression. RAD21 screening in 33 sporadic CIPO patients did not show coding mutations. *RUNX1* expression, a RAD21 target, was decreased in the patient's cell line. *Rad21* morpholino knockdown in zebrafish embryos resulted in incomplete or absent expression of *Runx1*, in severe reduction of enteric neurons and delayed intestinal transit. These defects could be rescued only by wild-type *Rad21* cDNA, indicating that the mutation found in the CIPO pedigree extinguishes RAD21 functionality. Several binding sites for RAD21 were present in apolipoprotein A/C gene cluster and its altered binding to the regions dysregulated apolipoprotein expression levels. We studied APOB promoter and identified two RAD21-putative binding sites. EMSA assays showed that RAD21 wild-type, but not mutant, binds these regions. We therefore measured APOB protein levels that were increased in all CIPO patients, including the syndromic patient carrying the RAD21 mutation. Conclusion: The study of these two previously unlinked pathways in patients and in model organisms is contributing to clarify the pathogenesis of CIPO and will bear clinical implications for the diagnosis and prognosis of this disabling condition.

P03.13-S

Genotype-phenotype discrepancy in congenital adrenal hyperplasia due to trimodular haplotype of the RCCX module: the case of two Italian families

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Congenital Adrenal Hyperplasia (CAH) due to 21-hydroxylase deficiency, encoded by CYP21A2 gene, is an autosomal recessive disorder with variable

incidence among the various groups. The CYP21A2 gene, is localized in a genetic unit defined RCCX module and is considered one of the most polymorphic of human genes.
We considered new evidences about the presence of a RCCX trimodular haplotype with a CYP21A2-like gene to explain the lack of a genotype-phenotype correlation in two patients referred to our centre for a suspected Non Classical form of CAH (NC21OH).
To identify the presence of deletions/duplications we used Multiplex Ligation Probe-Dependent Amplifications (MLPA) and to confirm the presence of a CYP21A2-like gene downstream TNXA gene we used previously described amplification and restriction strategy followed by the sequencing of the CYP21A2 gene downstream TNXB gene.
We validated the methods developed by recent studies and we found a good concordance with their results. The amplification strategy, direct sequencing and restriction analysis of CYP21A1P/CYP21A2-TNXA PCR product in association with MLPA assay and sequencing of CYP21A2 gene downstream TNXB gene, were able to identify the presence of more than one copy of CYP21A2 gene.
The strategy suggested is useful to screen cases where there is no genotype-phenotype correlation. We validated the modified screening strategy to facilitate molecular testing of CAH patients, considering the new evidence about possible different haplotypes.

P03.14-M
Familial mediterranean fever (FMF): genetics and role of S100A4

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Number of patients with FMF in Armenia is dramatically increasing due to genetic drift and better professional competence, treatment, genetic counselling. Clinical and genetic investigations of FMF are "forced" by the high social and public health problems. Common MEFV mutations account for 98,71% of FMF patients and 1:3 in healthy population. 18.72% of heterozygote carriers have abortive or mild features; no mutations detect in 1.29% of FMF patients. Particular MEFV mutations (M694V homozygotes) have significant correlation with renal amyloidosis (RA). The risk of male patients to develop RA is four times higher than that of female patients. Colchicinotherapy delays RA progression, but patients with M694V-homozygous genotype present a more severe phenotype and a limited response to colchicine at the nephrotic stage of RA. Patients with other genotypes have a good chance to escape the nephrotic syndrome and to maintain renal function.
Serum amyloid A1 (SAA1) α/α with M694V homozygous genotypes are associated with a seven-fold increased risk of developing RA, compared to other SAA1 genotypes. The presence of one SAA1 α/α allele does not suggest an increased susceptibility to RA.
Association of pro-inflammation with pathogenesis of neoplasia, inflammatory, autoinflammatory diseases is confirmed. Correlation of S100A4 concentration with a pattern of MEFV mutations has been analyzed. Data demonstrating a significant increase of S100A4 in plasma of 100 FMF patients likely implicating of S100A4 in the pathogenesis of the disease. These findings suggest that chronic inflammation is mediated by S100A4 and SAA proteins, thus therapeutic targeting of pro-inflammatory pathways should be effective.

P03.15-S
Mutations in PAX2 Are Associated with Adult-onset FSGS

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Focal and segmental glomerulosclerosis (FSGS) is a histologically defined form of kidney injury characterized by the presence of partial sclerosis of some but not all glomeruli. Studies of familial FSGS have been instrumental in identifying podocytes as critical elements in maintaining glomerular function. Exome sequencing in members of an index family with dominant FSGS revealed a nonconservative, disease-segregating variant in the PAX2 transcription factor gene. Sequencing in probands of a familial FSGS cohort revealed seven rare and private heterozygous single nucleotide substitutions (4% of cases). Further sequencing revealed seven private missense variants (8%) in a cohort of individuals with congenital abnormalities of the kidney and urinary tract (CAKUT). As predicted by in silico structural modeling analyses, in vitro functional studies documented that several of the FSGS-associated PAX2 mutations perturb protein function by either affecting proper binding to DNA and transactivation activity, or altering the interaction of the transcription factor with repressor proteins, resulting in enhanced repressor activity. Thus mutations in PAX2 contribute to adult-onset FSGS in the absence of overt extrarenal manifestations. We expand the phenotypic spectrum associated with PAX2 mutations, which have been shown to lead to CAKUT as part of papillorenal syndrome. PAX2 mutations can cause disease through haploinsufficiency and dominant negative effects, which could have implications for tailoring individualized drug therapy in the future.

P03.16-M
Genetic and bioinformatics analysis of four novel GCK missense variants detected in Caucasian families with GCK-MODY phenotype

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Heterozygous loss-of-function mutations in the glucokinase (GCK) gene cause a subtype of maturity-onset diabetes of the young (MODY) known as GCK-MODY/MODY2 . Diagnostic GCK sequencing identified 16 distinct mutations (13 missense, one nonsense and one splice-site substitutions, and one frameshift deletion) in 23 out of 25 unrelated GCK-MODY probands, all of which showed to co-segregate with hyperglycaemia. Four of the 13 missense substitutions (c.718A>G/p.Asn240Asp, c.757G>T/p.Val253Phe, c.872A>C/p.Lys291Thr, and c.1151C>T/p.Ala384Val) were novel, and the nonsense mutation (c.76C>T/p.Gln26*) segregated in seven families from north-eastern Italy, suggesting a founder effect in this geographical region. We focused on the novel missense variants to test whether an accurate and multi-level bioinformatics approach could satisfactorily strengthen family-genetic evidence for potential pathogenicity of such a type of variants in the routine diagnostic field, where wet-lab functional assays are generally not viable. In silico analyses of the novel missense variants (orthologous sequence conservation, SIFT, PolyPhen-2 and MutationTaster2 predictors, structural modeling, and splicing predictors) suggested that the encoded amino acid substitutions and/or the underlying nucleotide changes are likely to affect GCK structure/function. In conclusion, this study shows how a careful and multi-level bioinformatics analysis could provide effective suggestions to help a molecular-genetic diagnosis in the absence of wet-lab validations.

P03.17-S
Genetic spectrum of growth hormone deficiency: Findings from the Indian patient population

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Background: Isolated growth hormone deficiency (IGHD) and combined pituitary hormone deficiency (CPHD) result in significant short stature caused by mutations in *GH1, GHRHR PROP1, POUIF1* and *HESX1* genes. The present study describes the mutation profile of Growth Hormone Deficiency (GHD) patients from India. One hundred each of patients (60 IGHD, 40 CPHD) and controls were recruited for screening of G*H1, GHRHR, PROP1* and *POU1F1.*
Results: Consanguinity, family history and mutations were present in 20%, 8% and 35% of the cases respectively. Five novel and four reported mutations were identified (Table-1). *GHRHR* mutations were the most common change in IGHD followed by *GH1* cluster deletion. A novel *GH1* coding region deletion and three promoter SNPs were identified. These SNPs are reported to induce differential transcriptional activity resulting in reduced secretion of growth hormone. Novel insertion and nonsense *PROP1* mutations identified lead to frameshift and a truncated protein leading to GHD. Novel *POU1F1* splice site mutation in three patients from the same geographical region implies possible presence of founder effect.
Conclusion: This is the first report demonstrating genetic contribution to GHD in our population wherein 'Glu72Term', the most common change and five novel mutations were identified. Hotspot mutations reported worldwide were absent suggesting a distinct profile. Results of the study will facilitate timely diagnosis, counseling and intervention thereby reducing the stigma associated with GHD.

Table-1 Mutations/SNPs identified in the present study

S.No	Type of GHD (N=100)	Gene	cDNA position	Amino acid position	Nature of mutation/SNP	Frequency
1	IGHD (n=60)	*GH1* gene cluster	Gross deletion	-	Reported	15%
2		*GH1*	Deletion of exons 3-5	-	**Novel**	2%
3		*GH1* promoter	rs2005171	-	Reported	20%
4		*GH1* promoter	rs2005172	-	Reported	15%
5		*GH1* promoter	rs11568828	-	Reported	18%
6		*GHRHR*	c.214G>T	Glu72Term	Reported *(Nonsense)*	10%
7		*GHRHR*	c.527C>T	Ala176Val	Reported *(Missense)*	3%
8		*GHRHR*	c.920insC	-	**Novel** *(Insertion)*	2%
9	CPHD (n=40)	*PROP1*	c.112_124del13	-	Reported *(deletion)*	2.5%
10		*PROP1*	c.45C>T	Arg16Term	**Novel** *(Nonsense)*	2.5%
11		*PROP1*	c.128InsA	-	**Novel** *(Insertion)*	2.5%
12		*POU1F1*	c.605-1G->A	-	**Novel** *(Acceptor splice site)*	7.5%

P03.18-M

Exonic *de novo* Mutations in Sporadic Hirschsprung Disease

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Hirschsprung disease (HSCR) is a disorder of the enteric nervous system (ENS) and is characterized by the absence of enteric neurons along a variable length of the intestine. HSCR most commonly presents sporadically, although it is familial in 5-20% of the patients. The sporadic form of the disorder is believed to be a genetically complex disease. To assess the role of *de novo* mutations in sporadic HSCR, we performed exome sequencing on 20 HSCR patients, predominantly females with long segment HSCR and their unaffected parents. We identified and confirmed 24 *de novo* mutations (18 SNVs, 6 Indels) in 17 different genes (1.2 per trio). Non-synonymous *de novo* mutations were identified in RET in 8 out of 20 patients, corroborating previous findings that RET is the major genetic contributor in long-segment HSCR. A replication study in independent

HSCR patients, gene burden tests and functional analysis in both cell lines and zebra fish are currently being conducted. Interestingly, some of the genes harboring *de novo* mutations are members of pathways involved in the development of the ENS and the encoded proteins interact with known key signaling molecules. We will present all data which will enable us to make conclusion on whether, *de novo* mutations in genes other that RET also contribute to the development of sporadic HSCR.

P03.19-S

RET and EDNRB mutations screening in Indonesian patients with HSCR, functional study and its implication in diagnostic

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Hirschsprung disease (HSCR) is a majority cause of chronic constipation in children. HSCR is an inherited disease and germline mutations are frequently identified in *RET* and *EDNRB.* However, defining causality of mutations in this genetic complex disorder is difficult. Therefore, in this study we combined sequence analysis of *RET* and *EDNRB* with functional studies to determine causality. We screened a total of 61 HSCR children and identified 8 rare RET coding variants (R79W, R144H, P270L, R694Q, A756V, G533S, Y1062C,D489N) and one possible splice-site variant. No rare variant in EDNRB were identified. The mutation frequency (15%) is comparable to previous studies. Four missense variants and one possible spice site variant have never been reported before. Four of the nine arose *de novo*, while the remaining variants are all inherited from an unaffected parent. One patient harbor two RET coding variants, one coming from each unaffected parent. Functional studies showed that 7 out of 8 coding RET variants resulted in significant lower expression of the phosphorylated-RET protein. Only 4 RET variants (R144H, R694Q, A756V, Y1062C) resulted in significantly lower expression of phosphorylated-ERK, a downstream component of the RET pathway, when compare to wild type RET. The possible splice-site variant (IVS 1880-4A>G) did not disturb the splicing process and, therefore, is not considered pathogenic. Our data suggest that 7 of the 9 identified RET variants are pathogenic and that not all rare RET variants are pathogenic, hence functional studies are essential to prove pathogenicity.

P03.20-M

Translating genetic findings in functional defects: functionomics of hypomagnesemia-causing genes

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The recent improvements of next generation sequencing techniques allow relatively rapid and cheap identification of new gene mutations in patients with familial hypomagnesemia. However, translating the genetic findings into functional assays to examine the function of the affected genes remains challenging because of inadequate cell models, the absence of an Mg2+ radioisotope and limited availability of animal models.

For example, we have identified new mutations in the gene CNNM2 in five families suffering from mental retardation, seizures, and hypomagnesemia. For the first time, a recessive mode of inheritance of CNNM2 mutations was observed and mutations in CNNM2 are associated with mental disability. Using stable Mg2+ isotopes, we demonstrated that CNNM2 increases cellular Mg2+ uptake in HEK293 cells and that this process occurs through regulation of the Mg2+-permeable cation channel TRPM7. In contrast, cells expressing mutated CNNM2 proteins did not show increased Mg2+ uptake. Knockdown of cnnm2 isoforms in zebrafish resulted in disturbed brain development and reduced body Mg content. These phenotypes were rescued by injection of mammalian wild-type Cnnm2 cRNA, whereas mammalian mutant Cnnm2 cRNA did not improve the zebrafish knockdown phenotypes. Altogether these data show that CNNM2 is fundamental for brain development, neurological functioning and Mg2+ homeostasis.

By establishing a novel Mg2+ transport assay using stable Mg2+ isotopes and the loss-of-function zebrafish model, we provide a unique system to examine the function of novel genes in Mg2+ homeostasis. These new in vitro and in vivo models may aid to explain the function of electrolyte transporters in the future.

P03.21-S

Family genomics reveals disease genetics: Inflammatory bowel disease

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By definition, complex diseases are caused by many different genes, but this might hold true only at the population level. We hypothesize that within families with a high disease burden, complex diseases can arise from only a few high-effect risk variants. Assuming that all or most affected family members share the genomic region(s) that harbor the risk variant(s), whole-genome sequencing is the most precise method for determination of identical-by-descent segments to narrow down the search space for disease genes. Family genomics is especially powerful in multi-generation pedigrees. In some of the pedigrees we analyze diseased individuals are separated by more than 10 meioses each of which narrows the number of candidate alleles by approximately half, per Mendel's law of segregation. Family genomics furthermore permits identification of sequencing errors and detection of rare variants with high confidence.

In addition to our identical-by-descent detection tools we have created an analysis workflow to identify and score variants according to their inheritance pattern, population frequency and predicted function. We are currently analyzing over 200 families in over 30 studies that cover a wide range of diseases, from rare congenital diseases to common neurodegenerative and chronic inflammatory diseases. We present here our methods for identifying high confidence candidate variants for inflammatory bowel disease in families with high disease burden.

P03.22-M

A hemizygous mutation in retinitis pigmentosa GTPase regulator (*RPGR*) is a potential novel cause of congenital renal tract malformations

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Congenital anomalies of the kidney and urinary tract (CAKUT) comprise a range of structural malformations and constitute the principal cause of end-stage renal disease in children. Although several genes have been linked to CAKUT pathogenesis, the genetic background remains unclear in many patients. We performed whole exome sequencing in the patient's DNA (SureSelect, Agilent / SOLiD5500™ System, Life Technologies). Through whole exome sequencing we identified a novel hemizygous dinucleotide frameshift deletion in the X-linked retinitis pigmentosa GTPase regulator (*RPGR*) gene of a male patient with unilateral kidney dysplasia. This mutation was confirmed through Sanger sequencing and was found to be inherited from the mother. Mutations in *RPGR* are frequently reported in patients with progressive retinal degeneration, however its potential role in kidney pathogenesis has not previously been observed. At present, we investigate the effect of *RPGR* dysfunction on kidney development *in vivo*, by generating zebrafish mutants using transcription activator-like effector nucleases (TALENs). Subsequently, complementation assays will be performed to study the specific effect of the human *RPGR* mutation in this vertebrate system. Also, we aim to characterize both wild-type and mutant RPGR *in vitro*, using patient-derived cell-lines isolated from urine and IMCD3 (murine inner medullary collecting duct) 3D cellular spheroids. To conclude, by identifying novel players in the aetiology of CAKUT, we will enhance our knowledge on the molecular networks underlying normal and disrupted kidney development. Identification of the genetic aetiology of CAKUT will improve DNA diagnostics and genetic counselling for CAKUT patients.

P03.23-S

The Prognosis of Abnormal Fetal Kidneys

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Introduction: With the advent of high-resolution ultrasound scanners and the offer of a 20-week ultrasound scan to all pregnant women in Denmark, a suspicion of abnormal kidneys in the fetus is raised more frequently now than earlier. The prognosis of a fetus with abnormal kidneys is often not known, which complicates decision-making and causes anxiety to parents.
Methods: A population-based cohort of fetuses diagnosed with abnormal kidneys in the period 1st of January 2007 - 31st of December 2012 and a population-based matched comparison cohort of foetuses examined in the 20-week screening programme were established.
A national query identified fetuses with cystic kidneys, echogenic kidneys, multicystic kidneys, renal agenesia and/or severe hydronephrosis. Severe hydronephrosis was included in the query to avoid missing cases of multicystic kidneys, as the primary finding may have been interpreted as hydronephrosis.
All Danish departments of obstetrics and fetal medicine use the same software for prenatal diagnostics and ultrasound examination. These data were merged into a national dataset of around 1000 pregnancies. Prenatal fetal data were linked to their mothers' data using the mothers' civil registration numbers. This allows unambiguous individual-level linkage to all relevant data sources in Denmark, including the Danish National Patient Registry and medical journals.
Results: The prevalence of the various types of abnormal fetal kidneys, the prevalence of terminated pregnancies and measures of prognosis will be discussed. This study may facilitate that parents having a fetus with kidney abnormalities can be counselled based on valid and comprehensive data on prognosis.

P03.24-M

Targeted sequencing of 208 candidate genes in 460 CAKUT patients facilitates the inclusion of a novel gene set in diagnostics

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Congenital anomalies of the kidney and urinary tract (CAKUT) comprise a spectrum of structural malformations. CAKUT occur in 1:500 live-births and form the most common cause of end-stage renal failure in children. We aim to identify rare mutations in CAKUT candidate genes and elucidate involvement in CAKUT aetiology. After in-solution enrichment (SureSelect, Agilent), 208 candidate genes were sequenced (SOLiD5500™, Life Technologies) in 460 sporadic CAKUT patients. All genes were known to play a role in human CAKUT or to disrupt nephrogenesis in animal models. We demonstrated coverage depth of 120X and 65% enrichment efficiency on average. After variant calling, filtering was performed based on sequencing depth (≥15X) and allele frequency, excluding common variants. We identified 47 indels, 20 nonsense, 22 essential splice-site mutations, as well as 150 novel missense variants in 82 genes that were predicted to be pathogenic. Of these, 71 variants in 39 genes were previously reported in The Human Gene Mutation Database as causal mutations for kidney-related disorders. The variants are currently being validated in patients and parents by Sanger sequencing. Preliminary data show that the majority of variants are inherited, except for a well-known frameshift variant in *PAX2* and a novel variant in *LZTS2*, which occurred *de novo*. We conclude that our approach provides knowledge on the frequency of known disease-causing mutations and underlines the heterogeneity of CAKUT. Results indicate the value of including novel genes in an NGS based genetic test for CAKUT, facilitating early diagnostics and genetic counselling for CAKUT patients and their relatives.

P03.25-S

Renal fibrosis is the common feature of Autosomal Dominant Tubulointerstitial Kidney Diseases (ADTKD) caused by MUC1 or UMOD mutations

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For decades, clinically ill-defined autosomal-dominant renal diseases originating from tubular cells and leading to tubular atrophy and interstitial fibrosis were reported. Patients exhibit mutations in at least 4 genes: UMOD, HNF1B, REN, MUC1, but are clinically indistinguishable all associated with renal fibrosis and renal failure the 3rd and 6th decade of life. In contrast to what the frequently used term "Medullary Cystic Kidney Disease" (MCKD) implies, development of medullary cysts is neither an early, nor a typical feature, as analyzed by MRI.
We now investigated 10 such families. In two large families we performed genome-wide linkage and haplotype analyses confirming linkage to a known 3.4 Mb locus MCKD1 locus on chromosome 1q21. Targeted genomic sequencing of the complete linkage locus in affected and healthy individuals of these two families and affected individuals of further families failed to uncover any segregating variant in any of the genes, including MUC1. The VNTR region in the coding sequence of the MUC1 was masked in these analyses due to its high GC-content and repetitive nature. After the recent publication of one MUC1-VNTR insertion mutation we established SNaPshot-minisequencing confirming this MUC1 mutation in 4 families. Further 3 families carried an UMOD mutation, leaving 3 families unsolved to date. On the basis of clinical and pathological characteristics we propose the term "Autosomal Dominant Tubulointerstitial Kidney Disease" (ADTKD) as a new name for this entity. This new terminology should enhance recognition and correct diagnosis of affected individuals, facilitating genetic counseling and stimulating research into the underlying pathomechanisms.

P03.26-M

The promise and challenge of high throughput sequencing to discover genes involved in Medullary Sponge Kidney disease

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Medullary sponge kidney (MSK) is a rare, developmental congenital disease characterized by diffuse ectasia or dilation of precalyceal collecting tubules. It is generally considered a sporadic disorder, but an apparently autosomal dominant inheritance has also been observed. The diagnosis of MSK is radiographic: typical pictures reveal collections of contrast medium in dilated papillary ducts, giving the appearance of a blush or linear striations in the mildest cases, or of bouquets of flowers when cystic dilation of the collecting ducts is seen. We previously described the association of MSK with mutations in ATP6V1B1 and ATP6V0A4, responsible of distal renal tubular acidosis (dRTA), a condition characterized by hypokalemia, hyperchloremic metabolic acidosis, nephrocalcinosis, nephrolithiasis, osteomalacia and rikkets. In this disorder the α-intercalated cells in the collecting duct are unable to secrete H+ and to acidify urine. The aim of our study was to investigate, on the basis of family history and clinical presentation at various ages, the relationship between MSK and dRTA: we studied first the possible role of the B1 and a4 apical subunits of the H+ATPase pump (at present 8 patients show pathogenetic variations in these two subunits) and afterwards whether other genes could be causative of this condition. We investigated, by high throughput sequencing, 25 patients classified as having MSK and we studied, in addition to the role of the above mentioned genes, other genes encoding proteins expressed in the tubules and genes implicated in the collecting duct system development. We will show the results of this new pilot study.

P03.27-S
MODY/Type 2 Diabetes: molecular analysis of a large cohort of patients by Next Generation Sequencing

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Maturity-onset diabetes of the young (MODY) is a phenotypically and genetically heterogeneous group of diabetes caused by single defects in at least thirteen genes, characterised by autosomal dominant inheritance, a young age of onset and pancreatic β-cell dysfunction. Genetic testing for MODY has become a routine procedure allowing to set up proper treatment and discriminate from type 2 diabetes (T2D) whose symptoms are often overlapping. We analysed, in the last 5 years, more than 300 Italian families with MODY/T2D diagnosis by direct sequencing of genes GCK, HNF1α, HNF4α, IPF1 and HNF1β, and only about 40% of the cases resulted positive. Mutations in GCK gene account for up to 80% for all Italian MODY cases according to literature data.
We then reanalysed the negative subjects through Next Generation Sequencing technology. We excluded common, non-coding and synonymous gene variants, and performed in-depth analysis on filtered sequence variants in a pre-defined set of 102 genes implicated in glucose metabolism. We found, in association with known heterozygous SNPs associated with diabetes, rare and pathogenetic variants, demonstrating that this approach leads to a genetic diagnosis in most of patients. For example, we identified new variants in the RFX6 gene and in two of these cases we also detected rare variants in WFS1 and ABCC8. All patients showed a good therapeutic response to dipeptidyl peptidase-4 (DPP4) inhibitors.
This approach may help in understanding the molecular aetiology of diabetes and in providing a more personalised treatment for each genetic subtype.

P03.28-M
A molecular genetic analysis of nephrotic syndrome in a cohort of Tunisian families

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Background: Nephrotic syndrome (NS) is a renal disease characterized by heavy proteinuria, hypoalbuminemia, edema and hyperlipidemia. Its presentation within the first 3 months of life or in multiple family members suggests an underlying inherited cause. Mutations in the NPHS1 and NPHS2 genes are among the main causes of early-onset and familial steroid resistant nephrotic syndrome. This study was carried out to assess the frequencies of mutations in these two genes in a cohort of Tunisian pediatric NS patients.
Methods:Mutation analysis was carried out by direct sequencing of the NPHS1 and NPHS2 genes in 18 nephrotic syndrome (NS) families. This cohort included 6 families of congenital NS, and 12 infantile onset steroid resistant NS or familial cases.
Results: We detected likely causative mutations in 5 out of 6 families of congenital NS studied. A total of 5 different mutations were found in the NPHS1 gene. 3 homozygous mutations were found in the NPHS2 gene in 3 out of 12 infantile onset NS or familial cases.
Conclusions: Our results show a high prevalence of disease causing mutations in the NPHS1 (80% congenital, 30% overall) and NPHS2 (25% early onset and 16% overall) genes in the Tunisian NS children as compared to the European or Asian populations

P03.29-S
Gene Expressions In Glomerulopathies

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The nephrotic syndrome is connected with both primary and secondary glomerulopathies. The expressions of 47 genes associated with different kidney diseases in patients with primary and secondary glomerulopathies were studied. The aim of this study is to find prognostic factors from renal biopsy. Our study was performed on the set of 118 patients. There were five different biopsy-proven diagnoses: FSGS, IgAN, MCD, MGN and SLE. The expressions were analysed using the real-time PCR and normalized by the endocontrol GAPDH gene. The obtained data were analysed using software STATISTICA 10. At the beginning three genes had to be excluded for no patient's sample had showed the expression in these genes. The statistical analysis using Kruskal-Wallis ANOVA was divided into three parts. The p value of following genes was lower than 0.01. Firstly, all the diagnoses were compared with each other. The statistically significance was identified in nine genes mostly between FSGS and SLE patients. Secondly, patients with SLE and IgAN were sorted out according to their histological foundations and the gene expressions were compared in each diagnosis. The only significant gene was in the comparison of IgAN patients. Finally, it was made comparison between groups of SLE and IgAN patiens, MCD, FSGS and MGN patients. The statistically significant differences were identified in ten genes. The differences were mostly between one group of SLE patients and patients with FSGS. Our study demonstrated the differences in gene expressions not only inter-diagnosis, but also intra-diagnosis. Supported by the grant project PRVOUK- P25/LF1/2.

P03.30-M
Next-Generation-Sequencing-based molecular diagnostics for congenital and inherited kidney disease

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Congenital and inherited kidney diseases constitute the leading cause of chronic kidney disease in children. Molecular diagnostic analysis of heterogeneous renal disorders has long been hampered by the size and numbers of genes involved, but has become feasible with the advent of Next-Generation-Sequencing (NGS). We set up and implemented an NGS-based test in our ISO15189 certified laboratory to enrich and sequence 376 genes known to be causal in or associated with kidney and urinary tract disorders. Based on the enrichment 23 disease related genepanels covering 175 genes were formed, including renal cysts (50 genes), Bardet-Biedl syndrome (14 genes), Joubert syndrome (21 genes), nephronophthisis (15 genes), congenital anomalies of the kidney and urinary tract (40 genes) and nephrotic syndrome (16 genes). To reach a genotyping accuracy of at least 99%, a minimal vertical coverage of 15 individual reads per base is required. To deliver a comprehensive analysis of the genepanel, a horizontal coverage of at least 98% of targeted bases is requested. When coverage by NGS drops below requested coverage thresholds, additional "Sanger"-based sequencing is performed to fill in the gaps. In this fashion, a mutation detection rate of >95% is achieved for the genes analysed. Initial diagnostic sequencing results will be presented. To conclude, comprehensive genetic testing by NGS will boost the diagnostic yield to potentially >40-50% molecular diagnoses within one clinical genepanel analyzed. Thereby delivering a swift answer to the patient and a leap in efficacy and cost reduction for healthcare providers.

P03.31-S

Epistasic Association of CTRC and SPINK1 Gene Variants Combination with Recurrent Pancreatitis in Lipoprotein Lipase Deficiency

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Background: Lipoprotein Lipase deficiency (LPLD) is a rare autosomal recessive disease associated with severe hypertriglyceridemia and increased risk of pancreatitis or other co-morbidities. There are important unexplained inter-individual variations in the incidence and severity of acute pancreatitis episodes in LPLD patients. Several genes involved in proteolytic networks are associated with pancreas and lipoprotein functions and could influence pancreatitis risk in LPLD. **Objective:** To evaluate the association between two genes regulating serine proteases, chymotrypsin C (*CTRC*) and serine peptidase inhibitor kazal type1 (*SPINK1*), and recurrence of hospitalizations for acute pancreatitis among LPLD subjects. **Methods:** *CTRC* and *SPINK1* have been sequenced in a sample of 38 LPLD patients and 100 controls. In LPLD, 18 (47%) presented a history of recurrent (≥5) hospitalizations for acute and severe abdominal pain, whereas 8 (21%) had not yet been hospitalized for this condition. Comparisons between studied groups were done with chi-square tests and multinominal regression analyses. **Results:** Gene sequencing identified 15 SNPs. Genotype-stratified analyses in LPLD subjects and controls suggested an epistasic association between rs545634 (*CTRC)* and rs11319 (*SPINK1)* SNPs combination and recurrence of hospitalizations ($p<0.001$) in LPLD. Regression analyses, controlling for age, gender and smoking status, suggest that the risk of frequent and recurrent hospitalizations for acute pancreatitis is significantly increased in LPLD in presence of this *CTRC-SPINK1* SNPs combination (OR = 41.4 [CI: 2.0-848.0]; $p=0.016$). **Conclusion:** These results suggest that modifier genes involved in protease pathways could influence the trajectory of pancreatitis risk in LPLD.

P03.32-M

Towards introduction of exome sequencing in pediatric liver transplant program

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Pediatric cholestasis shows different etiology, clinical course and prognosis. Early recognition of the causes allows an appropriate treatment and management and plays a central role in decision-making about pediatric liver transplantation. Although each of genetic forms of cholestasis is rare, they collectively represent a frequent causes of liver transplantation. Starting in 2012, we enrolled and analyzed by exome sequencing 44 pediatric patients with isolated or syndromic forms of liver disease. The exonic region of 2,761 known disease-causing genes were assessed on patient and their parents using the TruSight Exome kit and 2X150 PE sequencing on MiSeq. Data analysis was focalized on phenotypic features and expected inheritance model. We obtained 32,799,211 reads/sample, the mean coverage was 200X and 98.54% of target regions were >20X. We identified the genetic causes in 21 patients (diagnostic yield:47.7%). Sixty seven percent of detected mutations were well known and previously described, while the remaining mutations satisfied criteria for molecular diagnosis. Alagille syndrome and progressive familial intrahepatic cholestasis were the most frequent diagnosis (42.8% and 28.6% respectively). Two patients were affected by a fructose intolerance while galactosemia, Niemann-Pick disease type C and Gaucher syndrome each have been diagnosed in a single patient. These results show that the diagnosis of children with intrahepatic cholestasis sometime is clinically challenging. Our data further expand the use of exome sequencing also in restricted subtype of pediatric patients, with a strong clinical impact also in liver transplant program.

P03.33-S

Success of liver transplantation in patients with progressive familial intrahepatic cholestasis: is there an association between genotype and outcome?

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Study aim
Characterization of the genotype-phenotype correlation in patients with progressive familial intrahepatic cholestasis (PFIC) and identification of prognostic genetic parameters for long term outcome and success of liver transplantation in order to improve individual treatment options.
Method
Genetic testing was performed for 57 PFIC index patients, including NGS panel diagnostics for 5 children with infantile cholestasis and atypical liver histopathology. Long term data (2-35 years) of 8 patients with genetically confirmed PFIC will be presented to illustrate their specific medical problems.
Results
Most pathogenic mutations were identified in ABCB11 (56%), followed by ATP8B1 (32%) and ABCB4 (12%); including 73% missense mutations, 17% truncating mutations, 7% splice mutations and 3% small deletions.
Response to pharmacological therapy was insufficient in 87% of patients, thus, biliary diversion and/or liver transplantation was performed in 75%. One additional patient is currently listed for transplantation.
Perioperative complications observed in patients with missense mutations were mild and included bleeding and coagulation problems (n=2). Life threatening complications (organ rejection requiring retransplantation, death) occurred in two patients with at least one truncating ABCB11 mutation. Severe long term complications were observed in 3 ATP8B1 mutations carriers including severe diarrhea, renal failure, deafness and polyneuropathy.
Conclusion
Truncating mutations may be associated with a higher rate of severe perioperative complications in liver transplantation, possibly due to a stronger immune reaction towards the wild type protein. With an ongoing study we are currently evaluating this hypothesis in a larger cohort including application of NGS panel sequencing to search for additional causative genes.

P03.34-M

Genetic analysis of *PKD1* and *PKD2* genes in Korean patients with autosomal dominant polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is most common inherited kidney disorder with progressive cyst growth and renal enlargement, resulting in renal failure. Mutations in the *PKD1* and *PKD2* genes account for 85% and 15% of all ADPKD cases, respectively. Although there are a few studies on the frequency and spectrum of mutations in the *PKD1* and *PKD2* genes in Korean patients with ADPKD, they did not sequenced entire exons of the *PKD1* gene but analyzed only for exon 36-46 excepting the pseudogene region, which made it difficult to evaluate the accurate frequency and the spectrum of mutations. Therefore, we performed sequence analysis of 20 consecutive unrelated ADPKD patients for kidney transplantation by using long-range PCR to avoid pseudogene amplification followed by exon-specific PCR and sequencing of the entire exons of the two genes. All patients met the diagnostic criteria of ADPKD based on the ultrasonographic findings and family history of PKD and 14 patients (70%) were revealed to have mutations; 11 with *PKD1* and 3 with *PKD2* mutations, respectively. Among 10 novel mutations, eight mutations were found in *PKD1* gene while two mutations in *PKD2* gene. All were deleterious mutations except one missense mutation. It is of note that 6 out of 11 *PKD1* mutations (54.5%) were located outside the range of exon 36-46. Considering the mutation spectrum in Korean patients with ADPKD, long-range PCR followed by direct sequence analysis of pseudogene region should be performed for accurate molecular diagnosis of the disease.

P03.35-S

Primary hyperoxaluria: analysis of GRHPR, HOGA1 genes and the promoter-sequence of AGXT gene in the Italian population

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Primary hyperoxaluria (PH) is a rare autosomal recessive disease, commonly arising in childhood with nephrolithiasis, nephrocalcinosis, chronic renal failure. Mutations in *AGXT, GRHPR* and *HOGA1* genes are responsible of type 1, 2 and 3 respectively. Our laboratory, member of the European PH consortium (OxalEurope), is the only Italian center offering these genetic analyses.
Currently, 81 *AGXT*-PH1 and 1 *GRHPR*-PH2 patient are known in Italy.
In this study the entire coding sequence of *GRHPR* and *HOGA1* genes and the *AGXT* promoter was sequenced in 15 patients with high clinical suspicion of PH, negative for *AGXT* mutations. No point mutations were detected.
One patient resulted homozygous for the c.341-81delT *HOGA1* variant in intron 2. This variant is not reported in literature and was evaluated as pathogenic by *in silico* prediction, generating a new acceptor splicing site (AG in c.341-79). The minigene *in-vitro* assay demonstrated that this variant did not interfere with splicing.

Two patients were heterozygous for two different *AGXT*-promoter variants (c.-647C>T, c.-424C>T), not reported in the scarce literature nor in 1000Genomes Database. These variants have been evaluated as not pathogenic because they do not lie in any known regulatory-transcription site.
The negative results in those patients with high clinical suspicion of PH could be explained by undetected deletions (investigable by MLPA analysis of the two major genes) or mutations in other genes involved in oxalate metabolism, or differential diagnosis.

P03.36-M
Screening of a large cohort of Italian patients with Albright hereditary osteodystrophy and/or Pseudohypoparathyroidism phenotype for subtelomeric deletions of chromosome 2

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Pseudohypoparathyroidism (PHP) is a heterogeneous group of rare genetic disorders due to end-organ resistance to the actions of PTH caused by genetic and/or epigenetic defects within or upstream the GNAS locus. The classification in different subtypes is based on the presence of specific somatic and developmental abnormalities, referred to as Albright hereditary osteodystrophy (AHO), and of resistance to other hormones acting via G protein coupled receptors.
Despite the advances in the study of PHP molecular determinants, about 30% of patients still lack a molecular diagnosis and, in the last years, independent groups found in a subset of PHP/AHO patients causative defects classically associated to diseases with partially common phenotype, such as deletions of 2q37.2 associated with the AHO-like syndrome (or brachydactyly-mental retardation syndrome, BDMR).
In this study, we screened by a multiplex ligand-dependent probe amplification (MLPA) assay targeting the chromosome region 2q our series of AHO/PHP pts negative for GNAS defects (n=56) and we detected 3 different deletions of 2q37, overlapping but smaller than those previously described. Ongoing studies will define the inheritance pattern of such deletions and will allow to narrow the common critical region associated with the AHO phenotype.
In conclusion, our data further confirm the molecular and clinical overlap between PHP/AHO and BDMR and will hopefully help to define genes involved in the AHO phenotype. Furthermore, all PHP/AHO pts negative for GNAS genetic/epigenetic defects should be considered for further molecular investigations to optimize genetic counselling.

P03.37-S
Exome sequencing reveals TPO mutations in Pseudo-Pendred syndrome

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Pseudo-Pendred syndrome (PPDS) is defined by the association of sensorineural deafness, hypothyroidism due to iodide organification defect, absence of inner ear malformation and absence of mutation in SLC26A4, the gene responsible for classical PDS.
In order to determine the cause of PPDS, we performed whole exome sequencing (WES) in a family with two children affected with hypothyroidism, developmental delay, positive perchlorate test and absence of inner ear malformation on CT-scan. Parents were healthy and non-consanguineous and direct sequencing of SLC26A4 was normal in both patients.
WES was performed in both patients and their father. Variants were ranked by segregation, allele frequency, protein change and degree of conservation to assess likelihood of causation. Both patients were found to be compound heterozygous for missense mutations (Y453D and W233C) in TPO and the father was heterozygous for the Y453D mutation.
In silico prediction tools determined a deleterious mutational impact of both variants on protein function. Sanger sequencing confirmed the mutation segregation and found that the mother was heterozygous W233C. The latter mutation was novel and both were absent in the control population.
TPO encodes thyroperoxidase which catalyzes key reactions in thyroid hormone synthesis and mutations in TPO are responsible for thyroid dyshormonogenesis 2A. Mutations in TPO have been reported in 4 patients with hypothyroidism and deafness in a series of Israeli patients with iodide organification defect (Tenenbaum-Rakover, 2007) but their phenotype was not described as PPDS.
These cases together with the present report suggest that mutations in TPO can be responsible for pseudo-PDS.

P03.38-M
Renal function decay after nephrectomy: role of surgery and impact of hypertension gene polymorphisms

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The loss of nephronic mass leads to the development of hypertension and chronic renal failure, but the frequency and the rate at which this takes place after radical nephrectomy (RN) or nephron sparing surgery (NSS) is extremely variable and the mechanisms have not been clarified to date.
To evaluate the progression rate of renal function decay within a population undergoing kidney surgery and to assess the influence of polymorphisms of genes involved in essential hypertension (HT).
162 patients were followed at the Outpatient Clinic of Nephrology after a surgery for renal carcinoma, 75 RN and 87 NSS. The eGFR (estimated Glomerular Filtration Rate), an indicator of renal function, was evaluated for a mean follow-up of 23.5 months. 128 genetic polymorphisms located in 70 loci candidate for HT have been tested by TaqMan OpenArray system. Statistical analysis was performed using a General Linear Model covarying for sex, age, BMI, blood, therapy, basal renal function and type of surgery.
About the different surgical techniques, NSS patients displayed a significant decrease of eGFR variation significantly lower than that observed in RN ones (-5.7 ml/min vs. -23.9 ml/min P <0.05). Two SNPs, located in SIK1 and in PRKG1 genes, associated with a quicker decay of renal function (p=0.009) -20 mL/min/1.73m2 after 12 months in carriers of SIK1 T/PRKG1 GG (n=8) vs. -4 at the same time in all the other groups (n=154).
The progression of renal function decline after kidney surgery depends both on surgery type (80%) and on genetic background (15-20%).

P03.39-S
Hereditary renal hypouricemia causing by defect in URAT1: a new insight into molecular pathology

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Renal hypouricemia is a rare heterogeneous inherited disorder characterized by impaired tubular uric acid transport with severe complications, such as acute kidney injury. So far, more than 100 patients with a loss-of-function mutation in the *SLC22A12* gene (URAT1, OMIM #220150) and more than ten patients with defects in the *SLC2A9* gene (GLUT9, OMIM #612076) have been described. The serum uric acid concentration in the proband was 1.1 mg/dL and expressed as an increase in the fractional excretion of uric acid 43%. The *SLC22A12* gene analysis for the patient revealed compound heterozygous variants of p.G366R and p.R477H. Functional and immunocytochemical analysis of URAT1 mutants was performed in *Xenopus laevis* oocytes. The urate uptake ability decreased to similar levels seen in mock samples in p.G366R mutant expressed oocytes. The p.R477H variant showed almost the same activity as the URAT1 wild type. In the co-expression samples, both variants p.WT/G366R and p.G366R/R477H lost their urate uptake activities. Variants p.WT/R477H tended to decrease urate transport compared to WT single expression; however, it was superior to the other two co-expression patterns (significant to WT/G366R, $^{\dagger}P<0.05$). Co-localization studies showed an accumulation of URAT1 in the endoplasmic reticulum of the p.G366R variant and mainly retention of wild type protein by variants p.G366R and p.R477H. The findings suggest that not only a loss-of-function mutation of URAT1 but also the dominant-negative effect cause renal hypouricemia *via* loss of uric acid absorption, partly due to protein misfolding caused by accumulation of URAT1 protein in the endoplasmic reticulum.
Support: LH13245 and PRVOUK-P25LF1/2.

P03.40-M
Clinical reappraisal of SHORT syndrome at the light of the PIK3R1 gene discovery

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SHORT syndrome is defined by its acronym: short stature (S), hyperextensibility of joints and/or inguinal hernia (H), ocular depression (O), Rieger abnormality (R) and teething delay (T). We and others recently identified the *PIK3R1* gene, encoding for the regulatory subunits of phosphatidylinositol-3-kinase (PI3K) and playing a key role in insulin signaling, as responsible of SHORT syndrome. In total, 22 cases with a *PIK3R1* and a variable phenotype have been reported. Only 15/22 patients presented at least 3 of 5 signs of the SHORT acronym. Indeed, the Rieger abnormality was found in less than half patients (8/21), but other ophthalmological manifestations such as hypermetropia can be present. Hyperextensibility of joints and/or inguinal hernia (3/18) were also rarely reported. At contrary, some features not part of the acronym are found at a high frequency: facial dysmorphism was typical with enophtalmia (20/20), lipodystrophy (19/19), insulin resistance (11/12), as well as diabetes mellitus in adolescence or adulthood (8/11). In conclusion, clinical reappraisal of SHORT syndrome at the light of the *PIK3R1* gene discovery permitted to revise the diagnostic criteria for SHORT syndrome. The presence of lipodystrophy and insulin resistance permit to classify SHORT syndrome among the rare syndromic forms of insulin resistance.

P03.41-S

Multiple SNP score associated to chronic renal disease risk predicts the eGFR of patients with newly diagnosed type 2 diabetes

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Genome-wide association studies (GWAS) have identified several loci associated with risk of cardiovascular disease (CVD), nephropathy and reduction of glomerular filtration rate (eGFR). Moreover, albuminuria and eGFR reduction are independent predictors of CVD.
The aim of this study is to explore the relationship between SNPs and CVD and impaired renal function phenotype in Verona Newly Diagnosed type 2 Diabetes Study (VNDS) patients.
We studied 45 CVD and 44 eGFR and/or CKD predictors SNPs in 529 GAD-Ab negative subjects, (mean±SEM age: 58.8±0.41 years; BMI: 30.0±0.22 kg/m2; FPG: 7.17±0.08 mmol/L; HbA1c: 6.86±0.05%). The association analysis was performed on 5 phenotypes: 3 CVD phenotypes (carotid arteries ultrasound, lower-limb arterial ultrasound, ECG) and 2 kidney disease phenotypes (eGFR, microalbuminuria). For both genetic risks was generated a genetic load score (GLS), by summing up the number of risk alleles carried by each patient. Both GLS were considered in the analysis as natural values and as tertiles. Cardiovascular GLS was not associated with any of the cardiovascular phenotypes (p = 0.30-0.70). Nephropathic GLS, was not associated with albuminuria, while was significantly associated with decreased eGFR, with the score expressed both as natural value (p <0.01) and as tertiles (respectively: 84.3±1.3, 81.7±1.5 and 78.9±1.5 ml/min/1.73 m2 p<0.02), even after age, sex, BMI, antihypertensive therapy and HbA1c adjustment.
Conclusions: In patients with type 2 diabetes at diagnosis, genotype predicts the residual glomerular function, suggesting that nephropathic genetic risk clock starts ticking long before hyperglycemia.

P03.42-M

A genome-wide search for factors contributing to thyroid hemiagenesis

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Thyroid dysgenesis is the major cause of congenital hypothyroidism in humans. Hemiagenesis of the thyroid (TH) is a rare congenital malformation presenting developmental failure of only one thyroid lobe, and an incidence of 1/2000 for general population. Majority of cases are sporadic, but the reported familial cases are suggestive for existence of genetic, heritable contributors. Molecular background of the disorder remains unclear. The aim of the project was a screening for abnormalities in genes playing an important role in thyroid organogenesis. A microarray and exome sequencing approaches were employed to identify novel genetic factors contributing to the disorder. Thirty-nine TH patients participated in the study. The genetic analysis were conducted in all patients and encompasses sequencing of TPO, PAX8, FOXE1, NKX2-1 and TSHR genes as well as copy number investigation using Multiplex Ligation Dependent Probe Amplification (MLPA). The MLPA analysis did not reveal any unbalanced rearrangements. In selected cohort of patients a missense mutations in PAX8 and TBX1 sequences were found. Microarray examination resulted in identification of a several regions potentially involved TH aetiology. Two genomic regions show recurrence in the cohort of sporadic, unrelated TH patients. This is a first report presenting comprehensive screening of genes contributing to severe forms of thyroid dysgenesis, in TH patients. Mutations in those genes confirm their importance for organ development but cannot explain the abnormal phenotype and bilobation disturbance for majority of TH sporadic cases. Further studies on molecular background of the disorder are necessary.

P03.43-S

Further delineation of Tenascin-X-related Ehlers-Danlos Syndrome in patients with Congenital Adrenal Hyperplasia

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Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is an autosomal recessive disorder characterized by cortisol with/without aldosterone deficiency, and androgen excess. The severity of clinical manifestations depends on the degree of 21-hydroxylase impairment caused by mutations in the CYP21A2 gene. Its classic form has an overall incidence of 1 per 15000 live births. CYP21A2 is flanked by the TNXB gene, encoding the extracellular matrix protein tenascin-X. Both, haploinsufficiency and complete loss of tenascin-X have been associated with phenotypes similar to Ehlers-Danlos syndrome (EDS). A significant portion of CAH-patients presents deletion of mostly one CYP21A2-allele, and up to 13% of these might be a contiguous deletion extending into TNXB.
We report two brothers (20 and 29 years old) with classic CAH and homozygous deletion encompassing CYP21A2 and TNXB, who were evaluated for clinical evidence of EDS: both present skin hyperextensibility, joint hypermobility and clinical history of multiple joint subluxations, without evidence of atrophic scars or other classic EDS features. The younger brother underwent surgery for inguinal hernia and rectal prolapse during adolescence. Cardiac ultrasound examination revealed bicuspid aortic valve, mild aortic dilatation and mitral valve insufficiency in the older brother; cardiologic follow-up was advised.
The parents, heterozygous carriers of the contiguous deletion of CYP21A2 and TNXB, don't show clear features of EDS.
To our knowledge, bicuspid aortic valve has not been observed in CAH with tenascin-X-related EDS yet. Our findings confirm that clinical evaluation for connective tissue pathology should be considered in CAH patients, especially those harboring CYP21A2 deletion.

P03.44-M

Tricho-rhino-phalangeal syndrome type III: an autonomous clinical entity or a clinical variant of type I ? Further report of an italian affected girl and her mother.

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Trichorhinophalangeal syndrome is an autosomal dominant condition characterized by craniofacial and skeletal anomalies and three different clinical subtypes: type I, carrying mutations in TRPS1 gene, type II, a 8q23 microdeletion syndrome with additional features such as mental retardation and exostoses, type III, with mutations in TRPSI gene and severe short stature, short metacarpals and normal intelligence. We report on clinical evaluation in a 10 year old girl presenting with disharmonious short stature, facial dysmorphisms (sparse hair, bulbous tip of the nose, long upper lip), severe metacarpophalangeal shortening and normal intelligence. Hormonal and meta-

bolic screening evaluation (including mucopolysaccaridosis disease) did not reveal pathologic patterns. Since few months she suffered from pelvic pain: an X-ray of the hip revealed left coxa vara and a round area of osteoporosis in the femural external side ,sclerosis of femural nucleus and of the overlying acetabular roof. Spine X-ray brought out convex scoliosis and marked left iliac crest dysmetria, with an overcoming external subluxation of the right hip. The CT scan confirmed these data and an orthopedic follow up started. The peculiar face with short stature, brachidactyly, scoliosis, hip dislocation led us to consider in differential diagnosis Trichorhinophalangeal syndrome type 3: molecular analysis of TRPS 1 gene proved a new missense mutation (exon 7 H1233P), that was also carried by her mother, who presented a minimum clinical phenotype (face anomalies, short metacarpals,not severe short stature). Our study could be a further clinical contribute to the delineation of the Thricorhinophalangeal subtype III syndrome.

P03.45-S
Renal and urinary system malformations in girls with Turner syndrome

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Turner Syndrome (TS), in which there is a loss of all or part of one X chromosome, occurs in 1 in 2500 born femals. Renal and urinary system malformations with their posterior complications such as urinary tract infections or proteinuria have been recognized to increase in patients with TS. In this retrospective study we report a detailed clinical history and analyzed renal and urinary system pathology in 32 girls with TS observed between 2000-2010. All 32 TS patients were evaluated by renal and collecting system utrasonography and if structural renal or urinary malformations were found, cystourethrography and centellography (DMSA or DTPA) was used. Patients mean age at renal and urological studies was 9,8 years (2-18 years). The cytogenetic findings in 32 patients with TS were: classic : 45,X in 18 patients (56,25%), mosaic and structural aberrations of X chromosome: in 14 patients (43,75%). The prevalence of renal and urinary system pathology was 43,75% (14 patients) . The most frequent findings were urinary system malformations 21,87% (7 patients) , associated with renal malformations 9,38% (3 patients), while 4 patients (12,5%) had renal malformations alone. Horseshoe kidney, malrotation or other position abnormalities, duplication of the collecting system, and different ureterovesicular obstruccion were found. Conclusion: The early diagnosis of renal and urinary system malformations in TS and their follow-up is crucial to reduce the morbility in these patients. There appears to be no correlation between karyotype and the presence or type of renal or urinary system malformations.

P03.46-M
Does summation of alleles account for genetic risk and genotype-phenotype association in Type 2 Diabetes Mellitus?

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Introduction: Type 2 diabetes (T2DM) and metabolic syndrome are common complex disorders with a high prevalence in the Maltese population. The aim of this study is to further define the genetic interplay between cognate genes from metabolic and inflammatory pathways on the likelihood of developing T2DM in adulthood and to relate the association of certain genetic profiles with defined biological and clinical endpoints. **Method**: Eight hundred carefully characterised T2DM cases were recruited. Anthropometric and biochemical parameters, including serum high-sensitivity C-Reactive protein (hsCRP) levels were determined, and genotyping of 43 cognate genes carried out. Neonatal cord blood samples were used as the control reference population in this study. **Results**: Ten polymorphisms in metabolic/inflammatory pathways showed significant association with T2DM. Three loci showed significant association with lipid profile, body weight and hsCRP levels. hsCRP levels demonstrated a strong positive correlation with body mass index. Genetic score analysis showed that combining multiple genetic markers results in higher relative risks. The functional significance of these polymorphisms is being further evaluated using targeted siRNA-mediated silencing in cultured monocytes. **Conclusion**: A panel of ten candidate genes has consistently demonstrated significant association with type 2 diabetes and metabolic syndrome in the Maltese population. These gene variants serve functional roles in inflammation and adipose tissue function. A recruited cohort of untreated newly-diagnosed T2DM serves to identify and explore genotype-phenotype association. The strong effect sizes of these alleles could be used to develop personal genetic susceptibility profiles for T2DM leading to personalization of care and prevention of chronic complications.

P03.47-S
XX male sex reversal in an azoospermic proband

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The basis of conception of genetic sex determination was initially, at least in mammals, limited to a form of chromosomal currency. However, the presence of an entire Y chromosome is not essential for the development of the male phenotype and transfer of the SRY-gene alone could be a sufficient condition. Critically, mutations in the *SRY* gene are linked with XY-female sex reversal in mice and humans indicating that *SRY* is essential for male development. It appears that the only role of SRY is to upregulate the SRY-related HMG box containing gene 9 (SOX9) bipotential gonads, which results in Sertoli cell differentiation and, ultimately, in testes differentiation. XX male sex reversal is rare and in most cases is the result of translocation of the SRY gene to the X-chromosome during male meiosis. However, a recent report by Cox et al. [2011] described a family, two brothers and an uncle, with 46, XX male sex reversal who lacked the SRY gene and had a 600 kb duplication upstream of *SOX9*. We report on a case of a 38 infertile male (sent by the fertility unit) with azoospemia and hypergonadotrophic hypogonadism a 46 XX male sex reversal (without SRY). A 244K aCGH in the proband showed a novel and apparently, de novo duplication within *SOX3* gene (at Xq27.1 chromosomal region) in a complex genomic rearrangement. These data provide additional evidence that *SOX3* gain-of-function in the XX bipotential gonad causes XX male sex reversal.

P03.49-S
Identification of 65 novel mutations and genotype-phenotype correlations in patients with Alport syndrome (ATS) and thin basement membrane nephropathy (TBMN)

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ATS is characterized by hematuria, proteinuria with progression to end-stage renal disease (ESRD), eye abnormalities and sensorineural deafness. Mutations in COL4A3/COL4A4 (autosomal recessive/dominant), and COL4A5 (X-linked) have been identified as underlying cause. Compared to ATS, autosomal dominant TBMN is characterized by hematuria, minimal proteinuria and preserved renal function. Dominant mutations have been identified in COL4A3 and COL4A4.
A cohort of 167 patients and 49 relatives were genetically tested and clinically evaluated. In 145 patients mutations were identified, 65 of them were novel. 54% resp. 88% of the ATS patients showed only one mutation in COL4A3 or COL4A4. Three TBMN and eight ATS patients (COL4A3 or COL4A4) had hearing loss, but only two of the ATS patients carried two mutations. An ATS patient with one mutation in COL4A4 showed no hematuria, but proteinuria, ESRD, and hearing loss. Two patients (COL4A5) showed isolated proteinuria. 26% of the ATS patients (COL4A5) had hearing loss and 43% of these presented the mutation at the 5' end of the gene.
This study comprises one of the largest genotype-phenotype correlation in European ATS and TBMN patients. A large number of novel mutations were detected. Many patients had only one heterozygous mutation in COL4A3/COL4A4. This might be explained either by an autosomal dominant inheritance, the possibility of missed mutations, its function as a modifier additionally to so far unidentified causative mutations, or a combination of these possibilities. Interestingly, ATS/TBMN patients can also be affected by isolated proteinuria and TBMN patients rarely develop hearing loss.

P04.01-S
Screening of 1200 FDA-approved molecules to identify pharmacological modulators of expression of ACVR1, the gene mutated in Fibrodysplasia Ossificans Progressiva.

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ACVR1/ALK-2 encodes for a type I BMP receptor and is mutated in Fibrodysplasia Ossificans Progressiva (FOP, OMIM135100). FOP is a rare and severe disease of heterotopic ossification, with a progressive and episodic course. No treatment is available to the progression of the disease and great effort is devoted to better understand the pathogenic mechanisms underlying the dysregulated BMP pathway associated with FOP that may be targeted by in-

novative therapeutic approaches.
The characterization of the ACVR1 promoter region provide us with the molecular tools to generate a cell-based system exploitable for High-Throughput Screening (HTS) of chemical compounds with potential pharmacological effect on the ACVR1 expression at the transcriptional level. The cell system has been generated in ATDC cells by stable transfection of the Luciferase reporter gene under the control of the ACVR1 promoter.
We describe here in detail the HTS procedure we developed and report the results obtained with the screening of 1200 FDA-approved compounds (Prestwick Chemical Library). We identified 18 compounds, showing an inhibitory effect ≥ 60%, and 8 molecules with activating properties on the ACVR1 transcription. Identified hits belong to different pharmacological classes among which corticosteroids, PDE inhibitors, FANS. We are currently performing experimental validation of selected molecules with different assays.
In conclusion, we present a cell-based system suitable for HTS of small chemical compounds to target the ACVR1 transcriptional activity, thereby modulating the downstream pathway. Screening of compounds approved for clinical purposes, may provide candidates for a drug repositioning approach.

P04.02-M
Autosomal-recessive Adams-Oliver syndrome caused by homozygous mutation in EOGT, encoding an EGF domain-specific O-GlcNAc transferase

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Autosomal recessive Adams-Oliver syndrome was diagnosed in three remotely related Bedouin consanguineous families. Genome wide linkage analysis ruled out association with known Adams-Oliver syndrome genes, identifying a single homozygosity ~1.8 Mb novel locus common to affected individuals (LOD score 3.37). Whole exome sequencing followed by Sanger sequencing identified only a single mutation within this locus, shared by all affected individuals and found in patients from five additional apparently unrelated Bedouin families: a 1bp deletion mutation in a predicted alternative splice variant of EOGT, leading to a putative truncated protein. RT-PCR demonstrated that the EOGT predicted alternative splice variant is ubiquitously expressed. EOGT encodes EGF-domain-specific O-linked N-acetylglucosamine transferase, responsible for extracellular O-GlcNAcylation of epidermal growth factor-like domain containing proteins, and essential for epithelial cell-matrix interactions. F-actin staining in diseased fibroblasts showed apparently intact cell cytoskeleton and morphology, suggesting the EOGT mutation acts not through perturbation of cytoskeleton, but through other mechanisms yet to be elucidated.

P04.03-S
Immunomodulation in Atopic Dermatitis: Inherited, Environmental and Behavioral Risk Factors, and Evaluation of Biomarkers

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The barrier function of the skin is essentially maintained by the epidermal differentiation complex protein filaggrin, which is located in the stratum corneum of the epidermis. Loss-of-function (LoF) mutations of the filaggrin gene (*FLG*) lead to an increased risk of atopic dermatitis (AD), allergic sensitization, as well as asthma in the presence of AD. Patients with active AD, or clinically dry skin show a reduction of the epidermal filaggrin protein levels even in the absence of *FLG* LoF mutations.
Here we investigate the disease modification in AD with principal focus on *FLG* risk variants and their influence on disease severity. To identify additional susceptibility loci we analyze *CLDN1, CLDN4, CLDN23, OCLN, IVL, SPINK5, IVL, IL31, TLR2, CLDN20, SELP,* and *FLG2* variants possibly involved in AD. Altogether 80 variants are genotyped from 500 Finnish patients with a detailed AD history, and from 1000 population cohort controls.
The tight junction proteins claudin-1, claudin-4, claudin -23, occludin and involucrin are essential for proper barrier function. However the LoF state of the encoding genes remains largely unknown. Here we suggest that LoF variation of these genes may be detected especially in patients with very severe disease and a poor treatment response, such as the AD patient cohort with extreme IgE levels (>10 000). Three candidate genes (*CLDN20, SELP* and *FLG2*) are drawn from a systematic survey of LoF variants in human protein-coding genes, that identified rare and likely deleterious LoF alleles markedly enriched in the Finnish population at greater than 1% frequency.

P04.04-M
The benign joint hypermobility syndrome: guidelines for diagnosis and management.

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Introduction The hypermobility syndrome (HMS) or benign joint hypermobility syndrome (BJHS) is a frequent condition affecting many females, interfering with their normal activities of daily life. Hypermobility is associated with an increased risk to develop a chronic pain syndrome. Patients often seek medical help in later stages of this condition for therapy-resistant complaints. We designed a protocol to improve the referral and management strategy for this particular patient group. **Methods** We report data on patients (n=167) referred to the department of clinical genetics to exclude rare genetic connective tissue disorders such as the Ehlers Danlos syndrome. **Results** Based on the data of medical history, clinical examination and if indicated, DNA-investigations, the likelihood of an underlying hereditary disorder in the 167 counsellees was estimated. Further DNA-investigation was performed in 83 cases, revealing an underlying monogenetic disorder in two cases. **Conclusions** Based on our experience we provide tools to distinguish between the majority of patients with BJHS and those with a hereditary connective tissue disorder. For the referring physician, the modified scoring list according to Beighton is essential to decide whether to refer or not. Individuals with the BJHS (Beighton scoring (BS) 4-6) should be referred to a rehabilitation specialist for treatment. In case of a BS of 7 or more, or 6 or less with unusual features (f.e. male gender, age over 50, and/or a positive family history) referral to a clinical geneticist is recommended for further evaluation.

P04.05-S
A mutation in the LRP4 gene is associated with bone mineral density in Maltese postmenopausal women

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Background: Osteoporosis is a hereditary multifactorial skeletal disease characterised by low bone mass and increased fracture susceptibility. The low-density lipoprotein receptor-related protein 4 (LRP4) controls the actions of sclerostin which is a known inhibitor of the Wingless (Wnt)/Beta (β) catenin pathway involved in bone formation.
Objective: To evaluate the effect of two non-synonymous coding polymorphisms rs6485702 (A>G) and rs2306033 (C>T) in relation to Bone Mineral Density (BMD) and different low-trauma fractures in Maltese postmenopausal women.
Methods: Research subjects were 1045 women subdivided in three BMD groups if without history of fragility fracture: normal, osteopenic or osteoporotic. Women with a fracture history were classified as cases. Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism. Associations with BMD and fracture were analysed using odds ratios (OR) determined by logistic regression and adjusted for age.
Results: Homozygosity for the rs6485702 A allele was associated with a lower BMD at the lumbar spine, LS (OR=2.2 [95% confidence interval 1.1-4.4] p=0.03) relative to research subjects with a normal BMD. Heterozygotes for this allele had a lower BMD at the femoral neck, FN (OR=1.5 [1.1-2.2] p=0.02). The G-C haplotype was strongly associated with LS BMD (p=0.004) and to a lower extent FN BMD (p=0.04). No association with fracture risk was seen (p>0.05).**Conclusion:** The LRP4 rs6485702 variant plays a role in BMD regulation in Maltese postmenopausal women. This polymorphism is located in the β-propeller domain which is thought to interfere with the binding of sclerostin and hence will not affect Wnt signalling.

P04.06-M
A novel mutation in BMPR1B gene(R486L)in a Polish family with brachydactyly A2/C with symphalangism

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Two different substitutions at position 486 of the BMPR1B gene result in a phenotype of brachydactyly A2 or brachydactyly C with symphalangism. Here we report a Polish family presenting this rare phenotype.
On X-ray images in the 47 year old female the metacarpals are shortened and the proximal phalanges of the second fingers are hypoplastic. The left finger shows a transverse radiolucent defect consistent with pseudoarthrosis. The middle phalanges of the 2nd fingers are absent. The middle phalanges of the 3rd and 5th fingers are hypoplastic/dysplastic. In the feet there is hypoplasia of the 1st metatarsals with hypoplasia/dysplasia of the proximal phalanges. There is sclerosis of the second metatarsals.
In her 23 year old son the metacarpal of the first right finger is hypoplastic and that of the first left finger is hypoplastic/dysplastic. The proximal phalanges of both the 2nd fingers are hypoplastic and the middle phalanges are present as small bony remnants. In the left 2nd finger there is radial subluxation of the distal phalanx. There is also hypoplasia of the 1st metatarsals with hypoplasia/dysplasia of the proximal phalanges. The second metatarsals are of increased density. Clinical and radiological analysis of skeletal abnormalities suggested alteration of the BMPR1B gene. A novel mutation: c.1457G>T(R486L)segregates with brachydactyly in this family. Our data extends mutational and radiological spectrum associated with mutations in BMPR1B gene and confirms existence of a universal hotspot in the BMPR1B gene for this complex phenotype, that is clearly distinguishable on radiological basis.

P04.07-S
Targeted DNA sequencing of chromosome 8q22 identifies rare variants in *DCSTAMP* in patients with Paget's disease of bone

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Paget's disease of bone (PDB) is a common bone disease with strong genetic component. We previously identified a PDB-susceptibility locus on chromosome 8q22 tagged by a common SNP located within the *DCSTAMP* gene (Albagha *et al, Nat Genet* 2011). To identify functional susceptibility variants, we investigated this locus using a targeted DNA sequencing approach. A 700kb region containing four genes (*RIMS2*, *DCSTAMP, DPYS,* and *LRP12*) was captured using the Haloplex target enrichment kit followed by DNA sequencing using the Illumina Hiseq2000 platform. A total of 244 samples were sequenced (143 unrelated cases without *SQSTM1* mutation, 40 controls, 48 familial cases and 13 controls). Variants passing quality control were subjected to multiple filters to include missense variant, those in the 5' or 3'UTR or those predicted as functional by the ENCODE database. In *DCSTAMP*, we detected two novel variants (1 missense and 1 in 3'UTR) that were not present in our controls or publicly available databases including 1000 genomes. The novel missense mutation was detected in 3 cases and showed transmission in familial cases. Two other missense mutations were only found in cases (n= 7) but they are present in 1000Genome database with MAF<0.03. No disease-specific mutations were detected in *RIMS2, DPYS* or *LRP12*). *DCSTAMP*, which encodes a dendritic-cell-specific transmembrane protein, is a strong functional candidate gene for PDB because it is required for the fusion of osteoclast precursors to form mature osteoclasts. Our data suggest that rare variants within this gene could influence PDB-susceptibility.

P04.08-M
Identification of mutations in *DYNC2LI1*, a member of the mammalian cytoplasmic dynein 2 complex, expands the clinical spectrum of Jeune/ATD ciliopathies

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Disorders of brain malformations, polydactyly, kidney cysts, and skeletal abnormalities belong to the ciliopathy spectrum caused by defects in formation, maintenance and function of the primary cilium. This phenotypic spectrum is present among patients with short rib-polydactyly syndromes (SRPS), the asphyxiating thoracic dystrophy (ATD/Jeune) and Ellis-van Creveld syndromes (EVC). Underlying genes affect the dynein motor, intraflagellar transport complexes, or the basal body.
After excluding known causative genes we performed exome sequencing in a patient of non-consanguineous parents presenting an intermediate phenotype between ATD/Jeune and EVC. We selected variants based on potential ciliary function as identified in a yeast two-hybrid screen with NEK1, a basal body protein involved in SRPS II. This identified compound heterozygous nonsense (p.R208X) and missense (p.T221I) mutations in *DYNC2LI1* segregating in the family. *DYNC2LI1* is ubiquitously expressed and interacts with DYNC2H1 to form the dynein 2 complex important for retrograde intraflagellar transport. The hypothetical protein caused by the nonsense mutation lacks the coiled-coil domain involved in protein interaction and dimerization. The mutation p.T221I affects a highly conserved nucleoside triphosphate hydrolase domain responsible for GTPase driven dynein protein localization.
Mutations in both DYNC2LI1 interacting partners DYNC2H1 and NEK1 are associated with Jeune/ATD/SRP III and SRPS II/ATD, respectively. The intermediate phenotype in the patient can be explained by its dimerization with DYNC2H1 and the suggested interaction between DYNC2LI1 and the basal body protein NEK1. This is the first report of mutations in the light intermediate chain of the dynein 2 complex further expanding the clinical spectrum of ciliopathies.

P04.09-S
Exome sequencing in patients with Circumferential skin creases Kunze type: Evidence for locus heterogeneity

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Congenital circumferential skin creases are extremely rare and children born with this feature are referred to as 'Michelin tyre babies' based on the similarity with the mascot of the French tyre manufacturer. Some of these children have additional abnormalities including typical facial dysmorphism, cleft palate, short stature and intellectual disability. For this syndrome, our group proposed the term 'Circumferential skin creases Kunze type' (Wouters *et al.*, 2011). So far, less than 10 cases have been described in the literature and all occurrences are sporadic. In an international collaboration we collected DNA samples from 8 patients with Circumferential skin creases Kunze type. Exome sequencing was performed on the HiSeq2000 platform for two case-parent trios as well as two additional patients with this syndrome. Data analysis revealed the presence of pathogenic mutations in either one of two interacting genes, providing evidence for genetic heterogeneity. Three additional patients with the same phenotype have also been found to carry a mutation in one of these genes. While some patients carry a heterozygous *de novo* mutation, others present with homozygous mutations. Accurate genotype-phenotype correlations are being investigated. In addition, we are performing functional analyses at the protein level to elucidate the pathogenic mechanism of the mutations.

P04.10-M
The type II collagenopathies: a spectrum of disorders in 194 Italian families

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Mutations in the type 2 collagen gene (*COL2A1*) have been described in a group of dominant skeletal dysplasias with a wide degree of phenotypic severity ranging from perinatally lethal to mild conditions manifesting only in late adolescence or adulthood. In the past 6 years we performed molecular analysis of *COL2A1* in 194 Italian probands and we identified 78 different mutations: 25 known and 53 never described in the literature. The phenotype was quite variable among our patients, ranging from Stickler syndrome (STL), due mainly to nonsense and splice-site mutations, to more severe bone dysplasia phenotypes, usually due to a dominant negative effect (SED, SMED, SPD, ACG2). In 9 of our cases with a diagnosis of STL who were negative at *COL2A1* analysis we found mutations in *COL11A1*. Considering the broad spectrum of phenotypes, the absence of mutational hot spots and the large size of the genes implicated in "type II-collagenopathies", mutational analysis by standard techniques (DHPLC, HRM and/or direct Sanger sequencing), represent a very expensive and exhausting test. We therefore switched to a method coupling amplicon based gene capture to continue both the *COL2A1/ COL11A1* analysis and we are now extending the study

to less frequently mutated genes (*COL11A2*, *COL9A1-2*): this innovation has improved our analysis' reliability and turnaround times.

P04.11-S
About skeletal dysplasia patients carrying two Col2a1 mutations

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Background and objectives: Skeletal dysplasia are usually dominant disorders due to heterozygous mutations in COL2A1 mutations. To report on four cases carrying two COL2A1 mutations.
Methods: Bidirectional Sanger sequencing of the whole COL2A1 gene.
Results: Four patients carrying two different mutations were identified in a series of 136 skeletal dysplasia.
Patient 11 carried a p.Thr1439>Met paternal allele and a de novo c.1267-2A>G. This latter may explain that she presented with a more severe form of Kniest dysplasia than her affected father. The p.Ala145Val, predicted as benign, might not be contributive, as the p.Gly405Asp alone was shown associated with SEDC (Meredith SP, 2007). The association of p.Arg137His and p.Gly213Val seems to induce a different phenotype than previously observed in a patient carrying a heterozygous p.Gly213Val (Kannu M, 2011).
Conclusion: We report here the first cases of COL2A1 skeletal dysplasia with either compound heterozygous (recessive) or complex alleles. Recessive forms of type 2 Stickler syndrome were previously associated with COL11A1 (Richards AJ, 2013). Parents' samples from families 41, 47, and 49 are under evaluation to phase the mutations and define whether they are de novo.

a- Barat-Houari M, unpublished data, b- Unger S 2001, c- Meredith SP 2007, d- Kannu P 2011, e- Score

Family ID	Phenotype (MIM number)	Mutation 1	Score(e)	Mutation 2	Score (e)
11	Kniest dysplasia (156550)	c.1267-2A>G (a)	68%	p.Thr1439Met (b)	3
41	Spondylo Epiphyseal Dysplasia Congenita, SEDC (183900)	p.Ala145Val (a)	1	p.Gly405Asp (c)	3
47	Stickler type 1; syndromic; non syndromic ocular (108300; 609508)	p.Arg137His (a)	2	p.Gly213Val (d)	3
49	Spondyloepimetaphyseal Dysplasia Strudwick type (184250)	p.Arg940Gln (a)	2	p.Gly1089Arg (a)	3

P04.12-M
Identification of a novel locus for a recessive congenital myopathy by linkage analysis in an Israeli Bedouin family

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Congenital myopathy disorders (CMDs) are heterogeneous inherited diseases of muscle characterized by a range of distinctive histologic abnormalities. We have studied a consanguineous family with a non progressive congenital myopathy. In order to pursue a molecular diagnosis in this family, we performed genotyping on four patients, their parents and a healthy sibling using the Affymetrix GeneChip Human SNP5 array. We determined the genotype calls by using Affymetrix GeneChip Genotyping Analysis Software (GTYPE) and KinSNP software. Based on the consanguinity in the family, we hypothesized homozygosity by descent of a recessive mutation as the likely cause of the disorder. Therefore, we searched for homozygous regions consistent with linkage. Three homozygous blocks (on chromosomes 6, 10 and 14) shared by the three affected individuals, heterozygous in the parents, and not homozygous in the unaffected sib were identified. Exome sequencing revealed a highly suggestive mutation in a gene not previously reported to be associated with myopathy in humans. The variation segregated as expected in the family, it did not appear in dbSNP, evs or the 1000 genome project, nor was it found in 134 Bedouin control individuals.

P04.13-S
Diagnostics of connective tissue disorders: NGS gets the target when clinics wanders around

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Introduction Next-generation sequencing (NGS) offers a valuable tool for the diagnostics of connective tissue disorders (CTDs), in view of the large size of most of the genes involved and the complex spectrum of, often overlapping, phenotypes, which makes differential diagnosis difficult. Recently, we designed a platform for the targeted analysis of CTDs, including Marfan syndrome, Ehlers-Danlos syndrome, Osteogenesis imperfecta, Stickler syndrome and related disorders. In total, 42 genes were included. Based on the associated phenotypes, six gene panels were defined. **Material and methods** A solution-based target enrichment kit was designed to capture all exons and flanking splice sites of 42 genes. Data were analysed using an in-house pipeline (based on freely available software) and Cartagenia. In total, 309 gene panels were analysed on 290 patients referred to our Diagnostics. The aortic/arterial aneurysms/dissections and the Ehlers-Danlos syndromes were the most requested gene panels (90%). **Results** (Likely) pathogenic mutations were identified in 14.5% of the patients. In most of these patients DNA findings confirmed the clinical diagnosis or were in line with the proposed differential diagnosis. Altogether, NGS strongly facilitated pinpointing the correct diagnosis. Importantly, in a few cases NGS led to a different diagnosis, which had not been considered based on clinical presentation. **Conclusions** The CTDs-NGS platform offers advantages in terms of time-efficiency, in view of the complex spectrum of phenotypes and the difficult differential diagnosis. This methodology helps preventing misdiagnosis, especially in young patient with incomplete phenotypic expression. The clinical phenotype of certain CTDs will possibly expand in the future.

P04.14-M
Functional role of the Bardet Biedl Syndrome-associated gene 9 in the pathogenesis of nonsyndromic craniosynostosis: disrupted primary cilium in craniofacial ossification

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Sagittal craniosynostosis (sNSC) is a highly prevalent craniofacial malformation, with a widely unclear etiopathogenesis. A possible involvement of the BBS9 gene, encoding a protein located in the transition zone of the primary cilium, has been proposed as a result of the first GWAS carried out in 2012. Through microarray genome-wide expression profiling we have shown an altered expression of cilium-associated genes, including BBS9, in calvarial tissues and cells of sNSC patients. We aimed at investigating the role of BBS9 in the aberrant osteogenic phenotype of calvarial cells isolated from sNSC patients, through gene expression analysis, immunofluorescence, gene silencing, and differentiation assays. BBS9 expression was significantly upregulated in cells isolated from fused sutures (syn-cells) compared to cells isolated from matched patent sutures (control cells), and increased upon 5 days of osteogenic induction. Confocal microscopy showed that: syn-cells produced less primary cilia compared to control cells; BBS9 expression was spread throughout the cytoplasm in syn-cells, while appeared organized in polarized structures surrounding the cilium basal body in control cells. Upon BBS9 silencing in syn-cells, the expression of osteo-specific transcription factors (RUNX2 and OSX), and of SMO (key molecule of the hedgehog pathway), were significantly down-regulated. The osteogenic potential of BBS9-silenced syn-cells decreased and reverted to the physiological behaviour observed in controls. Our results suggest a functional involvement of BBS9 and primary cilium signalling, in the aberrant osteogenic signalling occurring at the site of premature suture closure in sNSC, proving a possible novel role of this molecular machinery in osteogenesis and craniofacial malformations.

P04.15-S
Chromosomal aberrations in complex craniosynostosis: genetic heterogeneity helps identifying biological pathways

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Craniosynostosis (CRS) represent the most prevalent craniofacial malformation, occurring in 1 out of 2500 livebirths, either as an isolated feature

(nonsyndromic CRS) or in complex phenotypes. A genetic basis can be found in 20-30% cases of complex CRS, with extremely high heterogeneity. The aim of this study is to describe the inventory of structural chromosomal aberrations identified through cytogenetic and molecular cytogenetic testing (as a part of the diagnostic algorithm) in a sample of complex CRS patients. The possible role of the genes involved in the genomic rearrangements in CRS etiopathogenesis have been investigated in vitro, using gene expression analysis, immunofluorescence and gene silencing assays, on calvarial stem cells isolated from CRS patients.
In our center we have enrolled 253 patients affected by CRS, including 13 cases with complex phenotypes that did not resemble any clear known syndrome. In 9 out of 13 cases, standard cytogenetics and array CGH allowed evidencing a chromosomal structural mutation, encompassing. multiple alternative genomic loci (2p, 9q, 2q, 22q, 8q, 9, 10, 5, 17, and 7p, among others). Genes involved in craniofacial development have been mapped to those loci enabling a biological interpretation of the abnormalities. In particular, the role of developmental genes involved in the structure and function of the primary cilium has been demonstrated in selected cases.

P04.16-M
The prostaglandinE2-pathway as a key player in the pathogenesis of non-syndromic craniosynostosis

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The etiopathogenesis of midline nonsyndromic craniosynostosis remains still largely unclear. We attempted to clarify this issue using microarray comparative gene expression profiling. Among the differentially expressed genes, we focused particularly on the hydroxyprostaglandin dehydrogenase (HPGD) gene, which encodes the prostaglandin E-2 (PGE2) catabolizing enzyme, whose pathway is known to be involved in osteogenic differentiation. Mutations in this gene result in primary autosomal recessive hypertrophic osteoarthropathy and craniosteoarthropathy. Total RNA and calvarial cells were isolated from calvarial specimens of both sutures and synostoses of NSC patients, collected during surgery. RNA was used for exon-level microarray analysis; gene expression and alternative splicing events were confirmed using real time PCR and RT-PCR. For functional validation, calvarial cells isolated in primary culture were treated with scalar concentrations of PGE2; after 10 days of treatment cells were alternatively lysed to extract RNA or stained with Alizarin Red to analyze osteogenic differentiation. Gene expression profiling allowed the identification of 114 significantly modulated genes and 150 alternatively spliced genes, including HPGD. Exon level analysis of HPGD revealed that the gene encoding the active isoform of the enzyme was significantly downregulated in synostosis-derived tissues. Upon PGE2 treatment, cells isolated from synostoses displayed a higher amount of osteogenic differentiation compared to patent suture-derived cells, as a result of the reduced HPGD levels. The results of this study may provide the original description of an impairment in the PGE2-signaling pathway in the pathogenesis of premature suture fusion in NSC patients. Translational implications may further derive from these data.

P04.17-S
Single-gene testing and Next Generation Sequencing in a Dutch cohort of syndromic craniosynostosis patients

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Introduction According to literature, the genetic cause of craniosynostosis can be identified in approximately 24% of the patients. Often, mutations are found in *FGFR2*, *FGFR3* and *TWIST1*. But mutations are also identified in many other genes (like *IL11RA*, *TCF12* and *ERF*). Those genes can be sequenced all in parallel by Next Generation Sequencing (NGS), leading to more complete diagnostics. **Methods** Syndromic craniosynostosis was defined as multisuture or unicoronal synostosis, familial craniosynostosis, or as craniosynostosis in combination with other congenital malformations and/or mental retardation. According to protocol, these syndromic craniosynostosis patients were tested by single-gene testing for mutations in *FGFR2*, *FGFR3*, and *TWIST1*. If these genes tested negative, other genes were analyzed if applicable. In addition, a subgroup of 8 families (15 patients, 11 clinically unaffected relatives) and 8 isolated patients were sequenced by NGS and checked for mutations in known craniosynostosis genes. **Results** Of our patient cohort, 705 patients were tested by single-gene testing. Mutations were found in 280 patients. By NGS, mutations were identified in *TCF12* (1 patient, 1 unaffected carrier, 1 relative), *IL11RA* (1 patient, 2 relatives), *ZIC1* (three patients, 1 relative) and in *IDS* (1 isolated patient). **Conclusion** By single-gene testing, mutations were identified in only 15% of the syndromic craniosynostosis patients. Possibly, because the group of tested patients was defined differently, but also because only a subset of genes was tested. By NGS of a small cohort, mutations were identified in additional genes in 6/23 patients, illustrating the power of parallel sequencing.

P04.18-M
CYLD and Brooke-Spiegler syndrome: mutations in Hungarian patients, a review of published variants and a database update

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Brooke-Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant condition characterized by skin appendageal neoplasms including cylindromas, trichoepitheliomas, and/or spiradenomas. In 2000, the gene locus for BSS was mapped to 16q12-13, and, in the same year, variants of the cylindromatosis gene (CYLD) were identified in BSS, familial cylindromatosis (FC; OMIM 132700) and/or multiple familial trichoepithelioma type 1 (MFT1; OMIM 601606). The gene codes for an enzyme with deubiquitinase activity. To date, a total of 81 different disease-causing mutations have been published for the CYLD gene. A summary of recurrent mutations identified in Hungarian patients and a review of published mutations is presented in this update. Comparison of clinical features in affected families with the same mutation strongly confirms that identical mutations of the CYLD gene can give rise to different phenotypes, making genotype-phenotype correlations difficult. Variable expression of the phenotype associated with the same CYLD mutation may reflect the influence of other genetic and/or environmental factors. Most mutations are frameshift (43%), nonsense (24%), splicing (16%) and missense ones (12%), but there are some reported rare variants as well (5%). The vast majority of the mutations (99%) are located between exon 9-20, which encodes the 3rd Cap-Glycin and the ubiquitin-specific protease domains of the CYLD protein, suggesting that these domains are important for CYLD deubiquitinating activity.
This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'.

P04.19-S
An atypical form of progressive extreme heterotopic calcification in a patient with a de novo insertional translocation der(X)ins(X;2)(q26.1;p13.3)

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We report on a girl with widespread, rapidly progressive ectopic calcifications detected shortly after birth. Calcifications became present around most joints, involving tendons and ligaments, but no internal organs or skin, and eventually caused almost complete immobility of the child at 2 years. Laboratory evaluation failed to identify autoimmune disorders as well as calcium metabolism or other biochemical abnormalities; molecular studies did not identify any mutation in disease genes known to be involved in ectopic calcifications. Further analysis identified a de novo insertional translocation (IT). Array-CGH analysis showed a 2p13.3 duplication which was validated by qRT-PCR. Fluorescence in situ hybridization (FISH) confirmed the rearrangement, a der(X)ins(X;2)(q26.1;p13.3). The two breakpoints were characterized at nucleotide level by inverse PCR. The duplication on chromosome 2 encompassed nine coding genes, seven completely duplicated and two (ANTXR1 and MXD1) partially duplicated. ANTXR1 is interrupted in IVS10; the MXD1 coding sequence is maintained, but its 3'-UTR is almost completely lost. The chromosome 2p13.3 duplication is inserted into a gene desert on chromosome Xp26.1, between ARHGAP36 and IGSF1 genes. We suggest the phenotype in our patient is due to a likely gain of function mechanism. We hypothesize that the de novo insertional translocation causes a fusion transcript or an altered regulation of a gene by position effect. Further studies are in progress to identify the pathogenic mechanism triggering this unique phenotype.

P04.20-M
An 8-year-old Iranian girl with the FKBP14- related form of the Ehlers-Danlos syndrome with severe myopathy, scoliosis but normal hearing

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Recently mutations in *FKBP14* have been identified in patients with a novel variant of Ehlers-Danlos syndrome (EDS) characterized by progressive kyphoscoliosis, myopathy and hearing loss (MIM #614557). This disorder shares many clinical features with the kyphoscoliotic type of EDS (EDS VIA; MIM 225400) such as congenital hypotonia, progressive kyphoscoliosis, hyperelastic skin and hypermobility of joints. However, there are also some distinctive features like myopathy and hearing loss. While an increased ratio of urinary lysyl pyridinoline (LP) to hydroxylysyl pyridinoline (HP) is diagnostic for EDS VIA, measurement is normal in patients with EDS caused by mutations in *FKBP14*.
Here we report on an 8-year-old girl born to first cousin parents, who presented with severe congenital hypotonia, psychomotor retardation, severe muscle weakness, progressive scoliosis, severe joint hypermobility of fingers, wrists and toes, soft skin, easy bruising, bilateral clubfoot, prominent heel and pes planus, normal hearing, nasal speech and dysarthria. Because of a suggestive clinical history by absence of hearing impairment our initial clinical diagnosis was EDS VIA, but normal LP/HP in urine ruled out this condition. Therefore we sequenced *FKBP14* and detected a novel homozygous c.143T>A substitution in exon 1 causing a p.Met48Lys mutation. Hearing loss, was consistently reported in the initial cohort of patients with this novel form of EDS, in whom nonsense mutations were found. It is compelling to hypothesize that absence of hearing impairment in our patient might reflect a partial loss of function of FKBP14 caused by the identified p.Met48Lys missense mutation, thus suggesting a possible genotype-phenotype correlation.

P04.21-S
Zebrafish modeling of β3GalT6-deficient Type of Ehlers-Danlos Syndrome Stresses the Importance of Glycosaminoglycans in Development

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Proteoglycans are important components of cell plasma membranes and extracellular matrices. They are composed of glycosaminoglycan (GAG) chains attached to a core protein through a tetrasaccharide linker region. The addition of the third residue in this linker is catalysed by galactosyltransferase II (β3GalT6), encoded by B3GALT6.
We recently identified bi-allelic mutations in B3GALT6 in several individuals from independent families with a severe autosomal recessive pleiotropic connective tissue disorder characterized by skin fragility, delayed wound healing, joint hypermobility and contractures, muscle hypotonia, intellectual disability and a spondyloepimetaphyseal dysplasia with bone fragility and severe kyphoscoliosis. To characterize the function of β3GalT6 we employed zebrafish as an in vivo model. Whole mount in situ hybridization of zebrafish embryos showed high b3galt6 expression levels in brain, retina, pharyngeal arches and notochord epithelium, corresponding to tissues that are affected in the human patients. A morpholino-based approach was used to characterize the developmental effects of b3galt6 knockdown in zebrafish embryos. Complete knockdown of b3galt6 is lethal, but partial knockdown results in an abnormal pharyngeal cartilage phenotype and a notably reduced head and eye size. These morphological changes were accompanied by a significant reduction in the total amount of sulfated GAG chains.
In conclusion, our results emphasize a crucial role for β3GalT6 in GAG synthesis and development. Ongoing and future experiments aim to extensively analyze the changes in GAG composition as well as to further characterize the way in which these changes impact embryonic development of e.g. cartilage and heart using different fluorescent transgenic reporter lines.

P04.22-M
Familial vascular Ehlers-Danlos syndrome caused by a mutation in *COL5A1*

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Different forms of Ehlers-Danlos Syndrome (EDS) exist, with specific phenotypes and genes associated. Vascular EDS, caused by mutations in *COL3A1*, is characterized by a fragile vasculature with a high risk of catastrophic vascular events at young age. Classic EDS, characterized by fragile, hyperextensible skin and joint laxity, is caused by mutations in *COL5A1* and *COL5A2*. To date, vessel rupture in four unrelated classic EDS patients with a confirmed *COL5A1* mutation was reported.
In the current report, we describe a familial form of clinically vascular EDS, diagnosed in a mother and her two sons, who all three died at an early age from arterial ruptures. Diagnostic Sanger sequencing failed to detect a *COL3A1* mutation in the eldest son, our index case. Next, our probands DNA was analyzed using a next-generation sequencing approach targeting approximately 550 genes (possibly) linked to vascular disease (VASCULOME project). This revealed a heterozygous pathogenic mutation in *COL5A1*, resulting in an essential glycine substitution in the triple helix domain, nearby the C-terminus of the protein (c.4610G>T; p.Gly1537Val). This novel mutation was present in DNA isolated from autopsy material of his brother. No autopsy material was available from the mother, but the mutation was excluded in her parents, siblings and in the father of her sons, suggesting that the *COL5A1* mutation occurred in the mother's genome de novo.
This is the first describtion of familial vascular EDS with a pathogenic mutation in *COL5A1*. We conclude that this mutation can give a similar vascular EDS phenotype as a *COL3A1* mutation.

P04.23-S
Phenotypic features of knockout mice for dermatan 4-O-sulfotransferase 1 (D4ST1)-deficient Ehlers-Danlos Syndrome (DDEDS)

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Dermatan 4-O-sulfotransferase 1 (D4ST1)-deficient Ehlers-Danlos syndrome (DDEDS), caused by recessive loss-of-function mutations in CHST14, is a recently delineated form of EDS [Dündar et al., 2009; Malfait et al., 2010; Miyake et al., 2010; Kosho et al., 2011], characterized by a unique set of clinical features consisting of progressive multisystem fragility-related manifestations (skin hyperextensibilty and fragility, progressive spinal and foot deformities, large subcutaneous hematoma) and various malformations (facial features, congenital multiple contractures). Multisystem connective tissue fragility is caused by impaired assembly of collagen fibrils through loss of dermatan sulfate (DS) replaced by chondroitin sulfate in the decorin glycosaminoglycan sidechains. Complete loss of DS in patients' urine suggests systemic loss of DS to be the basis of the disorder. Because the patients suffer from progressive multisystem fragility-related complications, appropriate disease modeling is indispensable in view of developing etiology-based therapy. In this study, we report phenotypic features of knockout (Chst14-/-) mice. Frozen sperm from Chst14+/- male mice were obtained from the Mutant Mouse Regional Resource Center, Chst14+/- mice were reproduced, and Chst14-/- mice were generated. Complete loss of DS in urine from Chst14-/- mice was demonstrated, suggesting these mice to reflect glycobiological abnormalities in DDEDS. Chst14-/- mice lacked congenital multiple contractures, but showed perinatal lethality, reduced postnatal weight gain, mild facial asymmetricity, thoracic kyphosis, and reduced grip power and skin tenacity, compared with Chst14+/+ or Chst14+/- mice. These features could be therapeutic targets for etiology-based therapy such as AAV vector-mediated gene therapy.

P04.24-M

FGF16 nonsense mutations as the underlying cause of X-linked recessive metacarpals 4 / 5 fusion

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Metacarpal 4-5 fusion (MF4; MIM%309630) is a rare congenital malformation of the hand characterized by the partial or complete fusion of the 4th and 5th metacarpals. The anomaly occurs either as an isolated trait or part of a genetic syndrome. Recently, using whole exome sequencing (WES), we have uncovered the genetic cause of isolated MF4. WES was performed on samples form a single trio of Polish ethnicity, i.e. sporadic male proband presenting with isolated MF4 and his unaffected parents. The study revealed a nonsense mutation (c.C535T; p.R179X) in exon 3 of the FGF16 gene, which maps to chromosome Xq21.1. The result was than confirmed by Sanger sequencing. The link between FGF16 gene mutations and MF4 was further supported by the identification of two other truncating FGF16 mutation in two unrelated male probands. Sanger sequencing showed that one index case carried a nonsense mutation (c.C470A; p.S157X), whereas the other harbored a truncating frameshift variant (c.474_477del; p.E158DfsX25). Two out of three mothers of the probands were available for testing. Upon assessment, they were clinically and radiologically unaffected and both carried FGF16 heterozygous mutations. All of the FGF16 disease-causing variants identified by us were located in the last exon (exon 3) of the gene, therefore they are unlikely to result in a nonsense mediated decay. Our study shows that truncating mutations of FGF16 are associated with X-linked recessive metacarpal 4-5 fusion and provides evidence for the involvement of FGF16 in the fine patterning of the human skeleton of the hand.

P04.25-S

Giant cell tumor of the bone is caused by somatic mutations in H3F3A gene while patients with giant cell tumor arising on Paget's disease of bone shared a distinct genetic alteration

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Giant cell tumor of bone (GCT) is an aggressive bone tumor caused by the uncontrolled proliferation of the spindle-like stromal cells which promote osteoclast-like giant cells formation responsible for the osteolitic lesions. A recent study showed that GCT is due to recurrent somatic mutations in *H3F3A* gene in the stromal cells. We analysed a cohort of giant cell tumor of bone for the presence of H3F3A mutations identifying somatic mutations in 38 out of 44 cases (86%). In contrast, the analysis of patients with Paget's disease of bone (PDB) associated with giant cell tumor did not show any mutation in *H3F3A* gene, at both somatic and germline level, suggesting a different genetic background. We recently reported an extended Italian family in which 4 out of 14 PDB affected members developed multiple GCTs at pagetic skeletal sites. Clinically, all affected members had polyostotic PDB, but subjects developing giant cell tumors showed an increased disease severity with a reduced clinical response to bisphosphonate treatment and an increased prevalence of bone pain, deformities, and fractures. Whole exome sequencing, in this family identifies a missense mutation in a novel uncharacterized gene. Additional genetic analysis in 7 independent affected patients with the same clinical phenotype, discloses the same mutation in all patients, strongly suggesting that this clinical phenotype is due to a founder effect. Therefore, we conclude that the GCT phenotypes associated or not with PDB are due to mutations in different genes, suggesting that different molecular signatures are responsible for these two phenotypes.

P04.26-M

Identification and characterization of a novel susceptibility locus for nonsyndromic cleft lip and palate at chromosome 15q13

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations worldwide and considered to be of multifactorial etiology. NsCL/P shows considerable phenotypic variability and can be subdivided into nonsyndromic cleft lip only (nsCLO) and nonsyndromic cleft lip and palate (nsCLP).

Genome-wide and replication studies have recently led to the identification of 15 nsCL/P susceptibility loci. However, a number of additional genetic risk factors still await elucidation. Here we used data from a recent genome-wide meta-analysis (Ludwig et al. 2012, Nature Genetics) and combined these with results from an independent European trio cohort (n=793). Integration of subgroup-information on nsCLO or nsCLP revealed rs1258763 on chr. 15q13 as a novel genome-wide significant locus associated with nsCLP (P=1.04×10-08). The associated region maps in intergenically, between the Gremlin-1 (GREM1) and Formin-1 (FMN1) genes. GREM1 is a known antagonist in bone-morphogenetic-protein pathways which are relevant to craniofacial genesis. Sequencing the entire GREM1 coding region in 196 patients and 196 controls did not reveal a causal variant, however, a significant overrepresentation of rare variants within patients was observed (P=0.02). Analyses of murine Grem1 expression during embryonic craniofacial development might suggest a functional role of Grem1 in lip and secondary palate formation. Notably, the top variant rs1258763 has previously been shown to influence variation in facial morphology (Boehringer et al. 2011, EJHG, Liu et al. 2012, PloS Genetics).

Our results demonstrate that increasing sample sizes and precise phenotypic information might help identifying further risk loci for genetically complex traits.

P04.27-S

Study of clinical and mutational findings in 45 Russian families with ectodermal hypohidrotic dysplasia

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Ectodermal dysplasia is a group of syndromes involving abnormalities of the ectodermal structures and is comprised of more than 150 different forms. This work is devoted to hypohidrotic ectodermal dysplasia (HED). Main causes of HED are mutations in three genes: EDA, a ligand that belongs to the tumor necrosis factor (TNF)-α family, EDAR, a receptor related to the TNFα receptors, and EDARADD, a specific adaptor.

The study cohort consisted of 45 Russian patients with HED was performed by us, using direct sequencing of coding region EDA gene and MRC-Holland MLPA kit for search of large deletion in EDA, EDAR and EDARADD genes.

In result EDA gene mutations were found in 76% (34 patients) of cases. Twenty new allelic variants of HED were described by us. Repeating mutations are revealed, but the analysis of the polymorphic markers linked to EDA gene showed absence of the founder effect. Large deletion in EDAR and EDARADD genes aren't found. No correlations were revealed between clinical features and specific mutations within a EDA gene.

We present the first in Russia large hypohidrotic ectodermal dysplasia cohort focusing on clinical manifestations in combination with mutational analysis.

P04.28-M

Different genetic defects in four females with characteristic features of Incontinentia Pigmenti

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Introduction *IKBKG/NEMO* gene mutations are a well-known cause of Incontinentia Pigmenti (IP), an X-linked dominant neuroectodermal disorder (OMIM #308300). IP is characterized by characteristic skin lesions and ectodermal, ocular and central nervous system features. We collected four female patients with a clinical IP phenotype to study their molecular and phenotypical features.

Methods Direct genomic PCR using primers in the *NEMO* gene was used

to test for the classical exon 4-10 deletion. Sequence analysis of *NEMO* was performed using standard Sanger sequencing. X-inactivation was measured using a methylation-sensitive restriction enzyme. An Affymetrix Cytoscan HD array was used to detect copy number variations (CNVs) according to the manufacturer's protocol.

Results The classical *NEMO* exon 4-10 deletion was detected in one patient and her mother. In another patient, a 130 kb interstitial gain in chromosome 17q25.3 was identified, containing five genes (*CD7, SECTM1, GPR14, TEX19* and *OGFOD3)*. There was no skewed X-inactivation. The fourth patient was not molecularly investigated.

Discussion We show four female patients with a clinical IP phenotype and different genetic defects. *NEMO* deletions impair NF-kB activation, causing IP cells to be highly sensitive to apoptosis. Interestingly, a novel 130 kb gain of chromosome 17q25.3 was identified in another patient, including *SECTM1*. *SECTM1* is shown to induce IFN-γ production *in vitro*; IFN-γ stimulates apoptosis. We hypothesize that a dosis effect, caused by the *SECTM1* duplication, induces cell death and thereby causes IP. This finding suggests heterogeneity of IP and supports the role of apoptotic pathway CNVs in this disorder.

P04.29-S

CMG2/ANTRX2 gene mutation analysis in 9 families suffering from Infantile Systemic Hyalinosis

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Infantile Systemic hyalinosis (ISH) is a severe rare autosomal recessive inheritance disease characterized by accumulation of amorphous, hyaline material in skin and other organs. It leads to skin lesions, gingival hypertrophy, flexion contractures of the joints, digestive tract and lymph node impairment with severe phenotypes in newborns. Beginning within the first few months of life, it is characterized by painful joints, generalized skin thickening, papules, periorificial nodes, hyperpigmentation over the joints, osseous nodes, failure to thrive. This disorder is progressive and usually leads to death, due to recurrent chest infections and diarrhea, before two years of age. The diagnosis is made by histology on skin biopsy showing hyaline deposits. Deleterious mutations have been reported in Capillary Morphogenesis Gene-2 (*CMG2*) alias Anthrax Toxin Receptor 2 (*ANTRX2*). *CMG2/ANTRX2* (4q21) is composed of 16 exons and the mutations reported in several families with ISH mostly concern exons 13 to 15. We report the causal mutations in 9 families found using Sanger sequencing. Mutations were found in 6 affected newborns, 22 relatives, among them 6 foetus with 6 homozygote mutations, 1 composite heterozygote and 1 associated to a supposed deletion. The 8 different mutations found will be described. 5 of them are non-sens mutations and 3 are 1-2 base(s) deletions or duplications leading to frameshift, Half of them are unknown in literature with 3 mutations in exon 1 and 10 and 1 between intron 8 and exon 9 affecting splicing. There is no treatment for this severe disease and the prenatal diagnosis is available.

P04.30-M

Whole-exome sequencing identifies a TTN mutation in a multiplex family with inguinal hernia

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Inguinal hernia repair is one of the most frequently performed gastrointestinal surgical procedures. Over 700,000 groin hernias are repaired annually both in the United States and Europe. Males are seven times more likely than females to develop a hernia and have a 27% lifetime risk of developing an inguinal hernia. Risk factors that have been associated with developing inguinal hernias include male gender, aging, pulmonary disease with chronic cough, and other conditions that cause constant increase of intra-abdominal pressure. Connective tissue disorders, such as Ehlers-Danlos and Marfan syndromes, and systemic collagen subtype imbalances have also been associated with increased risk of hernia. Furthermore, several studies have demonstrated that a positive family history is an important risk factor for the development of primary inguinal hernia.

The aim of this study was to investigate a multiplex Estonian family with inguinal hernia in four generations. Whole-exome sequencing was carried out in three affected family members and subsequent mutation screening using Sanger sequencing was performed in 8 family members (five affected and three unaffected). A heterozygous missense mutation p. Lys29627Thr was identified in the highly conserved A-band of the titin gene (*TTN*). The mutation co-segregated with the disease in the family, and was not present in 875 ethnically matched control subjects.

P04.31-S

Sibs with microdeletion in maternal 14q32.2 and phenotype congruent to paternal isodisomy 14

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A girl born to healthy non-consanguineous parents and her three-year-younger brother showed at birth moderate to severe respiratory insufficiency which required long-term tracheostomy in the boy. Both neonates were large with plump facial features, narrow thoracic cage with radiographic bell-shaped configuration. Diastasis of the abdominal wall musculature and signs of distal arthrogryposis were mild in the girl and pronounced in the boy. Neuromotor development was delayed with independent walking achieved at 18 months and at 5½ years respectively. The mild intellectual disability in the boy, who has persisting contractures at the wrists and ankles, may be correlated with bouts of therapy-resistant hypoxia during the NICU admission of six months duration. At night he still needs mechanical support with airflow.

The clinical and radiographic pattern in both sibs was compatible with paternal UPD 14. However, an imprinting defect was ruled out using microsatellite marker analysis. Recently, the lady's complaints about long lasting respiratory infections and physical tiredness prompted reexamination of this family. SNP array analysis using an Illumina Cyto-SNP 12v2.1 chip detected a microdeletion on chromosome band 14q32.2 in both sibs. The deletion with a minimal size of 64 kb encompasses the lncRNA genes MEG3 and MEG8 and several miRNAs. Each of these genes is subject to maternal imprinting. The mother is mosaic for the same microdeletion. Molecular analysis regarding exact size, gene content and degree of methylation is ongoing and bound to contribute to more insights into the disease mechanisms involved in this imprinted region.

P04.32-M

Increased frequency of F5 and F2 thrombophilia predisposing variants in families with unilateral limb reduction defects - a pilot study

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Limb reduction defects (LRDs) are usually unilateral and affect 8 newborns per 10000 livebirths. Several authors hypothesized that vascular accidents may account for a substantial proportion of LRDs, however the basis of such events in the fetus remains unknown. Over the past two decades, a number of genetic thrombophilia risk factors (TRFs) have been identified and associated with the increased risk of peri/postnatal occlusive disease and with a higher rate of pregnancy loss. Mutations c.G1691A(p.R506Q) in F5 and c.*97G>A in F2 genes represent one of the most commonly tested genetic TRFs. In this study, a cohort of 45 proband-mother pairs, in which the child manifested unilateral LRDs, was recruited for the analyses. The patients were clinically evaluated and blood of the probands and their mothers was subjected to both F5 and F2 testing. We found either F2 or F5 heterozygous variant (or both) in 6 mothers (13.3%) and in 4 probands (8.9%). At least one individual carried a single (or both) mutation(s) in seven proband-mother pairs (15.6%). Within this group one proband and one mother carried both mutations. F5 and F2 are identified in a control population with the frequency of about 1-2%. Based on our findings, we hypothesize that there is an excess of genetic TRFs in probands affected by unilateral LRDs and in their healthy mothers in comparison with control population. This may contribute to the higher risk of antenatal vascular accidents and consequently to LRDs. Further studies based on bigger samples are needed to support our findings.

P04.33-S

Homozygous Ala529Val LMNA Mutation in Patients with Mandibuloacral Dysplasia in a Turkish family

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Mandibuloacral dysplasia is a rare autosomal recessive disorder characterized by acroosteolysis, hyperpigmentation, mandibular and clavicular hypoplasia, bird-like facies with beaked nose and prominent eyes, delayed closures of the cranial sutures and joint contractures. Homozygous Arg527His and Ala529Val mutationS in LMNA gene are previously reported in MAD patients with type A lipodystrophy. Ala529Val (c.1586 C>T) missense mutation resulting in substition of alanin with valine may distrupt the formation of salt bridge between arginine 527 and glutamate 537. Mutant lamin A and C proteins alters the nuclear envelope, chromatin organisation and effects the cellular processes.

Here we report a Turkish family with MAD. The proband was 36 years old male and the first child of consanginous parents who have 3 children. He had acroosteolysis, hyperpigmentation, mandibular and clavicular hypoplasia, beaked nose,joint contractures .Aunt of the proband has also detected as MAD with consangineous parents. LMNA gene Exon 9 sequencing revealed homozygous Ala529Val LMNA mutation in affected persons of this family. Proband's parents are also detected as carrier for Ala529Val LMNA mutation and the other family members were detected with normal genotype.

Previously homozygous Ala529Val mutation in patients with MAD was first reported by Garg et al. Up to date our study was the second report about patients with homozygous Ala529Val mutation. Molecular studies of MAD patients will help to determine the genotype-phenotype correlation, variability of phenotypic expressivity of mutations and molecular basis of disease.

P04.34-M

A combined workflow for FBN1 mutation detection using next generation and Sanger sequencing methods

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Marfan syndrome is an autosomal dominant disorder of connective tissue with skeletal, ocular and cardiovascular system involvement. Sequencing of the fibrillin-1 (FBN1) gene has a 70-90% mutation detection rate in patients with Marfan syndrome. The goal of this study was to establish a diagnostic workflow for rapid and reliable mutation detection in the FBN1 gene.

Exons and flanking regions of the FBN1 gene were amplified in 65 amplicons using published and newly designed primers. Our workflow was based on the followings: 1) exons containing homopolymer regions (n=16) were tested by Sanger sequencing method because of the well known high error rate of pyrosequencing-based next-generation sequencing (NGS) in such regions, 2) all other amplicons were sequenced using NGS (Roche GS Junior), where coverage criterion was above 40x. Pathogenic mutations detected by NGS method were confirmed by Sanger sequencing.

Eleven families (15 patients) were tested with a mutation detection rate of 8/11 unrelated patients. 7 missense (6 of them affecting cystein residues) were found and one base pair deletion causing frameshift. 5 novel mutations were detected.

By combining NGS and Sanger sequencing methods reliable diagnostic workflow could be established for Marfan syndrome.

P04.35-S

Melorheostosis and *LEMD3* related sclerosing bone diseases: clinical, radiological and molecular characterization of an Italian series

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Melorheostosis is an uncommon, relatively benign, sclerosing bone disease, characterized by long bones hyperostosis, resembling flowing of candle wax. Skin and subcutaneous tissue can be involved. The disease may be asyntomatic (incidental X-ray diagnosis, finding of the typical hyperostosis pattern) or cause pain, joint stiffness or deformity. Melorheostosis is sporadic and pathogenesis is largely unknown. Occasionally in the same family we observe individuals with melorheostosis and osteopoikilosis, disease characterized by multiple round foci of increased bone density. *LEMD3* gene mutations have been revealed in osteopoikilosis and Buschke-Ollendorff Syndrome (BOS), an association of skin lesions, as connective tissue naevi, and osteopoikilosis or melorheostosis. Sporadically *LEMD3* mutations have been identified in Melorheostosis. Recently it has been suggested that *LEMD3* mutation somatic mosaicism cause Melorheostosis. Most reports describe single cases or are focused on orthopedic management; only few and small series have been reported in medical literature and molecular analysis data are available only for few patients. We present clinical and radiological information of 23 patients with Melorheostosis (14), BOS (2), osteopoikilosis (3) or with findings of similar disease (4). Among patients with isolated melorheostosis until now analyzed, none has *LEMD3* germline mutation. Instead, we identified mutations in all BOS patients and in 2/3 patients with osteopoikilosis. No mutation has been identified in cases with unclear diagnosis. These results confirm literature data, indicating that *LEMD3* germline mutations are not a major cause of isolated melorheostosis and are mainly associated to BOS or osteopoikilosis. Further studies are needed to understand molecular bases of Melorheostosis.

P04.36-M

Intragenic deletion of the FBN1 gene in a child with mildly dilated aortic sinus: a retrotransposal event

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Marfan syndrome (MFS), an autosomal dominant connective tissue disorder, is mainly due to fibrillin 1 (*FBN1*) gene mutations. MFS primarily affects the skeletal, ocular and cardiovascular systems. The vast majority of MFS cases are caused by point mutations in *FBN1* with only 1-2% due to large deletions. Transposons are abundant in the human genome and retrotransposons have been reported to cause disease by inserting into genes. We describe the detection of an intragenic *FBN1* deletion by chromosomal microarray analysis (CMA) in a child with mildly dilated aortic sinus. Breakpoint sequencing showed that the deletion was due to a SVA retrotransposition. The child was first seen at 4 years of age. Echocardiogram showed a mildly dilated aortic sinus. He had a history of muscular VSD (closed spontaneously) and trivial mitral regurgitation. Phenotypes include frontal bossing, anteverted ears, pink striae and joint hyperlaxity. He also had a learning disability and attention deficit. CMA with an Agilent 1M array detected a 36 kb deletion at 15q21.1 which is predicted to result in a deletion of exons 7-9 of the *FBN1* gene. Long range PCR confirmed the deletion of exons 7-9 of the *FBN1* gene and the insertion of a MAST2 SVA transposon. CMA confirmed a diagnosis of MFS in a child who had a few MFS clinical symptoms but who did not fulfil the Ghent criteria. This diagnosis will enable appropriate surveillance, patient management and genetic counselling. To the best of our knowledge, this is the first report of MFS caused by retrotransposition.

P04.37-S

Panel-based Next Generation Sequencing - a powerful diagnostic tool for patients with genetic skin diseases

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Purpose: Genetic skin diseases (genodermatoses) represent a broad and heterogeneous spectrum of mainly rare conditions. Overlapping phenotypes additionally often hamper clear clinical diagnosis and straightforward genetic testing. As a precise genetic diagnosis is crucial for counseling of patients regarding the course of the disease, prognosis, recurrence risk, and therapeutic approach, we have developed a diagnostic panel based on Next Generation Sequencing (NGS).

Method: 241 genes were selected from the OMIM database and literature. After customized target enrichment, NGS was performed using the HiSeq 2500 platform (Illumina), followed by bioinformatics analysis. Variants with minor allele frequencies < 5 % were selected for further investigation. Validation of relevant variants was performed using Sanger sequencing.

Results: We subdivided the genodermatoses panel according to the most relevant phenotypes, e.g., ichthyosis, epidermolysis bullosa, ectodermal dysplasia, connective tissue defects, dyskeratosis congenita, albinism, and skin disorders associated with malignancy. In a pilot study we have identified clear pathogenic mutations within the genes *ALOX12B*, *TP63*, *PLOD1*, and *ABCC6*. The preliminary data indicate high detection rates: in 22 % of the cases we identified clearly pathogenic mutations, in 33 % likely pathogenic variants were detected, and 44 % remained unsolved. **Conclusion:** We have developed a comprehensive panel based diagnostic NGS tool for diagnosing genetic skin diseases. This highly reliable and very cost-efficient diagnostic panel enables us to molecularly diagnose patients with clinically heterogeneous and also very rare skin diseases. Furthermore, it contributes to a better understanding of genotype-phenotype correlations.

P04.38-M
Neurofibromatosis type 1 and Legius syndrome: differential molecular diagnosis in children

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Neurofibromatosis type 1 (NF1) and Legius syndrome (LS) are autosomal dominant disorders caused by germline mutations in *NF1* or *SPRED1* gene respectively. The NF1 phenotype partially overlaps the most benign of LS that can be just molecularly distinguished. Appearance of typical NF1-features, like neurofibromas and Lisch nodules, is age-related thus pigmentary manifestations (cafè-au-lait macules and/or freckling) can be the only clinical sign of NF1 in early childhood, making difficult to distinguish NF1 and LS. The occurrence of serious NF1-complications (i.e. MPNST, brain gliomas) emphasizes the usefulness of an early differential diagnosis. To address a proper diagnosis, we applied a specific clinical workflow and a comprehensive genetic test in a cohort of 149 children. Pigmentary manifestations were considered as the main sign combined with presence of typical NF1 features and/or affected first-degree, and with the age at medical examination. 128 (85.9%) had a clinical diagnosis of NF1. For 17 (11.4%) NF1 vs LS was undistinguishable and 4 (2.7%) had a diagnosis of familiar occurrence of pigmentary manifestations. A causative mutation was found in 122/128 patients (95.3%) confirming NF1 clinical diagnosis. 11 patients with ambiguous diagnosis carried *NF1* mutations (64.7%), 2 carried *SPRED1* mutations (11.8%). Among those thought to be LS, 3 had mutation in *SPRED1* (75.0%), one in *NF1* (25.0%). Children with just NF1 pigmentary manifestations would be clinically followed for long time waiting the age-related appearance of most typical features. Thus an early molecular diagnosis might be very useful, considering that the milder LS phenotype require a less intensive follow-up.

P04.39-S
Clinical description of NF1 patients and motivations to perform the genetic test

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Diagnosis of Neurofibromatosis I (NF1) is typically based on clinical criteria, with genetic testing being performed only in a fraction of cases. We reviewed the records of NF1 patients referred to our clinic to explore what factors may have conditioned the choice to undertake genetic testing.
Among 100 consultants attending for a family/personal history of NF1 in the period December 2002 to December 2013, clinical data were available for 94 patients: 68 patients satisfied the criteria for the clinical diagnosis of NF1 and in 44 of these (65%) NF1 gene analysis were performed, leading to mutation detection in 26 cases (60%). Tested patients differed from untested patients by median age at diagnosis (12 versus 23 years) and number of criteria satisfied (≥3 in 75% of tested and 30% of untested), while the rate of familial cases did not differ between the two groups (27% among tested, 26% of non-tested). Moreover, the rate of patients tested has increased in the years, from the 33-50% in the period 2003-2005 to the 50-100% of patients tested in the period 2006-2013. In conclusion, genetic testing was more likely to be undertaken in patients diagnosed in childhood and in those with a more severe phenotype, especially in recent years. Indeed NF1 analysis is demanding but has been improved across the years, which makes genetic test now more accessible.

P04.40-M
Tyrosinase gene mutations in Chinese Han population with OCA1

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Background/Aims: Oculocutaneous albinism (OCA) is a heterogeneous autosomal recessive genetic disorder which affects melanin synthesis. OCA results in reduced or absence of pigmentation in the hair, skin, and eyes. Type 1 OCA (OCA1) results from tyrosinase (*TYR*) gene mutation and is a severe disease type. This study investigated *TYR* mutations in a Chinese cohort with OCA1. **Methods:** This study included two parts: patient genetic study and prenatal genetic diagnosis. Thirty OCA1 patients were subjected to analyze *TYR* gene mutation. Ten pedigrees were included for prenatal genetic diagnosis. One hundred unrelated healthy Chinese individuals were characterized for controls. The coding sequence and the intron/exon junctions of *TYR* were analyzed by bidirectional DNA sequencing. **Results:** Twenty mutations were identified, four of which were novel. In these thirty OCA1 patients, twenty-five patients were *TYR* compound heterozygosity; two patients carried homozygous *TYR* mutations and three were heterozygous. Among the ten prenatal diagnostic fetuses, three fetuses carried compound heterogeneous mutations; seven carried no mutation or only one allele mutation in *TYR* and appeared normal at birth. **Conclusion:** We identified four novel *TYR* mutations and showed that molecular based prenatal screening to detect *TYR* mutation in a fetus at risk for OCA1 provided essential information for genetic counseling of at risk couples.

P04.41-S
Oligodontia segregating with a 7p21.2p21.1 ~1 Mb duplication in an Italian family with three affected siblings

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We report on a 60 yrs. patient with short stature (< 2nd centile), spontaneous eruption of 7 teeth only, fusion of C1 to C4 vertebrae with partial occipito-atlantal fusion. Two of her seven siblings presented oligodontia. Standard chromosome analysis in the proband showed a mosaic karyotype 45,X[3]/46,XX[27], which may relate with the short stature. Array-CGH (60K, Agilent Technologies) showed a ~1 Mb duplication encompassing chromosome 7p spanning eight genes (*ISPD, SOSTDC1*, *LRRC72*, *ANKMY*, *TSPAN13*, *AGR2* and *AGR3*): (arr[hg19] 7p21.2p21.1(15,926,980x2,15,99 4,233-17,074,396x3,17,222,770x2). Real-time PCR on available members showed the duplication segregated in the two siblings with oligodontia, but it was absent in one healthy sister. We hypothesize that oligodontia is associated with the duplication of *SOSTDC1* (OMIM* 609675), which is a member of the sclerostin family. The protein acts as a bone morphogenetic protein (BMP) antagonist. Mice overexpressing Sostdc1 have a reduced number of teeth, whereas *Sostdc1*$^{-/-}$ show an increased number of teeth number and anomalies in teeth morphology. The gene has been demonstrated to antagonize Wnt signaling in mice. Interestingly, *WNT10A* mutations have been associated with oligodontia in humans. In conclusion, we speculate that the duplication of *SOSTDC1* at chromosome 7p21.2p21.1 may increase its protein level leading to oligodontia in our family. *SOSTDC1* could be a new candidate gene to be screened in isolated oligodontia.

P04.42-M
Identification of a novel regulatory variant in *OPTN* in familial cases of Paget's disease of bone

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Paget's disease of bone (PDB) is a common bone disease with strong genetic component. Previous studies have identified a PDB-susceptibility locus on chr10p13 tagged by rs1561570 which is located within the *OPTN* gene (Albagha *et al, Nat Genet* 2011). *OPTN* encodes a protein that plays a role in multiple cellular processes including autophagy and NF-KB signalling but its role in bone metabolism is yet unclear. Here we investigated the 10p13 locus by targeted DNA sequencing of a 650kb region surrounding the rs1561570 which includes seven genes. A total of 260 samples were sequenced including unrelated cases and controls, 60 familial cases and controls. All cases were negative for *SQSTM1* mutations. Target capture was performed using Haloplex kit followed by DNA sequencing using the illumina Hiseq2000 platform. Variants passing quality control measures were filtered to include only rare coding (MAF<0.01 in 1000Genome) or regulatory variants (as predicted by ENCODE). No disease-specific missense mutations were detected in any of the seven genes located in this region including *OPTN*. However, we identified a novel rare variant located in the *OPTN* promoter which was found to alter NF-KB transcription factor binding site. This novel variant had a high call quality (Q>4200, read depth >150) and was not present in the public databases including 1000 genomes and showed transmission from an affected father to an affected daughter and the unaffected mother did not carry this mutation. In conclusion our data suggest that rare regulatory variants within OPTN influence PDB susceptibility.

P04.43-S
Evaluating susceptibility loci for nonsyndromic cleft lip with or without cleft palate in the Arabic population: Analysis of 15 risk loci in a new case-control sample recruited in Yemen

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) has a genetically complex etiology. In recent years, several genome-wide significant susceptibility loci for nsCL/P were identified, most of them by genome wide association studies. However, most studies have been performed in populations from Europe and Asia, and few data are available concerning genetic susceptibility to nsCL/P in Arabic populations. The present study investigated a newly recruited nsCL/P sample from Yemen. Twenty-four single nucleotide polymorphisms (SNPs) representing all 15 currently known nsCL/P risk loci were genotyped in 242 nsCL/P cases and 420 healthy controls. Single marker association analysis revealed significant associations for four loci (8q24, 9q22, 10q25, 13q31). The strongest association was for the European high risk locus at 8q24 ($P_{corrected}$ = 5.09x10^{-4}; heterozygous odds ratio (OR_{het}) = 1.74, 95% confidence interval (CI 95% CI) = 1.22-2.47, homozygous odds ratio (OR_{hom}) = 2.47, CI 95% = 1.55 - 3.93). Five additional loci (1q32.2, 3q12, 8q21, 17q22, 20q12) showed nominal significance that did not withstand correction for multiple testing. Two loci (1p36, 2p21) failed to reach nominal significance but displayed a trend towards association with $P < 0.1$. Although the four remaining loci (1p22, 3p11, 15q22, 17p13) failed to reach nominal significance, the risk alleles were in the same direction as in the discovery studies. Our results suggest that four of the 15 analyzed nsCL/P risk loci which were identified in European and Asian ethnicities significantly confer risk for nsCL/P in Arab populations.

P04.44-M

Defective proteolytic processing of fibrillar procollagens and other extracellular matrix proteins due to mutations in *BMP1* results in a severe form of Osteogenesis Imperfecta

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Bone morphogenetic protein 1 (BMP1) is an astacin metalloprotease with manifold roles in morphogenesis and diverse substrates. Besides its role as procollagen C-proteinase for the major fibrillar collagens (types I, II and III) and its participation in N-propeptide removal of the minor fibrillar collagens (types V and XI), BMP1 is involved in the proteolytic trimming of multiple other substrates including a.o. small leucine-rich proteoglycans (e.g. decorin). Mutations in *BMP1* have recently been identified in three families with a severe, autosomal recessive form of osteogenesis imperfecta (OI). We report novel, bi-allelic mutations in two unrelated adult patients with severe OI, characterized by severe osteoporosis with numerous fractures, short stature with limb deformities and severe kyphoscoliosis. Biochemical analysis of secreted (pro)collagens showed defective type I procollagen C-propeptide processing. Immunofluorescent staining of type I and V collagen secreted by the patients' dermal fibroblast cultures showed a reduced and abnormal collagen deposition in the extracellular matrix (ECM). In addition, the reduced BMP1-activity was shown to result in deficient proteolytic trimming of decorin, an important regulator of collagen fibril organisation, leading to the retention of its propeptide. Ultrastructural analysis showed marked variability in collagen fibril diameter and uneven interfibrillar spaces. Overall, this indicates that BMP1 defects result in disturbed collagen deposition in the ECM. Besides the defective procollagen processing, it is likely that deficient cleavage of other BMP1 substrates contributes to abnormal ECM assembly and signalling, and as such to the phenotypic severity.

P04.45-S

Genotype-phenotype correlation in patients with dominant Osteogenesis Imperfecta

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Osteogenesis Imperfecta (OI) is a rare genetic disorder of connective tissue that occurs in approximately one in 15000-20000 new born with mostly autosomal dominant inheritance caused by mutations in the type I collagen genes, COL1A1 and COL1A2. The clinical spectrum is strongly heterogeneous with a wide intrafamilial and interfamilial variability; a continuum ranging from nearly asymptomatic individuals with occasional fractures, normal stature and lifespan, to lethal phenotypes characterized by severe skeletal fragility, deformity and growth deficiency. Affected patients may also show blue sclerae, hearing deficit, dentinogenesis imperfecta, cardiac lesions and joint hyperlaxity.

Actually more than 1100 COL1A1/A2 distinct variants associated with dominant OI have been identified without hot spot regions; most of the mutations are glycine substitutions followed by splice site alterations and nonsense mutations. Despite the identification of many mutations, few genotype-phenotype correlation studies have been performed and there are no unambiguous and clear indications.

To evaluate whether the severity and specific clinical manifestations of disease are linked with a specific genetic background we performed a genotype-phenotype correlation study analyzing an Italian case study of 300 patients. All patients have a clinical and radiographic diagnosis of OI from mild to lethal perinatal forms, according with Sillence clinical classification. The presence of mutations in COL1A1/COL1A2 genes has been investigated using a molecular screening protocol with High Resolution Melting (HRM) and multiplex ligation-dependent probe amplification to detect either point mutations and big deletions-insertions.

P04.46-M

Osteogenesis imperfecta - a dominant mutation of COL1A2 gene c.964G>A causes in homozygote status more serious phenotype

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Osteogenesis imperfecta (OI) is a heterogeneous group of connective tissue disorders characterized by a predisposition to fractures and variable extraskeletal symptoms. Original classification distinguishes OI regarding clinical and RTG findings to OI types I-IV. Clinical manifestation of OI can result from mutations of minimal 12 various genes and in the most of OI cases (~90%) the mutations are identified in COL1A1- or COL1A2-gene. The mutations of these genes affect collagen type I synthesis (chains A1 and A2) and the vast majority of the mutations in these two genes follows AD pattern of inheritance.

We refer on a child from a consanguineous family (the parents are the first cousins) with clinical findings of OI type III-IV and molecular finding of homozygote pG322S mutation (c.964G>A). This mutation has been described only in heterozygote form, causing a mild form of OI - type I. The clinical features of 4,5 year-old boy represented a very low stature (18 cm below 3.Percentile), history of both femurs and upper arm fractures in early infancy, slight skeletal deformities and disproportions, inability to stand or walk without support, slightly blue scleras and dentinogenesis imperfecta. Mental status was age-appropriate.

Although the parents initially denied any health problems (both were 160 cm tall and healthy), after repeated questioning they admitted a history of possible femur and arm fractures in young age (the mother) and one fracture after fall (the father). The analysis confirmed heterozygosity for pG322S mutation in both parents.

P04.47-S

A novel mutation in IFITM5, encoding BRIL, impairs osteoblast production of PEDF and causes atypical type VI osteogenesis imperfecta

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Osteogenesis imperfecta (OI) type V is caused by a recurrent dominant mutation (c.-14C>T) in *IFITM5*, which encodes BRIL, a transmembrane ifitm-like protein strongly expressed in osteoblasts, while type VI OI is caused by recessive null mutations in *SERPINF1*, encoding pigment epithelium-derived factor (PEDF). We identified a 25-year-old woman with severe OI, with normal serum PEDF but absent PEDF secretion by cultured osteoblasts. Her *SERPINF1* sequences were normal despite bone histomorphometry typical of type VI OI. Whole exome sequencing of the proband, parents and an unaffected sibling revealed a *de novo IFITM5* mutation in one allele of the proband, causing a p.S40L substitution in the BRIL intracellular domain. *IFITM5* expression was normal in proband fibroblasts and osteoblasts, as was BRIL protein level in proband osteoblasts on western blot and in permeabilized proband osteoblasts by microscopy. Notably, *SERPINF1* expression was decreased in proband osteoblasts and PEDF was barely detectable in conditioned media of proband cells. Expression and secretion of type I collagen was similarly decreased in proband osteoblasts, confirming this OI as collagen-related. Osteoblasts with the S40L mutation also had decreased expression of ALP and osteocalcin, as seen in primary PEDF defects. In contrast, osteoblasts from classical type V OI have increased *SERPINF1* expression and PEDF secretion during differentiation. These data suggest (1) that the type V OI and p.S40L BRIL are gain- and loss-of-function mutations, respectively, and (2) that BRIL and PEDF have a relationship that connects the genes for types V and VI OI and their roles in bone mineralization.

P04.48-M

A novel deletion mutation involving TMEM38B associated with autosomal recessive osteogenesis imperfecta.

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Osteogenesis imperfecta (OI) is and hereditary bone disease characterized by decreased bone density and multiple fractures, usually inherited in an autosomal dominant manner. Recently several genes encoding proteins related to collagen metabolism have been described in some cases of autosomal recessive (AR) OI (like *CRTAP, LEPRE1, PPIB, FKBP65, SERPINF1, BMP1)*. More recently, *TMEM38B*, a gene involved in releasing of calcium from intracellular stores and in cell differentiation, has been associated with AR OI. We describe the second new deletion-mutation involving the *TMEM38B* gene in a 11 year-old Albanian female, born of apparently non consanguineous parents from the same small village, showing an OI clinical phenotype. Since molecular analysis of *COL1A1* and *COL1A2* genes were negative for the presence of causative mutations, a SNP array analysis was performed using the Illumina Infinium SNP genotyping platform (HumanOmniExpress - 12 chips and BeadStation Scanner) in order to detect regions of homozygosity encompassing genes known to be involved in AR OI. The analysis revealed one homozygous region larger than 2 Mb (chr9:107,793,426 - 109,935,841) overlapping with the *TMEM38B* locus and characterized by a 35 Kb homozygous deletion spanning from marker rs1567368 to rs9408800 and involving exons 1 and 2 of *TMEM38B* gene. A long PCR amplification confirmed this finding. Eventually, we postulated that the deletion was promoted by the presence of repeated sequences in the segments before and after the breaking points. Our finding contributes to expand the role of *TMEM38B*, thus supporting the role of deletions in generating this type of AR OI.

P04.49-S

High spontaneous osteoclastogenesis in pediatric osteogenesis imperfecta patients receiving and not receiving intravenous neridronate

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Background. Osteogenesis Imperfecta (OI) is a heritable disease of connective tissue characterized by abnormal bone mineralization and skeletal deformities. Objective. This study investigate the osteoclastogenic potential of unfractioned peripheral blood mononuclear cells (PBMCs) from OI patients (mean age 10,44+/- 3,48) who received cyclical neridronate infusions for at least 1 year, untreated OI patients and control subjects. **Methods.** PBMCs from 6 patients and 6 controls were cultured in presence/absence of M-CSF and RANKL. At the end of the culture period, mature multinucleated osteoclasts (Ocs) were identified as TRAP+ cells. By real time PCR we studied gene expression in freshly isolated PBMC. By flow cytometry we characterized the presence of OC precursors (CD14+/CD16+) and TNF-α expression. **Results.** Spontaneous OC formation, without adding M-CSF and RANKL, occurred in PBMC cultures from treated and untreated OI patients. In these patients, the percentage of circulating OC precursors, increased respect to the controls (12,5% vs 0,1%, p< 0,01). By real time PCR, we found high levels of RANKL, TNF-α and MSCF receptor, as well as decreased OPG levels, thus leading to the increase of RANKL/OPG ratio. High TNF-α levels were also found on monocytes through flow citometry. **Conclusion.** We showed for the first time the high osteoclastogenic potential of PBMCs from OI patients, treated and untreated with biphosphonate, which could be due to the high percentage of circulating OC precursors, to the elevated TNF-α levels as well as to the increased RANKL/OPG ratio. This condition could contribute to bone disease affecting these patients.

P04.50-M

Study of ATG2B and ATG16L1 polymorphisms in Spanish patients with Paget's disease of bone

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Paget's disease of bone (PDB) it's a chronic skeletal disorder, that is characterized by abnormal resorption associated with inadequate remodeling, that leads to deformity of the bone. It's the second most common metabolic bone disease after osteoporosis and affects approximately 3% of the population over 40 years old and approximately 10% of those over the age of 85 years.

Missense and truncating mutations have been reported within the SQSTM1 gene (25–50% of familial and 5–10% of sporadic PDB patients) encoding the p62 protein. This protein has a crucial role as an assembly factor for autophagy, important for cell survival. Recent investigations showed that Microtubule-associated protein 1A/1B-light chain 3 (LC3) directly interacts with p62 via a newly identified LC3-interacting region (LIR), located between its ZZ and ubiquitin-associated UBA domains.

We studied the potential association of the two variants of autophagy-related gene (ATG), ATG2B (rs3759601) and ATG16L1 (rs2241880), in 292 patients with PDB and 192 controls, using the TaqMan® Pre-designed SNP-Genotyping Assay(Applied Biosystems).

We detected a significant association between genotype GG and PDB in the ATG2B polymorphism rs3759601 (p=0.004, OR=0.434; 95% CI=0.246-0.769). Moreover, we found a statistical significance between the T allele variant in ATG16L1 polymorphism rs2241880 (TT: p=0.000 OR=5,102 CI=2.853-9.123; CT: P=0.030 OR=2.821 CI=1.850-4.300)

In conclusion, these findings suggest that allele G of ATG2B polymorphism might contribute to increase risk in Paget's disease development and allele T of ATG16LB1 polymorphism may play a protective role in Paget's disease of bone in Spanish patients.

P04.51-S

Common variants at 20q11 influence skin color in Europeans

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We carried out a genome-wide association study (GWAS) on skin color in 6,000 Dutch Europeans followed by successful replications in thousands of Europeans from Australia and UK. We identified a total of five distinct genomic regions showing genome-wide significant association with continuous or categorical skin color phenotypes - all including known pigmentation genes: 5p13.2 SLC45A2; 6p25.3 IRF4; 15q13.1 OCA2 and HERC2; 16q24.3 MC1R; and 20q11.22 ASIP. The role of 20q11.22 in skin coloration has been much less clear than other genes/regions. In this large region (spanning ~ 1.5Mb), the top-associated DNA variant was rs6059655 (P=4.2e-13) in RALY. All other associated SNPs in this region were all in linkage disequilibrium with rs6059655 (r2>0.4). Among these are rs1885120 in MYH7B and rs910873 in PIGU that have been previously associated with risk of melanoma. Additional transcriptional analysis of 20 genes from 20q11.2 in 29 human skin epidermis samples highlighted RALY as well as other regional genes such as EIF2S2, ITCH and GSS with significant (P<0.01) expression differences between light and dark pigmented skin samples. A multivariate analysis highlighted 9 pigmentation genes (i.e. OCA2-HERC2, IRF4, SLC45A2, MC1R, RALY/ASIP, TYR, BNC2, SLC24A4, and SLC24A5 in a descending order) jointly explaining 16.3% phenotypic variance of perceived skin darkness in Europeans. The weighted allele sums showed a spatial pattern that is clearly correlated with latitude in Europe, but much less so in the rest of the world, suggesting that variants responsible for skin color variation between Asians and Africans are not included here.

P04.52-M

A spectrum of disorders are associated with somatic mutations in *PIK3CA*, encoding the p110α catalytic subunit of phosphatidylinositol-4,5-bisphosphate 3-kinase

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Aim: Somatic activating mutations in *PIK3CA,* encoded by the p110α catalytic subunit of phosphatidylinositol-3-kinase (PI3K), have been identified in several different disorders of segmental overgrowth. PI3K is a critical mediator of cellular growth, survival and metabolism, and is frequently mutated in cancers. We assessed the prevalence of *PIK3CA* mutations in 40 patients with variable forms of mosaic overgrowth.

Methods: Screening of DNA derived from affected tissue was undertaken using a PCR-based target enrichment with the Ion AmpliSeq™ Cancer Hotspot Panel v2™, and sequenced on an Ion PGM platform. Exons 2,5,7,8,10,14,19 and 21 were screened, and the detection level was 5% at 1500x depth.
Results: We identified 20 patients with somatic variants in exons 8, 10 and 21 of *PIK3CA* exhibiting a range of clinical phenotypes. There were no reported cases of cancer or metabolic disturbance, and 1/3 of adult patients had minimally progressive disease from the third decade.
Discussion: Somatic activation of *PIK3CA* causes a diverse spectrum of disease, ranging from isolated digit enlargement to more extensive overgrowth of the limbs, abdomen, brain or blood vessels. The rate of malignancy appears to be low, although this will need to be substantiated in larger studies. In the future, identification of biomarkers to predict disease progression will provide critical prognostic information, whilst the possibility of therapy with inhibitors of the PI3K-AKT pathway warrants evaluation in clinical trials.

	Number of cases screened	p.His1047Arg	p.His1047Leu	p.Glu542Lys	p.Glu545Lys	p.Cys420Arg	p.Gly1049Arg
Macrodactyly	14	4	1	1	0	0	1
Fibroadipose hyperplasia	9	4	2	0	0	1	0
CLOVES	6	1	0	0	3	0	0
MCAP	3	1	0	0	0	0	0
Klippel-Trenaunay syndrome	2	0	0	0	0	0	0
Hemihyperplasia	3	0	0	0	0	0	0
Proteus	2	0	0	0	0	0	0
Complex epidermal naevus syndrome	1	0	1	0	0	0	0
TOTAL	40	10	4	1	3	1	1

Congenital Lipomatous Overgrowth, Vascular Malformations, Epidermal Naevi, Scoliosis/Skeletal and Spinal (CLOVES) syndrome), Megalencephaly-Capillary Malformation (MCAP) syndrome. Tissue screened included FFPE, fresh tissue and dermal fibroblast cell lines.

P04.54-M
PRINS, the psoriasis susceptibility related non-coding RNA contributes to various aspects of cellular stress response

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We identified PRINS (Psoriasis susceptibility Related non-coding RNA Induced by Stress) and showed that it is highest expressed in the psoriatic uninvolved epidermis. According to the results of in vitro experiments PRINS expression is induced by various stessors such as UVB, translation inhibition and microbial agents. It has been previously demonstarted by us and by others that keratinocytes of the psoriatic uninvolved epidermis possess an abnormal response to various stressors and respond with hyperproliferation. Therefore we aimed to understand how the altered expression of PRINS in the uninvolved epidermis contributes to the aberrant stress response of the keratinocytes thus to disease susceptibility. To this end we identified a chaperon protein, nucleophosmin (NPM) as a direct interacting partner of PRINS. We could demonstrate that UV-B irradiation induced the shuttling of NPM from the nucleolus to the nucleoplasm and silencing of the PRINS non-coding RNA in the UV-B-irradiated keratinocytes resulted in the retention of NPM in the nucleolus. These results suggest that PRINS is physically and functionally linked to NPM, thus it plays a role in the NPM-mediated cellular stress response. In an other set of experiments we have demonstrated that PRINS signals independently from the NF-kappaB signal trasduction pathway, however its expression was induced when the keratinocytes were treated with the inflammasome-activating poly(dA:dT).
Our results indicate that the PRINS non-coding RNA is part of a ribonucleo-complex. Its altered expression in psoriatic uninvolved epidermis could contribute to the well-established aberrant stress response of psoriatic keratinocytes and as a consequence to psoriasis susceptibility.

P04.55-S
Association of Pseudoxantoma Elasticum with renal nefrocalcinosis: a rare manifestation of the disease

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We describe a 40 years old man from Albania with clinically evident hematuria and proteinuria; renal function was normal. The peculiar renal CT findings were: small multiple calcifications at the cortical junction bilaterally. The pedigree analysis revealed two sisters affected by an unspecified skin disease; no consanguinity was reported in the family. The clinical genetic examination showed yellowish papules of the skin on the neck. The association of renal calcifications in a young patient with skin lesions and the recurrence of a skin disease in the family led us to take into account Pseudoxanthoma elasticum (PXE). Therefore an ocular examination looking for signs of the disease was performed and angioid streaks radiating from the peripapillary area without subretinal neovascularization signs were detected. Pseudoxanthoma elasticum (PXE), is a rare multisystem disease characterized by degeneration and calcification of elastic fibres and blood vessels. The causative gene is ABCC6, mapped on chromosome 16p13.1, which encodes an ABC transporter protein (ABCC6) expressed primarily in liver and the kidneys. Patients typically develop cutaneous, ocular, cardiovascular and gastrointestinal manifestations. The molecular analysis of the ABCC6 gene detected a homozygous deletion of exons 23-29 (del23_29); this is the most common deletion described in literature associated to PXE, resulting in a premature stop codon with loss of 505 amino acids of MRP6 protein. Although this renal pattern cannot be considered specific for the diagnosis of PXE, we recommend to test PXE when both renal and skin abnormalities are present in a patient.

P04.56-M
KIF3A is associated to arthopaty envolvement in psoriatic patients

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Psoriatic arthritis (PsA, OMIM #607507) is a chronic inflammatory disorder presenting a highly heterogeneous phenotype and clinical course. PsA is a multifactorial disease associated with psoriasis, affecting the 30% of patients with psoriasis vulgaris (PsV).
Some genetic risk factors are specific for the arthropaty, other are shared with PsV (HLA-Cw*06:02, LCE, TNFα and TRAF3IP2). On this subject, different European studies have recently found several susceptibility genes, which are involved in the inflammatory and immunologic pathways of the disease.
To date, no associations between PsA and genes involved in bone metabolism have been observed. Thus we decided to screen a list of genes involved in bone metabolism and/or osteogenesis. We have carried out this screening in 429 PsA patients and 417 health, 380 PsV cases and 389 controls. A single nucleotide polymorphism rs2897442 (A/G) showed a significant association in PsA cases (p = 0.006; OR = 0.73, 95% CI 0.59-0.92). Interestingly , it is not associated to PsV but only in PsA. The rs2897442 is located in the 8th intron of KIF3A gene (5q21), which encodes for a kinesin II complex subunit required for the assembly of primary ciclia and involved in bone formation and in keratinocyte differentiation. Full resequencing of coding and regulatory regions failed to reveal evidence of further association. LD pattern does not reveal significant haplotypes associated to PsA. Immunohistochemistry analyses, as well as replication in an independent data set of patients, are expected to clarify the functional role of KIF3A in the pathogenesis and development of PsA.

P04.57-S
Spectrum of phenotypic anomalies in four families with deletion of the SHOX enhancer region

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SHOX alterations have been reported in 67% of patients affected by Léri-Weill dyschondrosteosis (LWD), with a larger prevalence of gene deletions than point mutations. It has been recently demonstrated that these deletions can involve the SHOX enhancer region, rather that the coding region, with variable phenotype of the affected patients.
Here, we report a SHOX gene analysis carried out by MLPA in LWD patients from 4 families with variable phenotype. All patients presented a SHOX enhancer deletion. In particular, a patient with a severe bilateral Madelung deformity without short stature showed a homozygous alteration identical to the recently described 47.5 kb PAR1 deletion. Moreover, we identified,

for the first time, in three related patients with a severe bilateral Madelung deformity, a smaller deletion than the 47.5 kb PAR1 deletion encompassing the same enhancer region (ECR1/CNE7).
Data reported in this study provide new information about the spectrum of phenotypic alterations showed by LWD patients with different deletions of the SHOX enhancer region.

P04.58-M
Sjögren-Larsson syndrome: molecular characterization of the first reported Cypriot families

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Sjögren-Larsson syndrome (SLS; MIM #270200) is a rare autosomal recessive disorder caused by deficient fatty aldehyde dehydrogenase (FALDH) activity. This reduction in enzymatic activity is secondary to bi-allelic mutations in the ALDH3A2 gene and results in impaired fatty alcohol oxidation and accumulation of fatty alcohols and related lipid products. SLS is typically characterized by pruritic ichthyosis, spasticity, intellectual disability and seizures. In the present study we report, for the first time in the Cypriot population, three apparently unrelated SLS patients. All three were homozygous for a CCC deletion at nucleotide 941 to 943 coupled with a 21 nucleotide 5'-GGGCTAAAAGTACTGTTGGGG-3' insertion (c.941-942delins21-bp). This genetic alteration, identified previously in two Caucasian patients, leads to the substitution of Ala314 and Pro315 to Gly and Ala, respectively, accompanied by the addition of six amino acids, Ala-Lys-Ser-Thr-Val-Gly (p.Pro315AsnfsX7).

P04.59-S
Next step in collagenopathies and osteodysplasies diagnosis: NGS Targeted re-sequencing

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Collagenopathies and osteodysplasies constitute a genetically heterogeneous group of diseases with low incidence, representing however an important health problem because patients require continuous specialized care, due to their deteriorating quality of life. The molecular diagnosis is essential to confirm clinical suspicion, but is not straightforward due to the high number of candidate genes. NGS targeted re-sequencing is a fast and cost-effective alternative to Sanger sequencing, as it can sequence all the genes involved in disease development.
We designed a NGS targeted re-sequencing panel for 229 genes associated with skeletal dysplasias, which spans 1.5Mb comprising coding exons, splice sites and 5' and 3' untranslated regions. Once panel was validated for diagnostic use testing HapMap cell lines, these regions were sequenced in 40 patients with clinical suspicion of these pathologies. Target regions were enriched and captured using the Enrichment SureSelect system (Agilent) and sequenced either with a SOLiD 5500 (Life Technologies) or MiSeq (Illumina) platform. Results were confirmed by Sanger sequencing.
The analysis identified 47 variants in 24 patients, of which 8 were classified as disease-causing and 4 as probably pathogenic. Both types of variants were either truncating mutations or glycine substitutions in collagen genes (essential for collagen structure). The identification of these mutations allowed the molecular diagnosis and an appropriate genetic counseling in affected families. Targeted re-sequencing is especially suited to highly heterogeneous diseases such as skeletal dysplasias, because it allows the identification of mutations in genes whose study by traditional sequencing would be difficult and expensive, increasing diagnostic time.

P04.60-M
Mutations in DNASE1L3 and familial SLE/Hypocomplementemic Urticarial Vasculitis Syndrome: the first italian case

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease that causes substantial morbidity. Much of the heritability of SLE remains unknown. Here we present the case of a patient who showed typical sign of an autoimmune disorder (recurrent febrile episodes, arthritis, skin lesions) at age 17. The clinical phenotype has worsened with age as many other symptoms appeared (asthenia, severe anemia, C3 and C4 decrease; urticarial dermatitis; microscopic hematuria, petechiae; glomerulonephritis; splenic strokes; interstitial pneumopathy, pulmonary hypertension; mesenteric ischemia). Interestingly, we also observed joint hyperlaxity with a peculiar apparent contractions of interphalangeal joints (manually extendable without difficulties). Familial history revealed parents' consanguinity; moreover, two other siblings were reported to show signs of an autoimmune disorder. Molecular analysis of *DNASE1L3* revealed the homozygous mutation c.289_290delAC (Thr97Ilefs*2), previously reported in three sisters, born from consanguineous parents and affected with HUVS (Hypocomplementemic Urticarial Vasculitis Syndrome). This is the first italian reported case of autosomic recessive SLE/HUVS caused by a mutation in *DNASE1L3* gene.

P04.61-S
Spondyloenchondrodysplasia in Brazilian patients with intrafamilial variability and a pattern of pseudo-dominance

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Spondyloenchondrodysplasia (SPENCD) is an AR spondylometaphyseal dysplasia characterized by radiolucent spondylar and metaphyseal lesions, whose patients can also present neurological and immunological changes. It is caused by mutations in ACP5, a gene encoding the tartrate-resistant acid phophatase. Considering that just 24 individuals with mutations on this gene were described so far, and the great clinical heterogeneity of this condition, the unusual pattern of a new family prompted us to present these Brazilian patients. The proband is the third child of non-consanguineous parents. At birth he presented mild hydrocephalus, esophageal atresia without fistula, and absence of right kidney. None radiological evidence of skeletal dysplasia was present. At the age of 6 it was observed short stature (-3SD), mental retardation and skeletal changes compatible with SPENCD. Complementary investigations showed normal CGHa and sequencing of the TRPV4, no calcification in the CNS, and autoimmune hypothyroidism. The radiological evaluation of his sister (19 yo) and his maternal uncle (34 yo) due to mild disproportionate short stature showed similar aspect of the spine without, however, other skeletal involvement. The proband´s mother has vitiligo without skeletal changes. By direct sequencing of the ACP5 a homozygous mutation (c.791T>A) was identified in the proband, his sister and his maternal uncle. The parents were heterozygous. We conclude that the proband has a pattern of malformation not associated with the SPENCD diagnosis; unlike the metaphyseal, spine involvement seem to be permanent; the homozygosity strongly suggest a high inbreeding in this family, and the mother´s vitiligo could suggest a heterozygous manifestation.

P04.62-M
Identification of copy-number variations (CNVs) in patients affected by split hand/foot malformation (SHFM)

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Split-hand/foot malformation (SHFM) refers to the group of congenital limb malformations characterized by the absence or hypoplasia of the central rays of the autopod, especially the digits but also the metacarpals and/or metatarsals. The malformation is clinically and etiologically heterogeneous. It often shows reduced penetrance and variable expressivity. SHFM occurs either as an isolated trait or part of a multiple congenital anomaly syndrome. The genetic origin of approximately 50% cases is still unknown. The main objective of this study was to identify submicroscopic genomic rearrangements in a group of 30 probands affected by isolated or syndromic SHFM with use of high-resolution array based comparative genomic hybridization (aCGH). All probands were previously screened for the most common SHFM-related genetic lesions (i.e. 10q24.32 and 17p13.3 duplications, TP63 mutations). Results of aCGH in one family with isolated SHFM showed a pathogenic deletion in SHFM1 (7q21.3) locus, much smaller than typical causative changes identified in this region. In another proband with syndromic SHFM, we identified a novel duplication likely being responsible for the patient's phenotype. In four other probands we detected previously unreported copy number variations of unclear clinical significance.

P04.63-S
The impairment of MAGMAS function in human is responsible for a severe skeletal dysplasia

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Impairment of the tightly regulated ossification process leads to a wide ran-

ge of skeletal dysplasias and deciphering their molecular bases has contributed to the understanding of this complex process. Here, we report a homozygous mutation in the mitochondria-associated granulocyte macrophage colony stimulating factor-signaling gene (*MAGMAS*) in a novel and severe spondylodysplastic dysplasia. MAGMAS, also referred to as PAM16 (presequence translocase-associated motor 16), is a mitochondria-associated protein involved in preprotein translocation into the matrix. We show that *MAGMAS* is specifically expressed in trabecular bone and cartilage at early developmental stages and that the mutation leads to an instability of the protein. We further demonstrate that the mutation described here confers to yeast strains a temperature-sensitive phenotype, impairs the import of mitochondrial matrix pre-proteins and induces cell death. The finding of deleterious *MAGMAS* mutations in an early lethal skeletal dysplasia supports a key role for this mitochondrial protein in the ossification process.

P04.64-M
A novel missense mutation in ST14 in a patient with ichthyosis, follicular atrophoderma and hypotrichosis

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Congenital ichthyoses are a clinically and genetically heterogeneous group of genodermatoses characterized by disorders of cornification. Mutations in *ST14* are causative of two syndromes: Ichthyosis, Follicular Atrophoderma and Hypotrichosis (IFAH, OMIM 602400) and Autosomal Recessive Ichthyosis and Hypotrichosis (ARIH, OMIM 610765). These two syndromes are caused by different types of mutations in the gene: loss of function mutations (splice site or deletion) have been found in 2 IFAH families, while missense mutations have been found in 2 ARIH probands. We report on the identification of a new homozygous disease-causing mutation in *ST14* in a girl with diffuse ichthyosiform scaling of the trunk and arms, follicular atrophoderma of both forearms and sparse hair. The screening of *ST14* by Sanger sequencing revealed a homozygous missense mutation, p.Glu519Gln, inherited by healthy parents, both carriers of the variation described. The mutation is not present in 1000Genomes nor in ESP databases, involves a highly conserved aminoacid and is predicted to be pathogenic by Polyphen2 and SIFT. *ST14* encodes the matriptase, a type II transmembrane serine protease, expressed predominantly in the epithelial cells of the surface-lining epithelium. Our result supports the role of matriptase alterations in IFAH, but the finding of a missense mutation points out the need of other experiments to better define the functional role of different mutations in the pathogenesis of IFAH and ARIH.

P04.65-S
De Novo Mutation of the Latency-Associated Peptide Domain of TGFB3 in a Patient with Clinical Features of Loeys-Dietz Syndrome

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We report the first case of a patient with a disease-causing mutation in the LAP domain of TGFB3 [c.899G>A, p.Arg300Gln; no mutation detected in FBN1, TGFBR1, TGFBR2, TGFB2, and SMAD3]. Previously, TGFB mutation has been described in one patient only (Rienhoff et al. 2013, Am J Med Genet A 161A:2040-6). That mutation was hypomorphic while we cannot exclude that mutations of the LAP domain result in constitutive activation of TGFB3 as in case of LAP mutations of TGFB1 [Saito et al., 2001, J Biol Chem 276:11469-72]. There were phenotypic similarities between our patient and the patient reported by Rienhoff et al. (2013) such as low muscle mass, hypertelorism, cleft soft palate, pectus excavatum, and arachnodactyly, but also considerable differences such as overgrowth and generalized hyperextensibility of joints instead of growth retardation and digital contractures, respectively. Both the previous case and our present case emphasize the inclusion of TGFB3 in the comprehensive genetic testing of (Marfanoid) patients with LDS clinical features, such as bifid uvula and hypertelorism. Further studies are needed to clarify the phenotypic spectrum of TGFB3 mutations including the risk for vascular disease.

P04.66-M
Modelling Poikiloderma with Neutropenia in zebrafish: a start point to elucidate disease pathogenesis

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Poikiloderma with Neutropenia (PN; OMIM#604173) is a rare autosomal recessive genodermatosis characterized by early onset poikiloderma, hyperkeratosis, pachyonychia, bone alterations, craniofacial dysmorphisms and non-cyclic neutropenia, which accounts for recurrent infections in infancy and susceptibility to myelodysplastic syndrome and solid tumours.
C16orf57, disclosed in 2010 as the causative gene of PN, has been recently renamed USB1 (U six biogenesis 1) as its protein is a RNA exonuclease involved in the 3' end processing and stability of U6 snRNA, a core component of the active spliceosome. However, no evidence for splicing defects could be achieved in PN patients cells unlike in Usb1 defective yeast. As no multicellular organism has been modeled for PN, we enrolled zebrafish, a vertebrate model increasingly important for the study of human genetic diseases and haematological malignancies, to gain insights into the consequences of USB1 disruption during early embryogenesis.
The zebrafish genome harbours only one copy of the USB1 orthologue (NM_001003460.1) ubiquitously expressed and with a high degree of conservation in genomic structure and aminoacid sequence (73% similarity and 46% identity).
Loss-of-function experiments, performed injecting three different specific antisense, morpholinos in embryos at one cell stage, allowed to obtain embryos with an overall phenotype that recapitulates the major traits of PN.
Usb1 depletion causes the development of embryos with decreased skin pigmentation, small head with defects in early cartilages of pharyngeal arches, oedema in the pericardial area, defects in blood circulation with a reduction of myeloid and erythroid cells as highlighted by in-situ hybridization and real-time experiments.

P04.67-S
Whole-exome sequencing identifies polymorphic variants in a large Arab family with split-hand/foot malformation with long-bone deficiency

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Split-hand/foot malformation with long-bone deficiency (SHFLD) is a rare, severe limb deformity**.** This is characterized by tibia aplasia with or without split-hand/split-foot deformity. Using DNA microarray analysis and employing various statistical methods, we have mapped the SHFLD1 and SHFLD2 phenotypes to chromosomes 1q42.2-q43 and 6q14.1 regions respectively. Additionally, we have identified six suggestive loci with evidence of linkage on chromosomes 1p36.13, 1q31.1, 1q42.3, 4q34.3, and 6q14.1 and 17p13.1 regions in a large multigenerational Arab family (Am. J. Hum. Genet.2007; 80:105-111). Subsequently, we have reported microduplications on chromosome 17p13.3, suggesting the association of BHLHA9 gene within the duplication in the pathogenesis of SHFLD development (J Med Genet.2012 Feb;49:119-25). Our recently performed exome sequencing using the SOLiD™ system at x200 coverage followed by prioritized mutation search within the linkage region between SNP markers rs1124110/rs535043, and rs623155/rs1547251 in selected SHFLD subjects showed polymorphic variants within the coding regions of *FILIP1* gene on 6q14.1 regions. A heterozygous nucleotide substitution G>A (c.3476G>A) resulting in a change from arginine and histidine Arg1159His (R/H) was observed in heterozygous condition in selected affecteds. However the data on chromosome 1q31.1 region is yet to be analyzed. Our present analysis provides the understanding the pathophysiology of the SHFLD disease and also provides ultimate genetic diagnosis of the condition. Additionally, the data can also help us in developing non-invasive methods of screening for the disorder in at-risk family members, in order to reassure those are not carrying the mutation and to plan prophylactic measures for those who are/will be affected.

P04.68-M
Biomarkers and early stage drug development in ectodermal dysplasias

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EDI200 is a recombinant ectodysplasin currently in clinical trials for the correction of abnormal ectoderm development in neonates affected by X-linked hypohidrotic ectodermal dysplasia (XLHED). For early drug trials, there is a premium placed on identifying molecular markers of drug response likely to predict long-term clinical benefit, especially when endpoints in XLHED such as teeth, hair and sweat function may take months to years to ascertain. The ectodysplasin-deficient Tabby mouse model for XLHED demonstrates a consistent and sustained phenotype response to EDI200. Through a combined approach of qPCR and RNA-seq we have begun to map the Tabby ectodysplasin-responsive biological pathways. EDI200 at 2 mg/kg (or vehicle alone)

was administered by intraperitoneal injection to newborn Tabby mice, with tail, back and footpad tissues harvested at time points ranging from two hours to one month post-injection. Isolated RNA was subjected to analysis by qPCR for eight genes known to contribute to the development/structure of ectodermal placodes and appendages. Levels of expression for the ectodysplasin receptor EDAR and the downstream regulator sonic hedgehog (Shh) were upregulated specifically at 24 hrs post-injection in all tissues. RNA-seq analysis with DAVID bioinformatics software confirmed these results for EDAR and Shh and extended the gene set analysis to identify novel and unexpected response pathways including those for fat metabolism and solute transporters. This combined approach for biomarker validation and delineation of system response biology will provide an invaluable tool set in identifying pathways for drug targeting as well as in optimizing drug dosing in ectodermal dysplasias.

P04.69-S
A new col5a1 genomic variant identified in an italian patient with ehlers danlos syndrome classic type

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Ehlers Danlos syndrome (EDS) is a group of heterogeneous connective tissue disorders. The clinical classification recognizes six subtypes and the Classic type is the most frequent. Classic EDS is an autosomal dominant disorder, characterized by skin hyperextensibility, abnormal wound healing and joint hypermobility. It is estimated that approximately 50% of patients with classic EDS phenotype harbor mutations in COL5A1 and COL5A2 gene. We report a case of 43 Italian patient, presented to Ospedale Maggiore Policlinico (Milan) for clinical and genetic counselling. He showed: soft and hyperextensibility skin specially at the neck, face and hands, atrophic scars and joint hypermobility. The cDNA sequencing revealed a new single base variation in COL5A1 gene. The mutation was a change of C for T in exon63 and established an amminoacid substitution Leucine by Valine at the c.1656. This mutation was located on higly conservated domain of the protein. Sequencing of COL5A2 gene and study of the null allele of COL5A1 gene didn't showed any differences. CGH-Array platform, characterized by a higher density of probes in those chromosomal regions that are thought to be related to EDS, didn't revealed any variations. Bioinformatics analysis identified that this nucleotide sequence is extremely conserved in different species, indeed, up to date, no mutation or polymorphism have been described for this region. The identification of new genomic variant represent the first step towards the understanding of symptom causes. Moreover, further studies on protein structure could be necessary to understand the real properties of this mutation.

P04.70-M
Further phenotypic delineation of metatropic dysplasia

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Dominant mutations in *TRPV4* are responsible for a family of rare skeletal dysplasias that encompasses, in decreasing severity, metatropic dysplasia (MD), parastremmatic dysplasia, spondyloepiphyseal dysplasia Maroteaux type, spondylometaphyseal dysplasia Kozlowski type, autosomal dominant brachyolmia, and familial digital arthropathy with brachydactyly. Here, we present the case of two monozygotic twins, daughters of healthy, non consanguineous parents. They were referred to our clinic at age 5 months because of suspected skeletal dysplasia. They presented unspecific craniofacial dysmorphism, including congenital torcicolis associated to a C1-C2 subluxation, long narrow trunk and short extremities. Radiologically they presented marked platyspondyly, flared metaphyses, a halberd shaped pelvis and no carpal ossification. Now, aged 24 months, they show an inversion of the proportions due to progressive and severe kyphoscoliosis. *TRPV4* sequencing analysis revealed a c.2396C>T (p.Pro799Leu) heterozygous *de novo* mutation, conforming the diagnosis. This *TRPV4* mutations, that affects the cytoplasmic domain of the protein is one of the most common MTD mutation and usually associated with a moderate phenotype. In conclusion, MD is diagnosed by its characteristic clinical and radiographic features. Genetic testing can help confirm a diagnosis and to provide specific molecular prenatal genetic testing to couples at risk, as germline mutations has already been reported. Being a rare disorder with few reports in the medical literature, consultation with an experienced clinical geneticist may be required before a diagnosis is made. An early diagnosis is beneficial for early referral of the patients to the appropriate follow-up. These patients are best managed by a multidisciplinary team.

P04.71-S
A review of Molecular Genetic diagnosis of Epidermolysis bullosa cases from Southwest Iran

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Epidermolysis bullosa (EB) is a skin disorder that is divided regarding involved skin area into three main forms: Junctional epidermolysis bullosa (JEB), dystrophic epidermolysis bullosa (DEB), and epidermolysis bullosa simplex (EBS). EB ranges from mild to very severe forms with the age of onset form infancy to childhood. The landscape of EB is extensive blistering and scarring. Other signs include fused fingers and toes, joint deformities and alopecia. EB is a rare disorder with autosomal recessive and dominant genetic pattern. This disease is very rare in southwest Iran. From last decade to date, 12 individuals were diagnosed for DEB. But, more clinical differential diagnosis was not possible for patients. Mutations in 4 genes COL17A1, LAMA3, LAMB3, and LAMC2 cause the JEB disease, whereby the LAMB3 gene mutations are responsible for more than 70% of all JEB cases. In contract, mutation in the COL7A1 gene causes all three forms of DEB including autosomal recessive HS -RDEB, non HS-RDEB, and autosomal dominant DDEB. Mutations within exons 70-75 of the COL7A1 gene very frequent in the DEB patients. However, three patients from southwest Iran with DEB as preliminary diagnosis showed frame shift mutations within exons 73-74, and another individual was positive for a novel nonsense mutation in the LAMB3 gene. He was also affected by JEB, and not DEB. We were not able to detect any mutation in other 8 individuals with EB. Exom sequencing or next generation sequencing might be helpful to resolve their puzzle.

P04.72-M
Perturbation of Specific Pro-mineralizing Signalling Pathways in Human and Murine Pseudoxanthoma Elasticum

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Pseudoxanthoma elasticum (PXE) is characterized by skin, ocular and cardiovascular manifestations, due to calcification and fragmentation of elastic fibres. Caused by mutations in the ABCC6 gene, the mechanisms underlying this disease remain unknown. The knowledge on the molecular background of soft tissue mineralization largely comes from insights in vascular calcification, with involvement of the osteo-inductive Transforming Growth Factor beta (TGFβ) family (TGFβ1-3 and Bone Morphogenetic Proteins [BMP]), together with ectonucleotides (ENPP1), Wnt signalling and a variety of local and systemic calcification inhibitors. In this study, we have investigated the relevance of these signalling pathways as well as apoptosis and ER stress using immunohistochemistry and mRNA expression profiling in dermal tissues and fibroblasts of PXE patients, and the eyes and whiskers of the PXE knock-out mouse. Apoptosis was evaluated by TUNEL staining. We demonstrate upregulation of the BMP2-SMADs-RUNX2 and TGFβ-2-SMAD2/3 pathway, co-localizing with the mineralization sites, and the involvement of MSX2-canonical Wnt signalling. Further, involvement of apoptosis is shown with activation of Caspases and BCL-2. In contrast to vascular calcification, neither the other BMPs and TGFβs nor endoplasmic reticulum stress pathways were perturbed in PXE. Our study shows that we cannot extrapolate knowledge on cell signalling in vascular calcification to a multisystemic mineralization disease as PXE. Contrary, we demonstrate a specific set of perturbed signalling pathways in PXE patients and the mouse model, and propose a preliminary cell model of ECM calcification in PXE.

P04.73-S
Neonatal case of thumb duplication: case report and review

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Introduction. The preaxial polydactyly represents a complete or partial duplication of the thumb, usually is unilateral. The thumb duplication is

the most common anomaly of the hand. This congenital anomaly may be isolated and sporadic or expressed with a syndrome's phenotype. Within the Oberg, Manske, Tonkin (OMT) classification thumb duplications are a failure of formation and/or differentiation affecting the radial/ulnar axis of the hand plate. The primary signal centre involved is the zone of polarising activity (ZPA) in the posterior part of the developing limb bud. Sonic Hedgehog protein, which is expressed in the ZPA, plays a major role in determining radial-ulnar characteristics. Other morphogens are involved in the development of thumb duplications. Instead for an approach of management the Wassel description is used. Case report. We describe a case of a female newborn with a isolated hand left preaxial polydactyly. The weight at birth was 2050g, 45 cm of lenght and head circumference 33 cm, physical examination revealed preaxial hexadactyly with thumb duplication. Extra digit was mild hypoplasic and the patient was unable to move it independently. Radiographs showed extra thumb containing two proximal phalanges (type IV polydactyly, according to the Wassel's Classification). Conclusion. In this condition are recommended to value radiographs of the affected limb to show whether the rudimentary digit contains skeletal elements, and any associated non-hand anomalies. Surgical reconstruction of the radial polydactyly is indicated, not only for the obvious cosmetic improvement, but also to obtain a stable, mobile thumb of adequate size and appropriate shape.

P05.01-S

The gene variants in 3' end of prothrombin gene in patients with idiopathic thrombophilia in Serbian population

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Background: Thrombophilia is a multifactorial disorder which arises from the interaction of acquired and genetic risk factors. Despite the significant effort made to understand the etiology of this disease, there are still a certain number of patients suffering from idiopathic thrombophilia.
Objectives: The aim of this study was to screen 3' end of prothrombin gene, which is susceptible for gain-of-function mutations due to its non-canonical sequence elements, in patients with idiopathic thrombophilia and to determine its eventual role in the pathogenesis of thrombophilia.
Material and Methods: This study was carried out in100 patients with idiopathic thrombophilia and 100 healthy controls DNA variants in the 715bp long region of the 3'end of the prothrombin gene were identified by sequencing.
Results: In our study, we detected two variants: A19911G and C20068T. The frequency of A19911G gene variant was slightly increased in the group of patients compared to controls. Heterozygous carriers of FII C20068T were four times more frequent in the patients (4%) than in controls (1%), but this difference did not reach statistical significance.
Conclusions: Our findings suggest that variant A19911G is not a significant risk factor (OR=1.05; 95%CI 0.57-1.93), while C20068T may represent a potential risk factor for idiopathic thrombophilia (OR=4.12; 95%CI 0.57-1.93). To confirm our results, further studies should be conducted in a larger cohort of patients.

P05.02-M

Molecular analysis of aneurysm genes in familial and sporadic abdominal aortic aneurysm

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The role of aortic aneurysm syndromes genes in abdominal aorta aneurysms (AAA) was investigated by analysing the TGF-β pathway genes *TGFBR1*, *TGFBR2*, *SMAD3*, *FBN1, EFEMP2,* smooth muscle cells genes *MYH11*, *MYLK* and *ACTA2* and the vascular Ehlers-Danlos gene *COL3A1* in a large group of familial and sporadic AAA patients.
Sanger sequencing was performed of all coding exons and exon-intron boundaries of the aneurysm genes in AAA patients diagnosed. Patients with at least one affected first-degree relative with an aortic aneurysm were classified as familial AAA (fAAA). *In silico* analysis was used for assessment of the clinical significance of the variants.
We found 38 different variants of unknown clinical significance (VUS) in this study population of 72 AAA patients including 56 fAAA patients (78%) and 16 patients (22%) with sporadic AAA. In fAAA one null mutation in *COL3A1* and 48 VUS were observed in 26 (46%) patients. In sporadic AAA one *de novo TGFBR2* mutation and 11 VUS were observed in 8 (50%) patients. Four VUS (11%) were possibly pathogenic; *TGFBR2* c.1234G>A (Val412Met), *MYH11* c.760C>T (Arg254Cys), *MYH11* c.5697G>C (Glu1899Asp), and *MYLK* c.3403G>A (Gly1135Arg). Fifteen patients (13 familial and 2 two sporadic) had complex genotypes, including seven *in cis* variants. Altogether 3% of the AAA patients had a pathogenic mutation and 24 (47%) one or more VUS.
The results endorse the genetic heterogeneity of AAA, showing a modest contribution of *TGFBR2* and *COL3A1* in AAA. In addition a high prevalence of rare VUS suggests involvement of other aneurysm genes in AAA.

P05.03-S

Longer GT repeats and rs2071746T allele in the heme oxygenase-1 gene promoter are associated with abdominal aortic aneurysm

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Abdominal aortic aneurysm (AAA) is multi-factorial disease with life-threatening complications due to mainly asymptomatic course of development. Vascular inflammation induced by oxidative stress contribute to pathogenesis. Inter-individual differences in response to oxidative stress are partially under genetic control. In this study the associations between the functional SNPs and (GT)n repeat length polymorphisms in genes involved in the vascular response to hypoxia/ischemia: HIF1A (hypoxia inducible factor-1α) and HMOX1 (heme oxygenase-1) and the development of AAA were examined.
The study encompassed a series of 518 AAA patients, 345 patients with atherosclerotic aortoiliac occlusive disease (AOID) and 498 controls. The HIF1A rs11549465C>T, rs11549467G>A and HMOX1 rs2071746A>T SNP genotyping was performed by using predesigned TaqMan SNP-genotyping assays. For simultaneous assessment of the HIF1A and HMOX1 (GT)n polymorphisms, the method based on multiplex-PCR with fluorescent-labeled sense primers and fragment size analysis using DNA sequencer has been developed.
We found, that carriers of the HMOX1 (GT)n repeat long allele (n>27) had increased risk of developing AAA (OR=1.46 for dominant model, P=.034). The frequency of carriers of both HMOX1 risk alleles: rs2071746T and/or long (GT)n repeat in AAAs (58,5%) was higher as compared to AIOD (49,0%, P=.007). On the other hand, the frequency of noncarriers in AAAs was 0.0%, as compared to 1.3% in controls (P=.010) and 0.9% in AIOD (P=.066).
In conclusion, HMOX1 gene promoter long (GT)n repeat allele and rs2071746T, allele related to decreased anti-inflammatory and antioxidant capacity of heme oxygenase-1, are associated with abdominal aortic aneurysm. Supported by Polish Ministry of Sciences grant NN403_250440.

P05.04-M

Age- and sex-specific causal effects of adiposity on cardiovascular risk factors

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Observational studies have reported different effects of adiposity on cardiovascular risk factors between men and women and among different age groups, but these aspects have not been investigated with regards to causality.
We estimated the causal effect of adiposity on blood pressure, lipid concentrations, glycemic indices, and markers of inflammation and liver disease in up to 67,553 individuals using a non-weighted genetic score constituting of 32 genetic variants associated with body mass index (BMI) within a Mendelian randomization framework. All analyses were stratified by age (cut-off, 55 years) and sex.
We found similar effects of the genetic instrument on BMI in different strata. Each additional allele of the genetic score was associated with an increase in BMI of 0.03 SD (95% CI, 0.028-0.033; P=3x10^{-107}). We found evidence of a causal effect of adiposity in non-stratified analysis on blood pressure (systolic and diastolic), circulating lipids (high-density-lipoprotein cholesterol, triglycerides), glucose homeostasis (HbA1c, fasting insulin), and markers of inflammation (C-reactive protein, interleukin-6), and liver damage (alanine aminotransferase and gamma-glutamyl transferase) (all P<0.05). We observed significantly larger causal estimates in younger individuals than in older for low-density-lipoprotein cholesterol and total cholesterol (P_{diff}=0.04 and

0.02 respectively). For fasting insulin, in secondary analysis, we found a larger effect of adiposity in males than in females (P_{diff}=0.01 using *FTO* variant as a genetic instrument).
We provide evidence for a causal effect of adiposity on many cardiovascular risk factors, and observe differential estimates across age and sex for insulin and circulating lipids.

P05.05-S
Genetics in acquired Long QT Syndrome (aLQTS)

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Background: Long QT Syndrome (LQTS) is an inherited arrhytmogenic disease characterized by a prolonged QT interval in association with life-threatening arrhythmias. The LQTS can be congenital (cLQTS) or acquired (aLQTS) as an adverse response to drugs, hypokalemia or bradycardia. Mutations in 13 genes have been associated with cLQTS, while the genetic background of aLQTS remains unclear. The aim was therefore to evaluate if a genetic substrate may contribute to aLQTS. Methods: Through a multicentre study, 211 aLQTS probands were collected from Japan, Italy and France. They were clinically evaluated and divided into 3 groups according to the baseline QTc interval: "true-aLQTS" (females QTc<460ms, males QTc<450ms, n=112), "unmasked-LQTS" (females QTc ≥ 460ms, males QTc ≥ 450ms, n=74), and "unclassified-LQTS" (without ECG, n=25). Genetic screening of the 5 major cLQTS genes was performed and mutations were compared to those of 875 genotyped cLQTS families. Results: Genetic analysis led to the identification of mutations in LQTS-susceptibility genes in 27% of aLQTS probands (57/211). In the "true-aLQTS" and "unmasked-LQTS", a mutation was detected in 23% and 38% of cases, respectively (p=0.04). Interestingly, KCNH2 was the most mutated gene (62% vs 15% of KCNQ1), while the frequency of mutations in the cLQTS population were 39% and 48%, respectively. Conclusions: Genes implicated in cLQTS play an important role also in aLQTS. The likely underlying mechanism would be a critical loss in repolarization reserve even in the presence of just a modest QT interval prolongation. KCNH2 mutations seem to have a prominent role.

P05.06-M
+11525 C/A Polymorphism in MicroRNA Binding Site of Angiotensinogen is Associated with Occurrence of Restenosis in Patients After Myocardial Infarction

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Introduction:
Angiotensinogen (AGT) is a key component of the renin-angiotensin-aldosterone system that plays a crucial role in blood pressure (BP) regulation. Increased BP is a well-known risc factor for various cardiovascular diseases. With the discovery of microRNAs, polymorphisms in 3´-untranslated regions (3´-UTR) of known genes became of interest. The aim of our study was to investigate the possible association of rs7079 in 3´-UTR of AGT gene with the outcomes in patients after myocardial infarction (MI).
Methods:
Total of 652 patients (men) presenting at the emergency room with chest pain with suspicious MI underwent selective coronarography. Diagnosis of MI was confirmed in 571 patients. Peripheral blood for DNA isolation was sampled during hospitalization and the genotypes were determined using TaqMan Genotyping Assay.
Results:
Statistically significant differences in age at onset of the first MI were observed among the genotypes of investigated polymorphism, the homozygote genotypes preseting with MI at younger age (AA/CC vs. AC; 58.3±9.7 years vs. 59.91±9.62 years, p = 0.04). Furthermore, statistically significant association was found between the occurrence of restenosis and AGT genotypes (p = 0.008). The logistic regression model revealed that the CC and AA homozygotes were at the higher risk of restenosis before 45 years of age compared to AC heterozygotes.
Conclusion:
Our study shows that rs7079 homozygotes present with MI at younger age and that the risk of restenosis is higher in these patients. This may be partially explained by altered miR-30 and miR-584 binding to 3´UTR region of AGT, as shown previously.

P05.07-S
Targeted next generation sequencing in heritable aortopathies uncovers unexpected findings

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Aortopathies are characterized by aneurysms, dissections, dilation, and tortuosity of predominantly the thoracic aorta (TAA) but also the abdominal aorta. Inherited forms comprising both syndromic and non-syndromic entities are genetically heterogeneous and phenotypically overlapping. Currently, mutations in at least 15 genes are known to cause familial thoracic aortic aneurysms (AAT3-AAT8), as well as dominant and recessive syndromic aortopathies. Molecular testing is impeded by similar phenotypes caused by mutations in different genes and mutations in the same gene leading to a wide clinical variability. In this setting NGS of an aortopathy gene panel offers an efficient alternative to conventional gene-by-gene Sanger sequencing.
We developed a NGS aortopathy multigene panel encompassing 15 genes (ACTA2, COL3A1, EFEMP2, ELN, FBN1, FLNA, MYH11, MYLK, NOTCH1, PRKG1, SLC2A10, SMAD3, TGFB2, TGFBR1 and TGFBR2). Coding and adjacent intronic regions were analyzed using Nextera Rapid Capture Custom Enrichment and 2x150 bp paired-end sequencing on a MiSeq instrument (Illumina). So far, the analysis of 20 patients with both syndromic and non-syndromic aortic disease identified eight disease causing mutations in seven patients in the genes COL3A1, EFEMP2 (compound heterozygous), FBN1 (2x), SMAD3, TGFB2 and TGFBR2, representing a diagnostic sensitivity of 35%. Additionally, two rare variants of unknown clinical significance were identified in the MYH11 and TGFB2 gene. Surprisingly, two patients with FBN1 and TGFB2 mutations were clinically diagnosed with vascular Ehlers-Danlos syndrome supported by ultrastructural findings of a skin biopsy. In conclusion, patients with aortopathies will benefit from a parallel testing approach enabling efficient and appropriate surveillance and intervention measures.

P05.08-M
Association of Apolipoprotein E polymorphism with coronary heart disease in Bulgarian population

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Apolipoprotein E (ApoE) plays a role in the regulation of lipid metabolism in humans. The objective of this work was to examine the association between ApoE gene polymorphisms and the risk of coronary heart disease (CHD) in Bulgarians. The case-control study was carried out on a total of 725 samples including 104 patients with angiographically verified coronary artery disease,151 patients with myocardial infarction and 470 population controls without data for cardiovascular complications. ApoE gene polymorphisms were genotyped by High Resolution Melting Analysis. The differences in allele frequency between the CHD patients and controls were evaluated with chi-square test. The frequencies of ApoE alleles in the CHD subjects were 0.81 for E3, 0.14 for E4 and 0.06 for E2, and in the control group were 0.84 for E3, 0.09 for E4 and 0.07 for E2. The ApoE4 allele frequency was significantly higher in the CHD patients than in the control group (OR=1.68, p=0.002). The carriers of E4 containing genotypes (E2/E4, E3/E4 and E4/E4) had a higher risk to develop CHD than carriers of E2 and E3 containing genotypes (OR=1.72, p=0.004). There were no significant differences in patients between the mean of total cholesterol, triglycerides, low density lipoproteins and high density lipoproteins levels among different ApoE ge-

notypes. The current study data suggest that ApoE4 allele is a significant risk factor for CHD in Bulgarian population. Acknowledgements: This work was supported by Infrastructural Grant: DUNK01/2/28.12.2009 "National Complex in Biomedical and Translational Research", funded by National Science Fund, Ministry of Education and Science, Bulgaria

P05.09-S
Targeted next generation sequencing of 51 genes involved in primary electrical disease

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Primary electrical disease (PED) encompasses a diversity of syndromes including the Short QT Syndrome, Long QT Syndrome, Brugada Syndrome, Early Repolarization Syndrome, and Catecholaminergic Polymorphic Ventricular Tachycardia. Each of these disorders predisposes to ventricular arrhythmias (i.e. polymorphic ventricular tachycardia, Torsade de Pointes, bidirectional ventricular tachycardia) that can degenerate into ventricular fibrillation which often results in sudden cardiac death. These disorders are all genetically heterogeneous and a significant phenotypic and genetic overlap exists. This overlap might be a source of misdiagnosis resulting in negative genetic testing. Therefore we suggest to test these patients for all genes involved in these disorders.
We developed and optimized a MASTR (Multiplex Amplification of Specific Target for Resequencing) assay comprising 51 genes involved in PED. The MASTR protocol consists of a multiplex PCR whereby a first PCR is performed to amplify all target regions followed by a secondary PCR in which patient specific barcodes and sequencing adaptors are incorporated. The PED assay consists of 951 amplicons distributed over 11 multiplexes. Following the MASTR assay, 2x250bp sequencing is performed on MiSeq v2. Next, data-analysis and -interpretation is performed using our local Galaxy-instance and our in-house developed variant database. For validation purposes, 20 CEPH samples (variants present in 1000G) and 20 positive controls were analysed, and we have achieved 100% sensitivity. Currently, we are in the process of screening 100 PED patients with unknown genetic defect. The results of this analysis will be presented. Subsequently, this panel will be implemented in a genetic diagnostic setting.

P05.10-M
Homozygous founder mutation in desmocollin-2 gene causes arrhythmogenic cardiomyopathy

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Dominant mutations in genes encoding desmosomal proteins are reported to cause arrhythmogenic cardiomyopathy (AC), an inherited heart muscle disease characterized by lethal ventricular arrhythmias and heart failure, accounting for 15 to 25% of cases of sudden cardiac death in patients <35 years. Recessive mutations are infrequent and most of them cause cardiocutaneous syndromes.
We report here the identification of the first founder homozygous desmocollin-2 (DSC2) mutation in the Italian population, segregating in 4 AC families showing a cardiac-restricted phenotype.
We performed an exon-by-exon analysis of the DSC2 gene on 80 unrelated Italian index patients diagnosed affected with AC according to revised 2010 Task Force criteria. We identified the p.D179G homozygous mutation in DSC2 gene in 4 (5%) of them, all originating from the same north-eastern Italian region. One of them resulted to carry an additional plakophilin-2 frameshift mutation. Haplotype analysis revealed a conserved haplotype among the DSC2 mutation carriers, strongly indicating a common founder. A severe form of biventricular cardiomyopathy with typical electrical features of AC was diagnosed in all homozygous mutation carriers, whereas heterozygous family members were clinically asymptomatic. In vitro functional studies on HL-1 cells showed that mutated DSC2 protein correctly localizes at the intercalated discs.
In conclusion, this is the first founder homozygous mutation in DSC2 gene invariably associated with a severe AC phenotype in the Italian population. This finding could have important implications for mutation screening strategy and risk stratification.

P05.11-S
Added Diagnostic Value of Multiplex Ligation-Dependent Probe Amplification of plakophilin-2 in Arrhythmogenic Cardiomyopathy

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Background: Arrhythmogenic Cardiomyopathy (ACM) is an inherited cardiomyopathy characterized pathologically by fibro-fatty infiltration and clinically by ventricular arrhythmias and an increased risk of sudden death. Genetic testing is limited to genes encoding desmosomal components resulting in a diagnostic yield of about 50%.
Aim: The aim of this study is to search copy number variations (CNVs) in the plakophilin-2 gene (*PKP2*) in order to increase the mutational detection in ACM.
Methods: Genetic screening for all 5 desmosomal-encoding genes was carried out in 60 unrelated patients with a clinical diagnosis of ACM by Sanger sequencing on a ABI-PRISM 3730 (Life Technologies). Genotype-negative probands underwent Multiplex Ligation-dependent Probe Amplification (MLPA) using SALSA MLPA kit P168 ARVC-PKP2 (MRC-Holland) and quantitative Real-Time PCR (qPCR) on a Light Cycler 480 (Roche Applied Science) in search of large deletions/duplications in *PKP2.*
Results: 60% of ACM patients (n=36) resulted positive on direct sequencing, showing 5 mutations in Desmoglein-2 gene (8%), 14 in Desmoplakin (23%), 7 in PKP2 (12%), 2 in Desmocollin-2 (3%), 2 in Plakoglobin (3%) and 6 patients (10%) carried multiple mutations. Additional screening by MLPA in patients without mutations in ACM-genes successfully identified a large heterozygous *PKP2* deletion, further confirmed by qPCR showing a *PKP2* copy number reduction when compared to control samples.
Conclusions: This study improved the diagnostic genetic yield in our population by nearly 2% for one gene, highlighting the usefulness of performing additional analysis for CNVs in ACM patients.

P05.12-M
Molecular and clinical analysis of digenic inheritance in a family with left dominant Arrhythmogenic Cardiomyopathy

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Background. Compound/digenic heterozygosity has been identified as one of the most important determinants of malignant arrhythmic outcome in arrhythmogenic cardiomyopathy (AC). The impact of two single nucleotide variations (SNV) in the AC phenotype expression in a small family was assessed.
Methods. Autopsy identified a concealed left dominant AC in a 40- years old competitive athlete. Conventional genetic screening for all desmosomal-related AC genes and parallel exome sequencing was carried out. SNV segregation and disease penetrance was further assessed in the family members.
Results. By exome and conventional sequencing two SNVs in different desmosomal genes were identified in the proband: one in exon 14 of DSG2, c.2137 G>A (rs79241126, E713K), previously reported in AC cases as an 'uncertain' variant with minor allele frequency (MAF) equals to 0.037; and the other SNV in exon 16 of DSC2, c.2603 C>T (rs141873745, S868F), considered a variant 'likely to be pathogenic' since can alter the functional properties of the protein, has no available reported MAF and in silico analysis predicted a malignant outcome (Polyphen-2: malignant, SIFT: probably deleterious).
Cascade genetic screening showed that all relatives were carriers for only one of the two SNVs. The brother and the sister, SNV carriers, showed a less severe AC phenotype with septal and LV late-enhancement at cardiac magnetic resonance.
Conclusions. The data herein reported confirm that digenic heterozygosity predicts a more severe phenotype and arrhythmic outcome in AC. However, the risk conferred by SNV in family members needs to be evaluated further by follow-up studies.

P05.13-S
Desmoglein-2 propeptide cleavage-site mutations in Arrhythmogenic Right Ventricular Cardiomyopathy

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Purpose
Mutations in Desmoglein-2 (DSG2), a heart specific cadherin, are common

causes of Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC). A hot spot of DSG2 missense mutations targets the consensus cleavage-site of DSG2 pro-peptide (Arg-X-Arg/Lys-Arg) by Kex2-like proprotein-convertases. These mutations are responsible for severe phenotype associating frequent severe right ventricular dysfunction and left ventricular involvement. In the present work, we aim to explore the pathophysiological mechanisms of these mutations.

Methods and Results

We performed ex vivo analysis on heart samples from two mutation carriers, and in vitro analysis by expressing wild-type (WT) or mutants pro-DSG2-GFP fusion proteins in cellular models. First, we demonstrated that all mutations prevented N-terminal propeptide cleavage. Using Biacore technology, we demonstrated that the presence of propeptide led to the loss of interactions between EC1 domains of cadherins, known to interact to structure desmosomes. Uncleaved pro-DSG2 mutants were correctly addressed to the intercellular junctions (cellular models) or at the intercalated disks (human tissue). However, we observed at low calcium concentration a mis-incorporation of pro-DSG2 mutants into desmosomes associated with an EGFR-dependant internalization of pro-DSG2 mutants and of desmosomal partners (Plakophillin-2 and Plakoglobin) suggesting increased turn-over of the unprocessed pro-DSG2. Mutants mis-incorporation in desmosomes was further confirmed by western-blot in cells submitted to cyclic mechanical stress showing an increase in mutant pro-DSG2 in the desmosome-independent soluble fraction as compared to DSG2-WT.

Conclusion

Our results strongly suggest a loss in desmosomal adhesiveness due to mis-incorporation of uncleaved pro-DSG2 mutant into desmosomes that might play a central role in ARVC pathophysiology.

P05.14-M

Human Genetic Evidence that Common Variants near PIK3CG are Associated with Atherosclerotic Plaque Hemorrhage and Vessel Density

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Aim: Atherosclerotic plaques may vary among individuals, in part due to heritable factors. However, the genetic architecture of plaque phenotypes is largely unknown. A common variant near PIK3CG on 7q22.3 has previously been associated with carotid plaque presence. Animal models suggest that PIK3CG may play a role in plaque formation through neovascularization. We hypothesized that the PIK3CG variant is associated with intraplaque hemorrhage (IPH) and vessel density in human plaques. Secondarily we focused on characterizing the functional role of genetic variants near PIK3CG in advanced human atherosclerosis.

Methods: We collected 571 Athero-Express Biobank Study patients, and genotyped them using Affymetrix SNP 5.0. After quality control we tested rs17398575 for association to immunohistochemically scored IPH and vessel density, correcting for age, gender and 10 principal components. We used the BiKE cohort to assess the effect of PIK3CG variants on PIK3CG expression in circulating monocytes (n=95) and in carotid plaques (n=126).

Results: The reported PIK3CG variant, rs17398575 (risk allele A, frequency=0.72), was associated with IPH (OR=1.40 [1.10-1.69 95% CI], p=0.0271) and vessel density (β=0.095 [0.0415 s.e.m.], p=0.0221).

The SNP dependent PIK3CG expression demonstrated a differential effect in the vascular wall (p=0.783 for rs17398575) compared to monocytes (p=0.0261 for rs17398575).

Conclusion: To our knowledge this is the first report involving the association of genetic variants to histological plaque phenotypes in humans. Further research should focus on replicating these results and elucidating the etiology of plaque vessel formation and intraplaque hemorrhage, as epidemiological studies demonstrated these associate with cardiovascular disease.

P05.15-S

Assessment of genetic risk for cardiovascular disease in Azores and mainland Portugal: a healthy population-based study

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Cardiovascular diseases (CVD) are a major source of morbidity and mortality worldwide, including the Azores archipelago where CVD have a higher mortality rate when compared to mainland Portugal. In order to investigate this question, we characterized 15 SNPs in 4 genes and 1 genomic region associated with CVD risk: 4 in PCSK9 (rs11591147, rs11206510, rs562556, rs505151), 4 in APOE (rs405509, rs429358, rs7412, rs439401), 4 in LDLR (rs2228671, rs5927, rs1433099, rs2738466), 1 in USF1 (rs10908821) and 2 in 9p21 (rs10757274, rs1333049). Genotyping was performed by real time PCR using TaqMan Assays in a sample of 170 Azorean and 108 mainland Portuguese healthy individuals. Results demonstrate that allele frequencies were similar in both populations; however, there were statistically significant differences for two SNPs: rs10757274 (9p21) and rs405509 (APOE; $\chi 2$, $p<0.05$). Genotype evaluation of rs1333049 (9p21), the most replicated risk SNP for CVD, showed that 19.4% and 18.5% of Azoreans and mainland Portuguese, respectively, present homozygosity for this risk allele. Moreover, it is possible to observe that, although there is no statistical significance, Azoreans (9.1%) carry a higher frequency of APOE4 allele compared to mainland (8.8%). Overall, the results are suggestive of an increased risk for CVDs in Azoreans compared to mainland; however, the joint analysis of all variants is being carried out. Finally, the results validate the need to study regional differences in Portugal, information that should integrate the Portuguese National Health Plan, in order to achieve a more direct and accurate strategy in terms of prevention and community health.

P05.16-M

A comprehensive multidisciplinary approach for the validation of the 2013 diagnostic criteria in Brugada Syndrome

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Background: Brugada syndrome (BrS) is a cardiac channelopathy characterized by a coved-type ST-segment elevation in the right precordial leads. The controversy about the value of high intercostal spaces (ICSs) and the number of diagnostic leads has been settled by a recent consensus statement. We have tested the validity of the new ECG diagnostic criteria through a multidisciplinary approach, including the molecular one. Methods: We analyzed 114 BrS patients with a spontaneous or drug-induced type 1 pattern recorded in one or more right precordial leads in 4th, 3rd and 2nd ICSs. The right ventricular outflow tract (RVOT) was localized by echocardiography. Molecular screening of SCN5A was performed through DHPLC and direct sequencing. Results: In total, 23 patients (21%) were carriers of a disease-causing mutation. The percentage of mutation carriers (MCs) and the cardiac event rate were similar irrespective of the diagnostic ICS (4th vs high ICSs: MCs,23% vs 19%; cardiac event rate 22% vs 28%) and the number of diagnostic leads (1 vs ≥ 2: MCs 20% vs 22%; cardiac event rate 22% vs 27%) used. The concordance between RVOT anatomical location and the diagnostic ICSs was 86%. Conclusion: The percentage of SCN5A-MCs and the cardiac event rate are similar regardless of the diagnostic criteria used. The site where the diagnostic pattern is recorded is mainly due to the anatomical location of the RVOT. This study supports the robustness of BrS diagnosis as achieved through the new diagnostic criteria.

P05.17-S

Mutational analysis of mitochondrial DNA in Brugada Syndrome patients

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Brugada syndrome (BrS) is a cardiac disorder characterized by typical ECG alterations and a high risk for sudden death due to ventricular fibrillation. This pathology has nuclear heterogeneous genetic basis and at present molecular diagnostic tests on nuclear DNA cover only 25-30% of BrS pa-

tients. The aim of this study was to assess a possible involvement of mitochondrial (mt) DNA variants in Brugada syndrome since their etiological role has been already described in several cardiomyopathies.
To reach this goal, mitochondrial genome of BrS patients was sequenced and analyzed. A specific mtDNA mutation responsible for Brugada syndrome can be excluded. However the most severe spontaneous ECG type 1 symptomatic patients show a high substitution rate in their mitochondrial genome. These patients also share a combination of four mt single nucleotide polymorphisms (SNPs: T4216C, A11251G, C15452A and T16126C), not found in asymptomatic subjects (either spontaneous ECG type 1 or induced) with a low number on mtDNA SNPs.
Our evidences suggest that the detected mtDNA allelic combination and a high number of mtDNA SNPs could represent an important cofactor in manifestation of BrS phenotype and seem to be associated to a higher risk for a more severe clinical state of Brugada syndrome.

P05.18-M
SCN5A mutation analysis in 147 Brugada syndrome probands

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SCN5A mutation analysis in 147 Brugada syndrome (BrS) probands identified 27 variants possibly associated with cardiac channelopathies, which resulted in a genetic diagnostic yield of 17,4%. 18 (66,7%) SCN5A variants have already been associated with BrS, 4 (14,8%) with other cardiac arrhythmias and 5 (18,5%) are novel undescribed variants. When only taking into account the patients with a baseline BrS type I ECG (8,2%) the genetic diagnostic yield increased to 41.6%. Interestingly, this was not observed in patients with a BrS type II ECG (11,6%), in which SCN5A
variants seem to be either novel or associated with other arrhythmias. We also identified a substantial number of described SCN5A mutations (> 9%) in BrS patients clinically diagnosed by ajmaline positive testing and a family history of BrS and/or sudden cardiac death (80,2%), demonstrating the added value of sodium channel blocker-induced ECG testing.
Segregation analysis was performed in available families of the identified SCN5A positive BrS probands to determine genotype-phenotype correlations. In more than 66% of tested families there was an incomplete segregation of the discovered variant. Revision of all ECG data revealed that some ajmaline or baseline ECG negative BrS patients with SCN5A mutations did not meet the stringent diagnostic criteria but demonstrated conduction disease. These results also urge for the need to revise the clinical diagnostic criteria for BrS, in order to stratify BrS patient groups based on more detailed phenotypic data necessary to discover novel major disease associated genes.

P05.19-S
Identification of candidate genes for Brugada Syndrome by targeted next generation sequencing

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Recent developments of next generation sequencing (NGS) represent a great opportunity to identify new candidate genes in genetically heterogeneous pathologies such as Brugada Syndrome (BrS), an inherited cardiac arrhythmogenic disorder with a prevalence of 1:5000 in Western countries leading to sudden cardiac arrest in young asymptomatic adults. Until now BrS genetic background remained elusive, since mutations in known genes cover approximately 30% of patients. We thus aimed at identifying new candidate genes performing targeted NGS in a cohort of 91 BrS patients. The coding regions of 158 genes were sequenced using the Illumina GAIIx platform, yielding a mean target coverage of 99.16% and a mean sequencing depth of 327.22x among the samples. Excluding all common polymorphisms and considering only protein-coding variations, we overall identified 98 novel variants in 71 subjects in a total of 70 genes, including missense, nonsense, splice-site and INDELs, and 33 clinical rs annotated in dbSNP137. To select more promising BS candidate genes, we then compared the mutational rate of each gene to that observed in repeated random sampling of healthy controls from 1000 genomes project data. Besides confirming an important role for sodium, potassium and calcium ion channels, our results identified new candidate BrS genes previously associated with other forms of inherited cardiopathies, such as ANK2, RYR2, DSG2, LMNA, suggesting an overlap between different disorders; however, many patients still remained genetically uncharacterized, prompting more extensive studies and suggesting a possible multigenic aetiology.

P05.20-S
A protein network of common susceptibility genes provides a link between inflammation and cardiovascular disease

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Genome-wide association studies (GWAS) have identified hundreds of susceptibility loci for chronic and inflammatory disease phenotypes in humans. There is increasing evidence that chronic inflammation is a crucial driver in the pathogenesis of cardiovascular diseases (CVD), which may be genetically determined. To understand the genetic architecture underlying chronic inflammation and CVD we performed a systematic analysis of (1) common risk alleles coming from published GWAS, (2) of protein-protein interaction (PPI) networks informed by (3) gene expression data with a defined molecular target involved in the inflammatory processes promoting CVD, myeloid-related protein (MRP) 8. (4) Through analysis of integrated haplotype scores (iHS) and F_{ST} values in HapMap phase 2 data, we investigated whether recent selection pressure acting upon inflammatory genes affected CVD susceptibility loci. Our findings provide significant evidence for a PPI network (P = 0.033), which connects inflammatory and cardiovascular susceptibility genes, and establish a genetic framework of inflammatory CVD. 41.59% of PPI genes are associated with immune functions. 28.3% of integrated genes can be linked to both, an inflammatory and cardiovascular disease phenotype. Interestingly, *CDKN2B*, and *CELSR2/PSRC1/MYBPHL/SORT1*, unequivocally replicated CVD loci, are integrated within this network as are several SNPs located in transcription factor recognition sequences, i.e. NFKB1, STAT3, which are key factors in inflammation. Finally, we observed a significant enrichment of inflammatory variants within CVD loci that are targets of selection (P=2.001e-11 in CEU population), suggesting that recent selective sweeps may have affected the genomic architecture underlying CVD.

P05.21-S
Systematic screening of rare coding variants in genes involved in cardiac arrhythmias

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The development of new strategies based on next-generation sequencing enables the large-scale screening of genes involved in rare diseases. We have developed a custom design based on the HaloPlex™ technology (Agilent Technologies) to sequence the coding regions of 163 candidate genes, including all genes previously linked to cardiac arrhythmias.
In total, 570 individuals were included in this study. To validate our design, we first analysed 42 patients with inherited cardiac arrhythmias. Among the 69 genetic variants previously identified in these patients, 68 were detected automatically after HaloPlex library preparation and Illumina sequencing. The undetected variant is a substitution located in a low-coverage region. Subsequently, 361 additional patients were analysed (178 patients with Brugada syndrome; 89 patients with early repolarization syndrome; 94 cases of progressive cardiac conduction defects). We also analysed 167 controls, over 65 years of age and showing no signs of cardiac rhythm or conduction abnormalities. The mean coverage was 577X and we found 5 rare functional variants per patient on average. Then, burden tests were performed to detect genes significantly associated to cardiac arrhythmias. This approach also identified potential new disease genes, and replication in an independent cohort is in progress.
Our study will lead to a catalogue of mutations in genes linked to hereditary cases of sudden cardiac death. The systematic screening of our cohorts will also guide our future molecular investigations for these diseases and contribute towards improving the prevention of sudden cardiac death.

P05.22-M

Targeted oligonucleotide-selective sequencing of 101 genes from 150 patients with idiopathic dilated cardiomyopathy in Finland.

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The genetic basis of idiopathic dilated cardiomyopathy (DCM) is recognized but still not widely utilized in diagnostics. Hundreds of mutations in >50 genes have been reported to associate with DCM. Comprehensive genetic testing not only improves diagnostics, prognostics and treatment optimization, but also allows effective screening of asymptomatic family members. We adopted the novel oligonucleotide-selective sequencing (OS-Seq) and developed a custom data analysis pipeline to identify pathogenic variants in 101 genes associated with cardiomyopathies. Our sequencing panels covered >99% of the targeted regions with >15x coverage. Validation with reference samples showed >99% sensitivity and >99.9% specificity. The accuracy to detect short INDELs was 100%. We sequenced well-documented 150 DCM patient and identified pathogenic or likely pathogenic variants in >50% of cases. Truncating titin gene mutations were identified in >20% of patients with familial form of DCM. Other major findings were in genes of the nuclear lamina, desmosomal genes and sarcomeric genes. We performed statistical analyses to identify genotype-phenotype correlations and performed family segregation analysis for selected kindreds. This study represents one of the largest multi gene analyses of patients with DCM, to date. The utilized novel technology allows significant cost reduction and rapid turn-around-time from sample to clinical interpretation. These results demonstrate that the high-throughput targeted OS-Seq platform for cardiomyopathies meets the technical criteria of clinical diagnostics and is a cost-efficient tool in clinical research.

P05.23-S

Application of (targeted) next generation sequencing (NGS) in clinical genetic diagnostics of cardiomyopathies

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The strength of next generation sequencing (NGS) in both research and diagnostics is becoming increasingly evident. It can be successfully applied to find causal mutations and confirm the clinical diagnosis in genetic cardiomyopathies. However, exome sequencing (ES) show incomplete representation and coverage of several exons, leading to clinically relevant mutations being missed. Therefore ES will, at least for now, coexist in clinical genetic diagnostics with other NGS-based strategies, such as targeted resequencing. To this end, using an enrichment kit targeting 48 genes associated with hereditary cardiomyopathies and analysing 90+ patient samples, we demonstrated that the sensitivity, specificity and robustness of targeted NGS are equal to those of Sanger Sequencing (SS). Subsequently, we constructed an improved kit targeting 55 genes and implemented this into routine diagnostics. Using this kit, 700+ patients have been analysed, and additional haplotype and cosegregation analyses were performed to further support pathogenicity of potentially causal mutations. Our results show that: (1) our approach results in significant increase in diagnostic yield, as in up to 50% of patients (potentially) pathogenic mutations were identified; (2) critical evaluation of the clinical diagnoses showed that higher diagnostic yields are achieved for patients fulfilling the respective cardiomyopathy subtype criteria; (3) *TTN* mutations account for a significant part of the yield; (4) in >10% of cases two or more (potentially) pathogenic mutations were identified; (5) further haplotype and cosegregation analyses support the pathogenicity of a significant number of potentially causal mutations. Taken together, our gene-panel based approach largely improved genetic diagnostics in cardiomyopathies.

P05.24-M

The genetic basis of early-onset cardiomyopathies in Finland

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Introduction: Cardiomyopathies (CMPs) are a group of severe, genetically heterogeneous heart disorders with more than 100 disease-causing genes reported to date. By enabling comprehensive genetic screening, Next Generation Sequencing (NGS) is an appealing diagnostic approach for CMPs. In this study, we apply NGS to a cohort of genetically undiagnosed patients in order to identify the disease-causing mutations and characterize the molecular background of early-onset CMP in Finland. By detecting robust genotype-phenotype correlations, genetic data can be informative in prioritizing cases to cardiac transplantation.
Materials and methods: Our cohort consists of 57 Finnish early-onset CMP patients. The clinical presentation is diverse, ranging from heart-specific muscle diseases to multiorgan syndromes. Nine of the patients were screened for mutations by whole-exome sequencing (WES) and 48 by targeted sequencing using a custom-designed panel (HaloPlex) of 117 cardiac genes. Variants were prioritized in respect to frequency and pathogenicity prediction. The candidate mutations were further verified to match the disease segregation in the family and to be absent in Finnish controls.
Results: Mutations were confirmed in four of the WES-investigated patients. Among these, screening of family members revealed *de novo* mutations in three sporadic severe cases. For the patients investigated with targeted sequencing, strong candidate mutations were identified in 14 cases.
Conclusions: In the current stage of the study, WES has led to a genetic diagnosis success rate of approximately 45%, while for targeted sequencing a success rate of 30% is expected. *De novo* mutations were found to cause CMPs in the early-onset cohort under study.

P05.25-S

Application of the next generation sequencing technology in identification of mitochondrial mutations in patients with congenital heart defects

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Introduction: Inherited type of cardiomyopathy is caused by mutations not only in nuclear genes, but also in mitochondrial genes. Mitochondrial DNA mutations are involved in development of cardiomyopathy through disturbing oxidative energy metabolism. It has been shown that various types of cardiomyopathy can be attributed to disturbed mitochondrial oxidative energy metabolism. The goal of our study was to apply next generation sequencing (NGS) technology as a method to detect mtDNA mutations in patients with cardiomyopathy.
Methods: 18 patients were included in this study. The entire mitochondrial DNA was amplified in two overlapping polymerase chain reaction (PCR) fragments from the Cardiac tissue of the patients undergoing cardiac surgery. mtDNA was deep sequenced by NGS technology.
Results: Six newborns and 12 infant patients with cardiomyopathy were studied using NGS technology and bioinformatics analysis of the sequence data allowed determination of new and reported variation for each individual. Both known and unknown mutations were determined from 18 of patients. Eleven novel mtDNA mutations were identified at seven patients. Three of the patients have novel mutations together with reported cardiomyopathy mutations. LHON, Cyclic Vomiting Syndrome with Migraine, Multiple Sclerosis, and Breast cancer risk associated mutations also observed at three patients.
Discussion: In this report, we provide the results of mtDNA analysis for 18 patients with cardiomyopathy. All patients displayed at least one mtDNA mutation. Sixty mutations were found, and 13 of them were unreported. This study represents the most comprehensive mtDNA mutational analysis in congenital cardiac infant patients.

P05.26-M

Copy number variants in the 22q11.2 region of congenital heart disease patients from São Miguel Island, Azores, Portugal

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Chromosomal rearrangements of the 22q11.2 region, including the 22q11.2

deletion and microduplication syndromes, are frequently associated with congenital heart diseases (CHDs). The present work aimed to study copy number variants (CNVs) in the 22q11.2 region of 87 CHD patients from São Miguel Island, Azores.
The CNVs were searched in all patients using MLPA, according to MRC-Holland protocol. Results showed that four (4.6%) out of 87 CHD patients presented CNVs, which were confirmed by aCGH in patients 1 and 2, and by FISH in patients 3 and 4. Patients 1 and 3, both affected with a ventricular septal defect, carried a de novo 2.5 Mb deletion of the 22q11.2 region, whereas patient 2, with an atrial septal defect, carried a de novo 2.5 Mb microduplication (2:1). Finally, patient 4 showed a 2.5 Mb triplication (3:1) and presented dysmorphic facial features, cognitive deficit, and aortic stenosis, a clinical feature not reported in the first case described in the literature. Interestingly, the evaluation of this patient's parents revealed that her non-affected father had a 2.5 Mb microduplication (2:1). Now we are investigating by microsatellite analysis the mechanisms responsible for microduplication and triplication.
In summary, the present study allowed the identification of very rare deletion and microduplication syndromes in Azorean CHD patients. Moreover, we report the second patient with a 22q11.2 triplication, whose clinical features increase the symptoms that could be present. This work emphasizes the relevance of biomedical research, since it can help paediatricians and other professionals to better assess health care needs.

P05.27-S
Congenital heart malformations in patients with 22q11.2 deletion syndrome

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Congenital heart malformations represent one of the most common birth defects, affecting about 0.7 or 0.8% of all live births. The association of wide range of conotruncal heart malformations with 22q11.2 microdeletion is well recognized. 22q11.2 microdeletion is the most common deletion in human genome and more than 80 dysmorphism/malformations have been described in patients with 22q11.2 microdeletion syndrome. In this study we investigated the frequency of 22q11 microdeletion among patients with congenital heart defect and clinical features of 22q11.2 deletion syndrome. The study population was 57 individuals who underwent detailed clinical evaluation including assessment of cardiac morphology, facial appearance, lymphocyte immunophenotyping, presence of cleft palate and hypocalcemia/hypoparathyroidism screening. All tested patients have had congenital heart defect. Fluorescence in situ hybridization and multiplex ligation-dependent probe amplification analysis revealed 22q11.2 microdeletion in 42.1% of studied patients. Cardiac malformations observed in patients with 22q11.2 deletion were tetralogy of Fallot, pulmonary artery atresia, common arterial trunk, interrupted aortic arch, ventricular septal defect and mitral stenosis. Echocardiography accompanied by contrast computed tomography scan and magnetic resonance angiography revealed malposition of branch pulmonary arteries in two patients with 22q11.2 microdeletion. In conclusion, with this study we stress the need for multidisciplinary assessment of patients with congenital heart malformations which should include testing for 22q11.2 microdeletion.

P05.28-M
Conotruncal malformations and absent thymus due to a deleterious NKX2-6 mutation

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Background: Truncus arteriosus (TA) accounts for ~1% of all congenital heart defects (CHD) in live birth. The etiology of isolated TA is largely unknown; syndromic TA is mostly associated with chromosome 22q11 deletion. Hadassah Cardiogenetic Project includes a detailed registry and biobank. Within the frame of this Project we now report the results of a study of patients with multiple conotruncal malformations accompanied by athymia. **Methods and Results:** The subjects were patients originating from two unrelated families. Following the exclusion of 22q11 deletion, exome analysis was performed in one patient from each family. A homozygous mutation in chr8: 23560417InsA, p.Lys152fs*0, in the *NKX2-6* gene was identified in patients from both families. The mutation segregated with the disease in the families and was absent from large cohorts of controls. **Conclusions:** *NKX2-6* encodes a homeobox-containing protein which is expressed in mouse caudal pharyngeal arches and outflow tract at E8.0-E9.5. NKX2-6 was previously shown to be regulated by TBX1. The clear phenotype associated with a homozygous deleterious mutation in our patients, falls well within the spectrum of the cardiac defects seen in DiGeorge syndrome, is in agreement with NKX2-6 downstream location in the TBX1 signaling pathway and confirms *NKX2-6* role in human cardiogenesis..

P05.29-S
Is Type 2 diabetes gene ABCC8 coding a subunit of KATP channel associated with coronary artery disease in Turkish people?

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Coronary artery disease (CAD) is one of the most common cardiovascular diseases and is a major cause of morbidity and mortality worldwide. Classical risk factors for atherosclerosis, such as central obesity, arterial hypertension, and dyslipidemia, frequently coexist with type 2 diabetes and contribute to the increased prevalence of CAD. ATP-sensitive potassium (KATP) channels of pancreatic β-cells, which is assembled from two different subunits; a Kir6.2 and a sulfonylurea receptor 1(SUR1), play a key role in glucose-stimulated insulin secretion mechanism. ABCC8 gene which is located on chromosome 11p15.1 contains 39 exons and encodes SUR1. We performed a study to scan ABCC8 gene variants which we found significantly associated with type 2 diabetes previously, in patients with CAD. 125 individuals with CAD and 123 healthy individuals were included in the study. Genotyping was performed by PCR-RFLP technique for R1273R and exon 16/-3t→c substitutions using BslI and PstI, respectively. Statistical analysis was performed using SPSS18.0 program. p<0.05 was considered significant. Exon 16/-3t→c substitution showed association with disease (OR:17.13 [95% CI:4.84-60.62] p<0.001, under dominant model, while silent substitution R1273R in exon 31 had no effect on disease. According to our results, exon 16/-3t→c substitution accepted as having important role in type 2 diabetes genetic background has also associated with CAD in our population. Because relatively small sample size of our population is a limitation for the study, replicative studies in larger populations are needed.

P05.30-M
Genetic evaluation of plakophilin-2 and desmoplakin gene variants in ethnically different populations with dilated cardiomyopathy

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Background
Cardiomyopathies are a heterogeneous group of diseases with various etiologies. The potential involvement of genes encoding desmosomal proteins, usually associated with arrhythmogenic right ventricular cardiomyopathy (ARCV), was preliminarily evidenced in Caucasian patients with dilated cardiomyopathy (DCM). Accordingly, we investigated this potential genetic overlap in a large cohort of patients with clear diagnosis of DCM and belonging to different ethnicities.
Methods
DNA from 455 DCM patients, referred to our tertiary centres in Pavia and Cape Town, was collected following complete clinical evaluation. 290 samples (184 Caucasians; 84 black Africans; 5 Indians; 17 mixed ancestries) were screened for the two main ARVC genes: plakophilin-2 (PKP2) and desmoplakin (DSP).
Results
Of the 290 patients tested, 9 (3.1%) were found to carry a most likely pathogenic variant, being absent in the publicly available databases (nearly 20.000 controls) and functionally relevant through 6 bioinformatic tools. Respectively, 3 (3.6%) black Africans (1 in PKP2, 2 in DSP), 5 (2.7%) Caucasians (2 in PKP2, 3 in DSP), and 1 African patient of mixed-ancestry (1 in DSP) were positive. Variants of unknown significance (VUS) were found in 15 patients; interestingly, one Caucasian carried two VUS in DSP, suggesting a potential compound effect.
Conclusion

Our data confirm the presence of potentially damaging mutations in desmosomal protein genes in patients with DCM. The prevalence of these mutations is similar in black Africans and in Caucasians.

P05.31-S
eNOS as hypertension susceptibility gene: example from genome-wide association study to functional and clinical evidences

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Genome-wide association studies (GWAS) usually point to genomic regions of interest in relation to a trait, but seldom directly identify the causal or functional variant.
In a two-stage GWAS [Salvi et al, Hypertension 2012, PMID: 22184326], we revealed the association between rs3918226 polymorphism in the promoter of the endothelial nitric oxide synthase (eNOS) gene and hypertension (T allele, odds ratio 1.54; combined p = 2.58·10^-13). We identified *in-silico* a putative binding site for transcription factors of the ETS (E-twenty six) family only one nucleotide away from rs3918226.
We then confirmed our preliminary findings by target sequencing, in-vitro experiments, and a population study [Salvi et al, Hypertension 2013, PMID: 24019403]. Target sequencing and imputing of the eNOS region validated rs3918226 as the polymorphism most closely associated with hypertension. HeLa and HEK293T cells transfected with the eNOS promoter carrying the risk T allele had 20% to 40% (P<0.01) lower transcriptional activity than those carrying the C allele (luciferase reporter assays). In a general population of 2722 randomly recruited Europeans, TT homozygosity enhanced the age-related increase in blood pressure and risk of hypertension. The prevalence of TT homozygosity is low but the attributable risk is 51.0%.
Combined with other genetic markers, the rs3918226 polymorphism might, therefore, contribute to the stratification of cardiovascular risk. Further clinical research should establish whether eNOS might be a target for preventive or therapeutic intervention.

P05.32-M
The analysis of cytokine network gene expression profiles in peripheral blood leukocytes of patients with essential hypertension

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Essential hypertension (EH) is a multifactorial disease with obscure etiology and pathogenesis. Transcriptome of patients with EH is virtually unexplored. We performed the analysis of gene expression in peripheral blood leukocytes of patients with EH and healthy individuals using microarray technology (RT2ProfilerTM PCR Array, SABiosciences Corporation, Qiagen) with subsequent validation of the obtained results by quantitative real-time RT-PCR. We discovered a group of genes with altered transcriptional activity in hypertensive patients: CCL16, CCL17, CCL18, CCL19, CCL23, CCL8, CCR6, CCR8, CX3CR1, CXCL1, CXCL13, ICEBERG, IL17C, IL1F10, IL1F6, IL1F9, SPP1, CD40LG, XCR1, CCL2. Further quantitative analysis of genes with altered transcriptional activity was performed using cDNA samples of 32 EH patients and 31 control subjects. The results have confirmed significant differences of CCL18, CX3CR1, CXCL1, CXCL13, IL10, IL13, and CCR2 expression level between cases and controls (p=0.001). Relative expression level changes in EH patients were more pronounced for CX3CR1 gene (29.2-fold), CXCL13 (13.8-fold), IL1F6 (12.9-fold), CD40LG (8-fold), CXCL1 (7.2-fold). Functional analysis of differentially expressed genes was performed using Gene Ontology Biological Process and Kyoto Encyclopedia of Genes and Genomes databases. The genes with altered transcriptional activity in EH patients were found to encode for cytokines and cytokine receptors involved in immune response and inflammation, and their action was mediated via cytokine signaling pathway. These findings confirm both the hypothesis of implication of blood cells trancriptome in the pathogenesis of EH and the hypothesis of inflammatory basis of the development of EH. The study was supported by the RFBR grants 13-04-01561_a, 14-04-01169_a.

P05.33-S
Application of exome sequencing in differential diagnosis of pediatric hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is the most common monogenic cardiac disorder, usually caused by mutations in sarcomere genes. Little is known on genetic basis of infantile forms, since the etiology of HCM in this population is heterogeneous and includes inborn errors of metabolism, neuromuscular disorders, and malformation syndromes. Timely diagnosis is important for patients' management but it could be difficult at first clinical presentation, since the phenotype may not yet be clearly defined. Here we present the analysis of 8 patients presenting severe HCM at less than 1 month of age and their healthy parents by a panel of 2761 known disease genes (TruSight exome). We obtained 27,457,869 reads/sample, the mean coverage was 265X and 98% of target regions were covered at >20X. In one patient we identified a de novo mutation in a classical HCM-associated sarcomere gene (MYH7). Two infants were diagnosed with glycogen storage diseases, one cytoplasmic and one lysosomal, respectively caused by a de novo PRKAG2 heterozygous mutation and a GAA homozygous mutation, for which a specific enzyme replacement therapy exists. One patient carried a homozygous SLC22A5 mutation associated to carnitine deficiency. In the remaining 4 patients we identified 3 PTPN11 heterozygous missense mutations and one de novo heterozygous RAF1 deletion, suggesting the diagnosis of Noonan syndrome. Our results demonstrate the utility of exome sequencing in clinical practice, especially in infants who may benefit of prompt differential diagnosis for therapeutic management and follow-up planning.

P05.34-M
Early recognition of Familial defective apolipoprotein B-100 with identification of common p.Arg3527Glu mutation

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Background: Familial defective apolipoprotein B-100 (FDB) is a dominant inherited disorder most commonly caused by the substitution of glutamine to arginine at position 3527 in the ApoB-100 gene. The mutant p.Arg3527Glu causes a marked reduction in the affinity of low-density lipoproteins (LDL) for the LDL receptor, which leads to hypercholesterolemia and increased risk for cardiovascular diseases (CVD). The p.Arg3527Glu mutation among Caucasian patients have a wide geographic and population distribution. Its prevalence varies among various European populations ranging from 0.08 % to 1.4 %. To our knowledge, this mutation has not yet been studied in the Slovenian population.
Materials and Methods: Following clinical evaluation, p.Arg3527Glu mutation was analysed in a cohort of 102 paediatric patients recruited through nation-wide hypercholesterolemia screening, and 44 adult patients referred to specialised outpatient clinic due to hypercholesterolemia resulting in CVD.
Results: p.Arg3527Glu mutation gene was identified in 14 out of 102 paediatric and in 6 out of 44 adult patients.
Conclusions: Studied population represents two clinical extremes of the FDB, one with and one without manifestations of CVD. Similar frequencies of the mutation were detected in both studied groups, namely 13.7 % in paediatric and 13.6 % in adult patients. Early definitive identification of FDB through paediatric hypercholesterolemia screening and genetic testing is crucial for CVD prevention.

P05.35-S
Insight into genetic determinants of resting heart rate

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Recent studies suggested that resting heart rate (RHR) might be an independent predictor of cardiovascular mortality and morbidity. Nonetheless, the interrelation between RHR and cardiovascular diseases is not clear. In order to resolve this puzzle, the importance of genetic determinants of RHR has been recently suggested, but it needs to be further investigated.
The aim of this study was to estimate the contribution of common genetic variations on RHR using Genome Wide Association Study.
We performed a Genome Wide Association Study in an isolated population cohort of 1737 individuals, the Italian Network on Genetic Isolates - Friuli Venezia Giulia (INGI-FVG). Moreover, a haplotype analysis was performed. A regression tree analysis was run to highlight the effect of each haplotype combination on the phenotype. A significant level of association (p<5x10-8) was detected for Single Nucleotide Polymorphisms (SNPs) in two genes expressed in the heart: MAML1 and CANX. Founding that the three different variants of the haplotype, which encompass both genes, yielded a phenotypic correlation. Indeed, a haplotype in homozigosity is significantly associated with the lower quartile of RHR (RHR≤58 bpm). Moreover no significant association was found between cardiovascular risk factors and the different

haplotype combinations. Mastermind-like 1 and Calnexin were found to be associated with RHR. We demonstrated a relation between a haplotype and the lower quartile of RHR in our populations. Our findings highlight that genetic determinants of RHR may be implicated in determining cardiovascular diseases and could allow a better risk stratification.

P05.36-M
Different genetic background in the clinical onset of Hypertrophic Cardiomyopathy analyzed by Next-Generation Sequencing

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More than 20 genes have been correlated with Hypertrophic Cardiomyopathy (HCM), making the molecular diagnostic process time consuming, complex and often inconclusive. To better understand the molecular bases of HCM and to assess the utility of next-generation sequencing (NGS) into clinical laboratory practice we designed a panel to analyze 17 HCM related genes on PGM Ion Torrent. We selected 70 HCM patients, 35 early onset (≤35 years) and 35 late onset (≥65 years), with a well-defined hypertrophic phenotype.
All samples had on average 98% of target regions with coverage higher than 20X, with a mean coverage of 600X. Common variants (MAF>5%) were removed from the analysis, while potential splice variants and novel single nucleotide variants were analysed with bioinformatics pathogenicity prediction programs. We identified 40 different pathogenetic mutations (9 novel) in 9 genes: *MYBPC3* (16/40=40%); *MYH7* (14/40=35%); *TNNT2* (3/40=7.5%); *CAV3* (2/40=5%); and *GLA*, *MYH6, TNNI3*, *MYL2* and *MYL3* (1/40=2.5% each). The mutation detection rate was 9/35 (26%) in the late onset and 29/35 (83%) in the early onset group (p<0.0001). Considering only early onset patients with positive family history the detection rate was >90%.
Our results showed a strong difference in genetic background of HCM patients for age at onset, family history and clinical features of the disease. Genetic testing with NGS was reliable and allowed the molecular diagnosis especially for cases with early onset and positive family history. These results also demonstrated that an appropriate selection is required for molecular testing in patients with HCM.

P05.37-S
HDAC9 gene is overexpressed in stroke patients

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Aim: HDAC9 is a class IIa histone deacetylase family member and it regulates the epigenetic status of histones and therefore gene expression by catalyzing deacetylation. Risk variant rs11984041 of HDAC9 gene has been demonstrated to be associated with large vessels stroke. HDAC9 expression has been correlated with common carotid intima-media thickness, suggesting an association of HDAC9 gene with the atherosclerotic process, by accelerating atherosclerosis or promoting plaque instability. In this study we investigated the expression of HDAC9 in peripheral blood (PB) of stroke patients (large vessel and cardioembolic) and healthy controls. Aiming to understand the mechanism by which the risk allele is associated with large vessel stroke, we also evaluate the effect of rs11984041 polymorphism on gene expression.
Methods: HDAC9 gene expression analysis was performed on 26 atherothrombotic stroke patients, 26 cardioembolic stroke patients, and 20 healthy controls by Real Time PCR using Sybr Green. Polymorphism rs11984041 was genotyped by PCR-RFLP method.
Results: A significant increase of HDAC9 gene expression was observed in atherothrombotic and cardioembolic stroke patients compared to healthy controls (1.39±0.68 vs 0.61±0.36; p<0.001; 1.58±1.19 vs 0.60±0.36; p<0.001, respectively).
The genotyping of 70 individuals showed no gene expression differences between patients carrying CC and CT genotypes.
Conclusions: The gene HDAC9 is overexpressed in stroke patients compared to controls. This result indicated that this gene, that can regulate other genes implicated in the peripheral inflammatory reaction after stroke can represent a new target for the development of therapeutic agents against ischemic stroke injury.

P05.38-M
Novel mutations in the *ZIC3* gene cause X-linked heterotaxy

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Congenital heart defects (CHD) is one of the most common congenital abnormalities in newborns, affecting almost 1% of the general population and is associated with substantial morbidity and mortality. A small subset (3%) of this patient population fails to correctly establish left-right patterning during embryogenesis, resulting in abnormal lateralization of the abdominal and thoracic organs, a clinical phenotype called heterotaxy. As the heart is the first organ to develop asymmetrically, disturbances in the left-right axis lead to a variety of serious cardiac malformations in heterotaxy. One of the first genes linked to heterotaxy was *ZIC3*, a zinc transcription factor of the GLI superfamily, located on the X-chromosome. The *ZIC3* gene is a transcriptional regulator, based on the ability to activate transcription of target genes and to bind DNA.
Over the last 10 years we collected and diagnostically tested over 300 patients referred to our clinical genetics center with heterotaxy and/or a variety of heart defects for mutations in the *ZIC3* gene. We identified five potentially pathogenic mutations, as well as variations in the N-terminal polyalanine-repeat. The mutations were detected in families with a clear X-linked clinical profile. To support pathogenicity of detected mutations we performed several functional assays. We used confocal imaging to detect subcellular localization of mutated proteins and the *in vivo* zebra fish model to investigate the potential laterality and/or cardiac defects of these mutated proteins.

P05.39-S
Copy Number Polymorphism study in Essential Hypertension

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Essential Hypertension is a complex trait influenced by multiple susceptibility genes interacting with environmental factors. We used 2044 hypertensive cases and 1872 normotensive controls recruited over many years across European regions within Hypergenes project and genotyped with Illumina-1M arrays.
Cases and controls were genotyped together to avoid the batch effect for chips and time periods. The Log R ratio of the samples for each SNP was obtained from Genome Studio and the Copy Number (CN) call was performed in Golden Helix after quality controls. CN was estimated in each sample as a mere signal intensity interval measured at a particular locus. Thorough quality control to refine the signal intensity was essential before CN call. Approximately 9% of the samples uniformly distributed between cases and controls were removed for signal to noise ratio and waviness. The PCA analysis on wave corrected data doesn't show demarcation on sex/phenotype, whereas the distinguishable genotyping batches were corrected. Among the 824 segments identified and discretized into 3 state CN, 13 segments were significantly associated with the phenotype.
Odds ratio for these segments were calculated and we identified a top common variant around 1kb in 1st intron of LEPREL1 gene with an odds ratio of 1.7 for hypertension (95% CI 1.3-1.9, p-value = 3.01×10^{-12}). This result suggested that carriers of that CNP had a 1.7 times higher risk for hypertension than non-carriers. Further studies are needed to confirm the association of this CNP with hypertension in other dataset.

P05.40-M
Whole exome sequencing concentrating on the metabolome in familiar hypertrophic- and dilated cardiomyopathy: a pilot collaborative Czech study

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Hypertrophic- (HCM) or dilated (DCM) cardiomyopathies are associated with the risk of heart failure and sudden cardiac death. Molecular genetic diagnosis contributes to risk stratification in relatives and may allow for personalized therapy. Altogether, 35 Czech cases from 13 families (7 HCM / 6 DCM) with three or more affected individuals were analyzed by whole exome next generation sequencing (NGS) concentrated on the metabolome and utilizing TruSight Exome Gen Set on HiSeq1500 (Illumina). Potentially pathogenic gene variants were found in 11/13 families and comprised 17 genes that have been associated with all forms of CM, familiar arryth-

mogenic disease or myopathies (TTN, RYR2, CALR3, MYH 7, TPM1, JPH2, DSP, ACTC1, KCNH2, FLNC, SYNE2, CASQ2, VCL, PKP2). Three variants were detected in MYO1C, JAG1, SYNE1 that have not been associated with CM, thus far. Only 12/17 identified genes are included in the currently available targeted TruSight assay. In 3/11 families the potential causative variant was found only in one gene. The combination of two to three pathogenic variants in CM-associated genes were found in 8/11 families. Pathogenic potential of the identified variants must be substantiated by segregation and/or RNA/protein analyses. The failure to detect variants in two large families underscores the limitations of NGS. Nevertheless, our preliminary data suggest, that the use of metabolome NGS in complex families may have higher diagnostic yield than targeted approaches in CM.Supported by conceptual research of FNM 64203, CZ.2.16/3.1.00/24022 and IGA NT13770.

P05.41-S
Identification of Mexican-specific lipid variants using a novel cross-population GWAS approach

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Mexicans have a higher prevalence of dyslipidemia than Europeans which causes a serious health problem due to the increased risk for cardiovascular disease. However, complex population substructure in this admixed population leads to a decrease in statistical power hindering genetic research. Consequently, Mexicans are substantially underrepresented in genomic studies despite their high susceptibility to dyslipidemias. We hypothesized that part of the increased predisposition is explained by population-specific variants. To test our hypothesis, we performed a two-stage cross-population genome-wide association study (GWAS) of hypertriglyceridemia using 19,273 subjects of European and Mexican origin to screen for Mexican-specific variants. First, we compared the Mexican population with its European ancestry population represented by Finns, utilizing Mexican low triglyceride (TG) controls and Finnish low TG controls to screen for variants that differ in frequency between the two populations. Subsequently, we included only these variants in a Mexican TG case-control GWAS to identify Mexican-specific variants. Four Mexican-specific variants were discovered and replicated in an independent Mexican cohort (n=6,159), including signals near the lipoprotein lipase (LPL) and apolipoprotein A5 (APOA5) genes, suggesting that regulation of the two key TG genes, APOA5 and LPL, play a crucial role in elevated TGs in Mexicans. We validated the cross-population GWAS results with local ancestry analysis, and all replicated variants reside in highly Amerindian-ancestry enriched regions in Mexicans with high TGs, indicating that cross-population GWAS can effectively screen for population-specific variants. In summary, we present a novel approach, cross-population GWAS, which can be adapted for other admixed populations and different diseases.

P05.42-M
Whole gene deletion of MYBPC3 in Hypertrophic Cardiomyopathy

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Hypertrophic Cardiomyopathy (HCM) is characterized by left ventricular hypertrophy in the absence of a predisposing cardiovascular condition. Pathogenic variants in 12 genes that encode proteins of the sarcomere have been implicated in HCM. Most of the pathogenic variants act in a dominant negative fashion. However loss of function (haploinsufficiency) is the most common disease mechanism for pathogenic variants in MYBPC3 suggesting that large deletions of the MYBPC3 gene may play a role in HCM pathogenesis. Here, we describe an individual affected with HCM who carries a deletion of the entire MYBPC3 gene.
The patient was diagnosed with non-obstructive hypertrophic cardiomyopathy in his mid forties when undergoing assessment for palpitations and hypertension. Echocardiogram revealed moderately dilated left and right atria with mild mitral regurgitation. There was severe asymmetric left ventricular hypertrophy with interventricular septal hypertrophy. The left ventricular systolic function was normal with an ejection fraction of 65-70%.
MLPA analysis showed a deletion of all exons of the MYBPC3 gene. Subsequent microarray analysis confirmed this deletion and showed that it extended both 5' and 3' of MYBPC3 and included 3 additional known OMIM morbid genes (DDB2, SLC39A13 and RAPSN). Unlike MYBPC3, these additional genes are implicated in autosomal recessive disorders and therefore deletions of these genes are not expected to have clinical significance in this patient.
Our results suggest that although large copy number variation of HCM-related genes would still be considered rare, patients highly suspected of HCM may benefit from MLPA analysis if other deleterious variants have not been identified.

P05.43-S
The c.912_913delTT mutation in MYBPC3 gene is a founder mutation accounting for one-fifth of the Italian patients affected with hypertrophic cardiomyopathy

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Background - Hypertrophic cardiomyopathy (HCM) is considered the most common cause of sudden cardiac death (SCD) in young people. Over 18 genes have been associated with the disease. The aim of this study was to evaluate the clinical characteristics, penetrance and prognosis of HCM patients carrying a founder mutation in myosin binding protein C (MYBPC3) gene.
Methods and Results - Ninety seven HCM probands were screened for MYBPC3 mutations. The frameshift mutation c.912_913delTT (p.F305PfsX27) was found in 19 (19.5%) patients (14 males and 5 females). Among 81 relatives belonging to 14 apparently unrelated families, 45 (20 males and 25 females) resulted to be mutation carriers and 29 had HCM (17 males and 12 females). The family haplotype analysis confirmed a common founder ancestor.
Disease penetrance was incomplete (64.4%) and greater in males than females (85% versus 48%, p=0.009). Eleven (38%) affected mutation carriers were diagnosed between 30 and 40 years old. Probands carrying this founder mutation showed a worse prognosis for SCD or aborted SCD (p=0.01) compared with patients negative for MYBPC3 mutations.
Conclusions - The founder MYBPC3 mutation carriers have a high probability to develop the disease between 30 and 40 years of age, with an increased risk if they are men. They show a significantly reduced survival after the fourth decade of life when compared to patients without MYBPC3 mutations. These findings are of relevant importance for the genetic counseling and therapy, considering the high frequency and poor prognosis associated with this founder mutation.

P05.44-M
MYH7 and *MYBPC3* genetic characterization in Hypertrophic Cardiomyopathy (HCM)

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Hypertrophic cardiomyopathy (HCM) is a common disorder of the heart characterized by cardiac hypertrophy of variable degree, myocyte disarray and fibrosis and can cause severe disorders as angina, arrhythmias and heart failure. HCM affects approximately 1/500 subjects and is an autosomal dominant hereditary disease associated with mutations in genes encoding proteins of the contractile apparatus. As other inherited cardiomyopathies, HCM shows marked phenotypic variability, even within families. Mutations of *MYH7* and *MYBPC3* genes cover about 50% of cases. The *MYH7* gene (14q11.2), encoding the heavy chain of β-myosin protein, was the first gene whose mutations have been associated with hypertrophic cardiomyopathy and is responsible for about 25% of all currently known pathogenic variations. The *MYBPC3* gene (11p11.2) consists of 35 exons encoding the myosin binding protein-C, whose cardiac isoform consists of 1274 amino acids.
Here we report the data collected by our Unit since 2012. We tested 52 patients and found *MYBPC3* causative mutations in 11 affected subjects (21% of cases); six mutations are already described in literature while five result novel ones. *MYH7* genetic testing led to the identification of seven different

mutations in 7/34 patients (20,6%), 3 are novel mutations. Search for causative mutations is an integral part of the HCM diagnosis. The finding of a causative mutation in a patient, permits the identification of family members who may have the disease in an asymptomatic way or who may develop disorders in the future and may in turn transmit the mutation to their offspring.

P05.45-S
Semiconductor (Ion Torrent) massive parallel sequencing of the main Hypertrophic Cardiomyopathy genes based on two tubes multiplex amplification of DNA pools

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At least 30 different genes have been linked to Hypertrophic Cardiomyopathy (HCM), with MYBPC3 and MYH7 accounting for most of the mutations. Due to the large size of these genes, Sanger-sequencing of single amplicons is labor-intensive and expensive. Next generation sequencing (NGS) could facilitate the genetic screening of the HCM-genes in large cohorts. Our purpose was to designate and validate a procedure for sequencing the 9 most commonly mutated genes in HCM (*MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *ACTC1*, *TNNC1*, *MYL2*, *MYL3*, and *TPM1*), based on two-tubes multiplex amplification of DNA-pools (*Ampliseq;* 176 primer-pairs covering 15,690 bp, 98,62% of the target coding sequence) followed by semiconductor array sequencing with the *Ion Torrent* Personal Genome Machine (PGM; Life Technologies). We created a pool with DNA from 13 patients previously Sanger-sequenced for the coding exons plus at least 5 intronic flanking nucleotides of the nine genes. Each patient was heterozygous for a unique rare variant (including a small deletion); each unique allele (control variant) was thus diluted 1/26 inside the pool. We sequenced the DNA-pool in a medium capacity Ion chip 316 (semiconductor 100 Mb) array, and the data was analyzed with the *Torrent Suite* software optimized to detect insertion/deletion nucleotide changes. We successfully identified all the 13 control variants (including the indels). In conclusion, we developed a NGS protocol for the main HCM genes. The multiplex amplification of DNA pools would reduce the time and cost of screening these genes at a population scale.

P05.46-M
Role of genetic testing in diagnosis and management of idiopathic ventricular fibrillation cases

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Introduction: In approximately 5%-10% of the survivors of sudden cardiac death no underlying cardiac disease is identified and consequently the event is classified as idiopathic ventricular fibrillation (IVF). The genetic etiology of IVF remains mostly obscure and current consensus statements recommend the genetic screening only in presence of clear clinical indications. Methods: Using whole-exome sequencing (Illumina HiSeq 2000) we molecularly investigated 2 Italian unrelated infants with IVF together with the unaffected parents and 3 affected members of a German family with history of sudden unexpected deaths below age 40. Results: In one infant (2 yo) we identified a de novo missense variant in RYR2, the main gene responsible for cathecholaminergic polymorphic ventricular tachycardia (CPVT). The available clinical data were not sufficient to make the diagnosis, as the stress test, the leading clinical tool in CPVT diagnostic process, is not feasible in infancy. In the second infant (4 months), we detected a de novo missense variant in the PARN gene and the biological plausibility of this result is currently under investigation. In the German family we detected in all affected members a novel frameshift mutation in the desmoplakin gene, associated with ARVC with LV involvement (left-dominant arrhythmogenic cardiomyopathy, LDAC). As a matter of fact this finding allowed a better management and follow-up of affected members. Conclusion: Our approach suggests that a target screening for channelopathy and cardiomyopathy genes could be indicated in those IVF cases in which a complete clinical and familial evaluation is not feasible.

P05.47-S
FBN1 deep intronic mutations: Can they explain the molecularly unresolved Marfan cases?

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Marfan syndrome (MFS) is a multi-systemic autosomal dominant connective tissue disorder caused by mutations in the FBN1 (fibrillin-1) gene, but approximately 10% of MFS cases remain genetically unsolved. Here, we report a new FBN1 mutation in an MFS family that had remained negative after extensive molecular genomic DNA FBN1 testing, including denaturing high performance liquid chromatography (DHPLC), Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA) and micro-array analysis for copy number variation. In addition, four other aneurysm-linked genes were screened for mutations by Sanger sequencing, TGFBR1, TGFBR2, SMAD3 and ACTA2, without any result. Linkage analysis in the family revealed a large linked region on chromosome 15 which was confirmed by microsatellite analysis. Cultured proband fibroblasts and subsequent cDNA sequencing revealed a double peak pattern at the junction of exon 56 and 57. Sanger sequencing of intron 56 revealed a deep intronic point mutation generating a new splice donor site (c.6872-961A>G; ENST00000316623). Together with an existing cryptic splice acceptor site, this mutation results in the integration of a 90 bp pseudo-exon between exons 56 and 57 containing a stop codon and causing nonsense-mediated mRNA decay. Although more than 90% of FBN1 mutations can be identified with regular molecular testing at the genomic level, deep intronic mutations will be missed and require cDNA sequencing or whole genome sequencing.

P05.48-M
Compound-heterozygous Marfan syndrome?

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Introduction
Marfan syndrome is an autosomal-dominant condition often caused by mutations in the fibrilllin-1 (*FBN1*) gene. Here we report a family where the proband is compound-heterozygous for two mutations in *FBN1*.
Case
The proband (II:1) was on suspicion of Marfan syndrome at 21 years of age. An aortic dilation was found at age 17, during clinical investigation for rheumatoid arthritis. Family history was ambiguous: His father was operated for an aortic aneurism at age 30, allegedly caused by endocarditis. Sequencing using an NGS panel for Marfan and Marfan-like syndromes was undertaken. Two mutations were found in *FBN1;* one previously reported as causative for Marfan syndrome, the other novel. Results of family investigation and clinical findings are reported in table 1.
Interpretation
Compound heterozygousity has previously been reported in Marfan syndrome. Neither of the two mutations found are present in ESP, nor found among 2000 Danish exomes. In the present case we find it more likely that the previously reported supposed pathogenic mutation found in I:2 is of no/little phenotypical consequence. It was originally found in an isolated case of aortic dissection where no further details were given. The involved amino acid is not evolutionary conserved. Individual I:2 is without clinical symptoms of Marfan syndrome at age 55. The novel c.7694G>A mutation is *in silico* predicted to be likely pathogenic and segregates with the clinical phenotype.

Individual	Number	Mutation ***FBN1***	Clinical features	Medical history	Diagnosis
Father	I:1	c.7694G>A; p.Cys2565Tyr (novel)	Marfanoid face, wrist sign, arm-span to height ratio 1,05	Aortic dissection at age 36 Childhood surgery for pectus carinatum.	Marfan syndrome according to revised Ghent criteria
Mother	I:2	c.4727T>C; p.Met1576Thr (previously reported)	Normal		Healthy
Proband	II:1	c.7694G>A; p.Cys2565Tyr c.4727T>C; p.Met1576Thr	Marfanoid face, wrist sign, asymmetric chest, pes planus, myopia (-7,5 bilaterally), striae Height 200 cm	Aortic dilation	Marfan syndrome according to revised Ghent criteria

P05.49-S

Several loci enriched in lower frequency variants are associated to risk factor for metabolism and cardiovascular diseases in 4,000 Italian isolated individuals

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The main strength of genetic isolated populations is the enrichment in low frequency variants in respect to the general population. To date they have been used in large meta-analyses to highlight genetic association of common variants with several complex traits. Thanks to next-generation sequencing approaches we are now able to map population-specific low-frequency variants.
The Italian Network of Genetics Isolates (INGI) collects > 4,000 genotyped individuals from several isolated populations along Italy sampled for a large set of obesity and cardiovascular related traits. The whole Italian cohort has been imputed to UK10k sequenced reference set to highlight rare variants associations for ~ 30 traits in common that affect metabolism: several population-specific associated LOCI were enriched in rare variants, 33 suggestive LOCI were shared in 2 or more cohorts and most of UK10k outcomes were replicated by Italian cohorts.
A first random subset of 110 Italian isolated individuals was sequenced by a low-coverage approach: 39% were rare variants, 22% were private SNPs and 14% were population-specific variants. Most of lower-frequency SNPs match with 1000GP and UK10k called variants but many are population specific. To further enrich in lower frequency variants and design a Southern Europe reference panel with, ~ 1,000 samples were randomly selected from each isolated cohort and are being typed by low-coverage whole-genome sequencing and high-coverage exome-sequencing. The preliminary results will be presented.

P05.50-M
Differentially expressed miRNAs in the hepatic acute phase response

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Understanding gene regulation in the cardiovascular system provides a source of potential cardiovascular disease (CVD) mediators and therapeutic targets. It may also give functional evidence for associations between genetic variants and CVD. The link between inflammation and CVD is well established and Acute Phase Proteins (APPs) of the innate immune system are recognized CVD markers. Changes in the expression of APPs are primarily due to altered hepatic expression in response to circulating inflammatory cytokines such as interleukin-6 (IL-6). Whereas cytokines are rapidly cleared following induction of the Acute Phase Response (APR), the expression of APPs is maintained for several days and is diagnostically useful. MicroRNAs (miRNAs) also show potential as CVD biomarkers since many are stable in the circulation and their expression is altered in acute myocardial infarction and cancer.
To determine whether miRNAs are involved in modulating the expression of specific APPs in the APR, we performed RNAseq on small RNAs in HepG2 cells, and human and mouse primary hepatocytes stimulated with IL-6 for 0, 6 and 24h. Our data revealed 21 differentially expressed miRNAs upon IL-6 stimulation across all samples (10 up-regulated, 11 down-regulated), 13 of which are known circulating miRNAs. Interestingly, although no differentially expressed miRNAs were shared between the three hepatic cell types, groups of functionally related target genes (SOCS, ADAMTS, MMP, interleukins) are common to all three and are involved in cytokine signaling, coagulation, hypoxia and angiogenesis. Identifying new APR markers may provide important diagnostic tools and enhance our understanding of this complex system.

P05.51-S
No locus heterogeneity in familial microcephaly with or without chorioretinopathy, lymphedema, or mental retardation syndrome

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Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation syndrome (MCLMR, OMIM 152950) is a rare autosomal dominant disorder with variable expressivity. It is characterized by mild-to-severe microcephaly, often associated with developmental delay, ocular defects and lymphedema, usually on the dorsum of the feet. It can be sporadic or inherited. So far, 25 families (46 patients) have been described to carry a mutation in KIF11. This gene encodes a homotetrameric motor kinesin, EG5. Members of this protein family are involved in the establishment of a bipolar spindle during cell mitosis, in chromosome positioning and in centrosome separation. EG5 inhibition impairs endothelial cell proliferation and migration, and angiogenesis. We tested a series of 23 unreported MCLMR index patients for KIF11 and found 14 mutations, 12 of which are novel. We detected a mutation in all 7 familial cases and 7 of the 16 sporadic patients. The inherited mutations were found in an additional 12 family members. We subsequently reviewed the clinical phenotypes of all the patients with a KIF11 mutation, including those already published (n=46+26=72). Microcephaly in frequency was present in at least 94%, eye anomalies in 69%, mental retardation in 68% and lymphedema in 53% of patients. Three mutation carriers are unaffected. We observed 14 de novo cases within 39 index patients (36%). As the remaining sporadic patients may be mosaic, KIF11 mutations likely cause the majority, if not all, of MCLMR.

P05.52-M
Next-generation sequencing as a diagnostic tool in sudden unexplained death victims and patients with a cardiac disease

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Objective
Sudden cardiac death (SCD) is responsible for a large proportion of deaths in young individuals. After routine post-mortem investigations, many cases are still unexplained. Implementation of genetic investigations in forensic medicine may increase the diagnostic rate – not least in sudden unexplained death (SUD). With next generation sequencing (NGS), genetic analysis has become faster and more efficient, enhancing the outcome. The purpose of the study was to explore the yield of genetic analysis using NGS in forensic pathology and to compare the genetic findings to routinely diagnosed patients.
Methods and results
Genetic investigation was performed in 44 unrelated individuals; 15 forensic SUD cases and 29 unrelated patients diagnosed with channelopathies. In-solution targeted sequencing capture probes were custom designed by Roche NimbleGen, including all exons of 31 genes, and sequenced on the Illumina MiSeq. Larger deletions and insertions in five genes were investigated with multiplex ligation-dependent probe amplification. Of the SUD cases, 21% were found to have a probably pathogenic variant. The corresponding hit-ratio in the patient cohort was 37%. Two patients (7%) had large deletions.
Conclusion
By NGS, it was possible to detect a probably pathogenic single nucleotide variant in one fifth of the SUD cases, disposing to a cardiac disease. In contrast, almost half of the patients were found to carry a probably pathogenic variant or larger deletion. Genetic investigation with NGS can be used as a diagnostic tool in both a forensic and clinical setting.

P05.53-S
PRDM16 and MIB1 mutations are rarely identified in nonsyndromic left ventricular noncompaction cardiomyopathy

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Background Left ventricular noncompaction cardiomyopathy (LVNC) is a subtype of cardiomyopathy characterised by an excessively thickened endocardial layer with deep intertrabecular recesses. It is familial in the majority of cases, with involvement of mainly sarcomeric genes. Recently mutations in MIB1 were associated with LVNC while the LVNC in 1p36 deletion syndrome was attributed to the PRDM16 gene. The MIB1 gene is involved in the NOTCH pathway, regulating endocytosis of Notch ligands. PRDM16 regulates leukamogenesis, palatogenesis, neurogenesis and brown fat development and is expressed in both embryonic and adult left ventricular myocardium. **Methods and results** To establish the contribution of PRDM16 and

MIB1 mutations to nonsyndromic LVNC we analysed the coding regions of PRDM16 and MIB1 in a cohort of 41 nonsyndromic LVNC patients (39 adults and 2 children). In PRDM16, we identified 5 synonymous variants, and five missense variants. Two of these missense variants were found in 40 and 14 patients respectively and are considered single nucleotide polymorphisms (SNPs). Two variants are considered likely pathogenic (c.89C>T; p.A30V and c.1882G>A; p.D628N) and one was classified as a variant of unknown significance (VOUS: c.2290G>A; p.V764M). We did not find any mutations in the MIB1 gene. **Conclusions** We identified two likely pathogenic variants and one VOUS in the PRDM16 gene, supporting previous evidence of mutations in the PRDM16 gene as a rare cause for LVNC. The MIB1 gene did not contribute to LVNC in our cohort.

P05.54-M

Cardiovascular malformations caused by *NOTCH1* mutations do not keep left: data on 52 mutation carriers

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Background: Bicuspid aortic valve (BAV), congenital aortic valve stenosis (AVS), coarctation of the aorta (COA) and hypoplastic left heart syndrome (HLHS) are part of the spectrum of left-sided ventricular outflow tract obstructions (LVOTO) and are highly heritable congenital heart malformations. Mutations in *NOTCH1* are associated with LVOTO, but since its discovery in 2006 only eight new *NOTCH1* mutations have been identified, mostly in sporadic LVOTO patients. **Patients and methods:** In 427 LVOTO probands (204 BAV/AVS, 135 COA, 75 HLHS, 13 other) *NOTCH1* mutation analysis was performed. Also family members of probands with *NOTCH1* mutations were screened for the mutation. We report on the clinical characteristics and natural history of *NOTCH1* mutation carriers. **Results**: Thirteen pathogenic *NOTCH1* mutations were detected in 427 (3%) LVOTO probands (8% of familial cases and 1% of sporadic cases). In total 52 *NOTCH1* (obligate) mutation carriers were identified. The phenotype included not only LVOTO, but a wide variety of cardiovascular malformations (CVM) including conotruncal heart malformations (12%), septal defects (8%), and thoracic aortic aneurysms (8%). The disease seems highly penetrant at adult age. **Conclusion**: *NOTCH1* mutations are present in 8% of familial LVOTO. The phenotypic spectrum is expanded and includes a wide variety of heart malformations, indicating an effect of these mutations on neural crest derived cells and epithelial to mesenchymal transition. The high penetrance at adult age highlights the importance of genetic testing of *NOTCH1* for early diagnosis, not only in LVOTO families but in a variety of familial congenital CVM.

P05.55-S

Targeted oligonucleotide-selective sequencing for diagnostics of pulmonary arterial hypertension

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Rationale: The genetic basis of idiopathic pulmonary arterial hypertension (iPAH) is well recognized but still rarely utilized in diagnostic setting. Hundreds of mutations in coding exons and introns of, at least, seven genes have been reported to associate with idiopathic and familial forms of PAH. Comprehensive genetic testing can improve diagnostics, prognostics and treatment optimization of the index patient but it also allows effective screening of asymptomatic family members, which is a basis for well-informed genetic counseling. Methods: We adopted the novel oligonucleotide-selective sequencing (OS-Seq) and developed a custom data analysis and interpretation pipeline to identify pathogenic base substitutions and insertions and deletions in seven genes associated with PAH: BMPR2, BMPR1B, ACVRL1, ENG, SMAD9, CAV1 and KCNK3. Our sequencing panels covered >99% of the targeted regions with >15x coverage. We validated our diagnostic panels using reference HapMap samples with known genomes and mutations. Results: The sensitivity of OS-Seq to detect base substitutions was >99% and specificity was >99.9%. The accuracy to detect short INDELs was 100%. We then analyzed 22 iPAH patient samples to identify known and novel variants potentially associating with the disease. We confirmed all candidate variants using direct sequencing. The utilized novel technology allows significant cost reduction and rapid turnaround time from sample to clinical interpretation. Conclusion: These results demonstrate that the developed high-throughput targeted OS-Seq platform for PAH meets the technical criteria of clinical diagnostics and is a cost-efficient tool in clinical research.

P05.56-M

Clinical and molecular characterization of Chilean patients with Noonan Syndrome- multiple lentigines

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Introduction: Noonan Syndrome-Multiple Lentigines (NS-ML) is a rare autosomal dominant disorder, and one of the conditions known as RASopathies. PTPN11, RAF1, and BRAF are the genes known to be associated with NS-ML. Sequence analysis of coding exons 7, 12, and 13 of PTPN11 detects missense mutations in about 90% of individuals tested.
Objective: The aim of this study was to characterize the clinical and molecular features of Chilean patients with NS-ML. Methods: collaborative and descriptive study. Results: thirteen patients have been confirmed molecularly. This includes two familial cases. The age at diagnosis ranged from 20 days to 33 years old. In six patients including one of the family cases, the recurrent mutation p.Tyr279Cys in exon 7 was identified. They showed interfamilial and intrafamiliar variable expressivity. Four patients have mutations in exon 12, three with the recurrent mutation p.Thr468Met and one with an infrecuently reported one, p.Ala461Thr. Two different mutations were identified in exon 13, in three patients. The uncommon mutation p.Gln510Glu, was found in two patients, both with prenatal cardiac manifestations, and a Hypertrophic cardiomyopathy (HCM) rapidly progressive. The other mutation at codon 510 is a Gln510Pro. Discussion: The diagnosis of this condition may be challenging because clinical features overlap with other RASopathies. Although NS-ML seems to be rare, we have diagnosed a considerable number of patients. We developed a high index of suspicion particularly in patients with HCM. Eventually specific mutations could be predictor of adverse cardiac events.
Grant sponsor: Pediatric Division , Pontificia Universidad Católica de Chile

P05.57-S

The SMAD-binding domain of SKI: a hotspot for de novo mutations causing Shprintzen-Goldberg syndrome

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Shprintzen-Goldberg syndrome (SGS) is a rare, systemic connective tissue disorder characterized by craniofacial, skeletal and cardiovascular manifestations that show significant overlap with the features observed in the Marfan (MFS) and Loeys-Dietz syndrome (LDS). A distinguishing observation in SGS patients is the presence of intellectual disability, although not all patients in this series present this finding. Recently, SGS was shown to

be due to mutations in the SKI gene, encoding the oncoprotein SKI, a repressor of TGFβ activity. Here we report eight recurrent and three novel SKI mutations in eleven SGS patients. All were heterozygous missense mutations located in the R-SMAD binding domain, except for one novel in-frame deletion affecting the DHD domain. Adding our new findings to the existing data clearly reveals a mutational hotspot, with 74% (23 out of 31) of the hitherto described unrelated patients having mutations in a stretch of five SKI-residues (from p.(Ser31) to p.(Pro35)). This implicates that the initial molecular testing could be focused on mutation analysis of the first half of exon 1 of SKI. As the majority of the known mutations are located in the R-SMAD binding domain of SKI, our study further emphasizes the importance of TGFβ signaling in the pathogenesis of SGS.

P05.58-M

Mutations in the *TSPYL1* gene are not associated with sudden infant death syndrome in a Swiss cohort of deceased infants

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Background: Sudden infant death syndrome (SIDS) is currently the major cause of an unexpected and unexplained death of infants in the first year of lifetime in industrialized countries. Besides environmental factors also genetic factors are supposed as risk factors for SIDS. Notably, a frameshift mutation (c.457dupG, p.Glu153Glyfs*17) in the *TSPYL1* gene has been reported as disease causing for an autosomal recessive sudden infant death with dysgenesis of the testes syndrome (SIDDT) in an Old Order Amish community in Pennsylvania. Because the Amish community was originally founded in the German speaking part of Switzerland, including people from Alsace and Palatinate, a mutation analysis of the entire *TSPYL1* gene was performed in a cohort of 166 SIDS cases originating from the Swiss population around Zurich in comparison to 163 controls.
Eight known SNP variants (rs61746509, rs3828743, rs3749895, rs61746508, rs56100880, rs3749894, rs45490498 and rs9400897) were detected in the analyzed SIDS cohort, none of which was significantly associated with SIDS. In this context we also found two potentially disease causing amino acid substitutions in three deceased girls. One SIDS affected girl was heterozygous for the novel *TSPYL1* variant c.106C>G (p.Leu36Val) and two affected girls were heterozygous for the rare known *TSPYL1* substitution rs140756663 (c.1098C>A, p.Phe366Leu). *In silico* analyses predicted rather non-pathogenic effects for pPhe366Leu and p.Leu36Val, although protein features might be affected. The founder nonsense mutation c.457dupG (p.Glu153Glyfs*17) was not detected in our analyzed SIDS cohort of Swiss origin.
Conclusions: Mutations in the *TSPYL1* gene are not associated with SIDS in Swiss infants.

P05.59-S

SMAD3-related aortic aneurysms & dissections: A new physical finding in a large affected family

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Objective: To expand the phenotype of *SMAD3*-related aortic aneurysm and dissections by investigation of a large family. **Methods**: Genetic testing of *SMAD3* was undertaken. Physical features of two male first cousins presenting to a Genetics Clinic with thoracic aortic aneurysm/dissection were compared followed by testing and clinical characterization of the extended family. **Results**: Molecular testing confirmed that the proband and his cousin were heterozygous carriers of a deletion of exons 4-12 in *SMAD3*. Each had several features typically seen in Marfan syndrome or Loeys-Dietz syndrome (marfanoid habitus, ocular hypertelorism in one case and mild marfanoid craniofacial features and excessive striae in the other). One feature not reported in either condition was the presence of multiple small, punctate, palmar keratoses. Additional members of the kindred were examined and tested. Eight more mutation carriers were identified. Of the adults 39 years or older who carried the mutation, all 5 had multiple punctate palmar keratoses. For the 5 carriers aged 13-22 years, one had definite palmar keratoses. None of the mutation-negative patients had palmar keratoses. There was considerable variability in age at onset for aneurysm/dissection and presence of non-vascular features. A summary of features will be presented. **Conclusions**: We conclude that punctate palmar keratoses may be a previously unreported feature of some families with *SMAD3*-related aortic aneurysms and dissections, and a useful sign in distinguishing SMAD3-related cases from other genes associated with aortic aneurysm/dissection.

P05.60-M

Are ALOX5AP SNPs a risk or protective factor for Stroke?

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ALOX5AP (5-lipoxygenase) has been recognized as a susceptibility gene for stroke. Using a case-control design, we sequenced the whole coding and adjoining intronic regions of *ALOX5AP* to study the role of SNPs and their interplay with other risk factors in Greek patients with stroke. 277 patients were included and classified by the Trial of Org 10172 in Acute Stroke Treatment (TOAST): large vessel disease (63), intracerebral hemorrhage (33), cardioembolic origin (14), undetermined (38), other causes (12) and lacunes (117). The mean age of patients was 58.9±14.64, comprising 191 males. A control group of 210 subjects, ethnicity, sex and age matched, with no stroke history were also genotyped. Risk factors (hyperlipidemia, hypertension, atrial fibrillation, migraine, CAD, diabetes, smoking and alcohol consumption) were assessed as confounding factors. SNPs rs4769055, rs3803277 and rs202068154 showed significantly different ($p<0.01$) frequencies between patients and controls. More specifically the genotype frequencies of AA (minor allele) of rs4769055, of genotype CA (A minor allele) of rs3803277 and of AC genotype (C minor allele) of rs202068154 were significantly higher ($p<0.01$) in controls than in patients. All the above SNPs are located in intronic regions of the gene and according to *in silico* programs EX_SKIP and HSF they affect splicing of exons 1 and 2 of *ALOX5AP*. The results were indicative of a protective role of the three SNPs either in homozygosity or heterozygosity for MAF. However, confounding factors as mentioned above have a strong impact on stroke occurrence and outweighed the protective role of the above SNPs.

P05.61-S

Postmortem genetic testing in a series of 36 young patients after sudden cardiac death

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The incidence of sudden cardiac death (SCD) increases with age in parallel with coronary's diseases' prevalence. In young persons and athletes, SCD occurs in half of the cases, in the setting of genetically transmitted disorders such as cardiomyopathies. Molecular testing performed after necropsy may help management of families but experience in this area appears very limited. The aim is to report our experience of post mortem molecular testing after SCD and necropsy. We studied 36 patients <40 years who died suddenly with a suspected diagnosis of cardiomyopathy, established either after autopsy or known before death , with 6 dilated cardiomyopathy (DCM), 12 hypertrophic (HCM), 2 HCM/DCM , 1 restrictive (CMR), 14 arrhythmogenic right ventricular cardiomyopathy (ARVC), 1 HMC and left ventricular noncompaction. Fifteen mutations have been identified in sarcomeric or desmosomal or lamin genes. The identification of these mutations had significant impact: assessing right diagnosis in a doubtful case (HCM without LVH), modifying the appropriate diagnosis in another case (HCM and not DCM), providing guidance for genetic counselling and predictive genetic testing in relatives in all situations. Technical, ethical and legal issues may however be encountered and will be discussed. This study is one of the first series of post-mortem molecular testing after SCD which suggests the feasibility, efficiency and the benefit of the approach to improve the management of families. Postmortem molecular testing must take its place in the strategy of family care after SCD, even if a cardiomyopathy is suspected at necropsy, since genetic findings provide additional information.

P05.62-M

Mutation detection rate and -characteristics in thoracic aortic aneurysm (TAA) related disorders: results from next generation sequencing (NGS) panel testing

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Background: Targeted gene testing for Heritable Thoracic Aneurysms and Dissections (H-TAD) is compromised due to overlapping clinical features and the identification of mutations in non-syndromic patients. Therefore panel testing of multiple genes has now emerged as the preferred approach. So far, no data on mutation detection rate with this technique have been reported. **Methods:** We implemented NGS based screening after targeted

PCR enrichment of the 7 most common H-TAD-associated genes (*FBN1*, *TGFBR1/2*, *SMAD3*, *TGFβ2*, *ACTA2* and *COL3A1)*. Between November 2012 and December 2013, 141 samples from unrelated probands presenting either TAD (N=119), arterial aneurysm/dissection outside the aorta (N=10) or syndromic features with a positive family history for TAD (N=12), were sequenced on an Illumina MiSeq sequencer. **Results:** The median age of the cohort was 41.7 years (IQR 29.3 - 52.7y). We found a causal mutation in 22 patients (16%). Clinical and genetic findings are summarized in the table below.

	FBN1	TGFBR1	TGFBR2	TGFB2	SMAD3	COL3A1	ACTA2
Total	10	1	2	3	2	3	1
Clinic	9MFS 1NS-HTAD	1LDS	1LDS 1NS-HTAD	2S-HTAD 1MFS	1S-HTAD 1MFS	1vEDS 2NS- HTAD	1NS-HTAD

MFS: Marfan Syndrome; LDS: Loeys-Dietz Syndrome; (N)S-HTAD: (Non) Syndromic HTAD; vEDS: vascular Ehlers Danlos Syndrome.

Conclusion: NGS based gene panel testing in patients with H-TAD efficiently reveals a mutation in 16% of patients. Causal mutations in patients not presenting clinical manifestations of syndromal H-TAD, as well as mutations in genes -other than *FBN1*- in patients meeting the diagnostic criteria for specific syndromes including Marfan syndrome are identified, justifying a widespread application of this technique.

P05.63-S

A new COL3A1 transgenic mouse model recapitulates the clinical features of vascular Ehlers-Danlos Syndrome

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Vascular Ehlers-Danlos Syndrome (vEDS) is a severe, life-threatening heritable connective tissue disorder, characterized by translucent skin, easy bruising, and propensity to rupture of arteries and hollow organs. The molecular basis of vEDS has been well-studied, showing a wide range of mutations in COL3A1, encoding type III procollagen. Most mutations result in a glycine substitutions, pivotal for correct folding of the triple helical domain. The mechanisms by which mutant type III collagen cause vascular fragility are not well understood, but factors, other than mechanical failure, are believed to contribute to the phenotype. A Col3a1 knock-out mouse model shows early lethality and is therefore not suitable for functional studies.
We generated a transgenic vEDS mouse model with a Col3a1 missense mutation. With a BAC transgenic approach, the Col3a1 c.547G>A (p.Gly183Ser) mutation, a typical helical glycine substitution, was introduced into the C57BL/6 mouse genome. Spontaneous development of open skin wounds was observed in all transgenic males, but not in females. Expression analysis on dermal fibroblasts revealed substantial higher expression of type III collagen in transgenic males compared to transgenic females. Biomechanical testing of both transgenic males and females showed significantly reduced tensile strength of the skin, aorta and colon. Transmission electron microscopy of skin and aortic tissues showed severely abnormal collagen fibrils, intracellular spaces between smooth muscle cells and dilation of the endoplasmic reticulum (ER) , indicating excessive protein load and ER-stress. This novel animal model provides new opportunities for in-depth analyses of the pathogenic basis of vEDS and for possible therapeutic interventions.

P05.64-M

Genetic tests in the diagnosis of congenital vascular malformations

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Vascular anomalies are a heterogeneous group of congenital malformations of the circulatory system characterized by morpho-structural and/or functional defects of various nature, severity and extent in any type of vessel (arteries, capillaries, veins and lymphatic vessels) and in any part of the body. Although most vascular malformations are sporadic (simple or combined), syndromic and familial forms also exist. Diagnosis may be complicated even for experienced specialists, because there is substantial clinical overlap between lesions and each can mimic the others. To our knowledge, there are currently 15 known genetic forms of Mendelian vascular malformation and mutations with autosomal recessive, autosomal dominant or paradominant inheritance have been identified in 25 genes. Sporadic cases with de novo mutations in causative genes of familial forms have also been described. In this study, we investigated all 25 known genes in 108 patients with sporadic congenital vascular anomalies by next-generation sequencing. We identified 24 variations (22%). To estimate their pathogenicity, each variation was looked up in dbSNP137, its frequency was compared using data from the Exome Variant Server, the effect of amino acid substitution on the protein was evaluated using *in silico* analysis, and where possible, it was investigated in the family. Our results confirm the utility and efficacy of genetic testing, even in sporadic cases. Identification of causative genes will be decisive for: determining genotype-phenotype relationships; determining transmission risk; organizing personalized follow-ups; characterizing new drug targets for experimental gene-specific therapies.

P05.65-S

Association of VAV2 and VAV3 polymorphisms with cardiovascular risk factors in hypertensive and diabetic patients

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Hypertension, diabetes and hypercholesterolemia are risk factors for the development of cardiovascular disease, one of the leading causes of death in the world. There are few data on the influence of genetic polymorphisms in these diseases. The guanine nucleotide exchange factors VAV2 and VAV3 play an important role in vascular homeostasis in vivo. Therefore, we evaluated the association of VAV2 (rs 602990) and VAV3 (rs7528153) polymorphisms with susceptibility to hypertension and diabetes-induced cardiovascular risk and cardiovascular damage. We extracted DNA from peripheral blood of 384 patients (152 hypertensive, 66 diabetic and 166 non-diabetic non-hypertensive). Polymorphisms were detected by qPCR with TaqMan® probes. We analyzed systolic, diastolic and pulse blood pressure, basal glycaemia, endothelial dysfunction (by measurement of pulse wave velocity), retinopathy, left ventricular hypertrophy and cardiovascular risk. VAV2 Val584Met polymorphism is associated with family history of hypercholesterolemia in non hypertensive patients carrying the TT genotype (p=0.034, OR=2.706, 1.205-6.321) and the CT genotype (p=0.034, OR=2.194, 1.059-4.545), with elevated pulse pressure in smoker patients carriers of the TT allele (p=0.016, OR=5.538, 1.022-30.019) and with reduced risk of developing metabolic syndrome in non-smokers patients carriers of the TT allele (p=0.05, OR=0,408, 0.180-0.927). VAV3 Sher298Thr heterozygous polymorphism is associated with patients with lower incidence of family history of diabetes (p=0.018, OR=0.470, 0.279-0.793) and with increased fasting glucose in non-smoking non-diabetic patients carrying the TT allele (p=0.043, OR=3.700, 1.266-10.815). Our results suggest that polymorphic variants of VAV2 and VAV3 genes may be involved as risk factors associated with cardiovascular disease in hypertensive and diabetic patients.

P05.66-M

Association of Cytochrome P450 2C9 (CYP2C9) and VKORC1 polymorphisms and warfarin dosage in Iranian patients refer to Shahid Rajaie Heart Center

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Background: Warfarin is a commonly prescribed oral anticoagulant for the treatment and prevention of thrombotic diseases. Warfarin dose has a large interindividual variation. An insufficient dose may fail to prevent thromboembolism, while an overdose increases the risk of bleeding. Patients with CYP2C9*2 and/or CYP2C9*3 polymorphisms need lower dose than wild-type patients. VKORC1 (vitamin K epoxide reductase complex 1) is another gene affecting warfarin metabolism. VKORC1 variants have been reported to be associated with a need for lower doses of warfarin compared with wild-type variants during long-term therapy.
Objective: This study was conducted to identify the associations between demographic characteristics (sex, height, weight, age, ethnicity), and genetic polymorphisms of CYP2C9 and VKORC1 (-1639G>A) with warfarin dose among Iranian patients.
Materials and Methods: Our study concluded 200 patients that reached to a stable dose of warfarin. By PCR-RFLP method CYP2C9*2 and CYP2C9*3 polymorphisms of CYP2C9 gene and VKORC1 (-1639G>A) was genotyped.
Results and Conclusion: Our study showed that CYP2C9 polymorphisms had significant influence on Iranian daily warfarin dose(P=). Our results

suggested that patients with AA genotype in VKORC1 (-1639G>A) require lower doses of warfarin than those with AG or GG genotype. Height and weight did not have a significant correlation with the warfarin maintenance dose. In addition there was no significant relationship between sex and ethnicity with the maintenance dose of warfarin (p< 0.05).

P05.67-S
Analysis of c.1166A>C polymorphism in 3´UTR region of AGTR1 gene in patients with Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH; OMIM 178600) is a progressive vascular disorder characterized by pulmonary vascular resistance increase, vascular remodelling and right heart failure. Angiotensin II is both a potent vasopressor hormone and a primary regulator of aldosterone secretion. It is an important factor that controls blood pressure and volume in the cardiovascular system. AGTR1 gene plays an integral role in blood pressure control, and is involved in the pathogenesis of hypertension. In this study, we demonstrated the association between c.1166A>C polymorphism and PAH. We included 55 PAH patients and 52 controls. Using specifically designed primers we amplified and sequenced the 3´-untraslated region of AGTR1.
We analyzed the c.1166A>C polymorphism located on 3´UTR of AGTR1 gene. By comparing genotype frequencies of controls and patients with PAH, we obtained statistically significant differences for this SNP between the two groups (p>0.001). The RR of developing PAH in patients with this SNP is 20.3; IC 95%; p<0.001. The statistical analysis of clinical and hemodynamics parameters between patients with or without this SNP showed significant differences in systolic pulmonary pressure (p=0.037), cardiac index (p=0.012) and PAH subtypes (IPAH vs APAH) (p=0.028). This SNP is present in 72.4% of IPAH patients and in 67.8% of APAH patients, producing a more severe phenotype. This SNP only appears in 25% of control cohort.
In conclusion, this polymorphism in AGTR1 gene is more frequent in IPAH than in APAH patients and predispose individuals to an increased risk of developing PAH associated with a more severe phenotype.

P06.01-S
10 novel HGD mutations identified in “black bone disease” patients

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Alkaptonuria (AKU, “black bone disease”) is caused by mutations in homogentisate-1,2-dioxygenase (*HGD*) gene leading to deficiency of HGD enzyme activity. Clinically it leads mainly to homogentisic aciduria, ochronosis and painful and disabling ochronotic arthritis. AKU was described by Sir Archibald Garrod as the first inborn error of metabolism in 1902, and now, more than 110 years later, we test nitisinone as the first possible treatment of this disorder. We analysed for *HGD* mutations 40 AKU patients enrolled for the first part of this 7FP-supported project DevelopAKUre run in two study centres in Piestany (Slovakia) and Liverpool (UK). In this cohort we observed 20 different pathogenic variants, two of which were novel. The first mutation (R53Q c.158G>A) was present in homozygous state in one patient of Indian origin. The second one (T167I,c.500C>T) was identified in one copy in Slovak AKU patient, further confirming the genetic heterogeneity of AKU in this small country with increased incidence of AKU, where now already 13 mutations are reported. Eight additional novel *HGD* mutations were found in AKU patients sent to our laboratory for a routine molecular diagnostics within a frame of different collaborations. The novel R53Q mutation identified in DevelopAKUre was found also in one Italian patient. One mutation was identified in a patient from Brasil (M186K, c.557T>A), in patient from India (ivs7+6T>C,c.469+6T>C), from France (F147S,c.440T>C), and five in cases from Italy (K248E,c.742A>G// G205D,c.614G>A// K353Q,c.1056A>G// G251D,c.752G>A// Y40S,c.119A>C)). The total number of different AKU-causing mutations as published in our *HGD* mutation database is now 126.

P06.02-M
Intrafamilial gene rearrangements in Barth syndrome leading to different TAZ mutations in siblings

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The X-linked infantile-onset Barth syndrome (BTHS; OMIM #302060) is caused by mutations in the *TAZ* gene and generally manifests in hemizygous males. The major clinical manifestations are cardiomyopathy with or without left ventricular noncompaction, skeletal myopathy, hypotonia, growth delay and neutropenia. Confirmatory tests are provided by mutational analysis of the *TAZ* gene and/or the biochemical dosage of the monolysocardiolipin/ tetralinoleoyl cardiolipin ratio. Heterozygous females do not normally manifest clinically but may undergo molecular prenatal diagnosis during pregnancy.
Here we report the unusual case of a family in whom the male proband and his mother carry different *TAZ* mutations. The ten year-old proband harboured a novel g.4552364_4562302del9937ins705 complex rearrangement which serves to remove *TAZ* exons 1-5. However, during a subsequent maternal pregnancy, molecular prenatal diagnosis of the male fetus revealed that he carried a different *TAZ* gene lesion, g.4558047_4558278del232. The mother was subsequently confirmed to be heterozygous for this novel g.4558047_4558278del232 deletion (which removes only *TAZ* exon 1) but negative for the g.4552364_4562302del9937ins705. The g.4552364_4562302del9937ins705 mutation must therefore have occurred *de novo* in the male proband. The g.4558047_4558278del232 must have occurred *de novo* in the mother since it was not detected in either her mother or grandmother. Sequencing of the breakpoint junctions revealed microhomologies which could have prompted both rearrangements by serial replication slippage.
In conclusion, a mother carrying a gross *TAZ* gene deletion can undergo further germline gene rearrangement and generate children with a different mutation. Such a situation can complicate the pre- and post-natal molecular diagnosis of BTHS.

P06.03-S
Diagnosis of GM1-gangliosidosis or galactosialidosis by enzymology and molecular genetic testing from a single dried blood spot

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GM1-gangliosidosis and galactosialidosis are rare autosomal recessive lysosomal disorders associated with defects in the same protein complex. GM1 gangliosidosis is caused by a deficiency of β-galactosidase, whilst galactosialidosis is associated with deficiencies of both β-galactosidase and α-neuraminidase activity secondary to a defect in the protective protein cathepsin A. We have developed a dried blood spot enzyme assay for β-galactosidase and have added a reference enzyme (α-galactosidase) which is measured simultaneously as a measure of sample integrity. A panel of 300 unaffected individuals were tested to establish a normal reference range; all 5 affected GM1-gangliosidosis patients tested showed clear enzyme deficiency. α-neuraminidase is an unstable enzyme which is therefore unsuitable for dried blood spot analysis and difficult to interpret in leucocyte samples. Differential diagnosis of GM1-gangliosidosis and galactosialidosis has traditionally been carried out by measurement of α-neuraminidase in cultured fibroblasts; however skin biopsy and cell culture can take many weeks. Nested PCR and Sanger sequencing analysis of the GLB1 and CTSA genes can be carried out at this centre from the same dried blood spot sample as enzyme testing, providing a rapid diagnosis from a single convenient sample.

P06.04-M
Unraveling Boucher-Neuhauser syndrome: the multifaceted consequences of PNPLA6 gene mutations

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Boucher-Neuhauser syndrome (BNS) is a rare genetic entity belonging to the group of hereditary cerebellar ataxias with hypogonadism. The combination of chorioretinal dystrophy, hypogonadotropic hypogonadism and progressive spinocerebellar ataxia are the hallmarks of the disease. Here we report a new phospholipid disorder in a Brazilian BNS family caused by mutations in the PNPLA6 gene (Patatin-like phospholipase domain con-

taining 6) and its role in the metabolism of lysophospholipids. Biochemical and molecular investigations were undertook in a Brazilian kindred affected by a complex neurological disorder, presenting mainly as a spinocerebellar ataxia with hypogonadism and early vision loss. After molecular analysis of genes associated with spinocerebellar ataxia and exclusion of other inborn errors of metabolism (IEMs) associated with cerebellar disease, whole exome sequencing (WES) was performed. Mutations in the PNPLA6 gene were identified in all affected patients. They presented with very early onset visual loss (chorioretinal dystrophy) accompanied by progressive cerebellar ataxia and primary hypogonadism. Brain MRI showed cerebellar and pons atrophy. Peripheral neuropathy (motor axonal neuropathy) was identified in all of them. Mutant mice with mutations in PNPLA6 gene have shown a relentless neurological disease. PNPLA6 codifies a neuropathy target esterase (NTE) hydrolase, an integral membrane protein located on the endoplasmic reticulum. It shows phospholipase activity, being involved in the hydrolysis of lysophosphatidylcholine to yield glycerophosphocholine. Boucher-Neuhauser syndrome is a clear example of the profound biological consequences of mutations in such gene in the central and peripheral nervous system.

P06.05-S

Dissecting the genetic architecture of loci with established effects on multiple cardiometabolic phenotypes

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Genome-wide association studies (GWAS) have identified hundreds of loci associated with cardiometabolic phenotypes, many of which overlap or lie in close proximity. Variants associated with multiple phenotypes, such as at *IRS1*, can provide insight into biology of correlated cardiometabolic traits. However, the genetic architecture of these loci is frequently complex and needs further investigation. To disentangle association patterns of 630 associated SNPs (Dec 2012) from GWAS meta-analyses in Europeans for 19 quantitative phenotypes and two cardiometabolic diseases, we defined sets of adjacent variants located less than 500kb apart and harboring 446 associated SNPs within 151 genomic regions (range=2-8 SNPs/region). We examined, whether associations with multiple phenotypes within each region could be explained by LD through approximate conditional analysis (ApCA) using the GCTA tool.
Across the 151 regions, we observed 14 (10%) loci in which the same SNP was associated with multiple phenotypes. Associations in 11 of these 14 loci were with epidemiologically highly correlated traits. Through ApCA, we identified 41 (27%) regions with multiple associated variants that underlie the same association signals, thus suggesting multi-phenotype effects. Within 45 (30%) regions, multiple signals were explained by independent variants. Thirty-two (21%) regions showed complex architecture, and for the remaining 19 (13%), the association with one phenotype partially explained the effect on another.
Overall, a substantial number (87 or 57.62%) of cardiometabolic loci shows potential pleiotropic effects on multiple phenotypes, which might contribute to their shared biology. Within other regions, distinct genetic effects or more complex architecture could underlie independent regulatory mechanisms.

P06.06-M

Severe thrombocytopenia in CDG1a

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Congenital disorders of glycosylation type 1a (CGD1a), caused by mutations in the PMM2 gene, is a multi-systemic disorder characterized by the abnormal glycosylation of serum glycoproteins. There is a variable disease course, with the mildest form being virtually asymptomatic, to the most severe form presenting as lethal hydrops fetalis. The classical presentation is that of failure to thrive, abnormal fat distribution, and inverted nipples, with developmental delay, seizures and ataxia. Other systemic features can include protein-losing enteropathy, hepatomegaly, cardiomyopathy, renal cysts, and coagulopathy. Aside from coagulopathy, there are few case reports of hematologic abnormalities in CDG1a. We report a three and a half year old girl with CDG1a diagnosed at 4 months of age. She presented with feeding difficulties and typical physical features. At 6 months she developed progressive hepatosplenomegaly, liver cirrhosis, and ascites. After a life-threatening deterioration, she responded to aggressive management with diuretics, albumin infusions, and repeated paracentesis. She was noted to have pancytopenia with marked thrombocytopenia at 2 years of age, with platelet counts in the range of 10-20x10^9/liter. She did not improve with IVIG or steroids. She had a normocellular marrow with mild decrease of megakaryocytes and granulocytes, which suggested a primary consumptive thrombocytopenia, with some component of reduced megakaryopoesis. As she had become refractory to transfusion, splenectomy was performed in this medically fragile child and resulted in a moderate recovery of platelet levels. This is the second reported case of severe thrombocytopenia in CDG1a, and the only reported case of splenectomy with this disorder.

P06.07-S

Citrin deficiency caused by a novel mutation in the SLC25A13 gene - clinical, biochemical and genetic characterization of new Caucasian case

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Citrin deficiency is an autosomal recessive disorder caused by mutation in the SLC25A13 gene and has three different age-dependent clinical phenotypes: neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) in newborn, failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) in older children, and recurrent hyperammonemia with neuropsychiatric symptoms in citrullinemia type II (CTLN2) in adults. To date almost all reported patients were from East Asia and only few cases from Caucasian origin have been described.
We report the first Bulgarian case of NICCD - a male infant with a mild neonatal jaundice which resolved spontaneously. The patient became jaundice again at 40 days old.
Initial investigations revealed conjugated hyperbilirubinemia and evidence of liver dysfunction, hypoproteinemia, mild hypoglycemia and mild anemia. Metabolic evaluation showed lactate acidemia, galactosemia and significant elevation of citrulline, methionine and arginine.
Mutation study of the SLC25A13 gene showed the compound heterozygote, c.1081C>T (p.R361X) and c.74C>A (p. A25E), which confirmed the diagnosis of NICCD. c.1081C>T (p.R361X) is previously unreported nonsense mutation.
After galactose restriction and supplementation with fat-soluble vitamins liver functions were normalized and cath-up growth was achieved before 8 months of age.
In conclusion, we presented a new genetically confirmed case of NICCD patient from non-Asian origin. We detected a previously undescribed nonsense mutation in SLC25A13 which further expanded the genotypic spectrum and genotype-phenotype correlations of citrin deficiency.
NICCD has been underdiagnosed and should be considered in infants with intrahepatic cholestasis, especially when associated with elevated plasma galactose and citrulline, regardless of ethnicity.

P06.08-M

Mmachc is required for pre-implantation in the mouse

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Mutations in *MMACHC* cause the most common inborn error of vitamin B_{12} (cobalamin) metabolism - *cblC.* Patients with this disease are unable to convert cobalamin into the two active forms, methylcobalamin and adenosylcobalamin; consequently, they have elevated homocysteine and methylmalonic acid levels in blood and urine. Some *cblC* patients also have structural abnormalities, including congenital heart defects. In the mouse, *Mmachc* has tissue and stage-specific expression during organogenesis (Pupavac, M. *et al. Mol Genet Metab.* **103**, 401-405 (2011)). We generated mice with a gene-trap insertion in intron 1 of the *Mmachc* gene, (*Mmachc*$^{Gt(AZ0348)Wtsi}$). Mice heterozygous for this gene-trap allele were viable and fertile, but showed a 50% reduction in Mmachc protein compared to wild-type littermates. The *Mmachc*^{Gt} allele was inherited with a transmission ratio distortion in a subset of matings. Homozygous *Mmachc*^{Gt} embryos were not found after embryonic day 3.5. These studies indicate that the *Mmachc* gene product is essential for early mouse development.

P06.09-S
POLG mutations in Polish patients with mitochondrial disease of unknown etiology - preliminary data

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POLG mutations are considered as a major cause of mitochondrial diseases (MD) including AHS, MCHC, MEMSA, MIRAS, SCAE, SANDO, PEO, LS, MNGIE, MERRF, MELAS, and SMA-like phenotypes. Epidemiological data point the widespread occurrence of recurrent c.1399G>A (p.A467T), c.2243G>C (p.W748S), and c.2542G>A (p.G848S) mutations in European populations and emphasize significant differences depending on carriers' ethnicity.
A large group of 210 Polish patients with clinical suspicion of MD, and excluded common point mtDNA mutations, large-scale mtDNA rearrangements, and nuclear encoded *SURF1* and *SCO2* mutations, was recruited for *POLG* screening. DNA samples isolated from blood, saliva, urine, muscle and liver biopsies were genotyped for the presence of three common mutations by Real-time PCR with specific TaqMan allele discrimination assays (Light Cycler 480 II, Roche), and were then verified using ABI PRISM dye terminator cycle sequencing kits (Applied Biosystems).
The only mutation identified in the studied group was p.W748S; one case - in homozygous, and seven cases in heterozygous form. Six patients presented with AHS, one adult demonstrated sensory motor neuropathy, and one child psychomotor retardation and stroke-like episodes. In three children despite sequencing the whole gene we did not confirm the presence of the second mutation. Significant predominance of p.W748S mutation indicates similarity to the genotype identified in Nordic patients, that is not frequent in another parts of the world.
Evaluation of the carrier frequency of the common *POLG* mutations among MD patients are being continued.
The study was financed by NCN projects 2012/05/B/NZ2/01627 and 2857/B/P01/2010/39, CMHI projects S126/12 and S217/12.

P06.10-M
A targeted resequencing approach for diagnostics of Congenital Disorders of Glycosylation

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Congenital Disorders of Glycosylation (CDG) are a genetically heterogeneous group of disorders caused by the alteration in synthesis and structure of protein and lipid glycosylation. To date, more than 60 different causes of CDG have been defined genetically. CDG is also characterised by extremely variable phenotypes with manifestations ranging from severe developmental delay and hypotonia beginning in infancy, to hypoglycemia and protein-losing enteropathy with normal development. Till recently, finding the causative mutation of CDG patients has been impaired by this extreme genetic heterogeneity: it was virtually impossible to Sanger sequence all candidate genes. Instead, one or few genes were tentatively selected based on a combination of biochemical, cell biological and glycobiological investigations. In order to obtain a better diagnostic yield, we designed a capture assay for a panel of 79 genes associated with CDG type I, CDG type II and congenital muscular dystrophy-dystroglycanopathy. To evaluate the assay, targeted sequencing was performed for 16 CDG type I and 15 CDG type II patients. The mean coverage in the target region was about 600x and a genotype was called for more than 97% of the targeted bases. A diagnosis was confirmed in 8 cases. Interestingly, mutations could also be detected (and confirmed) in the gene ALG1 that could not be assayed by genomic Sanger sequencing due to the abundance of pseudogenes. After our targeted assay enabled an increased diagnostic yield, the the most interesting, unsolved cases will be subjected to exome sequencing for gene discovery.

P06.11-S
A patient with congenital disorders of glycosylation type 1q due to mutation in SRD5A3

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Congenital disorders of glycosylation type I (CDG1) are inherited metabolic diseases with an extremely broad spectrum of clinical presentations caused by defective glycosylation of glycoproteins and glycolipids. Recently, mutations in the SRD5A3 gene was found in patients with cerebellar ataxia, and eye malformations. Serum transferrin isoelectric focusing (IEF) demonstrated a type 1 glycosylation defect, thus this disorder was classified as CDG1q. We present here a 4 month old male with coarse face, hypertrichosis and developmental delay. The baby was the fourth child of first-cousin parents. He had narrow forehead, depressed nasal bridge, proptotic eyes, large fontanelle (6x8cm), long philtrum, thin lips, loose skin, hepatosplenomegaly and bilateral inguinal hernia. He also had iris coloboma, glaucoma, nystagmus, corneal clouding and elevated serum transaminases. He was followed up until 27 months of age. He had severe hypotonia and no head control at this age. Dark pigmentation of dorsum of feet was developed. His cranial MR imaging revealed cerebellar hypoplasia, enlargement of lateral ventricules and frontal atrophy. Serum transferring IEF showed a type 1 pattern. Serum IGF1 and antithrombin level also were decreased. Using homozygosity mapping followed by whole-exome genotyping, we described a homozygous missense mutation (c.320G>A; p.Trp107X) in the SRD5A3 gene in the patient. This mutation was reported previously in a Turkish patient with hypotonia and similar eye and cranial findings.

P06.12-M
Congenital Hyperinsulinism of Infancy (CHI): hunt for new genes

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Congenital Hyperinsulinism of Infancy (CHI) is a rare disorder, characterized by heterogeneity in clinical and genetic features. An inappropriate insulin secretion is responsible of hypoglycaemia, which can result in serious neurological damage and life-long handicap. The genetic causes of CHI have been found in genes regulating insulin secretion from pancreatic beta-cells but in about 50 % of CHI patients the molecular defect remain unknown. Hunting for novel CHI-causing genes we performed whole-exome sequencing (WES) on 10 CHI patients [Proverbio MC et al, 2013; doi:10.1371/journal.pone.0068740]. To pinpoint the causal mutation in a small number of samples and to select the most promising candidate genes, we implemented a computational strategy including: 1) a bionformatics pipeline to identify a high quality single nucleotide variants (SNV); 2) an exome homozygosity mapping using a novel algorithm H3M2 [Pippucci T el al, 2011; doi: 10.1159/000330164] and 3) a prioritization analysis using the list of rare and novel coding variants by mean of web tools such as ToppGene suite, Endeavour and Gene Distiller. Our approach resulted in a selection of small number of genes that significantly correlate with human phenotype and molecular pathways associated to congenital hyperinsulinism and insulin signalling. Among them we selected missense mutations affecting CDKAL1 and PIK3R1 for further investigations either at the level of protein structure (in silico protein modelling) and in respect to their role in beta cell using INS1-E cellular model and human pancreatic islets.

P06.13-S
Whole-exome sequencing for genetic diagnosis in congenital hypothyroidism

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Congenital hypothyroidism (CH) is the most common endocrine disorder among newborns but its genetic aetiology is still largely unknown, with genetic defects identified in less than 20% of the cases. Mutations in genes for thyroid hormone synthesis (TG, TPO, DUOX2, IYD, SLC5A5, SLC26A4) have been implicated in dyshormonogenesis, while defects in thyroid transcription factors (PAX8, Nkx2.1, Nkx2.5 and FOXE1) and in the TSH receptor have been linked to thyroid dysgenesis. To identify novel mutations in CH, 42 patients and 21 unaffected relatives (25 families) were whole-exome sequenced (WES) as part of the UK10K project. A further 29 patients and 10 unaffected relatives (24 families) were sequenced across a customized panel of genes. We searched for functional de novo or inherited variants that were rare in affected individuals, using 1000 Genomes, NHLBI ESP and UK10K as control datasets. A total of 15 mutations in known causative genes were identified in 10 dyshormonogenesis families. Ten of these variants were novel and predicted to be damaging (TG: 509X, C707Y, W1031L, C1474Y, W2666L, 140X, Q1625E, T1397RfsX30, G638+5; DUOX2: Q570L) and the remaining five were previously reported in CH cases (TG: R277X; DUOX2: Q686X, R354W; TPO: R665Q, R491H).
Our initial findings highlight the potential of WES as a diagnostic tool: five

families harbouring known TG/TPO/DUOX2 mutations have a conclusive genetic diagnosis and novel recessive nonsense mutations here identified are also likely causative. Wider pedigree-genotype correlation is needed to confirm the pathogenicity of the remaining recessive missense mutations here identified.

P06.14-M
Efficiency of the integrated Danon disease diagnostic protocol can be demonstrated in families affected by LAMP2 exon-copy number variations

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Danon disease (DD) is a X-chromosome-linked disorder that manifests by cognitive deficit, myopathy and cardiomyopathy in males. The phenotype in females is variable and mitigated as a likely consequence of tissue-specific X-chromosome inactivation (XCI) ratios. DD is caused by mutations in the lysosomal-associated membrane protein 2 (*LAMP2*) gene. Majority of the mutations abolish the protein expression due to truncation of LAMP2 open reading frame. 10-15% of the mutations are exon-copy number variations (eCNV) stemming primarily from recombination events in *LAMP2* intron 3. DD laboratory testing relies on identification of the absence of the protein and characterization of the mutation within the *LAMP2* gene. Importantly, the diagnostic protocol must reflect the following: (i) gender of the proband/patient, (ii) expression patterns of LAMP2 protein, (iii) alternative splicing of *LAMP2* pre-mRNA, (iii) mosaic *LAMP2* expression determined by XCI in female patients, (iv) germinal/somatic mosaicism phenomena. LAMP2 protein testing should be performed by flow cytometry in peripheral white blood cells as this approach offers both minimal invasiveness and detection sensitivity down to 0.008% of deficient granulocytes. The latter is of critical importance in samples from suspect XCI mosaic female patients and/or family members who are potentially germinal/somatic mosaics. Molecular genetics methods should universally assess full-length *LAMP2* mRNA isoforms (2B, A and C) and consequently compare the abnormal findings to gDNA changes. This integrative approach has major advantages in genetic setups when qualitative PCR based methods failed to identify the mutation. Model examples of such situations will be provided in families affected by *LAMP2* eCNVs.

P06.15-S
Radiographic features of the skeleton in disorders of postsqualene cholesterol biosynthesis

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Disorders of postsqualene cholesterol biosynthesis (DCB) are a group of inborn errors of metabolism characterized by multiple congenital abnormalities, including significant skeletal involvement. The most frequent and best characterized example is the Smith-Lemli-Opitz syndrome (SLOS). Nine other disorders are known to date, namely autosomal recessive Antley-Bixler syndrome, Greenberg dysplasia, X-linked dominant chondrodysplasia punctata, X-linked recessive male emopamil-binding protein (EBP) deficiency, congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome, CK syndrome, SC4MOL deficiency and the SLOS-like desmosterolosis and lathosterolosis. This study provides an overview of the skeletal radiology of DCB: we report the radiological features of 14 previously unreported patients and review the literature. Our study shows that the DCB have a common pattern of limb abnormalities including polydactyly, which is typically postaxial and rarely interdigital and can involve all four limbs, and syndactyly of the toes. Chondrodysplasia punctata (CDP) is specifically associated with a subgroup of DCB (Greenberg dysplasia, CHILD syndrome, X-linked dominant chondrodysplasia punctata, male EBP deficiency); the possible occurrence of epiphyseal stippling in SLOS, initially reported, does not appear to be confirmed. CDP is also associated with other congenital disorders such as chromosomal abnormalities, brachytelephalangic CDP (X-linked recessive CDP, disruption of vitamin K metabolism, maternal autoimmune diseases), peroxisomal disorders (rhizomelic CDP) and lysosomal storage disorders. In the differential diagnosis of epiphyseal stippling, a moth-eaten appearance of bones, asymmetry or the presence of the common pattern of limb abnormalities are further indicators of DCB. In conclusion, the specific differentiating radiological features of DCB are highlighted.

P06.16-M
Regional reference range lysosomal storage diseases in the Kazakhstan

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Objective of the study was investigating the criteria's of enzyme activity of alpha-L-iduronidase, iduronate-2-sulfatase, N-acetylgalactosamine-6-sulfatase, β-galactosidase, arylsulfatase B and providing diagnostics of patients with mucopolysaccharidosis from Kazakhstan.
The materials used for the study were dry blood spots of 2500 healthy newborns to study the enzyme activity of lysosomal storage diseases (LSD) by tandem mass spectrometry and fluorimetry. Reference ranges of enzymes activity were the following: alpha-L-iduronidase was 450-2614 nmol / spot* 20 h., iduronate-2-sulfatase was 0.02-0. 25 nmol / spot* 21 h., arylsulfatase B was 0.14-0.7 nmol / spot* 21 h., β-galactosidase was 35-126 nmol/h/ml., N-acetylgalactosamine-6-sulfatase was 5.7-33 nmol/24h/ml.
13 out of 2500 patients had lysosomal enzyme below the reference range. Two patients were defined with alpha-L-iduronidase activity which were below the reference range and constituted 33.06-187. 26 nmol / spot* 20 h. Activity iduronate-2-sulfatase was determined in 6 patients with suspected MPS type II. Decrease in enzyme activity was observed in all cases and was 0 nmol / spot* 21 h. In 2 cases, a decrease activity of to β-galactosidase 25-30 nmol/h/ml, the 1 patient had a decreased activity of N-acetylgalactosamine-6-sulfatase to 2.2 nmol/24h/ml. Arylsulfatase B activity was determined in 3 patients, a decrease of enzyme activity was observed 5-20 times, and was 0.03-0.07 nmol / spot* 21 h. The reference ranges of enzyme activity of LSD were calculated.

P06.17-S
Massive parallel sequencing in suspected familial hypercholesterolemia patients of Latvia

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Familial hypercholesterolemia (FH) is one of the most common single gene disorder mostly inherited as autosomal dominant trait. The physical sign of FH is elevated low density lipoprotein cholesterol (LDL-C), elevated total cholesterol (TC) levels and tendon xantomas. Identification and early treatment of affected individuals is desirable and in lack of physical symptoms DNA based diagnosis provides confirmations of diagnosis and enables early patient management.
Majority of FH cases are caused by mutations in four genes (APOB, LADLR, PCSK9 and LDLRAP1). There are commercial kits available for testing of most common FH causing mutations, but the spectrum of disease causing mutations is quite diverse in various populations.
Here we report mutations found in 64 patients with suspected FH in a sample from the Genome Database of Latvian population. We used targeted next generation sequencing approach in order to determine the full spectrum of mutations in coding regions of LDLR, APOB, PCSK9 and LDLRAP1. In total we found 28 missense one nucleotide mutations from which two rs5742904 (Arg3527Gln) in APOB gene and rs147509697 (Gly20Arg) in LDLR gene has been previously described as FH causing mutation confirming the FH in three patients (4.6 %). Possible FH causing mutations however were identified in majority of patients.
Conclusion: most commonly employed commercial mutation panel is not sufficient for diagnosis of FH patients and NGS can help to identify FH causing mutations in Latvian population. Our results also provide an example that DNA testing can identify FH patients before they develop serious physical symptoms.

P06.18-M
Development of a cell-based reporter assay suited for small-molecule drug discovery in FGF23-inducible HEK293 cells stably expressing Klotho

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Fibroblast growth factor 23 (FGF23) is a key regulator of phosphate homeostasis. It is of crucial importance in hereditary and acquired hypo- and hyperphosphatemic disorders. Moreover, FGF23 has emerged as a promising biomarker for the prediction of adverse clinical outcomes in patients with chronic kidney disease (CKD), as it might be related to mortality, cardiovascular abnormalities and disease progression. FGF23 is a bone-derived endocrine factor, which inhibits renal tubular phosphate reabsorption by activating receptor complexes composed of FGF receptor (FGFR) 1c and the co-receptor Klotho. As a major signalling pathway mitogen-activated protein kinase (MAPK) pathway is employed. For the investigation of FGF23 in an in vitro model we established FGF23-inducible HEK293 cells that stably express Klotho (HEK293-KL). The induction of HEK293-KL cells by FGF23 was shown by detecting the activation of MAPK pathway, which could be reduced by the use of two known small-molecule inhibitors of MAPK pathway: SU5402 and U0126. To identify novel small-molecule compounds that modulate FGF23/FGFR1c/Klotho signalling, we developed a cell-based reporter assay that is suited for high-throughput screening (HTS). The assay is based on the AlphaScreen SureFire platform of Perkin Elmer to monitor the phosphorylation of endogenous extracellular signal-regulated kinase 1 and 2 (ERK1/2) in cellular lysates of HEK293-KL after the induction with FGF23 in the presence of small-molecule compounds. Since increased plasma concentrations of FGF23 are the main cause of many phosphatemic disorders, a modulation of its effect could be a potential strategy for drug discovery and new therapeutic approaches in disorders affecting phosphate homeostasis.

P06.19-S
Gaucher disease and Langerhans cell histiocytosis

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Gaucher disease [GD] is an autosomal recessive lysosomal storage disease, with deficient glucocerebrosidase activity caused by mutations in the *GBA* gene. Patients are often categorized as manifesting three distinct phenotypes: non-neuronopathic [type 1]; severe, infantile neurologic [type 2]; and chronic, progressive neurologic [type 3]. We describe a male infant with neurologic GD as well as concomitant Langerhans cell histiocytosis [LCH]. The child presented at age 9 months with weight loss, hematochezia, pallor, and irritability. He had hepatosplenomegaly, anemia, thrombocytopenia, as well as a right upper lobe nodule. Excisional biopsy of the nodule led to a histologic diagnosis of LCH. Bone marrow and rectal biopsies did not reveal findings of LCH, but the presence of macrophages concerning for a storage disease. Enzyme assay confirmed a diagnosis of GD and gene testing revealed apparent homozygosity for a novel mutation in *GBA*, c.488C>T [p.A163V]. His presentation was intermediate between 'classical' type 2 and type 3 GD. Upon physical examination, he demonstrated opisthotonic posturing, hypotonia, horizontal oculomotor apraxia, and swallowing difficulties but was also able to sit with support, was attentive to environmental stimuli, and had not lost previously gained developmental skills. To our knowledge, this is the first GD patient reported with concomitant LCH. Even though the simultaneous occurrence of GD and LCH could be coincidental, it is also possible that LCH is secondary to the specific *GBA* mutation identified in the patient and thus LCH should be added to the list of lymphoreticular neoplasms associated with GD.

P06.20-M
Glucocerebrosidase enhancement in selected Gaucher disease fibroblasts by a series of DIX compounds

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Gaucher disease (GD) is a lysosomal storage disorder due to an inherited deficiency of the lysososmal enzyme glucocerebrosidase (GBA1). It produces the accumulation of glucosylceramide in cells of the reticuloentothelial system and causes multisystemic manifestations. The limited efficacy of the current treatments has led to the development of new strategies, including the use of pharmacological chaperones. These are small molecules, generally competitive inhibitors of the target enzyme, which at sub-inhibitory concentrations may induce the proper folding and trafficking of the mutated enzyme resulting in a concomitant increase in the residual activity. The aim of this work is to report on the ability of seven compounds to increase the residual GBA1 activity on fibroblasts from six Gaucher patients bearing different genotypes. This series of compounds, named DIX, has been designed by combination of the iminoxylitol scaffold of parent **1C9DIX** with triazolylalkyl side chains as GBA1 enhancers as potential pharmacological chaperones in GD. Most of the **DIX** compounds showed a preferential GBA1 enhancement towards genotypes bearing the G202R mutation, responsible for a neuronopathic phenotype of the disease. In particular, DIX-28 was the one with the best activity enhancement, reaching a 4-5 fold increase at100 nM. Moreover this compound showed also a remarkable effect, around 2-fold increase, on the N370S/N370S genotype at the same concentration. Finally, immunofluorescence staining and confocal microscopy imaging were used to confirm that **DIX28** increases the trafficking of the G202R mutant enzyme to the lysosome.

P06.21-S
Two New Cases of Myopathy with Cataracts and Combined Respiratory Chain Deficiency caused by Mutations in GFER gene

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Myopathy with cataracts and combined respiratory chain deficiency (MPMCHD, OMIM#613076) is caused by mutations in GFER gene and has been described in a single consanguineous family (Di Fonzo et al, AJHG 2009, 84:594). We report two additional cases of MPMCHD in a non-consanguineous Caucasian family: one patient presented at 6 months with cataracts, mild developmental delays (DD) and hypotonia and at 16 months suffered severe episodes of hypoglycemia and lactic acidosis. Despite intermittent periods of relative metabolic stability, the condition is progressing. At 13 years he is wheel-chair bound, has failure to thrive (requiring tube feeding), global DD, autistic features, central hypotonia, and dystonia. Urine organic acid analyses reveal persistent 3-methylglutaconic aciduria. Muscle biopsy showed ragged red fibers and decreased staining for cytochrom C oxidase. Muscle analysis demonstrated reduced respiratory chain complex IV activity and increased mtDNA content in the absence of mutations or deletions. This patient's sister has clinical presentation characterized by later onset and milder, but similar clinical course which includes congenital cataracts, global DD and autistic features. Bouts of lethargy associated with lactic acidosis are frequent and require hospital admissions. Whole exome sequencing [done at Baylor College of Medicine] revealed two mutations in GFER gene: c.581G>A (p.R194H, previously reported) and c.215delG (p.A73Pfs*73).
These cases further describe the phenotype associated with GFER gene mutations. Although both patients have features similar to the other 3 reported patients with MPMCHD, the clinical course in our patients is more severe and includes prominent lactic acidosis and 3-methylglutaconic aciduria but not deafness.

P06.22-M
Digenic Glycogen Storage Disease type I?

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We report a patient referred to our service due to a clinical diagnosis of Glycogen Storage Disease type 1. She is the only child of a healthy and non consanguineous couple, seen at the age of six months when hepatomegaly was detected during a routine pediatric evaluation. Laboratorial investigation revealed glycemia after 4-hour fasting ranging from 33 to 60 mg/dl, hypertriglyceridemia (252 mg/dl), and abnormal values of aspartate aminotransferase (132 mg/dl) and alanine transaminase (98 mg/dl). Gamma-GT, urea, creatinine, uric acid, total cholesterol, coagulation tests, blood gases, and CBC resulted normal. After starting treatment with frequent meals, cornstarch, and dietary restriction of lipids and sugars, she normalized neuromotor and somatic development but still presents with hepatomegaly. Genomic DNA was extracted from peripheral blood leukocytes of the patient and both parents by using the standard phenol/chloroform method. Exons of G6PC and SLC37A4 genes and their flanking intron/exon junctions were amplified by PCR using previously described primers. The fragments were directly sequenced using a MegaBACE1000® DYEnamic ET (Amersham Biosciences) apparatus, twice on both strands, and the obtained sequences were compa-

red with the sequences available in the Ensembl genome browser. Mutation analysis revealed that the patient is a double heterozygote for the previously known nonsense mutation p.Arg415Ter (rs121908979; c.1243C>T) in SLC37A4, inherited from the mother, and an undescribed missense mutation p.Gly222Glu (c.665G>A) in G6PC, inherited from the father. The molecular analysis of the two genes suggests that the clinical picture of this patient could be caused by digenic inheritance of GSDI.

P06.23-S
High frequency of the c. 3980 G>A (p.W1327X) mutation in AGL gene of Tunisian patients with hepatic presentation of glycogen storage disease type III syndrome

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BACKGROUND: Glycogen storage disease type III (GSD-III) is an inborn error of glycogen metabolism caused by a deficiency of the glycogen debranching enzyme (AGL) .Some of the mutations appear to be population specific, whereas others are found in probands from a variety of different ethnic backgrounds. The recurrent mutation W1327X in exon 31 was identified in the Tunisian population, suggesting a founder effect. In this present study, we report a phenotype-genotype correlation of this frequent mutation. METHODS: Seven unrelated Tunisian families (from MAHDIA); including 10GSD type III patients were presented with hepatomegaly, progressive severe myopathy and cardiomyopathy. The routine laboratory findings showed an elevated serum aspartate aminotransferase, alanine aminotransferase, creatine kinase and triglyceride levels. The blood lactate and uric acid levels were within normal limits. RESULTS: The biochemical results of ten patients indicated a striking elevation of glycogen content in the erythrocytes after several hours fasting favours type III GSD and completely decrease of debranching enzyme activity was measured in leucocytes. Mutational analysis of the AGL gene showed a homozygous p.W1327X mutation. Study of genotyping method, in 30 families, using four polymorphic microsatellite markers on chromosome arm 1p21, we identified a common haplotype for all patients originated from MAHDIA.
CONCLUSIONS: p.W1327X is the most characteristic mutation for Tunisian patients with GSD IIIa. A common haplotype which shows the existence of a specific effect founder of this population confirmation of GSD-IIIa in Tunisisan (from MAHDIA) patients by clinical and genetic findings.

P06.24-M
A yeast model to evaluate the pathogenicity of missense mutations causing HHH Syndrome

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Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome is an autosomal recessive multisystemic disorder characterized by mental retardation and myoclonic seizures. It is caused by mutations in ORNT1 encoding the mitochondrial ornithine transporter. In placentate mammals there is a second gene, ORNT2, that originated from a retro-transposition event, whose precise function has not been clarified. In yeast the major function of this transporter (encoded by ARG11) is to shuttle ornithine from the mitochondrial matrix to the cytosol.
We employed a yeast model to study the function of human ORNT2 and to test the pathogenicity of mutations found in HHH patients. The two human Ornithine transporters were expressed into the ΔARG11 strain. We found that ORNT1 but not ORNT2 complements the deletion of the yeast gene. Three ORNT1 residues, conserved from yeast to humans, are not conserved in ORNT2. We could recover ORNT2 activity by replacing these three residues with those found in ORNT1. This result suggests that, despite the high level of homology between the two transporters, their function is not overlapping. We used our yeast model to test the effect of missense mutations carried by patients with HHH syndrome. All missense mutations tested have a detrimental effect on the function of the human gene indicating that yeast is a simple and effective system to validate missense mutations occurring in patients with HHH.

P06.25-S
A case of siblings with Leigh-like disease caused by 3-hydroxyisobutyryl-CoA hydrolase deficiency

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3-Hydroxyisobutyryl-CoA hydrolase (HIBCH) is an enzyme specific for removing CoA in the catabolic pathway for valine. So far, only 4 cases of HIBCH deficiency have been reported. In 1 case, a homozygous null mutation in *HIBCH* caused congenital anomalies including a characteristic facial appearance, congenital heart disease, and multiple vertebral anomalies and led to death in infancy. The 3 other cases, which harbored missense or splice mutations, presented with hypotonia, neurological regression, developmental delay in infancy, episodes of ketoacidosis, and abnormal MRI findings in the basal ganglia. Here, we describe a case of Leigh-like disease in Japanese siblings with HIBCH deficiency who presented with developmental delay in infancy, abnormal MRI findings in the globus pallidus, and remarkable ketoacidosis without highly increased levels of pyruvate and lactate in the CNS during infections. The patients died before the age of 5. A new homozygous missense mutation was identified at the substrate binging site in these patients, and their parents were found to be heterozygous for this mutation. The levels of HIBCH activity in patient lymphoblastoid cells and HEK293 cells transiently expressing a mutant HIBCH confirmed that the patients had HIBCH deficiency. HIBCH deficiency leads to the accumulation of the HIBCH substrate 3-hydroxyisobutyryl-CoA and subsequently an increase in the amount of methacrylyl-CoA in the mitochondrial matrix. The methacrylyl-CoA then strongly binds to thiol compounds (cysteamine, cysteine, glutathione) and reduces the activity of mitochondrial enzymes by binding to their SH residues, thus leading to a dramatically decreased reduction state and reduced ATP production.

P06.26-M
Deleterious mutations in mitochondrial NADH dehydrogenase subunit 4 and ATPase subunit 6 in two siblings of Leigh Syndrome with progressive motor retardation and loss of visual function

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We report a mitochondrial NADH dehydrogenase subunit 4 and ATPase subunit 6 mutations in two siblings from first degree relative couple with Leigh Syndrome. Peripheral blood-EDTA samples were used for DNA isolation and target mitochondrial genes were analysed by MLPA technique (MRC-Holland). A girl of 16 years old presented with a episode of progressive mental and motor reardation. Until seven year she had normal apperance but at the age of 7 year, she had developed neurological and imaging features such as; loss of visual function, walking and speech impairment and epilepsy complaints that compatible with Leigh syndrome. We also present a 12-year-old boy with muscle weakness, severe neurological problems, hyperactivity, developmental retardation and complete visual loss. Screening of both siblings, based on their clinical phenotype revealed the homoplasmic deleterious mutation in the mitochondrial DNA MT-ATP6(ligation site of probe 12068-12069) and heteroplasmic deletion in NADH subunit 4(ligation site of probe 8993-8992R). The maternal mtDNA screening for target deletorious genes is still going on. These findings are consistent with infantile Leigh syndrome for the presented siblings.

P06.27-S
Lysosomal Storage Disorders (LSDs): Clinical and Genetic Spectrum: Local Experience at The Eastern Province of Saudi Arabia

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Lysosomal Storage Disorders (LSDs) are clinically and genetically heterogeneous a group of more than 50 inherited disorders, each one is caused by a deficiency of a particular lysosomal enzyme resulting in a progressive accumulation of specific macromolecules within the lysosomes. This eventually leads to an irreversible cell damage, and multi-organs dysfunction. The majority of LSDs are inherited in an autosomal recessive manner, with the exception of Fabry disease and Mucopolysaccharidosis type II which follow the X-Linked mode of inheritance. The majority of LSDs are pan-ethnic, but some are more prevalent in specific ethnic groups.
Here, we report our local experience of LSDs at Johns Hopkins Healthcare Main Hospital, Dhahran, The Eastern Province of Saudi Arabia from January, 1st, 1984 to December, 31st, 2013. A total of 86 patients were diagnosed with different LSDs within this time period. ucopolysaccharidosis type VI was found to be the most common disorder (18 %), followed by juvenile Neuronal Lipfuscinosis (13 %). The patients are distributed according to

the geographic location or their tribe origin where particular disorders and specific genotypes were identified. This information had been utilized for asymptomatic carrier testing for those selective disorders among the high risk population. However, this valued data could be further utilized for establishing the newborn screening of LSDs in Saudi Arabia.

P06.28-M
Changes of metabolome and lipidome in lysinuric protein intolerance (LPI)

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Lysinuric protein intolerance (LPI; MIM222700) is a rare autosomal recessive disorder caused by a defect of cationic amino acid transport in the small intestine and kidney tubules. All the Finnish patients share the same homozygous mutation c.1181-2A□T in the SLC7A7 gene which encodes the y+LAT1 amino acid transporter. LPI can be considered a multisystem disease which can be life-threatening. The main symptoms of LPI include protein aversion, failure to thrive, osteoporosis and hepatosplenomegaly. However, despite the homogenous mutation in the Finnish LPI patients, symptoms may vary markedly even within one family and may include severe complications, such as alveolar proteinosis and end-stage renal disease. Some LPI patients also suffer from combined hyperlipidemia.
Our recent microarray study revealed that also other amino acid transporter genes than SLC7A7, including non-cationic amino acid transporters, have changes in their expression levels in LPI patients compared to the controls. Therefore, LPI patients seem to have wide and persistent changes in their amino acid balance. This finding together with the fact that patients have combined hyperlipidemia let us hypothesize that there may be hitherto uncharacterized systemic metabolic and lipid alterations in LPI. We studied these alterations in the whole blood plasma samples of 26 Finnish LPI patients and 19 gender and age-matched controls. Global metabolomic and lipidomic analyses were performed with GCxGC-TOF and Q-TOF mass spectrometry, respectively, combined with ultra-performance liquid chromatography (UPLC). Taken together, this study will reveal the nature of system-wide dysregulation of metabolites and lipids associated with the Finnish founder mutation of LPI.

P06.29-S
Establishment of Zbtb16 role in metabolic syndrome by means of single-gene congenic rat strain derivation

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In the process of positional cloning of apparently pleiotropic locus on rat chromosome 8 affecting major features of metabolic syndrome we have derived the congenic SHR.PD-(D8Rat42-D8Arb23)/Cub (SHR-Lx) strain carrying only 7 genes of polydactylous rat strain (PD) origin on spontaneously hypertensive rat (SHR) genetic background. In this study, we have derived 2 new minimal congenic sublines in order to determine the role of candidate *Zbtb16* gene of PD/Cub origin carrying 2.9kbp deletion in intron 2.
Adult male rats of SHR.PD(*Zbtb16*) and SHR.PD(*Htr3*) strains were fed standard diet (STD) and subsequently treated with dexamethasone in drinking water (0.026 mg/ml) for 3 days. We contrasted morphometric and metabolic profiles between the two strains.
The differential segment of SHR.PD(*Zbtb16*) subline containing only *Zbtb16* gene spans 254kb, while the one in SHR.PD(Htr3) subline spans 563kb and contains 6 genes: *Htr3a*, *Htr3b*, *Usp28*, *Zw10*, *Tmprss5*, and *Drd2*. SHR.PD(*Zbtb16*) were slightly heavier and showed significantly higher fasting levels of glucose and triacylglycerides, increased area under the glycemic curve during oral glucose tolerance test in comparison to SHR.PD(*Htr3*). The insulin sensitivity of visceral adipose tissue was comparable in the two strains, however, both basal and insulin-stimulated glycogenesis was substantially impaired in SHR.PD(*Zbtb16*) skeletal muscle in comparison to SHR.PD(*Htr3*).
The metabolic disturbances including dyslipidemia, impaired glucose tolerance and insulin resistance of skeletal muscle observed in the original SHR-Lx strain are present in the single-gene congenic SHR.PD(*Zbtb16*) strain, establishing the *Zbtb16* as a pleiotropic hub of metabolic syndrome features.

P06.30-M
DNA diagnostics of MIDD and MELAS syndromes in Slovakia

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The two syndromes, MIDD (Maternally Inherited Diabetes and Deafness) and MELAS (Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes) arise on a common genetic cause - a mutation in mtDNA, most often m.3243A>G in the gene for tRNALeu. This mutation leads to different clinical symptoms according to heteroplasmy levels in different tissues. Usually, the first presentation of the MIDD syndrome is a progressive bilateral sensorineural hearing loss emerging in adolescence. Diabetes develops mostly in 30 - 40 years of life with clinical picture resembling type 2 diabetes. The MELAS syndrome has a more severe progression with further neurological and metabolic symptoms.
We tested 317 patients from 257 families with suspected MIDD or MELAS syndromes fulfilling criteria of matrilineal inheritance, conjoint diabetes and hearing impairment, diabetes development after 25th year of life, or progressive hearing loss. Patients´ DNA was extracted from peripheral blood and/or buccal mucosa and analysed for presence of m.3243A>G variant using RFLP and/or Real-Time PCR.
The m.3243A>G mutation was found in 18 patients from 8 families. The heteroplasmy was higher in DNA samples from buccal swabs compared to blood DNA samples. In one case, the heteroplasmy was detected in the buccal DNA only, while the DNA sample gave negative results repeatedly. Patients´ phenotypes varied from diabetes as the sole symptom to a complex picture of the MELAS syndrome.
DNA testing helped to determine the diagnosis, thus permiting correct patient management and intensified surveillance of yet healthy mutation cariers.
Supported by APVV 0148-10, „Transendogen“ (ITMS 26240220051)

P06.31-S
Hostspot mutation regions in a consanguineous population - Leptin, Leptin receptor and Melanocortin 4 receptor genes

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The monogenic forms of obesity have so far been demonstrated in only about 3-5% of the severely obese subjects. No systematic study has been carried out to assess the genetic spectrum of early onset extreme obesity in a single population. The present on-going study was undertaken to identify genetic variants in a cohort of 46 unrelated children with early onset severe obesity. All subjects were first screened for mutations in the leptin (LEP) and melanocortin 4 receptor (MC4R) genes by direct sequencing. Subjects found negative for these two genes, were subsequently screened using microdroplet PCR-enrichment (RainDance) combined with NGS. Of the 46 severely obese subjects, we identified 14 subjects carrying loss of function homozygous mutations in three genes that are directly involved in leptin-melancortin signaling. Of these, 9 subjects carried mutations in LEP, 3 in LEPR and 2 in MC4R. The genetic variants identified here included a novel LEP and LEPR mutation. All mutants had a BMI SDS for age > 3 and were extremely hyperphagic. However, the phenotypic features of MC4R mutants were less severe compared to those carrying LEP and LEPR mutations. These results together with the findings reported by us previously for the same consanguineous population, add up to 28 (25%) of a total of 110 severely obese children, with known or novel homozygous mutations in the 3 genes. Novel genetic findings leading to severe obesity in the remaining probands through screening by whole exome sequencing, is to be anticipated.

P06.32-M
Did lightning strike twice?

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Mitochondrial (mt) DNA disorders are a large group of multisystem con-

ditions with a very heterogeneous clinical presentation, initiated by a maternally inherited pathogenic aberration residing in the mtDNA. Over 400 mtDNA alterations (including point mutations and deletions) implicated in a deleterious OXPHOS are already identified, with tRNA leucine and lysine genes being hot spot mutation regions (www.mitomap.org, updated 15th November 2013). A UK study for the occurrence of 10 frequent point mutations in cordial blood of newborns, revealed an unexpected carrier frequency of 1 in 200. Usually, mtDNA aberrations are heteroplasmic, a term referring to the co-existence of both wild type and variant mtDNA molecules within the same cell of an individual and opposite to homoplasmy. We present here the case of a young male patient with a cerebral-vascular accident, but also suffering from migraine, epilepsy and transient lactic acidosis and renal insufficiency. This clinical picture was compatible with a respiratory chain disease caused by a mtDNA mutation. First line analysis of his tRNA leucine and lysine genes identified both the common MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke like episodes) and MERRF (Myoclonic Epilepsy with Ragged Red Fibers) mutations, in our patient's blood. This is a rather unique and unusual situation. To our knowledge, the presence of both pathogenic mtDNA mutations, at clinical relevant levels, within the same patient has never been reported before. Further molecular investigation with sequencing technologies of the leukocytes, urine epithelial cells and other tissues of the patient and maternal relatives is in progress.

P06.33-S
A unique combination of mitochondrial ribosomal RNA variants responsible for a form of mitochondriopathy with respiratory complex I deficit and hypoacusia

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Mitochondrial DNA mutations are known to cause highly heterogeneous phenotypes and their functional effects are often difficult to assess. Here we present a novel pathogenicity mechanism for two mtDNA variants found in ribosomal RNA genes. The whole mtDNA was screened in a patient who displayed clinical indications of mitochondriopathy, including hypoacusia and 37% deficit of mitochondrial complex I activity in muscle. No protein-coding mutations were identified, albeit two rare variants in ribosomal RNA genes, namely m.1452T>C/RNR1 and m.2397C>T/RNR2, both homoplasmic and present in the proband mother, who also displayed hypoacusia. Low population frequency was revealed for both variants by using the HmtDB database, which collects nearly 16,000 human mitochondrial genomes. Although the two variants were present in tips of the haplogroups tree, they were never found to co-occur in the same individual, implicating a potential mutual exclusivity. Therefore, it was hypothesized that their co-existence might lead to perturbations in mitochondrial ribosomal assembly. To this aim, since the patient's fibroblasts displayed a normal basal respiration and complex I function, mitochondrial proteins translation kinetics was analyzed in cybrids derived from patient and control fibroblasts. Patient-derived cybrids showed reduced synthesis of mitochondrial proteins and less efficient recovery of complex I activity after treatment with mitochondrial translation inhibitors. These results imply that the combination of the two variants might lead to mitochondrial translation defects, mainly affecting complex I function, especially in cells with a high energy demand.

P06.34-M
A new heterozygous compound mutation of the Galns Gene as a cause of mucopolysaccharidosis type IV in two brothers of the south-west of Colombia

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Mucopolysaccharidosis type IVA (MPS IVA), or Morquio syndrome type A is an autosomal recessive disease caused by deficiency of the lysosomal enzyme N-acetylgalactosamine-6-sulfatase (GALNS).
clinical, biochemical and molecular two brothers with MPS IVA was characterized.
Decreased enzymatic activity was found to GALNS, and molecular study, we found a mutation in exon 3 (c.280C> T p.R94C) and not reported a new mutation in exon 9 (c.998G> A p. G333D). Pending outcome of parents and brother.
Was analyzed with Polyphen-2 (2.2.2) software and this variant is predicted as probably harmful.
Two different heterozygous mutations were found to cause the MPS IVA patient presented a new mutation was found and another had been previously described by Ogawa in 1995.

P06.36-M
Combined deletions of GALNS and PIEZO1 genes in two patients affected by MorquioA syndrome

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Mucopolysaccharidosis IVA (Morquio A syndrome) is an autosomal recessive lysosomal storage disorder, caused by the deficiency of the enzyme N-Acetylgalactosamine-6 sulfate sulfatase (GALNS). The disease, albeit multisystemic, is characterized by prevalent skeletal involvement, short stature, and cardiorespiratory complications. Here we report two new large genetic rearrangements detected at the heterozygous level in two patients (Pt1 and Pt2) affected by Morquio A. The deletions include part of the PIEZO1 gene (piezo-type mechanosensitive ion channel component 1) whose mutations were found in the dehydrated hereditary stomatocitosis, a dominant autosomal recessive syndrome. This syndrome is typically associated with silent to mild hemolysis, perinatal edema, splenomegaly, elevated bilirubin levels and iron overload. Pt1 was found to be seemingly homozygous for the c.1485C>G (p.Asn495Lys) missense mutation in the exon 14 of the GALNS gene. Parents' genetic analysis, haplotyping some loci in exon 14 of the GALNS gene (in the context of a prenatal diagnosis of a patient's relative), and CGH-array revealed that Pt1 has a large deletion encompassing exons 10-14 of the GALNS gene and three upstream genes, including a part of the PIEZO1 gene. In Pt2, heterozygous for the c.346G>A (p.Gly116Ser) missense mutation, a large deletion ablating exons 9-14 of the GALNS gene and exon 1 of the PIEZO1 gene was also identified. The breakpoint regions were characterised in both patients and the eventual contribution of the partial deletion of the PIEZO1 gene on such patients' phenotype was considered at a biochemical and clinical point of view.

P06.37-S
Mucopolysaccharidosis type II: molecular analysis in a cohort of male patients and in a manifesting female carrier

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Mucopolysaccharidosis II (Hunter syndrome, MPS II) is a rare X-linked recessive disorder caused by a deficiency of the lysosomal enzyme iduronate-2- sulfatase. Major clinical manifestations of the severe form of MPSII include coarse facial features, short stature with joint stiffness, hepatosplenomegaly, cardiomyopathy and mental retardation.
The diagnosis of MPSII based on clinical symptoms and increased excretion of glycosaminoglycans in urine is confirmed by enzymatic assay and mutation analysis. We performed mutation analysis in 24 unrelated male patients and found 21 different mutations: two large and four small deletions, three small insertions, two splicing defects, two nonsense mutations, seven missense mutations and a recombination between *IDS* gene and pseudogene *IDS2*. Eleven of the found mutations are novel.
Even though the MPSII is expected to be found in males, few symptomatic females have been reported. We present a case of four-year-old girl with severe phenotype of MPSII. Molecular analysis of gDNA revealed a missense mutation c.1403G>A (p.R468Q) in heterozygous state, but only the mutant allele was detected at the cDNA level. X-inactivation was nonrandom and silencing preferentially the maternal X-chromosome. The mutation was not found in periferal blood samples of both parents. These findings support the assumption of either a *de novo* mutation in affected girl or a germinal mozaicism in the girl's father.
In spite of extremely rare manifestation of MPSII in females, an unrelated female patient with skewed X-inactivation and p.R468Q inherited from her mother was reported in the literature.
Support: IGA MZ ČR NT 14015, RVO-VFN64165/2012, MZ ČR-RVO (EÚ,00023761)

P06.38-M
Rare mutation in a patient with MPSII (Hunter disease)

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Mucopolysaccharidosis type II (MPS II) is an X-linked recessive multisystem disorder characterized by glycosaminoglycans (GAG) accumulation. Different severity of the disease has been described depending on the mutation in the iduronat sulphatase gene (IDS). Here we present a case, with severe MPS II detected early with an unusual duplication of the exon 7.
The child presented with a delay in reaching milestones of the first year and recurrent febrile episodes. At 11 months he had most of the major features of the MPSII: hoarse and hairy face, significant thoracic kyphosis, large scull, hepatomegaly and neurodevelopmental delay. X rays of the spine were typical for MPSII. Urinary mucopolysacharides were increased as well as the iduronatsulphatase in the urine (80nmol/h/ml).
Genetic analysis confirmed duplication of the exon 7 of the IDS gene in the child and his mother who does not have any signs of the disease.
The patient has been followed for 5 years. His growth during this period was unusual, he had height 4 SDS above the average for the age, head circumpherence was 3SD above the average. Values of growth hormone were normal, as well as the MRI of the pituitary region.
Therapy with Elaprase (Idursulphatase) improved the joint mobility, however, the boy has severe neuro-developmental delay.
Although no significant genotype/phenotype relation has been shown in children with MPSII, it seems that this rare gene change is associated with a severe form of the disease.
Prenatal diagnosis in the mother is planned for the second pregnancy.

P06.39-S
Exome sequencing identifies mutations in coenzyme A synthase causing neurodegeneration with brain iron accumulation

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The common feature of a group of genetic disorders collectively identified as Neurodegeneration with Brain Iron Accumulation (NBIA) is brain iron overload visualized by radiological and histopathological examinations. The clinical spectrum of NBIA is wide and includes early-onset neurodegeneration, with a fatal outcome, and adult-onset parkinsonisms-dystonia. Pantothenate Kinase Associated Neurodegeneration and Infantile Neuroaxonal Dystrophy are the most frequent forms of the disease due to recessive mutations in PANK2 and PLA2G6. Recently it was shown that NBIA is also caused by mutations in FA2H, ATP13A2 and C19orf12 genes, but still in a large proportion of patients, no genetic alteration can be found. Using exome-sequencing strategy we identified, in a NBIA patient, a homozygous missense mutation in the gene coding for Coenzyme A Synthase (CoASy). By performing traditional Sanger sequencing in a cohort of NBIA cases, we found another mutant patient. CoASy is a mitochondrial enzyme involved in the last step of Coenzyme A biosynthesis, a important molecule for several metabolic pathways. The missense mutation affects a highly conserved aminoacid residue in the catalytic site of the enzyme, a region conserved from yeast to human. Western-blot analysis showed that CoASy protein was absent in patient fibroblasts, whereas RT-PCR revealed that mRNA was reduced only in the patient carrying the non-sense mutation. HPLC analysis demonstrated reduced CoA concentration in mitochondria isolated from mutant yeast and patient fibroblasts. Together with mutations in PANK2, coding for the first enzyme in CoA biosynthesis, mutations in CoA synthase impinge on the same biosynthetic pathway causing NBIA.

P06.40-M
Novel mutations in the PNLPA2 gene causing late one-set of neutral lipid storage disease with myopathy in an Italian family

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Neutral Lipid Storage Disease with Myopathy (NLSDM), is a rare autosomal recessive disorder characterized by an abnormal accumulation of triacylglycerol into cytoplasmic lipid droplets (LDs). Mutations in the PNPLA2 gene cause the onset of NLSDM. PNPLA2 codes for adipose triglyceride lipase (ATGL), an enzyme that hydrolyses fatty acids from triacylglycerol. NLSDM patients are mainly affected by progressive myopathy, cardiomyopathy and hepatomegaly. Other clinical symptoms may include diabetes, chronic pancreatitis and short stature. To our best knowledge, twenty six different PNPLA2 mutations have been described in thirty two NLSDM patients. Here we report the clinical and genetic findings of a NLSDM Italian family with different affected members. In our patients we identified two novel PNPLA2 missense mutations (pL56R and pI193F). Since age of 38 years, the oldest brother had weakness and hypotrophy of right upper arm and kyphosis. He is now unable to raise arms in horizontal position (61 years old). The second brother, since 44 years of age, had exercise intolerance, cramps and pain in lower limbs. He currently has a distal amyotrophy. Genetic analysis revealed that also one of the two sisters presents the pL56R and pI193F mutations, but she is still barely symptomatic. Using a functional in vitro assay, we have observed that these mutations caused the production of ATGL proteins with diminish lipase activity, but able to bind to LDs. This is a very interesting family since it shows heterogeneity of clinical presentation from relatively asymptomatic phenotype to full expression of a severe myopathy.

P06.41-S
Producing hiPS cells for disease modeling of NLSD-M

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NLSD-M (Neutral Lipid Storage Disease with Myopathy) is a rare autosomal recessive disorder characterized by an abnormal intracellular accumulation of triacylglycerol into cytoplasmic lipid droplets (LDs). In most tissues the lipid droplets (LDs) are cellular organelles for the triacylglycerol storage. LDs metabolic functions are mediated by proteins bound to their surface. In particular, the lipase that catalyzes the removal of the first acyl chain from triacylglycerol is the patatin-like phospholipase domain-containing protein 2 (PNPLA2). This protein is coded by the PNPLA2 gene whose mutations cause the onset of Neutral Lipid Storage Disease with Myopathy. NLSD-M patients are affected by progressive myopathy, cardiomyopathy and hepatomegaly. Other clinical symptoms may include diabetes, chronic pancreatitis and short stature. NLSD-M has, at present, no specific therapy. We have previously reported clinical and genetic findings of some NLSD-M patients obtaining dermal biopsies from them. Here we report the development of hiPSc (human induced pluripotent stem cell) from patients' fibroblasts harboring different PNPLA2 mutations. Initial hiPSc colony selection was based on morphologic evaluation and on detection of pluripotency surface markers (SSEA-4 and TRA-1-81). HiPSc also expressed undifferentiated ES cell markers (NANOG, SOX2 and OCT4). Karyotypic analysis of hiPSc lines indicated a normal complement of chromosomes. Immunohystochemical evaluations of LDs on hiPSc revealed that they recapitulate pathological hallmark of the disease. We propose use of differentiated cells derived from hiPSc to study the pathogenetic mechanisms leading to NLSD-M and as a cellular model for therapeutic evaluation.

P06.42-M
Niemann Pick type C genetically verified in three Bulgarian patients

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Niemann-Pick type C (NPC) disease is a neurodegenerative lysosomal lipid storage disease, with autosomal recessive transmission, characterized by lysosomal/late-endosomal accumulation of endocytosed unesterified cholesterol. The clinical manifestations of NPC are heterogeneous. Most patients have a progressive neurologic disease, but both age at onset, which ranges from early infancy to adulthood, and subsequent course vary.
We present the first three Bulgarian female patients with genetically verified NPC disease. Two of the patients are sisters. All three patients presented neurovisceral involvement with juvenile onset with a mean age of onset varying between 11 and 19 years. Vertical gaze palsy, ataxia, involvement of the upper motor neuron and cognitive decline were prominent features of the whole group, while cataplexy was present in both sibs. Clinical and echographic examinations revealed hepatomegaly in one of them and splenomegaly in all of the affected. Neuroimaging changes encompass cerebellar atrophy and white matter changes. In two of them chitotriozidase was moderately elevated, while in the third patient it had a borderline value. The molecular genetic testing detected missense mutation in the NPC1 gene. The sisters are compound heterozygous carriers of the mutations c.3019C>G, p.Pro1007Ala and c.3718G>A, pGly1240Arg. The third patient is a compound heterozygous carrier of the mutations c.1421C>T, p.(Pro474Leu) and c.2974G>A, p.(Gly992Arg).
In conclusion, the detected mutations in our patients are already known in the literature. All detected mutations were missense and caused the same classical phenotype in Bulgarian patients. These cases are the first genetically confirmed NPC cases in Bulgaria.

P06.43-S

An exome-wide study for obesity in Singaporean East-Asian samples

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Aims:Previous genome wide-association studies(GWAS) have identified over 40 obesity-associated variants; however, these are primarily non-coding common variants and account for &lt3% obesity heritability. We performed an exome-wide association study(EWAS) to identify obesity-associated coding variants using the Illumina HumanExome array.

Methods:192 East-Asian(Singaporean Chinese and Malay) early-onset obese cases(body weight &gt150% of ideal weight for height and onset &lt10 years of age) and normal BMI adult controls (18.5kg/m2 ≤BMI&lt23.0kg/m2) from the Singapore Chinese Eye Study (SCES) were genotyped. Samples-QC procedures were performed(Table 1). SNP-QC excluded SNPs with call-rate below 95%, rare(MAF&lt0.05%) and/or monomorphic SNPs in total samples/cases and SNPs with significant deviations from Hardy-Weinberg equilibrium(p-value&lt0.0001). 47,605 SNPs remained for statistical analysis. Fisher's exact test was performed for rare SNPs(3%&ltMAF≤0.05%) and logistic regression adjusting for population stratification(first 5 principle components) and sex was carried out for common SNPs(MAF≥3%) using PLINK(v1.07). 58 hits with study-wide p-value&lt1.05x10-6 were followed-up using samples at extremes of BMI distribution (cases= BMI&gt27.5kg/m2 and controls= BMI&lt18.5kg/m2) from 2 adult Singaporean Chinese datasets[SCES (321 cases/140 controls)and Singapore Prospective Study Program(SP2, 89cases/94controls)].

Results:A coding SNP (exm1271824) at FANCA showed strong associations in the discovery EWAS [OR=4.69(2.67-8.2895%CI),p-value=4.95x10-7) and was validated in the adult datasets(metap-value=0.0327). The risk allele(G) was enriched in Chinese and Malay early-onset cases(MAF=3.904% and 10.004%) as well as adult cases(mean MAF=2.232%) and was rare among controls(MAF=0.717%).

Conclusion:We identified an obesity-associated coding variant at FANCA in East-Asians.

Table1

Sample	SCES	Early-onset extreme obese children
Total samples	2576	192
Sample with call-rate &lt 0.99	67	0
Samples with extreme heterozygozity (&gt 3 SD)	5	1
1st degree relatedness	38	8
PCA outliers	50	5
Samples remaining	2416	178
Samples with BMI data	2403	NA
Controls (BMI between 18.5kg/m2 and 23.0kg/m2)	1083	NA

P06.44-M

Identification of new missense variations in the SH2B1 gene in obese and lean individuals

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Genetic evidence coming from animal studies, genome-wide association studies and studies looking for structural variation, has identified the *SH2B1* gene as a novel candidate gene for obesity. Moreover, the SH2B1 protein has been shown to act as a positive regulator of leptin signaling. As such, it seems plausible that rare *SH2B1* variants might exist that influence food intake and weight regulation. Therefore, we have designed an extensive mutation analysis investigating the prevalence of genetic variation in *SH2B1* in both Belgian obese and lean individuals. We have screened 500 obese children and adolescents and 500 healthy, lean individuals for mutations with high-resolution melting curve analysis. Direct sequencing was performed for samples with melting patterns deviating from wild-type. Screening of all coding exons and intron-exon boundaries in *SH2B1* identified thirteen different non-synonymous heterozygous variants in our populations. Several of these were found both in lean and obese subjects, suggesting these are common polymorphisms. However, five private, heterozygous, non-synonymous variations were present in obese children only. Furthermore, we also identified five variants solely in lean individuals. Our mutation analysis has demonstrated that variation in the *SH2B1* gene is frequent in both groups, with distinctive variations being present on either side of the weight spectrum. We therefore speculate that both weight-increasing and weight-decreasing variations exist in *SH2B1* for which different functional effects can be recognized. Further functional testing of the variants identified here will thus be necessary to fully understand the impact of these variants on SH2B1 and the leptin signaling pathway.

P06.45-S

Ornithine transcarbamylase deficiency and a novel OTC mutation in two Bulgarian families

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Disorders caused by affected urea cycle enzymes are characterized by hyperammonemia, encephalopathy, and respiratory alkalosis. Ornithine transcarbamylase (OTC) deficiency is an X-linked inborn error of metabolism of the urea cycle which causes hyperammonemia. The disorder is treatable with supplemental dietary arginine and low protein diet. Here we report two unrelated Bulgarian families with OTC deficiency, subjected for genetic testing. The first patient is a 3 years old female with persisting hyperammonemia, elevated transaminase activity and elevated orotic acid excretion in urine. The girl demonstrated behavioral and sleep disturbances. The genetic test revealed a novel frameshift mutation c.695dupT in exon 7 of the OTC gene. The mutation is de novo, both parents are not carriers. After the genetic verification of the disease, the patient was subjected to low protein diet and ammonaps with a very good outcome. The second patient is a 1 year old boy with abnormal profile of organic acids (organic aciduria), undetectable citruline level and hyperammonemia. The genetic test revealed a missense mutation c.622G>A, p.(Ala208Thr) in exon 6 of the OTC gene. The mutation is available in the literature and inherited from the mother. The carrier mother does not demonstrate any clinical symptoms of the disease. The presented data enlarges the spectrum of OTC gene mutations in patients with persisting hyperammonemia. The genetic testing provides the possibility for adequate genetic counseling and prenatal diagnostics in affected families.

P06.46-M

Biochemical diagnosis of Peroxisomal disorders by GC/MS: Egyptian patients with X-linked adrinoleukodystrophy

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Introduction: Peroxisomes are organelles responsible mainly for metabolism of lipids and peroxides. Lack of peroxisomes or dysfunction in any of their normal functions is the cellular basis for human peroxisomal disorders (PDs).

Aim of the Work: diagnosis of peroxisomal disorders among a high risk group of Egyptian patients using gas chromatography mass spectrometry.

Subjects and Methods: Forty six patients suspected to have peroxisomal disorders were included in this study. Their ages ranged from 2 to 20 years. They were referred to The Biochemical Genetics Department, National Research Centre from all over Egypt. Forty one (89%) were males while five were females (11%). Parental consanguinity was positive in 28 cases (61% out of 46). Very long chain fatty acids were quantified after extraction from plasma of all cases using gas chromatography/mass spectrometry (GC/MS) technique.

Results: The present study included 46 cases suspected clinically to have one of the peroxisomal disorders; four of them (8.7%) proved to have X-linked adrenoleukodystrophy by quantitative determination of the very long chain fatty acids after extraction from their plasma. The other 42 cases showed normal profile for very long chain fatty acids.

Conclusion: X-linked adrenoleukodystrophy is the only type diagnosed among the study group of the suspected Egyptian cases. This study showed that GC/MS analysis for VLCFA discriminates patients from controls, representing a non-invasive, reliable, specific and sensitive method for the diagnosis of peroxisomal disorders.

P06.47-S
X-linked adrenoleukodystrophy from Iran, reporting 8 cases and a novel mutation

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X-linked adrenoleukodystrophy (X-ALD) is a rare peroxisomal disorder resulting in progressive cerebral demyelination, axonal dysfunction, and adrenal insufficiency. It is the most common peroxisomal disorder with an estimated birth incidence of about 1 out of every 20,000. There is no ethnic predominance.
X-ALD most severely affects male hemizygotes. The age of onset and morbidity are highly variable and progression is unpredictable. Male hemizygotes may initially present with neurological symptoms in two different forms: (X-ALD) with childhood presentations, and Adrenomyeloneuropathy (AMN) that presents in adulthood.
X-ALD, affects 4 to 8 year old boys. Primary manifestations of X-ALD are moderate cognitive deficits followed by diminished visual acuity, central deafness, cerebellar ataxia, hemiplegia, convulsions and dementia leading to a neurovegetative state or death within several years.
X-ALD is inherited in an X-linked manner. About 93% of index cases have inherited the *ABCD1* mutation from one parent; and 7% of individuals have a *de novo* mutation.
The diagnosis of X-ALD is based on clinical findings. MRI is always abnormal in males with neurologic symptoms and often provides the first diagnostic lead. Plasma concentration of very long chain fatty acids (VLCFA) is abnormal in 99% of males with X-ALD. *ABCD1* is the only gene known to be associated with X-ALD.
Here we report 8 affected boy with X-ALD from Iran. VLCFA was increased strongly, and MRI images were typical in all of them. Molecular analysis of ABCD1 gene confirmed the diagnosis in 4 of the patients and we detected 1 novel mutation in one.

P06.48-M
Rhizomelic chondrodysplasia punctata due to a novel mutation in the *PEX5*

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Peroxisome biogenesis disorders (PBD) are caused by mutations in one of 13 PEX genes. Based on the clinical and biochemical presentation the patients are divided into: 1) Zellweger spectrum disorders (ZSD) and 2) Rhizomelic chondrodysplasia punctata type 1 (RCDP1). ZSD is characterized by multiple organ defects and elevated levels of very long chain fatty acids (VLCFA). The patients have mutations in one of 12 genes (not *PEX7*). 2) RCDP1 is characterized by congenital cataracts, multiple skeletal abnormalities, normal VLCFA, reduced plasmalogens and elevated phytanic acid levels. Most RCDP1 patients have mutations in *PEX7*, but a RCDP phenotype with slightly different biochemical profile is caused by deficiency of either of the two peroxisomal enzymes DHAPAT and ADHAPS involved in plasmalogen biosynthesis.
We identified a homozygous frame shift mutation in *PEX5* in three siblings with congenital cataracts, multiple skeletal abnormalities, moderate to severe intellectual disability, epilepsy, demyelinating neuropathy, normal VLCFA and high phytanic acid levels. This clinical and biochemical profile is in agreement with RCDP1. *PEX5* encodes two isoforms, PEX5S and PEX5L, and the mutation in our patients is located in a sequence only present in PEX5L and required for interaction with PEX7, which is important for peroxisomal import of three enzymes (ADHAPS, PHYH and thiolase 1), carrying a peroxisomal targeting signal 2 (PTS2). We believe that disruption of the interaction between PEX5L and PEX7 causes the RCDP-like phenotype in our patients. We currently study patient fibroblasts in order to further characterize the peroxisomal defect caused by this novel *PEX5* mutation.

P06.49-S
The frequency of POLG1 gene mutations in Hungarian patients with mitochondrial disorders and the analysis of phenotype - genotype correlation

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In the background of mitochondrial disorders both mtDNA and nuclear DNA mutations can be detected. POLG1 is one of the most important gene responsible for the intergenomial communication of the two genomes. POLG1 gene mutations may cause a wide range of clinical symptoms. However many POLG1 mutations have been published the phenotypic spectrum is increasing.
Aims: The frequency of the POLG1 mutations in patients with intergenomial communication disturbance and their phenotype-genotype correlation was analyzed.
Patients and methods: 100 Hungarian patients with mitochondrial diseases were investigated. Most of them had mitochondrial abnormalities and multiple mtDNA deletion in the muscle tissue. The mtDNA deletion was investigated by long PCR, the POLG1 gene was sequenced.
Results: In the POLG1 gene of our cohort 7 pathogenic mutation was detected in 6 families. Segregation analysis detected 9 further family members harbouring the pathogenic mutations. In our cohort 3 SNPs showed association with valproate toxicity, 1 SNP was a genetic modifying factor. The most common clinical symptoms were myopathy (50%), neuropathy (34%), ataxia (34%), depression (34%), PEO (17%), epilepsy (17%), ptosis (17%), lipomas (17%), hypoacusis (17%). In 1 case with Alper's syndrome valproate toxicity resulted in fatal outcome.
Conclusion POLG1 mutation is a common genetic cause of the mitochondrial disorders. It is recommended to investigate at first in patient with mitochondrial disease having mendelien inherited PEO, ataxia, myopathy, epilepsy and psychiatric disorders. In cases with epilepsy the predisposing SNPs to valproate toxicity in the POLG1 gene shall be screened to avoid the serious side effects.

P06.50-M
Very high prevalence of infantile Pompe disease in the Bushinengue population of French Guyana as a result of founder effect and endogamy

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Pompe disease (Glycogen storage disease type II or acid maltase deficiency) is an autosomal recessive metabolic disorder caused by an accumulation of glycogen in the lysosome due to deficiency of the lysosomal acid alpha-glucosidase enzyme. The infantile form of Pompe disease is a rare lysosomal storage disorder usually symptomatic before age 1, with universally fatal outcome before age 4 in absence of enzyme replacement therapy. Incidence of neonatal Pompe disase is usually 1/150000 birth in most country, except Taiwan, where its incidence is evaluated around 1/15000. We have identified 17 newborns with infantile Pompe disease born in 10 years in the same maternity to parents from the Bushinengue tribes living at the frontier between french Guyana, an overseas French department, and Suriname. This population descends from African-onset slaves who escaped to the Amazonian forest and settled along the lower Maroni River in the nineteen century, where they grew as a geographical and cultural isolate population till recently. Genetic investigation revealed that all patients were homozygous or compound heterozygous for 2 mutations: p. Arg854* that has already been reported in the african-american population, and p.Gly648Ser, previously considered to be a mild variant. Despite probable incomplete detection, the raw incidence of infantile Pompe disease in the area is at least 1/2000 births, corresponding to a heterozygote frequency of 1/22. Early detection allowed us to put under enzyme replacement therapy the last 3 cases, which where all CRIM positives. Implementation of systematic newborn screening will be implemented in Guyana by mid 2014.

P06.51-S
New insights into PPARG regulation mechanisms: the unexplored impact of alternative splicing on its function

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The nuclear receptor PPARγ is a key regulator of cell proliferation and differentiation, including adipogenesis. Defects in PPARγ signaling are implicated in metabolic syndrome, cardiovascular diseases and cancer. The wide number of ligands, coregulators and target genes, combined to different protein isoforms and alternative transcripts, determines the complexity of PPARγ biological role.
Notably, our group identified a new PPARG isoform (γORF4) with a dominant negative effect toward PPARγ, suggesting underestimated aspects of its regulation. We also recently described the differential contribution of PPARG canonical transcripts and ORF4 variants to adipocyte differentiation of human mesenchymal stem cells. The analysis demonstrated that PPARG transcription is regulated in a time-specific manner, through differential usage of distinct promoters, also indicating that dominant negative isoforms are actively transcribed and regulated throughout the adipogenesis. Moreover, during this study we identified another PPARG transcript, named Δ5, with features similar to ORF4. Through transfection experiments, the usage of a PPARγ agonist and luciferase assay, we demonstrated its dominant negative activity and an altered ability to regulate cellular proliferation. In order to understand, on a genome-wide scale, the effects of γΔ5 isoform on PPARγ target genes' expression, we also performed a transcriptome analysis by RNA-Sequencing in HEK293 cells over-expressing Δ5, in presence of a PPARγ agonist. Our results indicate that different dominant negative isoforms encoded by PPARG gene may have a relevant, and yet unexplored, role in the biological function of PPARγ, with a potential involvement in physiologic and pathologic conditions, of which PPARγ is a key player.

P06.52-M
Association of rs780094 in GCKR with Metabolic syndrome and related traits were confirmed in Tehran lipid and glucose study

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Background: The minor T-allele of rs780094 in intronic region of glucokinase regulator gene (GCKR) is reported to be associated with a number of metabolic traits including higher triglyceride levels and lipid metabolite factors in GWA studies mostly European ancestry. Here we report replicating these finding in Iranian population using samples from the Tehran lipid and glucose study (TLGS), a large population-based cohort study. Methods: Among TLGS participant 1777 cases with metabolic syndrome according to ATPIII criteria, were selected and compared to 3909 control. In GCKR gene, rs780094 genotyped using the Centaurus (Nanogen) platform in DeCODE genetics. Association of T-allele with metabolic syndrome and lipid related variables (e.g. triglyceride, cholesterol, HDL and LDL) was tested using plink software after age and sex adjustment. Results: Replicating previous findings TLGS participants showed that T-allele of rs780094 was associated with higher triglyceride levels ($p < 10^{-8}$), higher cholesterol levels ($p < 0.05$) and metabolic syndrome prevalence ($p < 0.0025$). Conclusion: Our findings showed the association between the presence of T allele in rs780094 and metabolic syndrome and related traits among Iranian population and confirmed previous result in other ethnicity.

P06.53-S
Sengers syndrome: case report and review of literature

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Sengers syndrome is a rare autosomal-recessive condition (prevalence < 1/1 000 000) associating congenital cataract, hypertrophic cardiomyopathy, skeletal myopathy and exercise-related lactic acidosis.
We report on a non-consanguineous, full-term, eutrophic child with bilateral congenital cataract. Metabolic investigations were normal. Cataract surgery was early performed. At three weeks and three months of age, during an infectious episode, the infant developed respiratory distress, and deceased from cardiac insufficiency. X-ray revealed cardiomegaly, and severe lactic acidosis was found in blood sample. No genetic analysis could be performed in the patient. Thus we decided to analyse acylglycerol kinase (AGK) gene in both parents in this context.
We identified heterozygous mutations in AGK gene in both parents allowing retrospective diagnosis of Sengers syndrome in the child and clear genetic counselling for the family.
The management of congenital cataract should be very early and exhaustively performed, to exclude Sengers syndrome, considering the risk of cardiac failure during cataract surgery or during any intercurrent infectious episode.
Literature does not propose systematic and consensual investigations for bilateral congenital cataract. However, the wide range of aetiologies of congenital cataracts implies various but specific and appropriate managements. This case highlights the interest of cardiac ultrasounds in congenital cataract investigation.

P06.54-M
Cellular model of polyprenol reductase deficiency: useful tool for SRD5A3-CDG study

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Polyprenol reductase encoded by *SRD5A3* gene plays crucial role in the dolichol cycle by catalyzing the conversion of polyprenol to dolichol. Phosphorylated derivatives of dolichol are key molecules in protein glycosylation and glycosylphosphatidylinositol anchor biosynthesis. Decreased content of dolichols due to the mutations in *SRD5A3* gene has catastrophic consequences for the cell since it results in hypoglycosylation of proteins and in human manifests as Congenital Disorders of Glycosylation. SRD5A3-CDG patients have shown a substantial phenotypic variability despite the presence of truncating mutations in all of them. Biochemical and molecular studies on the cellular model of SRD5A3 deficiency are necessary for better understanding of the biological consequences of decreased level of dolichols and identification of the correlation between mutations found in *SRD5A3* with clinical symptoms.
To this end we established an in vitro system of HEK293 cells expressing miRNA silencing endogenous *SRD5A3* in an inducible manner under a tetracycline regulated promoter complemented by mutated variants of *SRD5A3*. Selected cell lines will be used for the analysis of various cellular effects of dolichol deficiency: protein N-glycosylation, O-mannosylation and GPI-biosynthesis, isoprenoid lipid homeostasis, expression of selected genes. Furthermore, the model might be useful for SRD5A3-CDG diagnostics and possible therapy development by maintaining the cellular dolichol/dolichyl phosphate pool at optimal level.

P06.55-S
A homozygous mutation in the transcription factor THAP11 in a patient with methylmalonic aciduria and a severe neurological phenotype

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Inborn errors of vitamin B_{12} (cobalamin) metabolism result in homocystinuria and methylmalonic aciduria, either alone or in combination. The most common error, *cblC* caused by mutations in *MMACHC*, results in the combined metabolic phenotype. We recently described a biochemically similar inborn error, *cblX*, caused by mutations in the gene for the X-linked transcriptional coregulator HCFC1, which activates the transcription of *MMACHC*. The *cblX* patients were originally diagnosed as *cblC*; however they have a severe neurological and milder biochemical phenotype than most *cblC* patients (Yu *et al*. AJHG, 2013, 93:5). One patient, characterized as *cblC* by complementation analysis, had no mutations in either *MMACHC* or *HCFC1* by Sanger sequencing. Clinically, he had methylmalonic aciduria, encephalopathy, profound mental retardation, seizures and tetraplegia and died at age 10. His biochemical manifestations were mild, with higher cellular function of both cobalamin-dependent enzymes than normally seen from *cblC* patients and no homocysteine elevation. Sequencing of patient genomic DNA identified a homozygous mutation, c.240C>G (p.F80L) in *THAP11*, the gene encoding a zinc finger transcription factor that functions with HCFC1. The mutation affects a residue located in a conserved zinc finger-containing domain, two residues away from P78, responsible for interacting with the zinc ion, and is predicted to be probably damaging by Polyphen-2. F80 is conserved to zebrafish and in 9/12 human THAP proteins. Transcriptome profiling showed significant down-regulation of the same group of genes, including *MMACHC*, in fibroblasts from *cblX* and the *THAP11* patient.

P06.56-M
Rogers syndrome (thiamine responsive megaloblastic anemia syndrome): the success of multidisciplinary approach

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Thiamine responsive megaloblastic anemia syndrome is a rare autosomal recessive metabolic disorder. Only ~80 cases have been described mainly in consanguineous families. A gene SLC19A2 coding high affinity thiamine transporter mediating vitamin B1 uptake through cell membrane has been identified. Classic triad of features is characteristic to the disease - megaloblastic anemia, deafness and non-type I diabetes.
We report a 3y old boy born in nonconsanguineous family. From the early days the boy was easy irritable and suffered with common affecto-respiratory spasms. The psychomotor abilities developed according to age. The slowdown of speech development was noticed from the 7th month of life. Insulin dependent non-type 1 diabetes was diagnosed at the age of 1y. At the age of 1.5y the profound bilateral hearing loss was diagnosed and cochlear implantation performed with good auditory and speech outcomes. During 3rd year of life severe megaloblastic anemia without folic acid or vitamine B12 deficiency and bilateral maculopathy has developed. The coding sequence of GJB2 gene was analyzed and genotype c.[313_326delAAGTTCATCAAGG G];[=] (p.[(Lys105Glyfs*5)];[=]) was identified. MtDNA 1555A>G mutation was not revealed. The patient had slightly elevated branched chain amino acids (Leu, Ile, Val) in plasma. The clinical diagnosis of TRMA syndrome was suspected and daily supplementation with thiamine 100mg started. The condition of the patient markedly improved several days after the initiation of treatment - therapy has positive effect on anemia and glycemia, also psychological status of the child clearly improved. The results of SLC19A2 gene molecular testing will be achieved in the near future.

P06.57-S
Tyrosinemia type II (Richner-Hanhart syndrome): a new mutation in the TAT gene

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In the present study we report the clinical features and the molecular genetic investigation of the tyrosine aminotransferase (*TAT*) gene in a young girl from Croatia with Richner-Hanhart syndrome, mainly suffering from photophobia, hyperkeratosis of the palmes and soles and slight neurological abnormalities. Sequencing analysis of the *TAT* gene revealed a novel homozygous missense mutation c.1250G>A (p.R417Q) in exon 12, and herewith confirmed the clinical diagnosis. Showing the first symptoms in babyhood, at the age of 8 years it was for the first time clinically diagnosed that the patient suffers from tyrosinemia type II and a therapy with tyrosine and phenylalanine reduced diet has been started successfully. All symptoms disappeared within 2-4 weeks. Since that time, we have been following the girl until today for more than ten years. She is in a good condition, and attends the normal high school program.

P06.58-M
Neurological impairment is frequent among heterozygote women for X-linked Adrenoleukodystrophy - A clinical, neurophysiological and biochemical study

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Background: Neurologic impairments in female heterozygotes for X-linked Adrenoleukodystrophy (X-ALD) are poorly understood. Our aims were to describe the neurological and neurophysiological manifestations of a cohort of X-ALD heterozygotes, and to correlate them with age, disease duration, mutations, X-inactivation and serum concentrations of a marker of neuronal damage, NSE. Methods: All 45 heterozygotes of our institution, with previous VLCFA and molecular diagnosis, were invited to be evaluated through myelopathy scales JOA and SSPROM, nerve conduction studies and somatosensory evoked responses. X-inactivation pattern was tested by HUMARA methylation assay. Serum NSE was measured by eletrochemiluminescense. Results: Thirty-three heterozygotes were recruited: 29 (87%) were symptomatic. Symptomatic and asymptomatic women presented different m±sd ages (43.9 ± 10.2 versus 24.3 ± 4.6), JOA (14.5 ± 1.7 versus 16.6 ± 0.2) and SSPROM (86.6 ± 7.9 versus 98.4 ± 1.1) scores ($p<0.05$). Both JOA ($r = -0.68$) and SSPROM ($r = -0.65$) correlated with age ($p = 0.0001$, Spearman). Delayed central ascending conduction studies on the lower limbs were present in 72% of all heterozygotes, and correlated with SSPROM ($r = -0.47$, $p = 0.018$, Spearman). NSE values were higher in heterozygotes than in controls (12.9 ± 7 and 7.2 ± 7 ng/ml, $p = 0.012$, Mann-Whitney U).. Mutation severity and inactivation patterns were not associated with neurologic status. Discussion: Neurologic manifestations, clearly related to age, were more common than expected. JOA and SSPROM scales discriminated asymptomatic from symptomatic heterozygotes. Both might be useful tools to follow disease progression, in future studies.

P06.59-S
Should persons with intronic variations of α galactosidase A and stroke be considered ‚manifesting carriers' of Fabry disease?

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Persons with unexplained early-onset stroke have been targeted for screening surveys for Fabry disease, the most common of the three X-linked lysosomal disorder, because Fabry patients with stroke are more likely to have the life-threatening progressive cardiac and renal manifestations and would therefore most benefit from early diagnosis and intervention with enzyme replacement therapy (ERT). Among 173 Israeli patients with unexplained cryptogenic stroke screened for mutations in the Fabry α galactosidase A (GLA) gene, sequencing identified four with 2-4 GLA intronic variants, one of whose father and three sisters had the same variants. Two variants, c.640-16A>G (g.10115A>G) in intron 4 and c.1000-22C>T (g.10956C>T) in intron 6, were common to all patients; only one male with a deletion in intron 2 had low residual enzyme activity; both he and the father had decreased mRNA expression. X-chromosome inactivation was not highly skewed in the six females. Nonetheless, these cases differ largely from those in the literature of carriers of intronic variants with multi-system Fabry disease. These current patients may not benefit from ERT, raising the question whether intronic variants alone are disease-causing or whether these patients should be considered ‚manifesting carriers' of an X-linked recessive disease.

P06.60-M
A pilot study on an expanded newborn screening program in Palestine

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Objective: Until this day newborns in Palestine are being screened for phenylketonuria and congenital hypothyroidism only. However, a number of other metabolic diseases have been recognized amongst the population, including amino acid and urea cycle disorders. The study was conducted in an effort to investigate the possibility of finding such cases in newborns throughout the West Bank of Palestine. In addition, reference ranges for a number of amino acids and urea cycle intermediates will be established.
Study Design: A cross-sectional observational study design was used and the study was conducted in all 12 districts of the West Bank. Convenience sampling was used to recognize the study newly born participants. The sample size was 4240 and an informed consent form was collected from all parents. The study blood collection cards were collected over a one year period by Ministry of Health staff. The study cards were shipped to the University of Liege Human Genetics Department in Belgium for analysis using tandem mass spectrometry.
Results: Statistical analysis showed a significant relationship between weight and some amino acid levels. A significant difference between some amino acid levels and the districts was also observed. The reference range for each amino acid and urea cycle intermediates tested was calculated based on the non-parametric percentile method as indicated by CLSI C28-A3.
Conclusion: Based on our established reference ranges for each analyte the results showed that 22.5% of the tested samples (955) had at least one amino acid level in the upper 2.5% of the population.

P06.61-S
First experience of idursulfase enzyme replacement treatment in patients with Hunter syndrome in Bashkortostan Republic (Russia)

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Mucopolysaccharidosis type II (MPS II, Hunter syndrome, OMIM309900) is a rare X-linked recessive lysosomal storage disorder caused by deficiency of the enzyme iduronate-2-sulphatase, resulting in accumulation of glycosaminoglycans (heparin sulfate and dermatan sulfate), multisystem organ failure and early death.
In the Republic of Bashkortostan (Russia), enzyme replacement therapy with idursulfase (recombinant iduronate-2-sulphatase) was established since 2011. Five patients with confirmed Hunter syndrome, aged between 3.5 and 12 years, were registered for the program. Patients had been treated with weekly intravenous infusions of idursulfase for 24 months. At the beginning of the treatment, all patients showed moderate to severe neurological

abnormalities, including hydrocephaly, ventriculomegaly, and white matter lesions, as well as other symptoms, such as facial and thoracic deformities, joint stiffness, exudative otitis media, hepatosplenomegaly and intellectual disability. After at least 9 months of treatment, urinary glycosaminoglycans levels had decreased, and liver and spleen size were reduced. Idursulfase treatment was well tolerated (adverse reactions occurred in none of the patients). However, cognitive development had shown clear tendency to decline over time, resulting in severe mental retardation by the end of second year of treatment. Our experience of enzyme replacement therapy of Hunter syndrome provides an evidence of the beneficial somatic effects and also suggests the importance of early diagnosis of the disease which might lead to more favorable impact on cognitive development of patients.

P07.01-S
ACTN1 : identification of novel mutations in a cohort of Italian IMTP patients

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Inherited macrothrombocytopenia (IMTP) is a highly heterogeneous group of inherited disorders characterized by a low platelet count and abnormally platelet size. Even though IMTP-causing mutations have been reported in several genes, only 50% of patients have to date a molecular diagnosis.
In March 2013 Kunishima and colleagues identified alpha-actinin 1 (ACTN1) as a new gene responsible for IMTP which accounted for 5,5% of cases in Japanese population. To evaluate the frequency of ACTN1 mutations in the Italian population, we performed a screening in 160 probands in which all the known forms of IMTP were previously excluded. Ten, including 8 novel, different missense have been identified in 11 patients. All except one (p.D666V) segregated with the macrotrombocytopenia within the families. In vivo transfection experiments in Hela cells were performed to demonstrate the pathogenicity of the variants identified. Except for p.D666V, preliminary data indicate that the missense mutations are associated with disorganization of cytoskeleton, as determined different co-localization of mutant and wild type actinin-a with actin. Further studies will be needed to determine how these mutations may lead to the onset of disease.

P07.02-M
Simultaneous silencing of Mcl-1 and survivin expression by small interfering RNA and enhancement of chemosensitivity in human acute myeloid leukemia cells

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Anti-apoptotic genes such as Mcl-1 or survivin may be responsible for resistance to apoptosis induced by chemotherapeutic drugs. The aim of this study was to investigate whether the knockdown of Mcl-1 or survivin expression by small interfering RNA (siRNA) would sensitize HL-60 acute myeloid leukemia cells to etoposide. Knockdown of Mcl-1 or survivin expression was confirmed by quantitative real-time PCR and Western Blotting. The effects of Mcl-1 or survivin down-regulation on the chemosensitivity of the cells was assessed by the MTT assay. Cell viability and apoptosis were also determined using the trypan blue exclusion assay and the annexin V/PI double-staining method, respectively. Transfection of siRNAs markedly decreased the expression levels of both Mcl-1 and survivin genes in a time-dependent manner. Down-regulation of Mcl-1 or survivin significantly inhibited the proliferation and enhanced the chemosensitivity of the cells. Furthermore, pretreatment with siRNAs clearly enhanced the etoposide-mediated apoptosis of the leukemic cells. Surprisingly, siRNA co-transfection had a more antileukemic effect relative to the single siRNA transfection. Our study demonstrates that a triple approach involving siRNA-mediated silencing of the Mcl-1 and survivin along with chemotherapeutic drugs could potentially be used to lower the effective doses of the chemotherapeutic drugs and reduce drug-related toxicities.

P07.03-S
Role of the mutations identified in the 5'UTR of ANKRD26 responsible for an inherited form of thrombocytopenia

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The joint application of clinical and genetic investigations has greatly expanded the knowledge of inherited thrombocytopenias, leading to characterization of new forms, such as ANKRD26 related disease (ANKRD26-RD). ANKRD26-RD is characterized by thrombocytopenia and bleeding tendency, as well as an increased risk of leukemia. Since the identification of ANKRD26, as the gene responsible for the disease, 12 different mutations, all localized in a short stretch of 22 nucleotides in the 5'UTR, have been identified. To further investigate their effect, we tested the activity of the basal promoter together with the wt or the mutant 5'UTR in a reporter assay. In a megakaryocytic cell line, mutations generated a statistically significant increase of the luciferase activity, suggesting that the 5'UTR plays a role in inhibiting the expression of ANKRD26 during megakaryopoiesis. Consistent with this hypothesis, ANKRD26 is expressed in human CD34+ and BFU-E but it is hardly detectable in megakaryocytes. Bioinformatic analysis revealed putative binding sites for transcription factors in the region hit by mutations. In order to identify these factors, we performed electrophoretic mobility shift assay (EMSA). Among different complexes of the same size in both wt and mutant samples, we observed an additional band generated by two mutant probes. Experiments of super-shift EMSA are in progress to characterize the pathogenetic factor(s) that could interfere with the physiological control of the ANKRD26 expression.

P07.04-M
HBB gene mutation spectrum of beta-thalasemia patients from Turkey

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Beta-thalasemia is defined by the absence or decrease of beta globin via mutations of the HBB gene and is one of the most common hereditary disorders existing in Turkey. With the mean carrier frequency of β-thalassemia being 2.1% in the general population, and rates as high as 10% concentrated in certain regions of the country, hemoglobin electrophoresis of the individuals at premarital stage and molecular diagnosis of the carrier individuals for genetic counseling cannot be overstated. Targeted diagnosis of the HBB gene mutations can be readily obtained using commercially available reverse dot blotting kits. A sequence analysis of the complete HBB gene covering UTR and near-gene regions provides a 99% mutation detection rate. We report here a summary finding of HBB gene analysis for 163 Turkish patients, along with their family members totaling 248 individuals, referred with beta-thalassemia indications covering the period of 2010-2014. 39 were found to have homozygous, 31 possessed compound heterozygous and 63 possessed heterozygous mutations. Overall, a total of 205 alleles were found to have mutations. The first 15 frequented mutations covered 88% of the entirety of all mutations. The summary range is as follows: c.93-21G>A (IVS1+110G>A) 30.7%; c.135delC (p.ser45fs) 7.8%; c.92+1G>A (IVS-I-1) 7.8%; c.25_26delAA (p.Lys9Valfs) 5.9%; c.20A>T (p.Glu7Val) 4.9%; c.92+6T>C (IVS-I-6) 4.9%. We discuss that the commercial targeted kits detect up to 80% of the HBB mutations for our patients. Sequence analysis of the HBB gene from 5' promoter (-250bp) to 3' promoter region (*250bp) contributes 15% to the mutation detection rate.

P07.05-S
Identification of two novel mutations in NLRP3 gene in Italian patients with Cryopyrin-associated periodic syndrome (CAPS)

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Cryopyrin-associated periodic syndrome (CAPS) is an autoinflammatory syndrome caused by mutations in NLRP3 gene. Three clinical types exist: familial cold autoinflammatory syndrome (FCAS), Muckle-Wells Syndrome (MWS) and chronic infantile neurological, cutaneous and articular (CINCA) syndrome. More than 150 different diseases-causing mutations have been reported in NLRP3 with more than 80% of them localized in exon 3.
We report here two cases of CAPS in Italian patients harbouring two de novo mutations. The first case is a 20 years old boy with CINCA. He has severe phenotype with clinical features mimicking those of juvenile rheumatoid arthritis, including recurrent episodes of skin rash, fever, arthralgia, and cen-

tral nervous system involvement. In this patient was identified a c.913G>A (p.D305N) mutation in the exon 3 of the NRLP3 gene. The analysis of the parents did not show the same mutation. Mutations at the codon 305 of NRLP3 gene have been described but not the Asp >Asn substitution. The second case is a 35 years old woman with FCAS. She showed recurrent episodes of skin rash, fever, arthralgia and conjunctivitis after generalized exposure to cold. In this patient the c.2113C>A (p.Q705K) mutation in exon 3 of the NRLP3 gene has been identified. Also in this case the analysis of the parents did not show the same mutation. In conclusion, we report two de novo mutations in NLRP3 gene in CAPS italian patients and we suggest the molecular screening of NRLP3 gene in patients with a strong suspicious of autoinflammatory syndrome.

P07.06-M
IPEX-like syndrome: beyond FOXP3 analysis

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IPEX-like patients have clinical features resembling IPEX syndrome (Immune-dysregulation, Polyendocrinopathy, Enteropathy, X-linked) without mutations in the *FOXP3* gene (Xp11.23), that encodes a transcriptional regulator critical for the development and function of $CD4^+CD25^+$ regulatory T cells. These cells are indispensable for the maintenance of immune self-tolerance and homeostasis by suppressing aberrant or excessive immune responses.
IL2Ralfa and *STAT5b* are two genes whose deficiency has been associated with IPEX-like syndrome and both encode proteins involved in FOXP3 pathway. *IL2Ralfa* codes for the alfa subunit (CD25) of the receptor complex for IL2, and the interaction between IL2-IL2Ralfa is the first step that trigger *FOXP3* trascription.
In this study we describe the identification of three different point mutations affecting *IL2R*alfa one of them was recently published by our group and the other two were never reported in literature.
All variations are missense mutations leading to a single aminoacid substitution that modify the protein structure. Two are located in exon 4 and alter the "sushi" domain that seems to be fundamental for IL2-IL2Ralfa interaction, the third mutation determines the substitution of a cysteine that may be involved in the formation of a disulfide bond, thus causing the alteration of the tertiary structure of the receptor.
Cytofluorimetric analysis revealed total absence of CD25 expression in all three patients, strengthening the hypothesis that these mutations cause the disruption of the receptor structure thus leading to its degradation. Further studies are ongoing to better characterize the consequence of these mutations from the molecular and cellular point of view.

P07.07-S
Immunophenotypic profile of erythroid extracellular vesicles obtained from peripheral blood of patients with Diamond-Blackfan Anemia

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Diamond-Blackfan Anemia (DBA) is a rare inherited anemia. Heterozygous mutations in one of 11 ribosomal protein genes cause defective ribosome biogenesis. Erythroid progenitors (BFU-E and CFU-E) in bone marrow (BM) show a proapoptotic phenotype. Suspicion of DBA is reached after exclusion of other forms of BM failure syndromes and the diagnosis is confirmed by mutations analysis. To improve DBA diagnosis, we tested a new approach based on the study of extracellular vesicles (EVs). EVs have been isolated from plasma of patients with DBA and appropriate controls by differential centrifugations and analyzed by flow cytometry. To study erythroid EVs we evaluated three erythroid markers: CD34, CD71 and CD235a. EVs immunophenotypic profiles of 8 patients with DBA, 20 healthy controls and 10 patients with other hematological diseases have been characterized. We were able to identify different clusters: CD71+/CD34-, CD34+/CD71low and CD235a+. In all gates the absolute number of EVs/µl has been evaluated. Only the CD34+/CD71low population is significantly different between patients with DBA and controls ($p< 0,05$). This population that is always present in healthy controls, is absent in patients with DBA. The absence of CD34+/CD71low population in DBA patients plasma is in agreement with the low level of erythroid progenitors in the patients' BM. The area under the ROC curve that compares patients with DBA and healthy controls is 0.92. Further analyses are needed to ascertain whether this assay may be used in the clinics.

P07.08-M
Analysis of rRNA maturation in patients with Diamond-Blackfan anemia

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Diamond-Blackfan anemia (DBA MIM # 105650) is an inherited erythroid aplasia caused by mutations in 11 genes encoding ribosomal proteins of the small (RPS) or the large (RPL) subunit. The 28S, 5.8S and 18S rRNAs are transcribed as a single precursor that is processed into mature rRNAs, which form the ribosomal subunits with the RPs. Mutations in a RPS or a RPL alter the processing of 18S or 28S and 5.8S rRNAs, respectively. The analysis of pre-rRNA by Northern blot in cell models or lymphoblastoid cells from patients shows different patterns of precursors depending on which RP is mutated.
Mutation analysis of 11 genes is needed to confirm the diagnosis. We have looked whether rRNA analysis of activated lymphocytes could improve the DBA diagnostic approach. We extracted total RNA from activated lymphocytes of patients with DBA (4 mutated in RPS19, 1 in RPS17, 2 in RPL11, 4 in RPL5 and 5 with unknown mutations). Northern blot analysis was performed using appropriate probes. All the patients with a mutation in a RPL showed the accumulation of a 32S precursor that is visible also by ethidium bromide staining. Northern blot analysis allowed to discriminate patients with mutations in RPS19 from those with mutations in RPS17. We observed the rRNA maturation defect also in a patient with a mutation in RPL5 after stable remission from the disease.
This is the first demonstration of rRNA abnormalities in patient lymphocytes. rRNA processing analysis is a useful approach to direct the subsequent mutational screening.

P07.09-S
A case of factor X (FX) deficiency caused by novel mutations Q56K, Q104X

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Background
Inherited deficiency of coagulation factor X(FX) is a rare bleeding disorder with prevalence of 1 per 500,000 in general population. Mostly, the condition is associated with missense mutation, which is a valuable clue.
Case and results
A six month-old infant was hospitalized due to fever, lethargy and seizure with no significant medical history. Symptoms were attributable to brain abscess subsequently diagnosed. For operability evaluation, prothrombin time (PT) and activated partial thromboplastin time tests were performed, and the results were prolonged to 121.6 seconds and 109.1 seconds, respectively. Coagulation factor activities were also measured. Factor II, V, X activities were 80%, 99% and 1%, respectively. Factor VII, VIII, IX, XI and XII activities were all in normal ranges. FX activity of the parents was not tested due to refusal of family study. FX of the patient was sequenced on coding regions and exon-intron boundaries. We found 1 missense mutation and 1 nonsense mutation of FX (c.C343A in exon 4, c.C487T in exon 5) that correspond to amino acid substitution Q56K and stop codon at Q104, respectively. Both amino acids are located in EGF-like-domain 1.
The patient suffered hematochezia, hematuria, subdural hemorrhage and hemarthrosis which were all irrelevant to the operation. Bleeding was treated with tranexamic acid and fresh frozen plasma with monitoring of FX activity which increased from 1% to 22%.
Conclusion
The amino acid substitution of glutamine to lysine at EGF-like-domain 1 is probably related to functional inactivation of coagulation FX. Confirmation by expression study will be required.

P07.10-M
Study of IL-1β and IL-1RA gene polymorphisms in familial Mediterranean fever (FMF)

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Objectives. Familial Mediterranean fever (FMF) is a recessively transmitted autoinflammatory disease, caused by mutations in the MEFV gene. The pro-inflammatory cytokine IL-1β has been implicated in the pathogenesis of FMF and the balance between IL-1β and its receptor antagonist IL-1RA plays an important role in the development of the disease. Since polymorphisms in the IL-1 gene cluster have been suggested to have an effect on IL-1β and IL-1RA production, our aim was to determine a possible association of specific polymorphisms in IL-1β and IL-1RA genes with susceptibility to and/or severity of FMF.
Subjects and Methods. Forty-two genetically confirmed FMF patients and 42 controls were genotyped for IL-1β(-511C/T), IL-1β(-31T/C) and IL1-1β(+3954T/C) polymorphisms by PCR- digestion. The IL-1RA VNTR was identified by fragment-size analysis. IL-1β and IL-1RA levels were evaluated by Luminex in supernatants of PBMC cultures of 30 FMF patients with and without 24h stimulation of monocytes by LPS.
Results. The CC genotype and C allele at positions -31 and +3954 of IL-1β gene were observed more frequently in FMF patients than in controls. No significant difference was observed in the genotypic and allelic frequencies of the IL-1β(-511) polymorphism and IL-1RA VNTR. FMF patients carriers of IL-1β(-31) CC genotype were associated with a 2 fold increase in LPS-induced IL-1β secretion, compared to patients carrying other genotypes.
Conclusion. These results indicate that IL-1β gene polymorphisms at positions -31 and +3954 may be associated with susceptibility to FMF. IL-1β(-31) may also contribute to the severity of the disease, probably by modulating IL-1β secretion.

P07.11-S
Host genotype-pathogen interactions in the PBMC cells of healthy individuals identify critical immune regulators

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Immune response to infection or vaccination is significantly influenced by host genetic components and autoimmune disease associated genetic variants may have stronger influence on such a response. One way to investigate the role of genetics in differential susceptibility to infection is to correlate the gene expression levels response to stimulation with autoimmune disease associated SNPs. We performed a pilot experiment by stimulating peripheral blood mononuclear cells (PBMC) of 46 volunteers with four different pathogenes for 4 and 24 hours. Gene expression profile was determined with Illumina arrays and genotype of 366 autoimmune disease-associated SNPs were genotyped using Immunochip. Linear model with stimulation status, genotype and stimulation-genotype interaction term was fitted for each probe and SNP. Due to the small sample size, we were not able to identify globally significant pathogen-genotype interactions after multiple testing correction, so possibly relevant interactions were prioritized based on uncorrected p-values, allelic trends and effect size differences between unstimulated and stimulated conditions. 133 SNPs showed interaction effect for at least one probe. The most significant interaction was between rs6087990 and a gene encoding macrophage receptor (uncorrected P=3.72×10-5). We were also able to identify SNP-gene pairs showing similar stimulation-genotype interaction in case of several different stimulations. These genes may play role in the disease susceptibility and serve as possible candidates for functional studies. The methods and preliminary results from this study reveal novel insights into the role of autoimmune SNPs and underscore the importance of large scale genotype-environment interaction studies in future.

P07.12-M
The influence of a short-term gluten free diet on the human microbiome

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A gluten free diet (GFD) is the most common diet worldwide. It is not only an effective treatment for celiac disease, but also commonly followed by individuals with gut complaints. How a GFD affects the human microbiome is largely unknown. We studied changes in the gut microbiome in healthy individuals following a short-term GFD.
23 healthy volunteers followed a GFD for 4 weeks. Stool samples were collected before the start of the diet, then at weekly intervals during the GFD and again at weekly intervals for 4 weeks on normal diet after a wash-out period. The samples were sequenced using 454 sequencing of the 16s rDNA (hyper variable region 3 to 4). We used closed reference picking to cluster reads into OTUs using the 2013 GreenGenes reference. Function imputation of the OTUs, is performed used PiCRUSt and HUMAnN.
We observed a limited difference of the GFD intervention, with the intra-subject variation being larger than the effect from a short-term diet change. However, we did observe that family *Veillonellaceae* (class *Clostridia*) was significantly less present in the GFD samples (p=6.58e−05, q=0.0132). Based on richness of the samples we were able to define two groups which show a difference in response to a GFD period. The lower richness group showed more bacterial variation in composition during the diet change. Furthermore, sixteen gene pathways showed significant association to the change in diet. In conclusion, in this we observed changes in microbiome and gene composition associated to a GFD.

P07.13-S
A dominant-negative GFI1B mutation causes autosomal dominant gray platelet syndrome

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Gray platelet syndrome (GPS) is a hereditary, usually autosomal recessive bleeding disorder caused by defective production of α-granules in platelets. The α-granule deficiency may be attributed to the failure of megakaryocytes to efficiently route proteins into α-granules, thereby hampering their maturation. We describe a large family with an autosomal dominant type of GPS characterized by mild to severe bleeding complications. In addition to gray platelets lacking α-granules, affected individuals had other GPS-associated phenomena like thrombocytopenia, emperipolesis, myelofibrosis and decreased expression of platelet factor 4. To determine the disease causing mutation we performed linkage analysis and identified a candidate locus on chromosome 9q34 (LOD score 3.9). We considered GFI1B (Growth factor independence 1B), located within this region, an excellent candidate gene because of its function as a transcriptional repressor essential in erythroid and megakaryocyte lineages. We identified a nonsense mutation in exon 6 (c.859C>T, p.Gln287*) that co-segregated with the GPS. The mutated transcript was not targeted for nonsense-mediated mRNA decay, resulting in a protein truncated within its 5th zinc finger, a domain essential for DNA binding. Using luciferase reporter assays we demonstrated that the truncated GFI1B protein was unable to repress gene expression and that it inhibited wild type GFI1B in a dominant-negative manner. Subsequently, we performed an immunophenotypical analysis of affected family members. Myeloid and erythroid lineages were unaffected, but we observed aberrant platelets and dysplastic abnormalities and disturbed lineage marker expression in GFI1B-mutated megakaryocytes. These studies define GFI1B to be key to megakaryocyte development, platelet production and α-granule biogenesis.

P07.15-S
Deletion between HLA-A and HLA-G genes is associated to HLA-A*23~HLA*G01:04~UTR-3~HLA*E01:01 and HLA-A*24~HLA*G01:04~UTR-3~HLA*E01:03:01 haplotypes

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The genetic region between HLA-A and HLA-G covers 300 kb and is highly polymorphic. A large-scale deletion of 50 kb between these loci associated to HLA-A*23 and HLA-A*24 has been described. This deletion includes many genes and pseudogene, as HLA-H.
We showed in a study conducted in a Malian population that HLA-A alleles display LD with HLA-G alleles and UTR; HLA-A*23:01:01~HLA-G*01:04~UTR3 haplotype displaying the highest frequency. This strong LD could be emphasized by a deletion occurring between those two loci, thus reducing the recombination rate between HLA-A and HLA-G. This haplotype

conservation might also be the result of a specific biological effect, as HLA-G*01:04 has been associated with high sHLA-G production and that HLA-A*23:01~HLA-G*01:04 haplotype may constitute a risk factor for allograft rejection in renal transplantation. Interaction with other immune effectors as HLA-E could also be incriminated. We showed that 2 haplotypes in Tswa Pygmies, HLA-G*01:04~E*01:03:01 and G*01:04~E*01:01 exhibited LD values.

The aim of our study is to explore the region between HLA-A and -E. We genotyped HLA-A, -G, -H and -E alleles in 71 french samples and explore their association.

We found that 11 haplotypes represent 75% of all the haplotypes. HLA-H deletion was exclusevely associated with A*23~HLA*G01:04~UTR-3~HLA*E01:01 and HLA-A*24~HLA*G01:04~UTR-3~HLA*E01:03:01 haplotypes. A new HLA-H*02:04(:02) allele was descibed and associated with A*11~HLA*G01:01~UTR-7~HLA*E01:01.

This study suggests that the HLA region between HLA-A and -E loci displays a haplotype conservation that might be the results of biological function. This haplotype conservation has to be further studied to understand their clinical implication.

P07.16-M
HLA-DRB1 genotyping in romanian patients with early rheumatoid arthritis

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As being the most debated polygenic disease, rheumatoid arthritis elicits great interest in the study of association with genetic factors in various ethnic and racial groups. Some of the HLA-DRB1 alleles are encoding shared epitope amino acids that are not conferring the same risk in various populations.

Our study focuses on the evaluation of the distribution of HLA-DRB1 alleles in Romanian patients with early rheumatoid arthritis, along with controls by using PCR- SSP method.

HLA-DRB1 allele genotyping showed statistically significant differences given by a higher allele frequency for *04, *01 and *14. Also, in our study was observed a lower frequency for *03,*11,*13 and *15 alleles in patients group compared with controls. Using a high resolution kit for HLA-DRB1 *04 group we found a high frequency for *0404 and *0408 alleles, in contrast with *0401 and *0402 which were significantly lower in patients than in controls. *0403, *0405 were not associated with early rheumatoid arthritis in our group diagnosed according with new classification criteria ACR/EULAR 2010.

Results of our study are demonstrating the need of a continuous work of allele tracing and associating with rheumatoid arthritis, especially in cases early diagnosed in order to create sufficient premises for instituting a correct and possibly long term remissive treatment.

Keywords: early rheumatoid arthritis; HLA-DRB1; allele distribution

P07.17-S
Genetic association of childhood psoriasis to the IL22 promoter is linked to higher promoter activity and increased IL-22 production in T cells

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Psoriasis is a common, immune-mediated and genetically complex skin disease. Recent large-scale studies have reported a number of psoriasis susceptibility genes. Most of these genes were identified in large cohorts not defining the clinical phenotypes and exploration of genes in distinct subtypes of psoriasis is missing. We recently reported that stratification according to age at onset was helpful in dissecting the genetic profile of early onset psoriasis. Here we investigated the genetic association to *IL22* in three psoriasis populations: disease onset between 0-9, 10-20 and 21- 40 years. *IL22* encodes for a cytokine with an established role both in psoriasis skin pathology and in host defense, thus exemplifying delicate balance between autoimmunity and control of infection. Herein we report strong association to regulatory elements in the *IL22* promoter confined to onset of psoriasis before puberty. The associated *IL22* variants contain putative binding sites for the transcription factor aryl hydrocarbon receptor, which is a potent inducer of *IL22* expression in T cells. We next compared the transcriptional activity between a high-risk and a low-risk gene variant in a luciferase assay which consistently resulted in significantly higher activity from the high-risk construct. Furthermore, in children carrying a high risk variant, T cells from peripheral blood produced significantly more IL-22 after *ex vivo* stimulation compared to children with a low-risk genotype. These data indicate that genotypes in the *IL22* promoter enhancing IL-22 production is preferentially enriched in psoriasis with onset before puberty and may predispose to development of psoriasis at an early age.

P07.18-M
Whole exome sequencing as a tool for detection of the genetic basis of inherited thrombocytopenias

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Thrombocytopenia is a common aspect of platelet function disorders (PFDs) which account for a significant proportion of bleeding diatheses. Identification of the genetic basis of congenital thrombocytopenias is often a difficult task due to the variability in clinical presentation and the relative redundancy of known platelet receptors and signalling pathways. DNA-based analysis has therefore previously played a confirmatory role dependant on platelet function testing to validate candidate mutations. Using a novel approach underpinned through the Genotyping and Phenotyping of Platelets (GAPP) study we have extended the DNA-based analysis, especially in cases where a qualitative defect is observed, to unravel the underlying genetic defects in patients diagnosed with inherited thrombocytopenia. The combination of platelet function testing, including flow cytometry, with whole exome sequencing provides a complete and complementary platform for efficient and cost effective investigation. Additional integration of a database of 338 platelet-related genes into our analysis improves detection and allows for the identification of variants in novel genes for further functional studies. To date our unique workflow has confirmed 14 mutations in 21 index cases, including two in novel genes with previously unreported mechanisms within platelet formation or function. Whole exome sequencing is therefore an effective tool to study the molecular genetics of inherited thrombocytopenias with excellent applicability for patient investigation when coupled with platelet function testing.

P07.19-S
Mutation spectra of the ITGB2 gene in Iranian families with Leukocyte Adhesion Deficiency type 1

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Leukocyte adhesion deficiency (LAD) type 1 is a rare, autosomal recessive disorder characterized by the triad of symptoms including recurrent bacterial infections, impaired wound healing, primarily localized to skin and mucosal surfaces. Beta-2 integrin subunit (ITGB2) gene located on 21q22.3 responsible for neutrophil dysfunction and impaired leukocyte cell adhesion. A total of 19 consanguineous families with typical LAD1 were investigated. Blood samples were collected after informed and written consent was obtained. Isolated DNA derived from subjects was amplified using intronic primers. The entire sequence of the ITGB2 gene, including regulatory region, coding regions and exon-intron boundaries were analyzed for any alteration by PCR and Sanger sequencing. A total of 10 mutations scattered throughout the ITGB2 gene were ascertained in the 15 subjects. Six different types of mutations previously reported, including IVS4-6 C>A, c.382G>T (Asp128Tyr), c.715 G>A (Ala239Thr), IVS7+1G>A, c.843DelC (Asn282fsX41), c.1907DelA (Lys636fsX22) and four novel mutations consist of IVS5-11G>T, c.576dupC (Asn193GlnfsX72), c.706 G>A Gly236Arg, IVS7+1G>T were identified. Moreover, two compound heterozygote and five homozygote mutations were detected in exon 6, suggested this region of ITGB2 gene might be a hot spot. This is the first comprehensive report of ITGB2 gene analysis in Iranian families with LAD1. Our results indicated that mutations distributed across the ITGB2 gene. Every population should develop a mutation database for their own rare genetic disorders. However, we suggest that to make a private database, appropriate screening strategy could be started with common alleles initially.

P07.20-M
Identification of monoallelic forms of Bernard-Soulier syndrome

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Bernard-Soulier syndrome (BSS) is a rare inherited macrothrombocytope-

nia caused by mutations in *GP1BA, GP1BB* and *GP9*, the genes encoding for three subunits of the GPIb-IX-V complex, which is the platelet receptor for von Willebrand factor (vWF). In addition to the biallelic form, a less severe autosomal dominant form (monoallelic) of BSS is due to mutations identified so far in *GP1BA* or *GP1BB*. Except for p.Ala172Val (Noris et al, 2012), which is relatively frequent at least in the Italian population, other mutations have been reported only in single families. In order to evaluate the frequency of the monoallelic form in our thrombocytopenic cohort, we selected 120 probands with large platelets and no mutations in candidate genes. In 11 cases we found heterozygous mutations in *GP1BA* (n=4), *GPIBB* (n=6) and *GP9* (n=1). In addition to 4 variants previously identified in biallelic BSS patients, the others are all novel mutations. They are classified as missense (n=8), nonsense (n=1) or frame-shift (n=2) mutations. Segregation analysis within the families showed that only the affected individuals carry the mutations. Functional studies are in progress to determine the effect of the mutations on GPIb-IX-V complex formation and its capacity to interact with vWF. Considering that half of the patients with inherited thrombocytopenia remain without a molecular diagnosis, we could estimate that the frequency of the monoallelic form of BSS could account for 5% of the cases. This suggest that monoallelic BSS should be always taken into consideration in the differential diagnosis of inherited thrombocytopenia.

P07.21-S
MHC and the 1000 genomes: genotyping from exome data

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The publicly available 1000 genomes (1KG) data is still a valuable source of scientific discovery after years of its release. We have demonstrated previously that HLA typing of MHC-I genes is possible from 1KG exome data for HapMap samples. In this study we are aiming to determine types for MHC-II genes and for any 1KG exome samples passing certain quality control (QC) conditions. We are presenting the generation and interpretation of these QC values. Besides the HLA genes we are showing typing results for other polymorphic MHC genes like TAP1, TAP2, MICA and MICB. The HLA genotype assignations were compared to classical HLA typing obtain by sequencing techniques. Typing a diverse set of genes of MHC sheds light on linkage disequilibrium and makes it comparable to the previously known HapMap statistics. Furthermore, differences in results from different exome capturing kits are also to be presented.

P07.22-M
SNP variants in MHC are associated with sarcoidosis susceptibility and subgroups - a joint case-control association study in four European populations

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Sarcoidosis is a multiorgan inflammatory disorder of unknown aetiology. The most probable pathophysiology of sarcoidosis, the dysregulation of the immune response strongly suggests benefits from a better understanding of the role of the immune mediating genes (e.g. MHC genes) in sarcoidosis susceptibility.
We present results from a Finnish case-control discovery sample as well as three independent replication studies from the Swedish, Dutch and Czech populations. We studied four genes in the MHC Class III region (LTA, TNF, AGER, BTNL2) and HLA-DRA in relation to HLA-DRB1 alleles to detect variants predisposing to sarcoidosis and to identify genetic differences between patient subgroups.
Patients with sarcoidosis (n=805) were further subdivided based on the disease activity and the presence of Löfgren syndrome. In a meta-analysis, seven SNPs were associated with non-Löfgren sarcoidosis (NL; the strongest association with rs3177928 in HLA-DRA, P=1.79E-07, OR=1.9) and eight with Löfgren syndrome (LS; the strongest association with rs3129843 in BTNL2/HLA-DRA region, P= 3.44E-12, OR=3.4) when compared with healthy controls (n=870). The high LD between SNPs and an HLA-DRB1 challenged the result interpretation. In addition to these SNPs, population-specific associations for sarcoidosis were observed.
In conclusion, there is clear evidence that polymorphisms in the BTNL2 and HLA-DRA have a role in sarcoidosis susceptibility. Most importantly, our study revealed sarcoidosis-related variants that were shared across ethnicities as well as ethnicity-specific genetic markers. Future functional studies are required to reveal the causal variants of these associations and the immunogenetic basis related to sarcoidosis.

P07.23-S
Impact of immunogenetic polymorphisms in predisposition to lymphoid malignancies in NBS patients

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The Nijmegen breakage syndrome (NBS) is a recessive genetic disorder, resulting in high predisposition to developing a malignancy. The 5bp deletion (c.657-661del) in the NBN gene is founder mutation for Slavic populations. In Ukraine 40 NBS cases were diagnosed, and in 12 cases (30%) lymphoid malignancies has been developed at age 5-12 years. It remains unclear why the carriers of same mutation are implemented in a different morbidity. The purpose of this study was looking for immunogenetic criteria for tumor developing in NBS patients. Interleukin-10 (IL-10) and interferon-gamma (IFN-γ) play a key role in controlling the immune response and SNPs IL-10 -1082 A/G and IFN-γ +874A/T respectively can significantly affect their expression. Patients with severe outcome (multiple chronic recurrent inflammation, developing of tumor, death) and moderate course of disease were comparatively investigated. The distribution of IL-10 1082AA, 1082GG and 1082AG genotypes in cases of severe and mild outcome established as 42.9% and 33.3%, 35.7% and 33.3%, 21.4% and 33.3% respectively. The distribution of IFN-γ 874AA, 874AT and 874TT genotypes in groups of patients with severe and mild outcome was 20.0% and 29.4%, 40.0% and 58.82%, 40.0% and 11.86% respectively. The results allow to suggest, that severe outcome in NBS may depends on carrying of IL-10 1082AA genotype, associated with decreased anti-inflammatory activity, and IFN-γ 874TT genotype, known by increased pro-inflammatory properties. In conclusion, disruption in balance of pro- and anti- inflammatory cytokines may strongly affect NBS course.

P07.24-M
MEFV gene mutations in pediatric patients with PFAPA syndrome in Slovenia

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Introduction: PFAPA syndrome is the most common autoinflammatory fever disorder in childhood, characterized by recurrent fever, aphthous stomatitis, pharyngitis and adenitis. Mutations in the *MEFV* gene are known to cause syndrome with PFAPA overlapping symptoms (Familial Mediterranean Fever), which is common in eastern Mediterranean population, but rarely reported in patients from Slovenia.
Objective: The aim of this study was to assess the frequency of *MEFV* gene mutations in pediatric patients with PFAPA syndrome from Slovenia.
Methods: We collected clinical and laboratory data and results of genetic testing of *MEFV* gene of PFAPA patients under the age of 18, who were followed from the beginning of 2006 to the end of 2013. All 10 exons and intron/exon regions of gene were directly sequenced.
Results: In total, 91 PFAPA patients were tested for *MEFV* gene mutations. All of them were under the age of 18, mean age at diagnosis was 7 years. The ratio of women to men was 1:1.
15 patients (16%) were found to have at least one mutation. 11 patients (12%) were heterozygote and 4 patients (4%) were compound heterozygote, 3 with R408Q/P369S and 1 with K695R/I591T mutation. The overall number of mutation found was 19. The most frequent was K695R(26%), followed by R408Q(16%), P369S(16%), E148Q(10%), I591T(10%), M694V(5%), S730F(5%), A289V(5%) and A744S(5%).
Conclusion: In order to evaluate effect of these mutations on PFAPA phenotype, we are planning to determine the carrier rate in healthy Slovenian population and evaluate genotype-phenotype correlation in *MEFV* gene mutation positive patients.

P07.25-S

A new gene involved in an autosomal dominant form of common variable immunodeficiency (CVID)

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Common variable immunodeficiency (CVID, MIM#607594) is the most common symptomatic primary antibody deficiency in adults. It includes a heterogeneous group of disorders characterized by defects in the terminal stage of B lymphocyte differentiation, whose underlying genetic defects remain unknown in the majority of cases.
We studied a five-generation Italian family with an autosomal dominant form of CVID through whole exome sequencing, giving priority to the variants within a 9.2 Mb candidate genomic interval on chromosome 3q27.2-q29 previously identified by genome-wide linkage analysis. Since no causative mutation was identified by whole exome sequencing, we performed a whole genome high resolution SNP array analysis, which allowed to detect a ~ 880 kb tandem duplication in 3q27.3, located inside the candidate linkage region and involving 8 genes: *ST6GAL1*, *RPL39L*, *RTP1*, *MASP1*, *RTP4*, *SST*, *RTP2* and *BCL6*.
The expression pattern analysis in control peripheral blood lymphocytes (PBL) of all genes included in the duplicated region revealed that only *ST6GAL1*, *RPL39L*, *RTP4* and *BCL6* are expressed in mature circulating lymphocytes, allowing us to exclude the remaining four genes from further investigations. Preliminary qRT-PCR analyses conducted on affected vs unaffected subjects showed a significant upregulation of *RTP4* in affected members (t-test, $p < 0.0001$). This finding suggests *RTP4* overexpression as a possible pathogenetic mechanism underlying CVID in this family. RTP4 is a Golgi chaperone and we hypothesize that it might be involved in the regulation of the Unfolding Protein Response (UPR), a key process of plasma cell differentiation.

P07.26-M

A family based exome sequencing identifies ADA2 deficiency as a novel cause for PIDD

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Primary immunodeficiency diseases (PIDD) encompass a wide and heterogeneous group of disorders caused by mutations in genes with immune function. The molecular mechanisms behind many forms are not yet known. The significant clinical and immunological heterogeneity of PIDD often delay the diagnosis making treatment challenging. Few thousand individuals are expected to suffer from PIDD in Finland, where the unique genetic background has proven to be very useful in identifying genes for monogenic disorders. An exome sequencing analysis of a Finnish PIDD family characterized by recurrent prolonged bacterial infections complicated by basal cerebral and frontal infarcts, subarachnoidal hemorrhage and occasional small aneurysms, has identified compound heterozygous mutations in the CECR1 gene not previously implicated in PIDD. The affected individuals carried a splice donor site mutation leading to nonsense mediated decay (NMD) of the allele and a rare missense variant, highly conserved and predicted deleterious. Analysis of the patient sera confirmed that the protein product (ADA2) of the mutated gene had no measurable activity. ADA2 is a member of the adenosine deaminase family, which also includes ADA1, a known cause of severe combined immune deficiency (SCID). ADA2 is a secreted protein in which binds to cell surface via proteoglycans, may degrade extracellular adenosine and has been shown to induce T cell-dependent differentiation of monocytes into macrophages and stimulate macrophage proliferation. Thus ADA2 deficiency is a plausible cause for the immunodeficiency complicated by infarcts and subarachnoidal hemorrhage in this family. The ongoing functional analyses will shed further light on molecular defects of the disease.

P07.27-S

Genome-wide DNA methylation analysis in CD19+ B cells in Primary Sjögren's Syndrome

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Primary Sjögren's syndrome (pSS) is a complex chronic autoimmune disease characterized by the inflammation of the exocrine glands leading to dry eyes and impaired salivary flow. Apart from glandular symptoms, systemic manifestations such as arthritis and chronic fatigue are common, and 75% of the patients have autoantibodies in their sera. PSS patients have a 15 fold increased risk of developing B cell lymphoma. Increasing evidence suggests an epigenetic contribution in the pathogenesis of autoimmune diseases, including pSS. Epigenetic modifications represent a dynamic link between genotype, environment and phenotype, by for example modulating gene expression. As methylation of the DNA base cytosine is considered as a prototypic epigenetic mark, our aim was to investigate the role of DNA methylation in pSS. We performed a genome-wide DNA methylation study in purified CD19+ B cells from 17 pSS patients and 28 healthy controls using Illumina HumanMethylation450 BeadChip arrays which cover about 486,000 CpG sites across the genome. Significant differentially methylated CpG sites between patients and controls were identified. We found disease-associated changes in DNA methylation in pSS and highlight pathways that may contribute to the pathogenesis of this autoimmune disease.

P07.28-M

Palmoplantar pustular psoriasis and its genetic background

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Palmoplantar pustular psoriasis (PPP) is a chronic inflammatory skin disease characterized by sterile pustules, erythema and hyperkeratosis on palms and soles. In at least 25% of PPP cases, psoriasis vulgaris (PsV) is also present and many patients suffer from psoriatic arthritis. Smoking and female sex are more frequent in PPP compared to PsV. So far, there are no confirmed genetic risk factors for PPP. Recently, in generalized pustular psoriasis (GPP), an extreme manifestation of pustular psoriatic disease, homozygous and compound-heterozygous mutations in *IL36RN* have been identified to be causal.The same mutations have been described to be more frequent in a group of 139 European PPP patients.Here, we recruited a group of >140 PPP patients,most of them were female and smokers (>60%, respectively). About half of them had a manifestational age of ≤40 years.We could confirm previous data showing that the frequency of the HLA-C risk allele, the major genetic risk factor for PsV, was comparable to the frequency of controls, indicating that PPP is genetically different from PsV. We further analyzed *IL36RN* for mutations in coding exons and for intragenic deletions/duplications. We identified three heterozygous carriers of mutations and no carriers of copy number variants. Compared to a population-based control group of 4.300 European individuals, there was no significant difference in the frequency of *IL36RN* mutations.Our data indicate that PPP is genetically distinct both from PsV and GPP. Further effort is needed to identify genetic factors contributing to PPP.

P07.29-S

Genetic, functional and therapeutic insights into patients with rare recurrent viral infections

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Primary immunodeficiencies (PIDs) are inborn defects in the immune system affecting about 1 in 500 people. In this present study we show that understanding the functional and/or genetic defect in patients suffering from severe PIDs can provide us new leads for treatment. Here we studied three patients suffering from severe recurrent viral infections with Herpes Simplex virus (HSV). The infections were caused by HSV2 in two of the patients, with no history of other infections, while the third patient was initially diagnosed with HPV6 papillomatosis and she developed recurrent HSV infections later in life. We isolated and stimulated PBMCs from patients and controls, and stimulated them with various ligands among which the double

stranded RNA ligand poly:IC. Cytokine production was measured in patients and compared to controls, and a specific defect in poly I:C-induced interferon-gamma was detected. Substitution therapy with interferon-gamma was successfully administered to all patients, leading to impressive amelioration. Additionally, we performed exome sequencing in all 3 patients, aiming to understand the genetic basics of the underlying immunodeficiency. While this genetic analysis did not identify one common cause of the primary immunodeficiency, rare variants in candidate genes SYK, IFIH1 and EIF3E were identified that may have caused the aberrant interferon signaling. In summary. These preliminary results indicate that patients with recurrent viral HSV infections may have genetic heterogeneous forms of primary immunodeficiencies; however, they show a common functional defect in double stranded RNA-induced IFNgamma, and common replacement therapy with interferon-gamma was shown to be an effective treatment.

P07.30-M
Allele specific expression of HLA haplotypes associated to autoimmune diseases

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HLA is strongly associated with many autoimmune diseases, including rheumatoid arthritis (RA), celiac disease (CD) and type 1 diabetes (T1D). For many diseases the association is established, but the underlying mechanisms are unknown. For example, heterozygosity for DR3-DQ2/DR4-DQ8 is a stronger risk factors to T1D compared to homozygosity for both DR3-DQ2 and DR4-DQ8 alleles. We aimed to study the downstream effect of HLA haplotypes by looking for allele specific gene expression (ASE) and haplotype expression quantitative trait loci (eQTLs) in HLA alleles associated to autoimmune diseases. We selected individuals heterozygous for DR3-DQ2/DR4-DQ8 and homozygous for both alleles (T1D and CD risk haplotypes), and individuals heterozygous for DR4-DQ8/DR4-DQ7 and homozygous for these alleles (RA risk haplotypes). We run RNAseq to quantify gene expression and performed eQTL and ASE analysis in these individuals. In total, 90 individuals were selected for this analysis. We observed different expression of multiple genes in HLA locus in DR3-DQ2/DR4-DQ8 heterozygous individuals, in compare with both homozygous group. In particular, HLA-DQA2, HLA-DQB1 and HLA-DQB9 (P-value Wilcox test <0.001 for all three genes). ASE analysis of individuals heterozygous for DR3-DQ2/DR4-DQ8 indicated ASE effect for multiple variants located in TAP2 gene, which plays a role in antigen presentation. In DR4-DQ8/DR4-DQ7 analysis we identified multiple eQTLs in the HLA locus, as well as allele specific effect on SNPs located in DOA genes.

P07.31-S
Phenotypic analysis of Peptidylarginine deiminase type 4 knock-out mice

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Rheumatoid arthritis (RA) is well-known as an autoimmune disease and is a chronic inflammatory disorder characterized by the destruction of multiple joints. Many genome wide association studies were performed and multiple RA-susceptibility loci and autoimmune-susceptibility loci have been identified. These studies suggested that multiple genes and its functions were related with disease causing and development. Previouly, peptidylariginine deiminase type 4 **(**PADI4) was identified as a susceptibility gene for RA in a Japanese population by case-control association study (Ref 1). PADI4 is a member of the PADI gene family and converts arginine residue (peptidylarginine) to citrulline residue (peptidylcitrulline). PADI4 is highly expressed in bone marrow, macrophages, neutrophils and monocytes. PADI genes are important genes in RA, because only PADs (translated protein from PADI genes) can provide antigens of ACPA, which are specific antibodies of RA. To evaluate the importance of PADI4 gene in the progression of RA, we generated Padi4 -/- DBA1J mice. We used Padi4-/- mice to show that PAD4 is significantly affected to progress of collagen induced arthritis (CIA), well known as an RA model animal. In Padi4-/- CIA mice sera, the concentrations of serum anti-CII IgM, IgG, and levels of inflammatory cytokines decreased significantly rather than in WT CIA mice. Futhermore, the transcription levels of inflammatory cytokines in CD11b positive splenocytes from Padi4 -/- CIA mice are also significantly lower than those from WT CIA mice. As the results, we suggested that Padi4 enhanced collagen-initiated inflammatory responses. 1) Suzuki, A. et al Nat. Genet.34, 395-402 (2003)

P07.32-M
Identification of susceptibility loci associated with primary Sjögren's syndrome by genome-wide association study

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Primary Sjögren's syndrome (PSS) is one of the most common autoimmune diseases which primarily affect women, with a female-male ratio of 9:1. To identify the susceptibility genes predisposing individuals to PSS, we conducted genome-wide association analyses comparing 242 PSS female patients with 1444 female controls, recruited from the Han Chinese population residing in Taiwan. In discovery GWAS, SNPs in the MHC region of chromosome 6 and the GTF2I gene of chromosome 7 were found to be associated with PSS. In particular, SNP rs117026326 on chromosome 7 showed the highest association (P value = 6.71 × 10-13). The two loci were previously reported to be associated with PSS. Replication with additional samples is now under process. Our study confirmed the associations between the two loci and PSS.

P07.34-M
Functional analysis of genetic risk factors for canine SLE-related disease complex and identification of genetic risk factors for human SLE

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Dogs represent an excellent model to study complex genetic disorders including many autoimmune diseases that they share with humans. A systemic lupus erythematosus (SLE)-related disease complex, which shows similarities to human SLE, has high prevalence in Nova Scotia duck-tolling retriever (NSDTR) dogs. Genome-wide association analyses have identified five candidate loci in NSDTR containing several genes. Detailed analysis of the candidate loci identified variants that alter the expression of several of these genes. Multiple genes involved in T-cell activation show expression differences, but regulatory mutations also alter expression of genes such as BANK1, involved in human SLE. As a proof of concept, the genes and pathways identified in dogs and also genes relevant for human SLE were sequenced in 140 Swedish human SLE patients. Patients were divided in 9 pools according to disease manifestation and a group of healthy Swedish controls was also sequenced. Targeted Nimblegen + Illumina sequencing of 219 genes and their regulatory elements resulted in approximately 250x coverage per individual. We detected 4276 novel SNPs (not present in 1000genomes or dbSNP137) of which 1258 SNPs were found only in cases. Seventeen genes showed ≥ 5 novel case-only variants. Of these, three genes were previously associated to human SLE and 14 were novel candidate genes. From the top genes, ten variants that show strong regulatory potential have been selected and being evaluated for their importance for gene expression and correlation with clinical phenotypes. Several variants appear to be potential regulators of specific SLE sub-phenotypes.

P07.35-S
Deep analysis of TCR repertoires of twins by next generation sequencing

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Immune system interacts with great diversity of pathogens. Receptors involved in antigen recognition - BCRs (B-cell receptors) and TCRs (T-cell receptors) - are not encoded in genome due to its limited capacity, but generated by V(D)J recombination. For a long time, no instrument to estimate TCR diversity in individual organism has been available. Next generation sequencing methods (NGS) have created the possibility of deep TCR profiling. Here, we aimed to estimate the role of genetic factors in TCR repertoire formation by sequencing TCR repertoires of monozygotic and dizygotic twins.
16 cDNA TCR libraries were generated and sequenced on Illumina platform.
Surprisingly, the overlap of amino acid sequences of CDR3 region in TCR clonotypes was not greater in MZ twin pairs and depended on sample size

only. However, the number of identical clonotypes was higher for monozygotic twins in the abundant clonotypes subset, representing mainly antigen-experienced T cells. At the same time V-segment usage is more similar in twin pairs for clonotypes before and after thymic selection.
We also showed that the ratio of clonotypes with identical CDR3 and V-segments (coding for CDR1 and CDR2) to clonotypes with identical CDR3 is higher in twin pairs in the abundant clonotypes subset.
All these features were not observed in a dizygotic twin pair, which probably reflects the impact of genetic factors on TCR repertoire formation.

P07.36-M
A novel StripAssay identifies genetic variants modifying beta-thalassemia disease severity

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Background: The clinical phenotype of patients with beta-hemoglobinopathies is extremely heterogenous, ranging from nearly asymptomatic forms of thalassemia intermedia to severe transfusion-dependent thalassemia major. The wide phenotypical variability is associated with the type of beta-globin mutation, the co-inheritance of alpha-thalassemia and the ability for persistent production of fetal hemoglobin (HbF) in adult life. For the latter, three different quantitative trait loci, accounting for 20-50% of HbF variation, have been identified by now. Single nucleotide polymorphisms (SNPs) in the gamma-globin gene promoter (HBG2), in the BCL11A gene and the HBS1L-MYB intergenic region lead to increased residual HbF levels in adults. Methods: A teststrip-based reverse-hybridisation assay was developed for the simultaneous detection of SNPs in the HBG2 (g.-158 C>T), BCL11A (rs1447407, rs10189857), HBS1L-MYB (rs28384513, rs9399137) genes. Results: The new StripAssay enables the concomitant identification of genetic variants known to influence beta-thalassemia disease severity. Based on the presence of positively modifying alleles, and combined with alpha- and beta-globin genotyping, it allows the prediction of patients likely to display less severe phenotypes. Favourable properties, such as the rapid DNA extraction protocol, ready-to-use reagents and teststrips, as well as the potential for automation of the hybridisation/detection and interpretation steps, make the StripAssay convenient and easy to perform within less than six hours. Conclusions: Testing for genetic modifiers influencing disease severity will lead to more specific and effective treatment, and support clinical decisions regarding the beginning of transfusion therapy in beta-thalassemia patients. Furthermore, the knowledge about prognostic markers has implications for genetic counselling and prenatal diagnosis.

P07.37-S
Family with inherited thrombocytopenia and homozygous pathogenic variant in *FYB* gene

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Inherited thrombocytopenias (IT) are a heterogeneous
group of rare diseases characterized by a reduced number of blood platelets.The frequency of IT is probably underestimated because of diagnostic difficulties and because not all the existing forms have yet been identified, and some patients remain without a definitive diagnosis. We report a family with IT with small size platelets seen in several members of a highly consanguineous Kurdish family from Northern Iraq. Genotyping of all affected, their unaffected siblings and parents, followed by exome sequencing revealed a strong candidate pathogenic variant in a homozygous state: a frameshift mutation was detected in the *FYB* gene. The protein encoded by this gene is a cytosolic adaptor molecule expressed by T cells, natural killer (NK) cells, myeloid cells and platelets and is known to be involved in platelet activation and controls the expression of interleukin-2. This is the first report to hypothesize that pathogenic variants in *FYB* gene could cause thrombocytopenia in human.
We propose that *FYB* is the causative gene for this phenotype.

P07.38-M
Distribution of A736V variant of TMPRSS6 gene in beta-globin mutation carriers

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The genome-wide association studies on the genes related with iron metabolism has been showed that some of the genetic variants were associated with serum iron level and erythrocyte parameters. One of these common variants was A736V in TMPRSS6 gene which regulates serum iron level. Inactivation of TMPRSS6 gene causes iron-deficiency anemia and impairment of iron absorption. It has been found that A736V variant of TMPRSS6 gene is associated with lower levels of serum iron and erythrocyte MCV with higher levels of serum hepcidin protein. It was also shown that inhibition of TMPRSS6 has a therapeutic effect on Beta-thalassemic mice. However the frequency of this variant among Beta-thalassemia patients is unknown. Related with these data, we aimed to investigate frequency of A736V variant of TMPRSS6 gene among patients carrying beta-globin gene mutations. 93 patients investigated for beta-globin gene mutation with DNA sequencing method were enrolled in this study. A736V (rs855791) variant of TMPRSS6 gene was detected by real-time quantitative PCR method. Erythrocyte parameters and hemoglobin levels were measured and their distributions according to gene variants were analyzed. Frequency of TMPRSS6 gene A736V variant was 0.47 among all patients. There was no association between A736V variant of TMPRSS6 gene and beta-globin gene mutations, hemoglobin levels or erythrocyte parameters. However, frequency of A736V variant was higher among the patients having low levels of hemoglobin or erythrocyte MCV with a wild type beta-globin gene. Further studies are needed for better understanding the relationship between of A736V variant and Beta-thalassemia prognosis.

P07.41-S
Novel 21 nucleotide duplication in alpha1/alpha2 globin gene involves in a variety of hypochromic microcytic anemia from mild to Hb H Disease

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α-Thalassemia (α-thal) is a common genetic disorder in Iran and many parts of the world. Genetic defects in alpha-globin gene cluster can results in α-thal that may develop clinical phenotype varying from almost asymptomatic to a lethal hemolytic anemia. Loss of one functional α-gene, indicated as heterozygote α+-thal, show minor hematological abnormalities. Homozygosity for α+ or heterozygosity for α⍰-thal have more severe hematological abnormalities due to a markedly reduced α-chain output. At the molecular level, the absence of three α-globin genes resulting from the compound heterozygous state for α⍰- and α+-thal, lead to Hb H disease. Here we present 21 bp duplication consists of 6 amino acids and 3bp of intronic sequence at exon-intron boundary, in the both α-globin genes. This duplication was detected in three patients originated from two different Iranian ethnic groups and one Arab during of more than 12 years. This duplication was found by direct DNA sequencing. The clinical presentation of these patients varies widely from a mild asymptomatic anemia (heterozygote in alpha1globin gene) to a severely anemic state, requiring blood transfusions (duplication in alpha2 globin gene in combination with MED double alpha globin gene deletion) who was diagnosed as an Hb H patient. Third patient who was homozygote for this nucleotide duplication in alpha1 globin gene shows severe hypochromic microcytic anemia and splenomegaly. In the last decade numerous α-globin mutations have been demonstrated the necessity of prenatal diagnosis for α-thal, and this study have contributed another one as an important novel mutation to be considered.

P07.42-M
Recovery of SLAM-SAP signalling pathway factors mRNA expression and invariant NKT cell deficiency characterizes sarcoidosis remission

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Invariant Natural killer T Vα24-Jα18-Vβ11 (iNKT) cells play critical role in controlling the strength and character of immune responses and have shown to be important in disorders with increased Th1 responses, such as sarcoidosis. Their exact role as well as factors involved in regulating the development and recruitment of iNKT cells is still to be determined. There is growing evidence that the SLAM-SAP signalling pathway is essential for the development of iNKT cells. In our study we followed up the mRNA expression of SLAM-SAP signalling factors and iNKT cells together with detailed clinical data in newly diagnosed sarcoidosis patients over 4 years.
Detailed clinical, functional, and radiographic evaluation and determination of blood mRNA expression of SLAM-SAP signalling factors and iNKT cells was carried out at presentation and after 3 months, 1 year, and 4 years of disease follow-up in 29 patients with pulmonary sarcoidosis. We also included 28 healthy control subjects.
We demonstrated a decreased expression of SLAM-SAP signalling factors

and marked deficiency of blood and lung iNKT cells in patients with newly diagnosed sarcoidosis. During 4 years of disease follow-up, there was a significant increase in expression of SLAM-SAP signalling factors, mainly *SLAMF1*, *SLAMF6*, and *FYN,* and blood iNKT cells. This increase clearly correlated with improvement in patients' clinical symptoms as after 4 years the disease had gone into remission in the great majority of patients.
Our longitudinal study showed that an increase in expression of SLAM-SAP signalling factors and iNKT cells characterizes the clinical remission of pulmonary sarcoidosis.

P07.43-S
A late diagnosis of NOMID-syndrome /CINCA-syndrome - lessons to learn

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The case of a 10 ½ year old boy from Germany is presented with a new diagnosis of NOMID syndrome, also known as CINCA-syndrome, the rarest and most severe of the Cryopyrin-associated periodic syndromes (CAPS), which are genetic syndromes of autoinflammation. The diagnosis was delayed despite many clinical clues including the typical non-itching maculopapular rash which was present from the neonatal period, raised inflammatory markers on many occasions without detectable infectious pathogens, progressive macrocephaly with hydrocephalus, evolving short stature, progressive optic atrophy, progressive deafness, progressive arthropathy and other non-specific haematological and immunological abnormalities. In addition, the patient had episodic conjunctivitis and finger clubbing. Periodic fever does not always occur in this condition and in the case of this patient was completely absent. This patient was treated for several years with growth hormone in the absence of a diagnosis without any improvement of his short stature. Early diagnosis of these patients is vital because treatment with interleukin -1-receptor antagonists is now well established and very effective. Untreated, the patients may develop a destructive arthropathy, blindness, profound deafness and renal failure secondary to amyloidosis. The macrocephaly, hydrocephalus and short stature result from the chronic aseptic meningitis. The disease is caused by mutations (mostly new dominant) in the NLRP3 gene, which may be mosaic and thus are sometimes only detectable by next generation sequencing.

P08.01-S
Chromosome 15q11.2 imbalances associated with neuropsychiatric and developmental disorders - array-CGH findings in a cohort of 1000 patients

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Array-Comparative Genomic Hybridization has led to the knowledge that some copy number variants (CNVs) correspond to susceptibility loci for developmental disorders. CNVs at chromosomal region 15q11.2 involving 4 known genes, TUBGCP5, CYFIP1, NIPA2 and NIPA1, are of challenging interpretation due to their presence both in normal populations and in individuals with diverse developmental disorders. In a cohort of 1000 patients analyzed by Agilent 180K oligonucleotide array-CGH we identified 12 patients with 15q11.2 imbalances, 9 deletions and 3 duplications, 7 females and 5 males. Four of the 12 patients had additional genomic imbalances. The patients presented with global developmental delay, dysmorphisms, ID, epilepsy, mirocephaly, amongst others. To date, we were only able to determine inheritance in 4 patients, 2 deletions maternal in origin, 1 paternal, and a de novo duplication. The proximal breakpoint was common in 11 of the 12 patients, while the distal breakpoint was variable, but similar in some patients. The 4 previously mentioned genes were involved in the genomic imbalances of all the patients, except in the patient with the distinct proximal breakpoint, where TUBGCP5 gene was in normal copy number. Functional data have revealed that TUBGCP5, CYFIP1 and NIPA1 genes are expressed in developing mammalian brain and are involved in processes such as microtubule nucleation, interaction with other proteins and nervous system development and regulation, respectively To date, there is still no straight interpretation when a 15q11.2 genomic imbalance is detected, but in this cohort further evidence was given that this region is associated with neuropsychiatric disorders.

P08.02-M
Overgrowth and developmental delay associated with a 200 kb deletion in 16p11.2 in two families

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Due to a high density of segmental duplications, the short arm of chromosome 16 is prone to a number of recurrent rearrangements. A common 600 kb microdeletion or -duplication in 16p11.2 has been associated with autism, intellectual disability, schizophrenia, and mirrored weight and head circumference phenotypes. An adjacent, but separate distal 200 kb region in 16p11.2, which contains the SH2B1 gene, has been associated with isolated obesity as well as developmental delay. Both aberrations are associated with high variability and incomplete penetrance.
We now report on two families harboring the 200 kb microdeletion in 16p11.2. Both index patients were initially referred with suspected Sotos syndrome due to tall stature, obesity and developmental delay. After normal NSD1 testing molecular karyotyping showed the 200 kb deletion in 16p11.2 in both patients as well as in patient 2's brother who had mild obesity and unspecific moderate intellectual disability. In both families the deletion was maternally inherited. Interestingly, patient 2, but not her cognitively more severely affected brother, additionally harbored a microduplication 1q21.1, which has been recurrently associated with variable and incompletely penetrant developmental delay, intellectual disability, behavioural anomalies and large head circumference. Both aberrations were inherited from the mother, who was obese but otherwise healthy and without cognitive problems. These two families further characterize the variable spectrum of phenotypes associated with the 200 kb microdeletion in 16p11.2. Our findings in family 2 also show that not even the co-occurrence of two ID-associated microaberrations necessarily leads to cognitive impairment.

P08.03-S
Clinical and molecular characterization of a patient with de novo 2.8 Mb deletion of 17q24.2-q24.3

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Microarray methods contributed to the identification of many rare microdeletion syndromes including that associated with deletions of 17q24.2-q24.3, characterized mainly by growth retardation, microcephaly, developmental delay, speech delay, intellectual disability, feeding problems, and teeth and facial abnormalities. Seven patients have been described in detail since 2008, and additional cases with only limited clinical information are listed in databases. It becomes evident that not all 17q24.2-q24.3 deletions share a single common region of overlap.
We report a 12-year-old girl with a normal karyotype who shows clinical features consistent with the syndrome. SNP array analysis revealed a 2.8 Mb long deletion of 17q24.2-q24.3 (chr17:65,281,651-68,017,013; hg19). FISH analysis confirmed the deletion and showed its de novo origin. The deletion affects 18 protein-coding RefSeq genes including PRKAR1A, MAPK2K6, and the ABCA gene cluster. Some of the genes could be candidates for the clinical symptoms and some are predicted to exhibit haploinsufficiency, for instance PRKAR1A, encoding a postsynaptic density protein associated with Carney complex, or BPTF producing a transcript in fetal brain which binds FMRP and encodes a transcriptional regulator. Only much smaller population copy number polymorphisms are present in the region. The deletion of our patient overlaps two groups of previously reported deletions. Despite the proximity of the deleted regions, the candidate genes could be distinct. Identification of additional cases will help to define the genes involved and their role in predisposition to neuropsychiatric phenotypes, as well as the emerging genetic heterogeneity within the 17q24.2-q24.3 microdeletion syndrome.
Supported by CHERISH, NT/14200, 00064203 and CZ.2.16/3.1.00/24022.

P08.04-M
Deletions of 1p34.3, encompassing the *AGO1*, *AGO3,* and *AGO4* genes, in five children with microcephaly and intellectual disability

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Small RNAs (miRNA, siRNA, and piRNA) regulate gene expression through RNA interference (RNAi), a process that has emerged as a fundamental principle of normal cellular function. The Argonaute (AGO) proteins are critical mediators of the RNAi pathway and constitute a highly conserved family of genes found in almost all eukaryotes. Four AGO genes are present in humans, three of which (AGO 1, 3, and 4) reside in a cluster on chromosome 1p35p34.

The possible effects of germline AGO mutations or dosage alterations in humans is not known, however, animal models deficient for AGO proteins display developmental brain defects including a reduction in total number of neurons and glia. Moreover, different studies have established that miRNA and siRNA are prevalently or exclusively expressed in the brain, where they play an essential role in the development and function of the central nervous system (CNS).

We describe five patients with hypotonia, microcephaly, intellectual disability, and facial dysmorphisms, in whom array-Comparative Genomic Hybridization revealed overlapping de novo microdeletions of the chromosomal region 1p34.3. The minimal critical region is a segment of approximately 694 Kb that encompasses the AGO1, AGO3, and AGO4 genes.

We propose that the neurocognitive deficits present in these patients are due to deletion of the 1p34.3 region and resulting haploinsufficiency of several AGO genes. It seems plausible that the 1p34.3 deletion syndrome may eventually be recognized as a neurodevelopmental disorder associated with RNAi deregulation, however, further investigation is necessary to prove this.

P08.05-S
Two brothers with 2q23 microdeletion syndrome inherited from their mosaic father

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Patient 1, the older brother, is 13 years old and has moderate intellectual disability. He communicates by single words, pointing and some signing. He walked at 4 years. He uses diapers, suffers from constipation and manifests aggressive outbursts and stereotypic movements. His sleeping pattern is disturbed by periods of awakeness. He has teeth enamel erosions. His twin sister, with Apert syndrome, died at 2 years. Patient 2, the younger brother, is 6.5 years and has moderate intellectual disability. He speaks a few words and infrequently combines two words. He has a heart murmur, enamel teeth erosions and periodic sleep problems. He is restless and active.

ArrayCGH of the patients and father showed a deletion of chr2q23.1(148705701-148926786 bp), including the MBD5, considered critical in the 2q23.1 microdeletion syndrome. By FISH the deletion in the father was found to be a mosaicism found in 73% of lymphocytes. Assessment of the mosaicism level in additional tissues is ongoing. The father received speech therapy in childhood and has dyslexia. He completed secondary school with low-average marks, and did not finish high school. He has been working as a manual worker in the same factory for 18 years. In patient 2 and his father aCGH detected also a chr15q26.1(9022468-90255068 bp) deletion, including PLIN1. PLIN1 mutations cause dominant familial partial lipodystrophy type 4, but the effect of PLIN1 haploinsufficiency is unknown. Patient 2 and his father, but not Patient 1, are obese.

To our knowledge this is the first familial case of the 2q23 microdeletion syndrome.

P08.06-M
Microdeletion of 8q21 region: clinical and molecular analysis based on a new case

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Submicroscopic deletion of 8q21.11 is a rare cause of intellectual disability, developmental delay and craniofacial dysmorphy. Hypotonia, impaired balance, sensorineural hearing loss, abnormal behaviour as well as mild fingers and toes anomalies are frequently observed. To date, 13 cases, including 5 from the same family, have been clinically and molecularly characterized. Here we report on the case of a 14-year-old girl with moderate mental retardation, numerous dysmorphic features (a round face with full cheeks, high forehead, ptosis, long and downslanting palpebral fissures, short philtrum, Cupid's bow of the upper lip, down-turned corners of the mouth, micrognathia, high palate, low-set and prominent ears, and short neck), short stature and overweight. At birth, microtia of the right ear with external auditory duct stenosis and atrial septal defect were diagnosed. Muscle tone was unremarkable. Other features comprise small hands with camptodactyly of fifth and second fingers of the opposite hands, unilateral transversal crease, and valgus, flat feet. Auto-aggressive behaviour, autism and sleep problems were also noted. Whole-genome microarray analysis revealed a 5.19 Mb deletion of 8q21.11q21.13 region encompassing 19 genes. The phenotypic and genetic findings of our patient will be compared with those of previously reported patients. We indicate several candidate genes, providing new data supporting further genotype-phenotype studies. Our results suggest that haploinsufficient genes within the deleted region, e.g. ZFHX4/STMN2, FABP4/FABP5 and HEY1, could underlie the intellectual impairment, excess weight and cardiovascular disorders, observed in 8q21 microdeletion. This study was supported by MNiSW Grant No. 0193/IP1/2013/72.

P08.07-S
Clinical and molecular delineation of the emerging 8q22.3 microdeletion syndrome

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Background: Five patients with deletions that involving chromosome 8q22.2-q22.3 has been recently reported. Four patients shared a similar craniofacial phenotype with microcephaly, blepharophimosis, uni or bilateral ptosis and little facial expression. They have moderate to severe intellectual disability (ID) and impairment or absent speech. These carrying an overlapping region deletion of 3.87 Mb (hg19:100.69-104.56). The other patient not showed this facial gestalt but presented moderate ID, and speech impairment. She presented the shorter deletion of 1.92 Mb (hg19:102.01-103.93). **Methods and Results:** Here, we described on 18-year-old female patient with microcephaly, unilateral ptosis, moderate ID and speech impairment. CytoScan HD array analysis identified a 2.95 Mb 8q22.2-q22.3 deletion. The comparation of the present patient with the above five cases contribute to narrow down the critical region in 8q22.3 of ~1.47 Mb (hg19:101.95-103.42). Furthermore, we analyze the gene functional roles and probability of haploinsufficiency (HI) of this genomic region; resulting the ubiquitin protein ligase E3 component N-recognin-5 (*UBR5)* gene as the best candidate for the phenotype. *UBR5* belongs to ubiquitin/proteasome system (UPS). Neurodevelopmental genetic disorders involving alterations in UPS have been described such as Angelman syndrome (*UBE3A*), Johanson-Blizard syndrome (*UBR1*) and X-linked intellectual disability type Nascimento (*UBE2A*). Recently, mutations in ubiquitin protein ligase E3B *(UBE3B)* in a blepharophimosis-ptosis-intellectual disability syndrome, which fits Kaufman oculocerebrofacial syndrome have been recognized. **Conclusions:** We suggested that this novel 8q22.3 microdeletion syndrome had a variable expressivity and proposed that the HI of *UBR5* could lead to this peculiar craniofacial phenotype and ID of this novel genomic disorder.

P08.08-M
An atypical inherited ATR-16 syndrome unrelated to SOX8 haploinsufficiency

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We report the case of a girl affected by mild mental retardation, macrocephaly and microcytic anemia. Clinical examination did not reveal significant dysmorphic features, except for frontal bossing and mild hypertelorism. Also her mother presents with mild intellectual disability.

Array-CGH analysis detected a 493.1 Kb telomeric deletion on chromosome 16p13.3, which was found to be maternally inherited. Further examination of mother's medical history revealed that she was also affected by microcytic anemia, which had been treated with oral iron therapy without benefit. The deletion involves the two alpha-globin genes (HBA1 and HBA2), explaining the haematological defect.

Deletions of 1.5 and 2 Mb involving the telomeric short arm of chromosome 16 cause the contiguous gene syndrome ATR-16 (MIM #141750) characterized by alpha-thalassemia, mental retardation and variable dysmorphic features (downslanting palpebral fissures, mild hypertelorism, broad nasal bridge, small ears and a short neck with webbing). Haploinsufficiency of SOX8 (MIM #605923) is thought to be responsible for intellectual disability. The deleted region in our patient does not include SOX8, whereas it comprises 31 genes whose function is still partially unknown, except for HBA1 and HBA2.

To our knowledge this is the smallest 16p13.3 telomeric deletion characterized by mental retardation and microcytic anemia, narrowing the critical region and pointing at other candidate genes for intellectual disabilities. Moreover this is the first case of inherited ATR-16 syndrome.

P08.09-S

Intellectual disability and autistic behavior due to de novo microduplication of Xq28 involving part of the AFF2 (FMR2) gene. Is this a plausible explanation?

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FRAXE is an X-linked form of intellectual disability characterized by mild to moderate cognitive impairment, speech delay, hyperactivity, and autistic behavior. The folate-sensitive fragile site FRAXE is located in Xq28 approximately 600 kb distal to the fragile X syndrome fragile site (FRAXA) and harbors an unstable GCC triplet repeat adjacent to a CpG island in the 5' UTR (untranslated region) of the AFF2 (FMR2) gene. The disorder results from amplification and methylation of the GCC repeats and consequence silencing of AFF2. Although chromosome abnormalities (mostly deletions) that disrupt AFF2 have been reported with mild-moderate intellectual disability, microduplication of Xq28 that partially involved AFF2 has not been described as a potential cause of FRAXE. We performed clinical and molecular characterization of patient with 301 kb interstitial de novo duplication at Xq28 (chrX:147˙490˙437-147˙791˙737bp)(GRCh37/hg19). This genomic mutation leads to the duplication of part of 5' UTR region of the AFF2 (FMR2) gene which could be inactivated due to this genomic change. Namely, duplication of this segment potentially could have the same effects as amplification and methylation of an unstable GCC triplet repeats. That's why we are performing experiments aimed to study genic expression/metilation. As our patient had cognitive impairment, speech delay, hyperactivity and mild dysmorphism, we predict that partial duplication of AFF2 in our patient could be the cause of his phenotype.

P08.10-M

Functional studies of ARX mutants linked to neurophenotypes and Application of rescue strategies targeting KDM5C down-regulation

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Neurophenotypes linked to Aristaless-related homeobox (ARX) mutations present a huge spectrum of brain diseases including cortical malformations, malignant epilepsy and intellectual disability (ID). We have recently identified a crucial disease path, linking functionally ARX to another XLID/Epilepsy gene, Lysine-specific demethylase 5C (KDM5C). It encodes an H3K4me2/3 demethylase required in neuronal differentiation. We found that ARX mutations, affecting PolyAlanine tracts or HD domain, cause functional damages of the ARX-KDM5C interaction, which severity depends on the type of alteration. In Arx KO embryonic brain and ES-oriented GABAergic neurons, a defective ARX-KDM5C-H3K4me3 path has been found. We tested in vitro correction of KDM5C-H3K4me3 defects, ideally through endogenous physiological mechanisms. We explored three different approaches: transcription factor targeting, gene knock-up by SINEUP non-coding RNA technology and epigenetic modifications. To UP-regulate transcription, we forced KDM5C transcription by PHF8/ZNF711 stimulation, a transcriptional complex that seems to not synergize with the action of ARX. To UP-regulate translation, taken advantage of SINEB2 lncRNAs (SINEUPs) acting as activators of translation, we generated synthetic lncRNAs antisense to KDM5C gene. In SINEUP KDM5C-transfected neuronal cell lines, we obtained increased levels of endogenous KDM5C protein. To achieve epigenetic correction of KDM5C path, we screened also a number of compounds targeting chromatin enzymes. A strong compensation of KDM5C down-regulation has been obtained at crucial time-point of neuronal commitment upon treatment with an HDAC inhibitor. Ongoing efforts to define rescue strategies may help to identify useful tools towards drug discovery for ARX XLID/Epilepsy phenotypes and many other disorders with malignant seizure.

P08.11-S

Defining a link between retinoic acid and autism: molecular approximation for exome sequencing results

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Autism spectrum disorder (ASD) is a range of complex neurodevelopmental conditions principally characterized by dysfunctions linked to mental development. The understanding of its genetic basis is difficult, mainly because the high allelic and locus heterogeneity together with the variable symptomatology. Therefore, multiple approaches have been applied to understand its genetic basis. Thousands of clues have emerged from initial exome sequencing (ES) studies, identifying possible de novo Novel (DNN) mutations associated to ASD. Since most of the variants reported to date were found mostly in Caucasian or European descendant, we applied ES in a cohort of Colombian - South American (admixed population) trios. In this previous study, we found two DNN non-synonymous mutations in genes ALDH1A3 and FOXN1 in the same child. Using bioinformatic approximations, we were able to locate several Retinoic Acid Response Elements (RARE) upstream of both genes in humans, as well as in mice. Chromatin immunoprecipitation (ChIP) followed by QPCR determined the relationship between Retinoid Acid Receptor ß (RARß) in adult piriform cortex and in embryonic whole brain.

P08.12-M

Array Comparative Genome Hybridization (aCGH) in children with autistic spectrum phenotype

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Aim: Autism or autistic spectrum disorders (ASD), are a group of complex neurodevelopmental disabilities, affecting social interaction and communication skills (Rosti R O et al 2013). Only α few cases of chromosomal abnormalities are identified by conventional cytogenetic techniques while molecular karyotype allows for the detection of submicroscopic genomic rearrangements providing a diagnostic yield ranging from 10% to 18% (Shen Y 2010). **Material- Methods:**. A total of 629 patients were studied by the Department of Medical Genetics, from which 114 fulfilled the the autism/ ASD clinical criteria The arrays used were high definition1X244K and 4X180K with the additional ability of SNP identification for the detection of uniparental disomy UPD and /or copy neutral loss of homozygosity (LOH).. All patients were referred by Clinical Geneticists after detailed evaluation and normal conventional karyotype. **Results:** Submicroscopic genomic rearrangements (CNVs), 0.08-19.01 Mb in size were detected in 55/114 subjects with autism or ASD, with the following most important genes previously implicated in the etiology of autism or ASD: *NRXN1, SHANK3, DOCK8, ZNF92, ASMT, HSFX1, KCNH7, CHRFAM7A, CHRNA7, KCND2, CNTNAP3, MAOA, MAOB, STS, VCX* **Conlusions** The identification of genetic alterations (CNVs) in a affected child with autism/ ASD allows more specific consultaion to the family about diagnosis, prognosis and recurrent risks, while genetic reevaluation with aCGH is also warranted in adult autism patients.

P08.13-S

Phenotype of 20 novel autosomal recessive cognitive genes- a report from Iran

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After releasing 50 novel intellectual disability (ID) genes by Najmabadi et al. in 2011, most of researchers working on cognitive pathway in human and animal models were interested in phenotypically aspect of some of these genes to reach the function of changes in mouse, drosophila and human brain.

From 50 novel candidate genes of ID that have been reported, 20 families had additional features that some of them could have been related to the effect of these genes on neuronal networking. Ten out of 20syndromic families with microcephaly or neurological symptoms were selected and MRI was performed for one or two affected siblings in each family. In the previous study, homozygosity mapping, coupled by conventional sequencing or next generation sequencing (NGS) were used to define underlying genetic defects.

From total of 10 families, eight families showed different types of abnormalities as periventricular leukoencephalopathy, reduced cerebral cortex, pachygyria, polymicrogyria, small corpus callosum and cerebellar hypoplasia on MRI including subjects with mutations in CAPN10, TAF2, CNKSR1, WDR45L, ERLIN2, PARP1 and SLC31A1 genes. Three syndromic families had normal brain architecture (ZBTB40, KIF7 and TMEM135).

More detailed of syndromic families have been described based on the table of novel cognitive genes published by Najmabadi et al. in Nature Oct 2011.

P08.14-M

Mutation in CAPN10 Causing Intellectual Disability in Two Independent Iranian Families with Overlapping Phenotypes

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Intellectual disability (ID) is a broad diagnosis encompassing a wide variety of phenotypes and severities. There are many reasons why the genetics of intellectual disability have been difficult to unravel, but the most important are extensive genetic and phenotypic heterogeneity in autosomal-recessive (AR) inheritance of ID. Therefore, our joined researches on ID started in 2003 with abroad centers to elucidate the molecular causes of ARID in Iran. As a part of this collaboration, whole genome homozygosity mapping and exome sequencing was performed to identify novel genes and mutations in two Iranian families affected with ID.
In our previous study, published in Nature 2011, we reported on a mutation in CAPN10 gene In a consanguineous Iranian family with syndromic ID. In the current study, two novel nonsense mutations were discovered in two unrelated Iranian families with ID in CAPN10 gene, which encodes calpain10 protein eight isoforms and involves in brain function, NIDDM and other cellular activities. In this study, in addition to the above mentioned mutations, we observed overlapping clinical findings including microcephlay, ID and distinct brain MRI features in two independent Iranian families. Here, our aim was to introduce CAPN10 as a promising candidate gene in syndromic ID.

P08.15-S

De novo single exon deletion of AUTS2 in a patient with profound intellectual disability

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The autism susceptibility candidate 2 (AUTS2) gene has a critical role in early brain development with its strong expression in fetal and adult brain. Association of AUTS2 with intellectual disability (ID), autism spectrum disorders, and other neurodevelopmental disorders has recently gained more attention. Genomic rearrangements and copy number variations (CNVs) involving AUTS2 have been implicated in a range of neurodevelopmental disorders with or without congenital malformations and dysmorphic features. Here we report a 127 kb de novo deletion encompassing exon 5 of AUTS2 at 7q11.22 which result in inframe deletion of 10 amino acids. The deletion was detected by SNP-array analysis applying InfiniumHD whole-genome genotyping assay with the HumanCytoSNP-12 BeadChip (Illumina Inc.). Obtained data were analyzed with Illumina GenomeStudio and QuantiSNP software. The single exon deletion was detected in a 10 year-old female patient with severe speech disorder, intention tremor, fine motor activity deficit, behavior disturbance, residual lesion in the CNS, and intracranial hypertension. This is one of the smallest de novo intragenic deletions of AUTS2 described in patients with neurodevelopmental disorders. Along with the review of previously reported 20 cases with small pathogenic CNVs and 4 cases with different de novo balanced translocations of 7q11.2 interrupting the AUTS2 gene with severe profound to borderline ID without autistic features or delayed psychomotor development with mild to moderate autism, this report provides additional insight into the clinical spectrum of AUTS2 disruptions.

P08.16-M

Whole exome sequencing detected a novel PHF6 mutation as the cause of Börjeson-Forssman-Lehmann syndrome in four males in a Danish pedigree

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Börjeson-Forssman-Lehmann syndrome (BFLS) is a rare X-linked mental retardation syndrome. BFLS was first described in 1962, and in 2002 the PHF6 gene at Xq26 was identified as the responsible gene.
Approximately 20 cases have been reported with mutations in the PHF6 gene. At least 12 different PHF6 mutations have been found in both familial and sporadic cases, predominantly missense and truncation mutations. Five recurrent mutations have been reported, which arose independently.
The main clinical features are normal birth weight, feeding problems and hypotonia in infancy, developmental delay, mild/moderate mental retardation, large ears, short toes, small genitalia, gynaecomastia, truncal obesity, tapered fingers, and coarsening of facial features.
We report a Danish family with four boys with developmental delay aged 3-12 years. They were related as brothers/cousins with mothers being sisters. All four boys had normal birth weight, poor suck, hypotonia, developmental delay, no language, big ears, small penis and testes, tapered fingers, and broad distance between 1st and 2nd toes. The pedigree was consistent with X-linked inheritance. Whole-exome sequencing was performed by exome capture (AgilentSureselect) followed by sequencing of all exons and exon/intron boundaries at the X chromosome. A hemizygous mutation p.M1V in the PHF6 gene was found in all four boys and in heterozygosity in the two mothers and the grandmother. The mutation alters the start codon where a p.M1T mutation has been reported previously. The mutation caused BFLS in the four affected boys with three unaffected carrier females. BFLS might be underdiagnosed due to its rather unspecific phenotypic characteristics.

P08.17-S

TALEN-mediated mutagenesis as a tool to generate disease models for diseases caused by dominant de novo mutations

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Engineering of the mouse germline has long been used to create targeted mutants. The TALEN-mediated approach based on embryo microinjection of TALENs was shown to result in up to 50% efficiency of germline mutated offspring. As TALENs can modify one or two copies of the target gene either before or after genome replication, mutant genotypes identified in the founder generation may represent heterozygous, compound heterozygous or mosaic state. The high efficiency could allow the generation of mouse models for diseases caused by dominant de novo mutations. Here we tested this approach to generate a mouse model for X-linked dominant neurodegeneration caused by de novo mutations in WDR45. After microinjection of TALEN mRNA surviving Embryo's have been transferred in groups of 10 into the oviducts of 3 pseudopregnant females resulting in 19% mutated founders (5/26). Mutations are deletions between 10 and 57bp in length with a predicted frameshift in 80% of mutations. From the in situ design and delivery of the TALENs (~1month), over in vitro testing in cell culture to the in vivo injection into embryo's (~2months) until the genotyping of the founder mutants it only takes 4-5 months. Hence, this presents an innovative tool to investigate de novo arising mutations in mosaic status which would be embryonic lethal.

P08.18-M

NR2F1 mutations cause optic atrophy with intellectual disability

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Optic nerve atrophy and hypoplasia can be primary disorders or can result from trans-synaptic degeneration arising from cerebral visual impairment (CVI). CVI comprise a class of disorders of the projection and/or interpretation of visual input in the brain, and symptoms can consist of low vision, visual field defects and abnormal visual behavior. Here we report six individuals with CVI and/or optic nerve abnormalities, born after an uneventful pregnancy and delivery. The affected individuals show mild to moderate intellectual impairment, without specific facial dysmorphisms. In three patients large optic disc excavations were seen, which are, until now, only reported in CVI caused by perinatal damage. In four of the affected persons *de novo* heterozygous missense mutations in *NR2F1* were identified. In the two other affected individuals SNP array investigations revealed heterozygous deletions on 5q14.3q15 of 0.84 Mb and 2.83 Mb encompassing 4 and 5 genes, respectively, including *NR2F1*.
NR2F1 encodes a nuclear receptor protein that regulates transcription. A luciferase reporter assay showed that missense mutations in the zinc-finger DNA-binding domain and the putative ligand-binding domain decrease NR2F1 transcriptional activity. Additionally, previous studies in KO mice has indicated that the protein is important for neurodevelopment, including oligodendrocyte differentiation (partly in vitro experiment), cortical patterning, and guidance of thalamocortical axons, as well as eye and optic nerve development.

In conclusion, these findings indicate that *NR2F1* plays an important role in the neurodevelopment of the visual system and that its disruption can lead to optic atrophy with intellectual disability.

P08.19-S
Chromosomal microarray analysis of patients with intellectual disability, autism or multiple congenital anomalies presenting for genetic services

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Copy number variations (CNVs) are the most common identifiable causes of intellectual disability/developmental delay (ID/DD), autism spectrum disorders (ASDs), or multiple congenital anomalies (MCAs). Chromosomal microarray analysis (CMA), with a 10-20% diagnostic yield, can identify CNVs≤1 Mb. We report our experience with the use of the Affymetrix SNP Arrays in 1600 Italian patients during the past 6 years (2008-2013). We identified CNVs with a high score of pathogenicity in 415 (27%) patients. Among them 143 (34.4%) showed a CNV overlapping with a known syndrome, 272 (65.6%) a likely pathogenic rearrangement. Of particular interest, we found some CNVs useful to further delineate the clinical features associated with deletions in 8q12.1q12.3, in 15q25.2, in 17q21.31, in 2q24.1q24.2, in 22q11.2, and duplications in 16p13.3 and in 11p13. Some CNVs were useful to describe new syndromes such as a 1.7 Mb deletion in 3q13.2q13.31. Also, we have identified a large group of small CNVs (< 1.0 Mb) encompassing, either in whole or in part, functionally related genes to the phenotypes such as CASK, CNTN6, SNTG2, HIP1, DLG2, NRXN1, MCPH1 and CHL1 genes. Among these small CNVs, we have reported a FOXP1 gene microdeletion in a boy with autism and speech delay, and a de novo interstitial deletion of 0.122 Mb at 2q24.2 region harboring only TBR1 gene in a boy with moderate to severe intellectual disability. Variants of uncertain significance (VOUS) because unreported, containing genes of uncertain clinical significance or non-genic but potentially regulating nearby gene expression, were identify in 128 individuals (8%).

P08.20-M
The Cohen syndrome-associated protein COH1 functions as Golgi matrix protein required for Golgi integrity

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Mutations in the COH1 (VPS13B) gene cause autosomal recessive Cohen syndrome, which is mainly characterized by mental retardation, postnatal microcephaly, pigmentary retinopathy, and intermittent neutropenia. However, the biochemical characteristics, cellular localization, or functional role of the encoded protein COH1 (3997aa) have so far not been addressed. Our cell biological analysis showed strong co-localization of COH1 with the cis-Golgi marker protein GM130, which was preserved even upon chemical disruption of the Golgi architecture. Further biochemical analysis showed that COH1 is a peripheral membrane protein similar to its remote homologue, Vps13p in yeast. Vps13p has been found to regulate anterograde and retrograde vesicular transport of transmembrane proteins between the prevacuolar compartment and the trans-Golgi network. Consequently, we found that loss of COH1 upon RNAi impairs the ability of the Golgi ribbon to (re)assemble and thus induces fragmentation into mini-stacks. Moreover, we found that COH1 regulates the formation of Golgi-derived membrane tubules consistent with its predicted function in intracellular membrane traffic. Further protein-protein interaction studies identified COH1 as a potential effector protein of the Golgi-associated small GTPase RAB6, emphasizing a role of COH1 for Golgi-related vesicle transport. Thus, our accumulated evidence suggests COH1 as a molecular regulator of antero- and retrograde Golgi membrane trafficking. As RAB6-associated Golgi transport critically regulates neuronal development and neuron function the Cohen syndrome pathology is likely caused by a failure of intracellular vesicle transport.

P08.21-S
De novo heterozygous mutations in beta-catenin 1 (CTNNB1) appear to be a frequent cause of intellectual disability (ID)

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Intellectual disablity (IQ<70) affects up to 3% of the general population. Until recently, the underlying cause was unclear in about half of the affected individuals. The introduction of whole exome sequencing (WES) techniques enables elucidating the genetic background of ID.
We performed WES in a cohort of 250 individuals with unexplained ID and identified heterozygous de novo CTNNB1 mutations in five unrelated individuals (three frameshift, one stop, and one splice mutation). All five patients have severe motor delay, profound speech impairment, hypotonia of the trunk and hypertonia of the legs. The craniofacial phenotype comprises microcephaly (4/5) and some consistent facial features - a broad nasal tip, small alae nasi, long philtrum and thin upper lip vermillion.
Beta-catenin is a key downstream component of the canonical Wnt signaling pathway, and acts as a negative regulator of centrosome cohesion. Whereas somatic gain-of-function mutations have already been found in various tumor types, germline loss-of-function mutations were suspected in animal models to influence neuronal development and maturation. This was supported by the finding of dominant inactivating CTNNB1 mutations as a cause of ID in 3/865 patients (0.35%, deLigt etal., 2012). Their phenotype was additionally characterized by absent/limited speech, microcephaly and spasticity with severely impaired walking.
Our finding of five individuals in our cohort of 250 (2.0%) suggests that CTNNB1 loss-of-function mutations might be a more frequent cause of ID than estimated from the data of deLigt and colleagues. Our data further emphasize the importance of Wnt signalling in human brain development and/or function.

P08.22-M
Deletions limited to CTNND2 cause mild intellectual disability

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Using chromosomal microarray testing we detected a 113 kb de novo out of frame deletion encompassing exons 4-7 of CTNND2 in a patient with borderline ID. This gene was mapped to the cri-du-chat syndrome critical region in chromosome 5p15.2 and encodes a regulator of neuronal migration. CTNND2 was considered responsible for the severe intellectual disability in cri-du-chat syndrome patients with terminal deletions. Extended deletion mapping however indicated that interstitial deletions restricted to the CTNND2 locus produce a milder level of intellectual disability. The girl was born at term with no complication and normal measurements. Apart from 2 episodes of acute subglottic laryngitis there were no remarkable health problems. Developmental milestones were within normal limits. The patient was referred to developmental testing because of behavioural issues and was diagnosed with mild intellectual disability. She showed a dissociated cognitive profile with better language than nonverbal functions (full scale IQ 77) and suffered from short attention span, poor executive functioning and impaired working memory. Three other patients with deletions limited to CTNND2 were found in the DECIPHER database. One patient had a 413 kb deletion with mild intellectual disability, autism and hypotonia. The other patient showed a 479 kb deletion with learning difficulties, behavioral problems and autism spectrum disorder. In the third patient a 154 kb deletion was detected. He showed intellectual disability and neurological problems which may be caused by an additional unidentified disorder. We assume that CTNND2 haploinsufficiency is a novel cause of mild neurodevelopment features.

P08.23-S
A familial case of 15q26.3 microduplication

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The "15q overgrowth syndrome" has been associated with the very distal 15q duplication. At least 26 cases with trisomy of 15q25-26qter have been published, of which approximately 70% have presented with overgrowth and about 65% have been the result of a parental balanced translocation. Overgrowth has been associated with the dosage effect of *IGF1R* (insulin-like growth factor 1 receptor) gene located on 15q26. However, about 50% of

patients with larger duplications of distal 15q (15q21-24qter, including the *IGF1R* gene) on the contrary presented with growth retardation, although a few authors have reported overgrowth as well.
We report 12 and 8 years old brothers with identical 0.77 Mb microduplications in 15q26.3 region (chr15: 101,031,538-101,802,565, Hg19). The elder sib presents with postnatal overgrowth - at the age 12 his height is 175 cm (+4 SD), but his birth length was 52 cm (0 SD). The younger sib has normal growth (+1 SD). In addition, they both present with expressive speech disorder, some facial and hand microanomalies, and poor fine motor skills. Their father has overgrowth (+2.5 SD), prominent facial features and presented with dysarthria in childhood. Therefore, the duplication is with high probability of a paternal origin (analysis in work). This is the smallest "pure" 15q26 duplication reported so far. Interestingly, the *IGF1R* gene is not duplicated in our patients. Furthermore, they demonstrate variable clinical phenotype. Therefore, we give further evidence that a more complex pathogenesis for the development of somatic overgrowth should exist in case of distal 15q duplication.

P08.24-M
6p21.33 microdeletion associated with EHMT2 haploinsufficiency and intellectual disability

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Microdeletion of 6p21 is a rare condition that has been described in patients with multiple congenital malformation and intellectual disability. We report on a patient with a 0.4 Mb microdeletion in 6p21.33 and intellectual disability. The genomic loss contained 30 RefSeq genes ncluding *EHMT2* which is a primary enzyme for mono- and dimethylation at Lys 9 of histone H3 (H3K9me1/2), and plays critical roles in various biological processes. The 6-year-old boy was referred for evaluation of intellectual disability and macrocephaly. He was born at 37 weeks of gestation to a 32-year-old G1 P1 mother and 34-year-old father after uneventful pregnancy. The birth weight was 2418 g, length 45 cm, and head circumference 33 cm. His psychomotor development was delayed. He achieved head control at 4 months, sitting at 10 months, and walking alone at 2 years. He spoke his first word at 2.5 years. He never had seizures and his hearing was normal. His height was 106 cm (-1.7 SD), weight 20.3 kg (-0.3 SD), and head circumference 53.5 cm (+1.4 SD) respectively. Brain MRI at age of 4 years showed incomplete rotation of the bilateral hippocampus. *EHMT2* is a human homologue of mouse G9a that exists predominantly as a G9a-GLP heteromeric complex. The human homologue of the GLP is *EHMT1*, which is a causative gene responsible for Kleefstra syndrome with characteristic facial dysmorphism and severe intellectual disability. This case provides insight into the etiological mechanism of histone modification and human development.

P08.25-S
EPHA1 as a new candidate gene for autosomal recessive non-syndromic intellectual disability

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We report a Ukrainian family consisted of healthy and non-consanguineous parents and two affected children with moderate intellectual disability (ID) and similar psychoneurological symptoms. Biochemical and CGH-array investigations revealed no genetic abnormalities in children.
Whole exome sequencing performed in family members identified two non-synonymous variants c.1475G>A and c.1891G>A in the EPHA1 gene. Both affected siblings were compound heterozygotes while father and mother were heterozygous carriers for the c.1891A and c.1475A variants respectively. C.1475G>A and c.1891G>A frequencies analyses in 300 healthy Ukrainian controls revealed that the c.1475T allele frequency was 1,2% while the c.1891A was not found. EphA1 belongs to Eph receptors family implicated in axon guidance control but it was not previously associated with ID. To understand a possible effect of these substitutions the mutant EphA1 proteins' tertiary structures were predicted. As it turned out the substitutions are located in important functional domains of EphA1. The substitution of positive charged Arg492 to uncharged Gln492 (c.1475G>A) is in the fibronectin type III repeat of EphA1 ectodomain involved in signal transduction and binding with ligands or protein-partners. The substitution Gly631Arg (c.1891G>A) is in the glycine-rich region of EphA1 tyrosine kinase domain responsible for ATP binding. We assume the c.1475G>A and c.1891G>A mutations may cause changes in conformational flexibility and solubility of these EphA1 domains resulting in impaired Eph signal transduction.
Predicted structural EphA1 changes and low c.1475A and c.1891A frequencies allow us to hypothesize that missense mutations in the EPHA1 gene may be responsible for autosomal recessive non-syndromic intellectual disability.

P08.26-M
Mutations in FOXP1 result in a recognizable mental disability phenotype

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Mental retardation with or without minor dysmorphic features represents a major challenge in clinical genetics. Over a decade ago, the introduction of arrayCGH analysis introduced the concept of reverse genetics in clinical practice identifying recurrent phenotypes in patients with similar molecular defects. With the increasing resolution of arrayCGH analysis, monogenic defects can sometimes be readily identified. A careful clinical description of the concurrent phenotype may therefore direct specific gene analyses in patients with similar phenotypes ('gestalt' diagnosis) or may prioritize variant analysis through next-generation sequencing of gene panels or exomes.
We present three novel patients with FOXP1 mutations. All patients presented with moderate to severe intellectual disability with nearly absent speech, patient 3 additionally had tonic-clonic seizures, horizontal nystagmus, spastic tetraparesis and aggression. The facial features in these patients included a frontal upsweep, a broad forehead, broad and bent palpebral fissures, hypertelorism, a bulbous nasal tip, prominent nasolabial folds and a wide mouth. These striking features and the identification of a de novo 420kb intragenic deletion on arrayCGH analysis in patient 1 enabled us to identify a FOXP1 mutation (p.R525X) by direct sanger sequencing in patient 2 and to prioritize data analysis in a large gene panel analysis for mental disability in patient 3 (p.W508X mutation in FOXP1).
We further delineate the clinical phenotype due to FOXP1 mutations. Our and literature data evidence an emerging and recognizable syndrome. In the era of exome/genome analysis the clinical definition of phenotypes remains important to order to enable genotype-phenotype correlations.

P08.27-S
Triplet repeat-primed (TRP)-PCR changes the paradigm for Fragile X Syndrome (FXS) testing: Experience from the Greenwood Genetic Center

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Virtually all clinical testing for FXS revolves around the determination of the length of the CGG repeat tract in the 5'UTR of the Fragile X Mental Retardation (*FMR1*) gene. Repeat lengths generally fall into four categories, where <45 CGG repeats is accepted as normal, 45-54 repeats is the intermediate range (gray zone), 55-200 repeats is considered the premutation range, and >200 repeats is a full mutation. Diagnostic testing for FXS has traditionally employed both standard PCR and Southern blot techniques since they provide distinct, yet overlapping, levels of resolution and complementary pieces of information. However, newer PCR protocols such as TRP-PCR have the potential to allow amplification of full mutations, thereby providing a method of screening for expansions at the *FMR1* locus. The molecular diagnostic laboratory at the Greenwood Genetic Center has offered FXS testing for nearly 25 years. In 2010, we implemented a validated, lab-developed *FMR1* PCR assay utilizing commercially available TRP-PCR reagents (Abbott Molecular) into our routine diagnostic workflow. Here we summarize our experience with the TRP-PCR assay, discuss how the implementation of this assay has changed our diagnostic workflow, and compare the hands-on time, cost, and turn-around time for the samples tested over the past four years to these statistics for samples tested prior to the implementation of the TRP-PCR assay. In conclusion, the implementation of the TRP-PCR assay has allowed us to drastically reduce the hands-on time, turn-around time, and number of Southern blots performed for samples submitted to our laboratory for diagnostic FXS testing.

P08.28-M
A pilot study for prenatal and preconceptional Fragile-X syndrome screening in the Balearic Islands

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The Fragile X-syndrome (FXS) is caused by the expansion of a CGG triplet located in the 5'region of the FMR1 gene and is one of the most frequent

causes of hereditary intellectual disability. The estimated incidence of FXS in males in the Spanish population is 1 in 2500 with full mutation and about 1 in 250 with premutation (Fernandez-Carvajal et al. 2009). In women, although the prevalence in Spain of premutation has not been established, estimates in other western countries range from approximately 1/150 to 1/250. Given the severity of the disease, its high incidence in the general population, the exclusively maternal expansion, the familial and social impact of the FXS, and the high level of detection of current techniques (99%), we think that screening for FXS in women of reproductive age is a reliable and desirable option. Therefore, we have initiated a pilot study in the Balearic Islands to determine the feasibility and acceptability of prenatal and/or preconceptional screening in women of childbearing age. The results obtained so far, in a total of 3118 women (252 preconceptional and 2866 prenatal) indicate a high acceptability of testing both, in women that are referred for prenatal or preconceptional consultation. Surprisingly, the incidence of carriers of a premutation (55-200 repeats) is (to date) very high: 1 in 97, which may indicate a higher prevalence than previously thought. We will present updated results based on a total of approximately 3500 women.

P08.29-S
GPM6A is duplicated in a patient with learning disability and influences cholesterol response and long-term memory in *Drosophila melanogaster*

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In a patient with learning disability and behavioral anomalies we identified a *de novo* duplication of *GPM6A* by routine chromosomal microarray testing. Glycoprotein M6A (GPM6A) is a neuronal transmembrane protein of the PLP/DM20 family that associates with lipid rafts and promotes filopodia formation. GPM6A variants have not yet been implicated in cognitive impairment. An increase of membrane protrusions in our patient's lymphoblastoid cells supports a functional effect of this dosage alteration. To further study the function of GPM6A/m6 and the effects of m6 overexpression and knockdown, we employed *Drosophila melanogaster* as a model organism. We could show that, as described for other animal models, expression of *Drosophila* m6 is stress responsive. Using the courtship conditioning paradigm, we demonstrated that correct m6 levels are necessary for proper long term memory function, which indicates dosage sensitivity of m6 and supports a causative role of the *GPM6A* duplication for the cognitive impairment found in our patient. Defects in the close homolog *PLP1* are causative for Pelizaeus-Merzbacher disease (PMD), a severe demyelinating neurodevelopmental disorder. Prompted by recent results on successful therapy of phenotypes in PMD mice by the administration of a cholesterol-enriched diet, we investigated if the cellular phenotype of GPM6A/m6 dosage alterations could also be improved by cholesterol. Indeed, cholesterol supplementation partially improved the phenotypes observed in patient cells with GPM6A overexpression as well as in flies with m6 knockdown. Together with other recent findings, these data point to an involvement of cholesterol metabolism in the pathomechanisms of some ID forms.

P08.30-M
A Novel HCFC1 mutation associated with X-Linked Intellectual Disability

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X-Linked Intellectual Disability is a heterogeneous disorder with a variable phenotypic spectrum. Currently over 90 XLID genes have been found to be implicated in XLID. The host cell factor C1 (HCFC1) gene is located on chromosome Xq28 and is a member of the host cell factor family. A mutation in HCFC1 has been previously found to be associated with XLID in a non syndromic XLID family namely MRX3. Currently very few studies exist that further confirm HCFC1 as an XLID gene.
We present an XLID family with two affected sons having mild intellectual disability epilepsy and no congenital abnormalities. Fragile X analysis and array-CGH using a chromosome X exon-specific array were performed and revealed normal results. Subsequently, next generation whole exome sequencing analysis for both brothers was performed on Illumina HiSeq 2000 following Agilent SureSelect sample preparation (20x coverage). Reads were aligned using the Burrows-Wheeler Aligner and the Genome Analysis Tool Kit. Autosome variants and variants present in dbSNP 135 were filtered out. Analysis of rare X chromosome variants present in both brothers revealed a non-synonymous mutation in exon 4 of HCFC1 (p.Ala897Val). This mutation is located within GABP2 and ZBTB17 binding domains. Previous screening studies of patients have reported variants within the GABP2 binding domain p.Gly876Ser in an individual with autism spectrum disorder (Piton et al., 2011) and p.Ala864Thr in a patient with mental retardation (Tarpey et al., 2010). The mutation was confirmed by Sanger sequencing in both patients and their mother. Extended family studies are ongoing.

P08.31-S
HDAC8 duplication in a patient with de Lange -like phenotype

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K.K., female, was referred to our attention with a previous clinical diagnosis of Silver Russel syndrome. Prenatal ultrasound were normal. No prenatal cytogenetic examination was performed. She was born at 40+5 week of gestational age through cesarean section; birth weight was 2,320 (< 3rd pc), length was 45 cm (< 3rd pc), head circumference was 30 cm (< 3rd pc). The karyotype was normal (46,XX). Major malformations were excluded. We evaluated her at 6 years and a half; she showed prominent eyes, long thick eyelashes, posteriorly rotated ears, high nasal bridge; short and mild micrognathia and hirsutism. Her weight was 18,900 Kg (10th pc), length 110,5 cm (3-10th pc) and head circumference was 46,5 cm (<<3rd pc); she also presented severe mental retardation. Hands and feet X-rays showed hypoplasia of the 5th meacarpal and the 5th metatarsal. A CGH-array showed duplication of a part of HDAC8 gene (exon 5 and 6) from nt 71,630,467 to nt 71,697,179. HDAC8 gene was recently found implicated in Cornelia de Lange syndrome with variable clinical expressivity. Up now only complete deletion or mutation of the gene have been described. No reports of duplication of this gene are available. Our patient shows some clinical features related to de Lange syndrome also if her phenotype is not classic at all. Expression study are in progress in order to define the biological consequences of this finding and the relationships with the phenotype.

P08.32-M
Clinical characterization of a patient with a complex rearrangement involving duplication and deletion of 9p and 9q

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Rearrangements of the distal región of 9p are an infrequent chromosome imbalance in human beings. Trisomy 9p is the fourth most frequent chromosome anomaly in life-born and was characterized as a clinically recognizable syndrome, Rethoré syndrome. Kleefstra syndrome, previously named 9q subtelomeric deletion syndrome, is either caused by a submicroscopic deletion in 9q34.3 or an intragenic mutation of EHMT1. We report the first case with 9p duplication and 9q deletion in a Mexican patient. Patient was referred for dysmorphic facies and congenital heart disease. Clinical examination revealed brachycephaly, upslanting palpebral fissures, depressed nasal bridge, anteverted nares, long filtrum, downturned mouth, lingual protrusion, low set and cupped ears, short neck, small hands with clinodactyly and aberrant palmar creases, left testicle shrink and small feet. He presented an abnormal development. Conventional karyotyping reported 46, XY, add(9)(q34.3), 21 pstk+. Microarray analysis showed 9p24.3p23 (203,861-11-,842,172)x3, 9q34.3 (138,959,881-139,753,294)x3, 9q34.3 (139,784,913-141,020,389)x1. All procedures were normal in both parents. Partial duplication of 9p is one of the most commonly detected autosomal structural abnormalities in live born. It seems that the high frequency of the partial trisomy 9p may indicate a particular breakpoint sensitively of one or more regions of chromosome 9p. Patients with partial duplication of 9p display considerable phenotypic similarity. Our patient with a duplication of 11.6 Mb, from 9p24.2 to 9p23 displays some features like upslanting palpebral fissures, downturned corners of the mouth and developmental delay. In spite of the great duplication region, the major clinical findings in our patient corresponded to the deletion region.

P08.33-S
Exome sequencing in carriers of 1q21.1 CNV

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The 1q21.1 CNV is associated with a variable clinical phenotype and normal to delayed neurodevelopment. We performed whole genome transcription and exome sequencing analysis in 5 subjects from 2 families with 1q21.1 deletion (3 subjects) and duplication (2 subjects), in search for genetic changes that could affect the phenotype. The subjects had variable learning difficulties. A pathogenic variant in the ATF6 gene from 1q22-23, which plays a role in endoplasmatic reticulum stress response, was detected in the mildly affected father and his more severely affected child with 1q21.1 duplication. It was validated by Sanger sequencing and associated with reduced ATF6 RNA and protein expression in patient lymphoblast cell lines. However, the ER stress response, as measured by induction of known ER stress genes (GPR78, Dnajb9, Sdf2l1) in response to tunicamycin was not altered in patient vs control cell lines. No candidate pathogenic mutation that could be linked to altered gene expression status and/or phenotype was found in the 3 subjects with the 1q21.1 deletion, either in the CNV region or genome-wide. Interestingly, for all 5 subjects with 1q21.1 CNV a larger number of the 107 genes implicated in ER stress response showed altered expression in comparison to controls on the whole genome expression array (15-28% in subjects in comparison to 0-6% in controls). Perturbed ER stress response caused either by dysfunction of genes from 1q21.1, genome wide, or both, could play a role in the observed phenotypic variability and result in more severe developmental abnormalities in unfavourable environmental circumstances.

P08.34-M
Contribution of copy number variants (CNVs) in congenital unexplained intellectual and developmental disabilities in 149 Lebanese patients

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Chromosomal microarray analysis (CMA) is nowadays the most adopted clinical test for patients with unexplained intellectual disability (ID), developmental delay (DD), and congenital anomalies. Its use has revealed its capacity in detecting copy number variants (CNVs) as well as regions of homozygosity which, upon their distribution on chromosomes, indicate uniparental disomy or parental consanguinity that is suggestive of an increase of occurrence of recessive disease. We screened 149 Lebanese probands having ID/DD and 99 healthy controls using the Affymetrix Cyto 2.7M and SNP6.0 arrays. We reported all identified CNVs that we divided into groups and confirmed the utility of this CMA technique in the detection of parental consanguinity in 42 cases (28.2%). Pathogenic CNVs were identified in 11.4% of the patients. We reviewed and reported the genotype/phenotype correlation in a patient with a 1q44 microdeletion, as well as defined the minimal critical regions responsible for the 10q26 and the 16q monosomy syndromes. Several likely causative CNVs were also detected, of which, new homozygous microdeletions (9p23p24.1, 10q25.2, and 8p23.1) in 3 patients issued from consanguineous parents (patient's ROH size ≥ 66 Mb), involving genes that are reported as potential candidates. However, the clinical interpretation of several other CNVs remains uncertain. Among those, 2 microdeletions touching ATRNL1 and the 3'UTR of SOX5. These CNVs of unknown significance were inherited from the patients' normal parent, which requires a screening of more ethnically matched controls in order to obtain enough evidence for their classification.

P08.35-S
Unbalanced translocations involving chromosome 4p associated with complex phenotypes: report of 3 cases

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Large imbalances of chromosome 4p, either deletions (associated with Wolf-Hirschhorn syndrome, WHS) or duplications have a defined clinical phenotype. Here, we present 2 situations of genetic abnormalities involving 4p16.3 region. Three patients were referred for genetic testing: 2 siblings, a 16 years old boy and a 12 years old girl, both with the same phenotype, including dysmorphic features, severe mental retardation; severe speech delay; hyperkinesias, aggressivity; epilepsy; skin allergic reactions. An 8 years old girl, with pre- and postnatal growth retardation, severe psychomotor retardation, spastic quadriparesis, dysmorphic features, cleft palate, bilateral congenital cataract, severe epilepsy with status epilepticus episodes. aCGH on an 105K Agilent platform was done according to manufacturer's recommendations; the results were validated by FISH. In the two siblings, aCGH revealed the same genetic abnormality: arr 4p16.3p16.1(72,447-8,373,151)x3,10q26.3(130,977,858-135,434,178)x1 pat. The balanced t(4;10)(p16.1;q26.3) translocation was detected by FISH in the genome of the patients' father. In the second case, aCGH showed another genetic anomaly involving 4p16.3 region: arr 4p16.3p16.1(72,447-9,371,067) x1,8p23.3p23.1(176,452-8,094,773)x3. The genetic investigations of the parents were normal. The 4p region includes over 140 genes, many included in OMIM Database as involved in various pathologies. The 10q deleted region consists of over 60 genes, many also described in the OMIM Database. The 8p duplicated region has a size of 8 Mb and includes 116 genes. The complexity of the phenotype, both for deletion and duplication of 4p16.3 region, of these cases can be explained by the association of other genetic anomalies (10q deletion and 8q duplication, respectively). Acknowledgements: project PN 09.33.02.03.

P08.36-M
Recurrent CNVs in 15q11.2-q12 in Bulgarian patients with generalized epilepsy and intellectual disability

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Copy number variants are frequent in autism spectrum disorders and generalized epilepsy. In this study, we performed comparative genomic hybridization assay (aCGH) using Agilent Microarray Kit, 4x180K in a preselected sample of 36 Bulgarian patients with epilepsy and intellectual disability (ID). The region most often engaged in copy number changes included 15q11.2-15q12. In 7 patients (19.4%) CNVs were located in 15q12. Five of them harbored microduplications in GABRG3 gene, 1 patient showed microduplication in GABRB3 gene and in 1 patient both rearrangements were present. Aberrations in 15q11.2 region were observed in 2 patients. One of them was a 0.161 Mb deletion, including SNURF/SNRPN upstream reading frame. In the other patient, a 2.608 Mb duplication covering 36 genes was revealed. Genomic region on 15q11-13 is involved in many clinically important rearrangements. These include aberrations of various sizes which can affect the neuronal differentiation by disrupting normal epigenetic control and gene expression. All Bulgarian patients with CNVs harboring 15q12 shared a common clinical phenotype of severe ID, speech impairment or complete lack of speech, different types of seizures, facial dysmorphisms, microcephaly and behavior abnormalities of the autistic spectrum. In contrast, the two patients with 15q11.2-rearrangements displayed different clinical characteristics and milder forms of mental retardation. Our results are in line with the central role of GABAergic systems in ID and epilepsy. Further studies are needed to investigate the parental origin and elucidate the effect of the CNVs and the phenotype-genotype correlations. Acknowledgement: the study was supported by DTK02/67/2009, DUNK01-2/2009 funded by NSF.

P08.37-S
Disruption of the Methyltransferase-Like 23 Gene *METTL23* Causes Mild Autosomal Recessive Intellectual Disability

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We describe the characterization of a gene for mild non-syndromic autosomal recessive intellectual disability (ID) in two unrelated families, one from Austria, the other from Pakistan. Genome-wide single nucleotide polymorphism (SNP) microarray analysis enabled us to define a region of homozygo-

sity-by-descent (HBD) on chromosome 17q25.1. Whole exome sequencing and analysis of this region in an affected individual from the Austrian family identified a 5bp frameshifting deletion in the *METTL23* gene. By means of Sanger sequencing of *METTL23*, a nonsense mutation was detected in a consanguineous ID family from Pakistan for which homozygosity-by-descent mapping had identified a region on 17q25. Both changes lead to truncation of the METTL23 protein, which disrupts the predicted catalytic domain and alters the cellular localization. 3D-modelling of the protein indicates that METTL23 is strongly predicted to function as a S-adenosyl-methionine (SAM)-dependent methyltransferase. Expression analysis of METTL23 indicated a strong association with heat shock proteins, which suggests that these may act as a putative substrate for methylation by METTL23. A number of methyltransferases have been described recently in association with intellectual disability. Disruption of METTL23 presented here supports the importance of methylation processes for brain function and development.

P08.38-M
Targeted next generation sequencing in a new cohort of 996 individuals with Intellectual Disability

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We screened DNA from 996 individuals with Intellectual Disability (ID) for variants in known genes and candidate genes for ID in order to identify the cause of disease. Overall, we investigated the coding sequence of 565 candidate genes in individual cases affected with moderate to severe ID.
We observed 8225 non-synonymous variants passing quality control criteria and frequency filter (<1% in internal and population controls). The rate of loss of function (LoF) variants (frameshift, nonsense or canonical splice site) was 0.43 per person. Overall, we have identified pathogenic LoF mutations in 11% of the cases (110 out of 996) affecting known genes previously reported to harbour mutations causing ID. LoF mutations in ATRX, CC2D2A, ARID1B and CUL4B were the most commonly observed (frequencies varied from 0.4% to 0.6%). The interpretation of the vast number of missense mutations in known genes proved to be more challenging, as rarity of variant is insufficient to assign pathogenicity in such a genetically heterogeneous phenotype.
Although the cohort consisted of predominantly non-syndromic ID cases, the yield of mutations in genes associated with a syndromic phenotype was high, for example, ATRX, CC2D2A, CHD7 and ARID1B. This suggests that the distinction between syndromic and non-syndromic ID is an increasingly artificial concept in a genotype-driven diagnostic era.

P08.39-S
Discovery of new mutations using exome sequencing in adult patients with intellectual disability and psychiatric disorders

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Next Generation Sequencing offers the opportunity to identify new mutations and increasing the proportion of patients with ID receiving a genetic diagnosis. We have analyzed a cohort of 102 adult patients affected by ID, psychiatric diseases and minor dysmorphic features, identifying a genetic cause of ID in 28 (27,5%). We recruited 9 trio cases negative for fragile X syndrome, chromosomal and subtelomeric rearrengements and pathogenic copy number variants. The exome was captured with Agilent sure select technology, multiplexed, and sequenced in an Illumina HISeq2000 lane. Potential damaging variants detected were selected and filtered (condel score, EVS, 1000genomes, dbSNP). Variants were finally confirmed by Sanger sequencing. We detected an average of 8624 non-synonymous variants in the cases, of which an average of 23 were de novo. Frequency and functionality based filtering reduced the number of potential candidate ID genes harbouring de novo variants to 1-10 per case. In 5 cases, potential disease-causing variants were identified in genes previously implicated in ID syndromes: One case was compound heterozygous for two RPGRIP1L rare missense variants, two cases carried a de novo mutation in (an in-frame deletion in UBE3A and a missense variant in MLL), one familial case was carrying a rare variant in TCF4 and in the last case a frameshift deletion was identified in SATB2. Two cases were compound heterozygous for two very rare or novel variants in two different potential ID candidate genes, and in the remaining two cases, no candidates were identified. Work supported by FIS grants: PI080778 and PI10/01710.

P08.40-M
The degree of Intellectual Disability is significantly associated with an excess of Runs of Homozygosity (ROH)

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Several recent studies focused on the effect of extended homozygosity on highly complex and polygenic traits where recessive inheritance may play an important role. Since excess of homozygosity might increase the risk for disorders like schizophrenia, Alzheimer disease and autism, we have set out a study to investigate the effect of ROHs on the degree of Intellectual Disability (ID). About 370 unrelated individuals with ID were collected and classified into mild/moderate ID (MM-ID) for IQ ranging from 35-40 to 70-75 and severe/profound ID (SP-ID) for IQ below 35-40. High-density SNP array data were processed with the aim of detecting and analyze ROHs. Since different array platform were used, homozygosity and ROHs mean length were compared in MM-ID vs SP-ID separately in each dataset. Results were then combined for a meta-analysis. Our data revealed an association between the amount of homozygosity and the degree of ID, according to the recent findings on autism (Gamsiz et al., 2013). Accounting for principal components to control population stratification, we tested for ROHs mean length and detected significantly ($p<0.005$) longer stretches in SP-ID compared to MM-ID. Weaker association was detected in burden ROH analysis, showing an increase of the percentage of genome covered by ROHs for SP-ID cases. Extent of ROHs seems to contribute to the pathogenesis of ID, suggesting that autosomal recessive variants have a crucial role on the modulation of the severity of ID that still need to be investigated.

P08.41-S
A familial interstitial 14 Mb deletion of 5p13p14 associated with a mild phenotype challenges the current genotype-phenotype correlation attributed to the MR III region

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Varying degrees of developmental delay or intellectual disabilities and dysmorphic features appear to be strongly associated with deletions involving 5p. Most of these deletions are associated with cri du chat syndrome. These deletions can be terminal, interstitial, or associated with complex chromosomal rearrangements. To establish the genotype-phenotype of 5p deletions many efforts had be done in the last years in dissecting the phenotype and three different critical regions had been determined. But there had been controversaries about the relationship between MR and deletions of MRI-II encompassing bands 5p13.2p14.3. Deletions limited to this region were reported to show no phenotype, but to aggravate the phenotype in case of accompanying aberrations (Zang X., et al. 2005). We now observed a novel 3- generation family with a interstitial deletion del(5)(p13.2p14.3) limited to MRIII without additional rare CNVs in microarray testing. The index patient, a 15 years old girl, showed learning disability (IQ 75), microcephaly, high pitched voice and a subtle facial phenotype. The patients mother and grandmother had the same deletion and likewise the learning disabilities, the high pitched voice and subtle facial features, but no microcephaly. In contrast to the current literature we therefore propose that deletions limited to the MRIII region have a mild, but distinct phenotype with particular emphasis on the high pitched voice.

P08.42-M
Inverted triplication of 7q11.22 embedded within the 7q11.21q11.23 duplication segment in a child with stigmata dysplastica, developmental and speech delay

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Introduction: A q-arm of human chromosome 7 harbours many copy number variants (CNV) with known pathogenic significance. Chromosome 7q11.23 duplication syndrome (OMIM#609757) is a multisystem develop-

mental disorder with mild craniofacial anomalies and increased incidence of congenital anomalies. Williams-Beuren syndrome (WBS; OMIM#194050) is caused by ~ 1.8-Mb hemizygous deletion on chromosome 7q11.23. WBS triplication syndrome with similar but more severe clinical features has been also described.

Case presentation: We present a 3-years-old boy with developmental and severe speech delay, dysmorphic signs, bilateral cryptorhidism and hypoplasic corpus callosum. Cytogenetic and molecular analysis revealed a complex de novo chromosomal rearrangement which we characterize as inverted triplication of 7q11.22 segment embedded within larger 7q11.21q11.23 duplication.

Methods and results: Molecular characterization by array-CGH revealed a 1.93-Mb duplication of segment 7q11.21, 5.27-Mb triplication of segment 7q11.21q11.22 and 1.33-Mb duplication of segment 7q11.23. Further molecular cytogenetic investigation was performed with multiple combinations of specific FISH probes. Metaphase and interphase FISH confirmed the location of triplication 7q11.22 segment within the 7q11.21q11.23 duplication. Additional FISH analysis revealed that triplicated 7q11.22 segment on derivative chromosome 7 was inverted. Parental cytogenetic analysis demonstrated de novo origin of this complex chromosomal rearrangement.

Conclusions: Although chromosomal imbalances are the major cause of developmental delay, large de novo CNVs are relatively rare events. We have identified a novel complex rearrangement on chromosome 7, duplication with embedded inverted triplication also known as DUP-TRP/INV-DUP structure. Triplication of segment 7q11.22 is probably generated by long range inverted repeats which still need to be identified.

P08.43-S

Identification of novel variants in PIGQ, PGAP3 and PIGY further implicate the GPI pathway in the pathogenesis of neurodevelopmental abnormalities

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Around 30 genes are required for glycosylphosphatidylinositol (GPI) biosynthesis. Mutations in many of these are reported to cause a spectrum of neurodevelopmental abnormalities. Here we describe novel mutations in three members of this pathway.

Whole genome sequencing was applied to a sporadic patient with severe early-onset epilepsy. This approach identified a homozygous *PIGQ* splice mutation, resulting in exon skipping and defective GPI biosynthesis. Additionally, autozygosity mapping and exome sequencing were combined to investigate two consanguineous Pakistani families where multiple affected individuals presented with intellectual disability and microcephaly. In the first family, seizures were present in 2/3 affected individuals. A homozygous p.G92D mutation was detected in *PGAP3* that co-segregated with disease. Affected individuals had elevated alkaline phosphatase, a marker for defective GPI biosynthesis. Functional studies using CHO cells confirmed the mutation influenced GPI-anchor remodelling. For the second family, the concurrent release of ENCODE data prompted us to examine UTR variants and a c.-540G>A variant was detected in *PIGY* which co-segregated with disease. Disruption of an SP1 binding motif suggested that the variant might influence gene expression. IonTorrent sequencing confirmed that in heterozygote carriers ~9% of transcripts were expressed from the mutant allele.

Our work strengthens the role of the GPI pathway in the pathogenesis of neurodevelopmental abnormalities. Although *PIGQ* and *PIGY* both encode subunits of N-acetylglucosaminyltransferase, the range of phenotypes seen in the families described recapitulates the pleiotropic effects of defective GPI biosynthesis as a whole. This study also highlights the importance of assessing UTR variants during analysis of exome data.

P08.44-M

Careful consideration is necessary in *MECP2* testing and allele drop out

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Allele drop out (ADO) leads to the preferential amplification of one of two allele due a series of reasons among which the occurrence of sequence mismatch within a primer-binding site or a complex DNA motif. The drop out can cause false results during PCR amplification and be responsible of potential pitfall in diagnosis. It is known that genomic DNA sequences can assume different complex secondary structures which can prevent DNA replication and transcription and, if altered by mutations, may be responsible of ADO events in PCR amplification. An example of these genomic DNA motifs are repetitive guanine-rich sequences, known as G-quadruplexes, on one strand and the complementary cytosine-rich sequences on the opposite strand, known as i-motifs. G-quadruplexes and i-motifs are present within the coding sequence of the *MECP2* gene.

Here, we report a case of ADO detected during *MECP2* molecular analysis in two unrelated patients with Rett syndrome. In both girls, the mutations were in exon 4 of the *MECP2* gene (c.1137delC and c.1151_1201del50; c.1163C>T) and were detected as seemingly homozygous. The mutations are located in the *MECP2* WW binding domain and fall in a region where a number of small deletions/single point mutations have been reported in literature.

Under the assumption of ADO, we have investigated the presence of the wildtype allele with a change in PCR condition and the primers.

We prove that the complexity of exon 4 sequence leads to allele amplification failure and that allele drop out is a technical risk in *MECP2* molecular analysis.

P08.45-S

MECP2 duplication in France: delineation of brain MRI abnormalities in 30 affected patients

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Xq28 duplications encompassing MECP2 have been described in male patients with a severe neurodevelopmental disorder associated with hypotonia and spasticity, severe learning disability, stereotyped movements and recurrent pulmonary infections. We report on standardized brain magnetic resonance imaging (MRI) data of 30 affected patients, including 5 symptomatic females, carrying a MECP2 duplication, the size of which varied between 228 kb and 11.7 Mb. The aim of this study was to compare brain MRI results to seek recurrent malformations and attempt to determine whether variations in imaging features could be explained by differences in the size of the duplications. We showed that 90% of patients had brain MRI abnormalities, and that they shared certain non-specific brain malformations such as corpus callosum abnormalities (n=20), ventricular dilatation (n=9), reduced volume of the white matter (WM) (n=12), increased T2 signals in posterior periventricular WM (n=6), and vermis hypoplasia (n=5). The occipito-frontal circumference could be highly variable since it was >+2DS in 5 patient and <-2DS for 4 patients. Among the 9 patients with dilatation of the lateral ventricles, 6 (67%) had a duplication involving L1CAM. The only patient harbouring bilateral posterior subependymal nodular heterotopia also carried a FLNA gene duplication. We could not demonstrate a link between periventricular WM hypersignals / delayed myelinisation and duplication of IKBKG. These results show that patients with MECP2 duplications share certain common but non-specific brain abnormalities. These imaging features, therefore, do not constitute a diagnostic clue. We did not clearly demonstrate a genotype-imaging phenotype correlation.

P08.46-M

Clinical relevance of the genotype-phenotype correlation in a patient with a de novo monosomy 9pter-p24.1 and duplication Xq28-qter

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We report the case of a 12 years old boy admitted to our observation in

the first year of life with severe neonatal encephalopathy. Since birth, the clinical conditions have been worsening during time. Now he shows severe mental retardation, dysmorphic features, extreme generalized hypotonia, white matter leucomalacia, drug-resistant epilepsy, blindness, genitourinary and gastrointestinal abnormalities, generalized joint laxity and recurrent respiratory tract infections. High resolution karyotype was normal. Subtelomeric FISH probes identified a derivative 9 of an unbalanced translocation t (9p;Xq) with monosomy 9p and Xq disomy. FISH studies of the parents revealed that the alterations were de novo in the patient. Array CGH was performed and identified two rearrangements: a 6Mb deletion on chromosome 9 from p24.3 to p24.1 and a 5Mb duplication on chromosome X from q28 to qter. This double rearrangement has never been described in literature. Our patient presents urogenital birth defects, facial dysmorphism (possibly due to the 9p deletion) and developmental regression, axial hypotonia, Hirschsprung disease, feeding difficulties, recurrent infections, epilepsy, absence of language, white matter disease (typically related to the Xq28 duplication). Several genes, included in the deleted (KANK1, DMRT1, SLC1A) and in the duplicated region (MeCP2, L1CAM), are known to have an important role in the Central Nervous system development. This report allows to compare the phenomics of our patient to the other cases described in literature, to contribute to the knowledge about genotype-phenotype correlation and to provide new informations for future studies about the 9p24.3-pter and Xq28-qter regions and the genes included.

P08.47-S
Exome sequencing identifies candidate gene for MEHMO syndrome

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The patient is a 3,5 years young boy suffering from severe psychomotor delay, microcephaly, epilepsy, and multiple endocrine disorders (i.e. obesity, diabetes, hypogenitalism, and partial deficiency of pituitary hormones). There are no other living male members in the mother's family, as mother's brother and grandmother's brother died in the first months of life. The clinical picture and family history indicated towards the MEHMO syndrome, an X-linked disease which has been described in three families only.
Exome sequencing of proband's DNA revealed 23 previously unreported variants on X chromosome as confirmed also by Sanger sequencing. Haplotype analyses showed only 3 of them to be shared with proband's mother and his mother's mother. Only one of them, a variant in EIF2S3 gene, is in the region of X-chromosome previously described to be associated with MEHMO syndrome. EIF2S3 encodes a γ subunit of eukaryotic translation initiation factor 2 (eIF2) that is responsible for transporting the initiator Met-tRNAiMet to the 40S ribosomal subunit. Point mutations in this gene were previously described in two families with intellectual disability. The variant found in the patient is a frame-shift mutation with premature stop codon influencing 8 last amino acids of the protein. In-sillico analyses evaluate this change as disease causing.
Our results support the role of EIF2S3 as a candidate gene, disruption of which might significantly contribute to this severe clinical symptomatology.
Supported by APVV 0187-12, KCMM (ITMS 26240220071)

P08.48-M
New insights on cognitive and structural brain imaging phenotype in primary microcephaly due to ASPM mutations

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Primary recessive microcephaly (MCPH) caused by ASPM mutations is a model of abnormal brain development linked to neural progenitors proliferation defects. MCPH is defined as a homogenous reduction of cerebral volume principally affecting the cortex. The reduction of brain volume has been correlated to cognitive disabilities. However, correlation between cognitive functions and structural brain phenotype has not yet been well established in genetic microcephalies. Here, we provide evidence that specific cognitive functions are preserved in ASPM-related patients and this is correlated with their structural brain changes. General intelligence, memory scales and structural brain magnetic resonance imaging scans were acquired from 6 ASPM-related patients. Using cranial MRI, we measured the volume of the different brain structures and focused on the analyze of the cortical volume, surface and thickness. These microcephalic patients have a normal mnesic functioning and preserved hippocampal volume compared to other cortical areas. Unlike most previous data that linked microcephaly to mental retardation, these findings suggest i) that these microcephalic ASPM-related patients are able to learn despite their cognitive disabilities and ii) that other master genes than ASPM are necessary for the development of hippocampus formation and function.

P08.49-S
Microduplication 17q12 in a patient with microcephaly and moderate psychomotor delay

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17q12 microduplication is a very rare genomic rearrangement associated with a variable phenotype, consisting of intellectual disability/developmental delay of various degree, epilepsy, behavioral problems, brain abnormality, esophageal atresia, renal, heart and ocular anomalies.
We report a case of a 9 years-old boy born from no consanguineous healthy parents.
Array-CGH analysis has revealed a 1,8 Mb de novo microduplication of chromosome region 17q12 : arr[hg19]17q12 (34,906,638-36,756,170)x3 dn
The proband presents microcephaly without dysmorphic features, moderate mental retardation, language delay, learning disabilities and impulsive behavior. Brain Magnetic Resonance is normal. EEG shows multifocal spikes and waves.
The microduplication extends from gene GGNBP2 to gene SRCIN1 and encompasses 20 genes in 17q12.
The neurologic phenotype seems to be associated with gene LHX1. Lhx1 is expressed in the brain and is implicated in Purkinje cell differentiation in the developing cerebellum as well as in migration of motor axon to the limbs. Lhx1 knockout mice shows anencephaly. These data support the hypothesis that LHX1 is a dosage-sensitive gene, involved in neurological phenotype of patients with 17q12 microduplication. Additional studies are needed to further delineate the phenotypic impact of expression of this gene.
Clinical features of this syndrome may depend on the size of microduplication variable for different breakpoints, the insertion site and/or orientation of duplicated fragment in the genome as well as additional genetic factors such as incomplete penetrance. The survey of this case contributes to extend the correlations among the phenotype and the genotype of this peculiar syndrome.

P08.50-M
Syndromic intellectual disability diagnosis by combined use of MLPA kits

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Intellectual disability (ID) is a common disorder, with major consequences for individual, family and society. Due to clinical and genetic heterogeneity, in about 50% of cases an etiologic diagnosis cannot be established. The aim of this study was to evaluate the ability of a combination of MLPA kits to establish the diagnosis in 380 patients with syndromic ID. All patients were assessed for chromosome imbalance using standard karyotype and MLPA analysis using SALSA P064 or P096 kit, if the phenotype was suggestive for a microdeletion syndrome (subgroup A - 188 patients), or subtelomeric kits, if the phenotype was not suggestive for a microdeletion syndrome (subgroup B – 192 patients). Abnormal results detected by both MLPA kits were further characterized using appropriate follow-up MLPA kits (Telomere Follow-up set, P029-B1, P250-B2). In subgroup A we identified 27 patients with microdeletions (14.3%). In subgroup B 8 patients showed an aberrant telomeric signal detected by only one of the two MLPA kits, 31 patients showed abnormal results detected by both MLPA kits (~16%), and 153 patients had normal results. In summary, the combined use of MLPA kits led to the diagnosis in 58 out of 380 patients (15.2%). The use of follow-up MLPA kits allowed us both to confirm abnormalities and to determine their size, which facilitated the interpretation of the clinical significance of these rearrangements. For laboratories that do not have yet access to microarray technology, using several MLPA kits represents an effective strategy for establishing the diagnosis in ID patients.

P08.51-S
Expanding the phenotype of a recurrent *de novo* Mutation in *PACS1*

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The PACS (phosphofurin acidic cluster sorting) proteins represent a family of multifunctional membrane traffic regulators that mediate organ homeostasis. Recently, exome sequencing revealed identical *de novo* mutations in *PACS1* in two unrelated individuals with intellectual disability and remarkably similar facial features (Schuurs-Hoeijmakers et al. *AJHG* 2012). Functional experiments indicated that the mutation exerts a dominant-negative effect by affecting the ability of PACS1 to mediate the specification and migration of SOX10-positive cells in the neural crest.

Here, we describe a third patient carrying the identical heterozygous *de novo* missense mutation c.607C>T; p.R203W in exon 4 of *PACS1* (NM_018026.3). The patient is a 2 year-old boy with developmental delay, congenital heart defect (atrio-ventricular septal defect), low levels of immunoglobulins and borderline microcephaly. His facial features are strikingly similar to the two patients described by Schuurs-Hoeijmakers et al.: He has hypertelorism, long eyelashes, downslanting palpebral fissures, a wide mouth with thin upper lips, downturned corners and a flat philtrum. His ears are low-set and rotated.

The combination of his features (congenital heart defect, hypotonia, developmental delay, facial dysmorphisms) initially prompted us to analyse the genes of the RAS/MAPK pathway. However, this did not lead to establishing a diagnosis. Finally, exome sequencing revealed the previously described *de novo* missense mutation in *PACS1*.

In summary, the recurrent *de novo* mutation c.607C>T; p.R203W in *PACS1* causes a phenotype characterized by developmental delay/intellectual disability, variable organ malformations and highly recognisable facial features.

P08.52-M

Whole exome sequencing approach to reveal the genetic aspects of extreme phenotypic variability of Incontinentia Pigmenti

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Incontinentia pigmenti (IP, MIM308300, 1/10.000) is an X-linked dominant neuroectodermal disease associated with skin defects and with extracutaneous manifestation (ocular, dental, hair, nail and central nervous system_ CNS defects) at variable frequency. In 30% of IP patients CNS anomalies (seizures, encephalopathy, encephalomyelitis, ischemic stroke) are reported. IP patients carry a mutation in the IKBKG/NEMO gene (IKBKG/Nuclear Factor kappaB, Essential MOdulator) that encodes for NEMO/IKKgamma regulatory protein of the IKK complex, required for the activation of the canonical NF-kappaB pathway. We collected a large cohort of IP patients with an high variability of clinical phenotype. A variable CNS defect was observed, even in IP families with the identical NEMO mutation. The skewed X-chromosome inactivation could only partially explain this variability thus modifier loci may contribute to the severity of IP phenotype. Here we present an IP trios-family with both child and mother with NEMOdel4_10 deletion: in the mother only skin defects were present, in the child also a severe mental retardation with neuromuscular defects were reported. We have designed an exome-sequencing approach to identify the modifier genes. From exome-enriched library and sequencing a list of single nucleotide/indels variants were produced. We will present candidate genes selected by applying combined filtering method to exclude benign and inherited variants and prioritizing the genes implicated in well-established pathways associated to NF-kB and neurogenesis. The identification of modifier genes able to influence the severity of IP phenotype will be useful to anticipate the outcome of the IP disease in order to apply a personalized therapeutic iter.

P08.53-S

NGS based whole X-exome analysis reveals a familial WDR45 missense mutation in 3 males with intellectual disability and brain iron accumulation

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X-exome sequencing in an adult male patient with intellectual disability, epileptic seizures during childhood and otherwise normal somatic development revealed a novel missense mutation c.698G>A; p.R233H at a highly conserved position in WDR45 (Xp11.23). Segregation analysis confirmed the presence of the mutation in his two similarly affected younger monocygotic twin brothers and their healthy mother with skewed X-inactivation (15:75). The mutation could be excluded in the maternal grandfather and both healthy maternal uncles of the patients. De novo mutations in the autophagy gene WDR45 have been previously described in patients with BPAN (beta-propeller protein-associated neurodegeneration), a recently established subtype of neurodegeneration with brain iron accumulation (NBIA). Mutations in WDR45 have been associated with an X-linked dominant form of NBIA, predominantly affecting females and presumed to be nonviable in males with germline mutations. Similar phenotypes in few reported males and females have been attributed to somatic mosaicism in surviving males and skewing of X-inactivation of females. A retrospective evaluation of brain MRI in the index patient at the age of 10 years was not suspicious for iron deposition. Axial MR images (mesencephalon) at the age of 19 years show clear hypointensity of the substantia nigra on T2w images and also on T2*, indicative of pathological iron accumulation. The primary developmental delay and stable course of the disease until young adulthood and transmission of the mutation via a healthy carrier expends the clinical spectrum of the WDR45 associated phenotype in X-linked intellectual disability.

P08.54-M

TRPC5 et KLHL15 are candidate genes for X-linked intellectual disability

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High-resolution array comparative genomic hybridization (a-CGH) enables the detection of intragenic rearrangements, such as single exon deletion or duplication. This approach can lead to the identification of new disease genes. We report the analysis of 54 male patients presenting with intellectual deficiency (ID) and a family history suggesting X-linked (XL) inheritance or maternal skewed X-chromosome inactivation (XCI), using a home-made X-chromosome specific microarray covering the whole human X-chromosome at high resolution. The majority of patients had whole genome array-CGH prior to the selection and we did not include large rearrangements such as MECP2 and FMR1 duplications. We identified 4 rearrangements considered as causative or potentially pathogenic, corresponding to a detection rate of 8%. Two CNVs affected known XLID genes and were therefore considered as causative (IL1RAPL1 and OPHN1 intragenic deletions). Two new CNVs were considered as potentially pathogenic as they affected interesting candidates for ID. The first CNV is a deletion of the first exon of the TRPC5 gene, encoding a cation channel implicated in dendrite growth and patterning, in a child presenting with ID and an autism spectrum disorder (ASD). The second CNV is a partial deletion of KLHL15, in a patient suffering from severe ID, epilepsy and anomalies of cortical development. In both case, in spite of strong arguments for clinical relevance, we were not able at this stage to confirm pathogenicity of the mutations and the causality of the variants identified in XLID remains to be confirmed

P08.55-S

Central nervous system developmental disorder in Noonan syndrome: a genomic approach

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Noonan syndrome (NS – OMIM 163950) is a multisystemic dominant disorder with a prevalence of 1/1000-1/2500 live births. It is clinically and genetically heterogeneous, with mutations in several genes of the RAS/MAPK pathway detectable in up to 75% of cases. Pathogenesis of central nervous system (CNS) developmental anomalies has not been thus far fully enlightened. We have applied the *multiple hit hypothesis* to study in deep the pathogenic mechanisms implicated in NS related CNS anomalies. In this oligogenic model, point mutations and genomic rearrangements cooperate in an additive manner in the pathologic development of CNS. Using array CGH technology, we analyzed 15 samples of selected patients with molecularly confirmed diagnosis of NS and a severe impairment of CNS. We found 37 rare CNVs (one/several per patient) most of them inherited from an healthy parent. According to the Database of Genomic Variants 12/37 were never reported and 25/37 were reported in very few cases. Based on the function of the genes mapping in the identified CNVs, 10/37 (27%) were probably involved in the pathogenesis of CNS anomalies observed in our patients. We provided first data supporting the hypothesis that CNS involvement in NS does not depend exclusively on single gene mutations but also on the

concurrent presence of other genomic penetrant variants. These results represent an initial assumption for the application of the *multi-hit hypothesis* in the dissection of the NS pathogenesis. Further studies on larger cohorts are deserved to better define the meaning and the clinical implications of these findings.

P08.56-M
NPAS3-related copy number variants: a role in developmental delay?

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Neuronal PAS domain-containing protein 3 (NPAS3) is a transcription factor expressed primarily in developing and adult brain tissues. Current evidence suggests it is involved in neuronal development and maturation, and it has recently been highlighted as potentially playing a key role in the evolution of the human brain. Clinically, NPAS3 has been identified as a candidate gene for schizophrenia, and numerous studies have supported this association. In addition, several cases of learning disabilities in patients with disruptions of this gene are reported, but this association remains relatively unexplored. Here, we present four unrelated individuals with small copy number variants (CNVs) within NPAS3: two intragenic duplications and two intragenic deletions. These CNVs range between 111-460kb in length and encompass exonic sequences within the NPAS3 gene. All four patients had variable degrees of developmental delay, and three had subtle distinctive facial features. Two patients also had macrosomia and macrocephaly, while the other two had autistic features and behaviour issues, including psychosis in one individual. In contrast, CNVs involving exonic sequences in NPAS3 have only been seen in one of over 19,000 controls. In summary, these four cases support an association between NPAS3 and developmental delay. This association is compatible with this gene's postulated effects on neuronal development, and suggests that abnormal function of NPAS3 may be implicated in other cases of non-specific cognitive impairment.

P08.57-S
A 9q21.3 microdeletion involving the NTRK2 gene as a possible cause of intellectual disability

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Microarray analyses identify new common copy number variants (CNVs) and microdeletion/microduplication syndromes. However, some CNVs are unique and no comparison with similar genotypes (and phenotypes) is available to assist in deciding about their causality.
We identified a twelve-year old stigmatized female patient with growth failure, microcephaly, severe psychomotor retardation, hypotonia, muscular atrophy, hip dislocation, foot deformity, vesicoureteral reflux and congenital heart disease. Her karyotype was normal. SNP array analysis (Illumina HumanCytoSNP-12) revealed a unique 1.7 Mb long deletion in 9q21.3 (chr9:86,595,071-88,357,495; hg19) flanked by segmental duplications. FISH analysis of the family confirmed that the aberration was de novo. It removed 5 protein-encoding RefSeq genes. The NTRK2 gene encodes a neurotrophin receptor involved in the regulation of brain development, neurotransmission and synaptic function. Therefore NTRK2 is a good candidate gene for intellectual disability (ID). A missense NTRK2 mutation has been described in a boy with ID and obesity, and NTRK2 was considered also in autism and other psychiatric disorders. The mouse homologue of another deleted gene, AGTPBP1, is associated with neurodegeneration.
Just one literature report exists describing a much more severely affected patient with a slightly larger deletion involving NTRK2. Remarkably, the deletion in our patient corresponds to an inversion described in several unaffected individuals. The presence of the inversion and segmental duplications at the breakpoints could indicate a specific mechanism predisposing to rearrangements. However, the inversion could not be identified in any of the parents of the patient.
Supported by CHERISH, NT/14200, 00064203 and CZ.2.16/3.1.00/24022.

P08.58-M
Co-occurrence of *TCF4* and *FOXG1* genes deletions in a 15-year-old girl

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Both Pitt-Hopkins syndrome and a congenital variant of Rett syndrome are rare genetic syndromes with similar clinical features and development.
Here we report the first case of co-existence of Pitt-Hopkins syndrome and a congenital variant of Rett syndrome. A 15-year-old girl was born after a second uneventful pregnancy of healthy young non-consanguineous parents. Normal delivery and birth growth parameters (head circumference at the 10th percentile). Shortly after the birth the girl presented with hypotonia, poor sleep pattern, unexplained irritability and episodes of crying, delayed development and early closure of fontanel (at 3 months). During the infancy she developed absence seizures without EEG changes. At the age of 15 years she was found to have a postnatally developed microcephaly, seizures, ataxic gait, severe intellectual disability with absent speech, drooling, self-mutilation, and stereotypic behaviour. Patient's dysmorphic features were highly suggestive of Pitt-Hopkins syndrome: Deep set eyes, strabismus, prominent nasal bridge, wide mouth with everted lower lip, thenar hypoplasia, tapering fingers, fetal pads, and hypoplastic nails. MLPA analysis revealed a heterozygous deletion of exons 4b-6 of the *TCF4* gene and a heterozygous deletion of the *FOXG1* gene, both *de novo.* Chromosome analysis and SNP of the only exon array were normal. No changes were revealed by sequence analysis of *TCF4*.
We speculate that co-existence of two different gene mutations is not as exceptional as commonly thought in the field of monogenic disorders. Additionally, the contribution of each deletion to the phenotype is discussed.

P08.59-S
Exome sequencing reveals a rare TSEN54 mutation in an Iranian family with Ponto Cerebellar Hypoplasia

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Ponto Cerebellar Hypoplasia (PCH) is a heterogeneous group of autosomal recessive disorders characterized by an abnormally small cerebellum and brainstem. PCH type 2 (PCH2), the most frequently subtypes, is characterized by cerebellar hypoplasia affecting the hemispheres more severely than the vermis and progressive cerebral atrophy, microcephaly, dyskinesia, seizures progressive microcephaly from birth, extrapyramidal dyskinesia and dystonia. This study was designed to find the genetic defect in a consanguineous Iranian family with two affected children, by means of homozygosity mapping and exome sequencing. Clinical examination of the affected individuals in this family showed microcephaly and severe intellectual disability. Cerebellar hypoplasia in brain MRI was present in one affected individual. Linkage analysis with the use of Affymetrix Axiom® Array platformrevealed two promising intervals (LOD score 1.927) onchromosomes 17 and 20. Exome Sequencing with 96% at 20x depth of coverage in one affected individual, detected a previously rare known homozygous missense mutation in TSEN54 gene (c.371G>T, p.G124V) which was about 9 Mbps, located in the second interval on chromosome 17. Albeit this is a known mutation, but it is considered as a rare mutation in PCH patients. Co-segregation analysis of the identified mutation was confirmed with Sanger sequencing. This data shows that combination of homozygosity mapping and exome sequencing in populations with high rate of consanguinity could be applied as an efficient technique for molecular genetics practice of clinically and genetically heterogeneous diseases.

P08.60-M
Identified A Novel Mutation in CDK5RAP2 Gene in Iranian Family with Autosomal Recessive Primary Microcephaly Using Whole Exome Sequencing

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Introduction:Autosomal recessive primary microcephaly (MCPH) is a congenital disorder caused by impaired neurogenic mitosis lead to defect in brain development. This disorder is heterogeneous genetically and characterized by reduced head circumference (-2 SD or more) under age and sex-based mean and mild to severe intellectual disability. So far, 12 genetic loci (MCPH1-12) with twelve corresponding genes (MCPH1, WDR62, CDK5RAP2,CASC5, ASPM, CENPJ, STIL,CEP135, CEP152, ZNF335,PHC1and CDK6) have been identified for this disease.Using whole-exome sequencing (WES) as a new pioneer technology and diagnostic tool, we can provide an opportunity for identifying the causal mutations with more efficient and cost-effectiveness in Iranian patients with autosomal recessive primary microcephaly. Case presentation:We report an Iranian family with two affected individuals with moderate intellectual disability, large toes and primary microcephaly with consanguineous marriage. We performed WES for one affected and focused

on genes associated with microcephaly and homozygous variants. We identified a homozygous novel frameshift mutation in CDK5RAP2 in the affected individual. Sanger sequencing confirmed the presence of the homozygous mutation in the other affected and heterozygous state for parents and normal siblings. Discussion:WES led to cost-effectiveness and rapid identification of novel frameshift deletion in CDK5RAP2 in Iranian family with primary microcephaly. We can facilitate genetic counseling for this family. To date, only five different mutations have been reported for CDK5RAP2 gene which most of them were from Pakistan. Moreover, this study implies that WES is a suitable diagnostic tool for identifying mutations responsible for MCPH families.

P08.61-S
New case of biallelic *TRMT10A* deficiency identified by exome sequencing confirms the associated phenotype of primary microcephaly with intellectual disability and short stature

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Recently a mutation in the *TRMT10A* gene was identified in a large consanguineous family of Moroccan origin defining a new syndrome of young onset diabetes, short stature and microcephaly with intellectual disability. By linkage analysis and exome sequencing a homozygous nonsense mutation was identified in all three affected siblings. The protein encoded by *TRMT10A* (also *RG9MTD2*), which was proposed to have tRNA methyltransferase activity, was shown to be ubiquitously expressed with enriched levels in the affected tissues brain and pancreatic islets and to be absent in lymphoblasts from the affected siblings. We now report a new case of biallelic *TRMT10A* deficiency in a girl born to apparently non-consanguineous parents of Kosovo origin. By exome sequencing in our patient we identified a homozygous nonsense mutation (c.379C>T) in the *TRMT10A* gene. Of note, this is the same mutation as recently reported, introducing a premature stop codon at position 127 of the protein. Our patient presented with primary microcephaly, intrauterine onset borderline growth, mild intellectual disability and fine motor problems, a high palate with uvula bifida and minor facial features such as long narrow face with narrow palpebral fissures, long thin nose and small mouth. At age 4 years a seizure disorder started. Notably, at age 8 years, our patient did not yet manifest diabetes, which was of adolescent onset in the previously described family. In conclusion, our report of a novel patient confirms the phenotype of the novel syndrome associated with biallelic *TRMT10A* deficiency including short stature and microcephaly with intellectual disability.

P08.62-M
Analysis of MECP2, CDKL5, and FOXG1 genes in Czech patients with Rett syndrome and Rett-like features

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Background: Rett syndrome is a severe X-linked dominant neurodevelopmental disorder primarily caused by de novo MECP2 mutations. Clinical features include developmental regression at the age of 6-18 months, acquired microcephaly, autistic behavior, loss or severe impairment of speech and purposeful hand use, stereotypic hand movements, gait apraxia, and seizures. CDKL5 mutations have been identified in early-onset seizure variant and FOXG1 mutations in congenital variant of Rett syndrome. We report the results of mutation analysis of these genes in Czech patients with Rett syndrome and mental retardation with Rett-like features. **Materials and methods:** MECP2 was analyzed in 416 patients, CDKL5 was analyzed in 59 patients, and FOXG1 was analyzed in 20 patients. MECP2 and CDKL5 were analyzed by high-resolution melting analysis and DNA sequencing. FOXG1 was analyzed by DNA sequencing. Large deletions and duplications were analyzed by MLPA analysis (MRC-Holland). **Results:** Pathogenic mutations in the MECP2 gene in were identified in 45 patients with classic Rett syndrome, 7 patients with atypical Rett syndrome, 11 patients with Rett-like features, and 1 patient with autism. CDKL5 mutations were found in 2 patients with early-onset seizures and Rett-like phenotype. No FOXG1 mutation was detected in this study. **Conclusions:** MECP2 mutations are common in classic Rett syndrome patients, but they are less frequent in atypical or Rett-like phenotypes. However, analysis of MECP2 in these patients should not be discouraged. More patients should be examined to determine frequencies of CDKL5 and FOXG1 mutations in Czech Republic. Supported by grants NT 13120-4/2012, UNCE 204011/2012, MZCR RVO-VFN64165/2012.

P08.63-S
Ageing in Rett Syndrome: Characteristics of long term survivors reported through the British Isles Rett Syndrome Survey (BIRSS)

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We report what is known about the health and wellbeing of thirty women of at least 40 years with Rett syndrome (RTT). The study is based on longitudinal data from the British Isles Rett Syndrome Survey (BIRSS). These 30 women have a clinical diagnosis of RTT: 24 women of 40-49 years, five women of 50-59 years and one older woman of 64 years. Tweny nine women were diagnosed with classic RTT and one with atypical RTT (AR).
MECP2 mutations were identified in 14 of the 18 women tested. There were six missense mutations; one early truncating; two late truncating; three C-terminal deletions and two large deletions. A simplified Smeets severity score was calculated for every decade of the women's lives. Little increase in severity was observed and, for most, the severity rating was 'mild'.
Factors contributing to disease severity have been assessed. Findings include: (1) severity of phenotype is milder amomg older women, indicating survival advantage; (2) depression among middle-age RTT may be a substantial but under-recognised problem; (3) menopause does not seem to occur earlier than in other women; (4) nutrition standards from the general population will often be inapplicable; (5) multiple opportunities exist to prevent functional decline through detailed attention to the quality of the medical and social care.
There is a particular need to increase awareness of RTT amongst staff caring for older adults with disabilities so that they can identify and meet the needs of their adult patients with RTT.

P08.64-M
Italian brother and sister with familial Xp22.12 microduplication including RPS6KA3 gene and phenotype description

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RPS6KA3 gene is responsible of Coffin Lowry Syndrome, but recent studies have demonstrated that microduplication of this gene can cause nonsyndromic X-linked ID, ADHD and localization-related epilepsy. The RPS6KA3 gene encodes a member of the ribosomal S6 kinase family. RSK proteins are activated by MAPK proteins in response to growth factors, polypeptide hormones, and neurotransmitters. RSKs appear to have important roles in cell cycle progression, differentiation, and survival. Further studies have evidenced that the presence of a small amount of residual enzymatic activity may be sufficient to maintain normal osteoblast differentiation and has also been linked to cognitive performance, with higher level of intellectual function.
Our case describes a brother and a sister affected by intellectual disabilities, language delay and behavioural difficulties, with more severe clinical manifestations in the affected male. Both show some dysmorphic features as very large and long nose, mild hypotelorism and dental crowding. The boy performed some instrumental exams as cerebral NMR and CT, which didn't reveal any major cerebral malformation, but showed an absent pneumatisation of the sphenoid sinus, moreover no other skeletal abnormalities were found. No seizures were reported, except during pharmacological sleeping EEG, some low waves in the right frontal area were identified. The boy had previously performed some molecular exam as FMR1 and COH1 mutation screening and karyotype, all normal. We performed high density SNPs-array analysis, which revealed a 512 Kb microduplication on Xp22.12 (19.842.599-20.355.406) in both brother and sister. The duplication was inherited from the mother who is completely asymptomatic.

P08.65-S
Exploring the effect of adducins genetic variability on cognition in schizophrenia

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Schizophrenia is a chronic disease characterized by cognitive impairment. Biological bases of cognitive deficits are still poorly understood and may lie in insults in the neurodevelopmental process. Synapses structural proteins are claimed to have an ethiopathogenic role in schizophrenia and a more direct effect on core cognitive functions. Adducins family proteins seem of great interest, as they are fundamental constituents of synaptic structures, involved in actin cytoskeleton assembly-disassembly responsible of synaptic plasticity. In particular, ADD2 is prominently expressed in brain tissues and previous researches reported a role of this gene in memory and lear-

ning processes, commonly impaired in schizophrenia. Based on this rationale, we analyzed three common genetic variants of Adducins, ADD1 G1532T (rs4961), ADD2 C1797T (rs4984) and ADD3 IVS11+386A>G (rs3731566) in a sample of 342 patients with schizophrenia, assessed with a broad battery evaluating core cognitive domains that are typically impaired in schizophrenia. The analysis showed significant effects of ADD1 genotype on executive function (p<.025)and of ADD2 genotype on several domains: working memory (p<.003), verbal fluency (p<.018), verbal memory (p<.001), abstract reasoning (p<.009), cognitive flexibility (p<.02570) and sustained attention (p<.010). Moreover an interaction effect of ADD1 and ADD3 genotypes was observed on symbol coding (p<.029) and verbal memory (p<.027). Our findings suggest that genes involved in synaptic building and plasticity, such as Adducins, may have a key role in cognitive impairment in schizophrenia and may help us to better understand the ethiopathogenesis of the disease.

P08.66-M
The power of Next Generation Sequencing in identifying mutations in non-specific ASD-ID phenotypes: the example of SHANK3

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Autism Spectrum Disorders (ASDs) comprise a range of early onset neurodevelopmental conditions of varying severity, with or without Intellectual Disability (ID), characterized by impairments in relatedness and communication, accompanied by restricted interests and repetitive stereotyped behaviors. SHANK3 haploinsufficiency implicated in Phelan-McDermid 22q13 microdeletion is one of the more prevalent monogenic causes of ASD, explaining at least 0,5% of cases, but the indication of *SHANK3* sequencing in patients with ASD and ID remain difficult. Here we report on two patients with *de novo SHANK3* mutations identified by next generation sequencing (NGS). Patient 1, aged 15, was part of a cohort of 40 patients screened by exome sequencing for undiagnosed severe ID, and a truncating mutation was identified in *SHANK3* (c.4381C>T ; p.Gln1461*). Patient 2, aged 10, was part of a cohort of 106 patients with ID screened by targeted NGS of 220 ID genes, and a causative heterozygous truncating mutation of *SHANK3* was identified (c.2955_2970dup;p.Pro992Argfs*325). They both had normal measurements, severe ID, developmental and speech delay with acquisition of a few words and secondary regression with absence of speech, attention deficit and behavioral disorders necessitating treatment, autistic traits, insomnia and tantrum. Patient 1 had eating and digestive difficulties, patient 2 had distal spasticity, epilepsy from age 5, and facial dysmorphic features. These two clinical presentations appear nonspecific within the ASD-ID spectrum. After reviewed the other patients with *SHANK3* mutations, we argue that NGS will be helpful to determine patients with *SHANK3* mutations in the absence of clear distinctive clinical features.

P08.68-M
Interpretation of TCF4 Variants Requires mRNA Splicing Analysis in Patients with Pitt-Hopkins Syndrome

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Background: Pitt-Hopkins syndrome (PTHS) combines severe intellectual disability (ID), hyperventilation, and a characteristic facial gestalt. The disease-causing gene TCF4 encodes a basic helix-loop-helix (bHLH) transcription factor. Patients carry de novo TCF4 deletions, truncating mutations, or missense mutations located chiefly in the bHLH domain. Variants that do not fall into one of these categories are difficult to interpret. We describe a comprehensive mRNA analysis method for assessing TCF4 variants of uncertain significance (VUS). Methods and Results: Using leukocytes from patients and minigene assays, we documented impaired splicing for the synonymous variant c.1071A>G p.(Ala357Ala), and for the only two missense variants outside the bHLH domain that are described to date, c.1073G>T p.(Gly358Val) and c.1604A>G p.(Asp535Gly). All assessed variants result in aberrant splicing and premature termination codons (PTC). Conclusions: All TCF4 mutations reported so far in PHTS are either missense mutations located in the b-HLH domain or result in a PTC. Splicing tests ought to be performed in patients with de novo TCF4 VUS, especially missense mutations lying outside the bHLH-specifying domain, even in the absence of in silico prediction for a splicing defect.

P08.69-S
A novel X-linked trichothiodystrophy associated with a nonsense mutation in RNF113A

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Trichothiodystrophy (TTD) describes a group of rare autosomal recessive disorders that variably affect a wide range of organs derived from the neuroectoderm. The key diagnostic feature is sparse, brittle, sulphur deficient hair that has a "tiger-tail" banding pattern under polarizing light microscopy. At the molecular level, TTD genes have a role in DNA damage repair pathways.
We describe two male cousins affected by TTD with microcephaly, profound intellectual disability, sparse hair, aged appearance, short stature, facial dysmorphism, seizures, immunoglobulin deficiency, multiple endocrine abnormalities, cerebellar hypoplasia and partial absence of the corpus callosum and absence of photosensitivity. Mutations in known TTD genes were ruled out. Obligate female carriers showed 100% skewed X-chromosome inactivation suggesting a potentially X-linked disorder. Linkage analysis localised the disease allele to a 7.75 Mb interval from Xq23 - q25. Sanger sequencing of 737 X-chromosome, Vega genes and whole exome sequencing was used to identify a nonsense mutation in the highly conserved RNF113A gene (c.901 C>T, p.Gln301*) ruling out other possible X-linked variants. The mutation segregated with the disease in the family and was not observed in over 10,000 control X chromosomes from public and in-house data. The mutation markedly reduced RNF113A protein expression in extracts from lymphoblastoid cell lines derived from the affected individuals. Knockdown of orthologs of RNF113A in model organisms strongly support a role for the gene in DNA repair and neurogenesis. The association of RNF113A mutation with TTD identifies a new locus for these disorders on the X-chromosome.

P08.70-M
An hiPSCs based *in vitro* model of Angelman Syndrome and dup15 Autism

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UBE3A gene maps on 15q11q13 chromosome and encodes for the protein E6AP, an Ubiquitine-ligase involved in protein degradation process. The gene is maternally imprinted in central nervous system and different studies show that loss of expressed copy causes Angelman Sydrome (AS) while the duplication of 15q11q13 represents the genetic cause in 1-3% of autistic patients (dup15 Autism). Using 2 cohorts of AS and dup15 autism patients, we applied induced Pluripotent Stem cells (iPSCs) technology and neuronal differentiation to identify molecular targets of a dys-regulated dosage of UBE3A. To generate iPSCs we used 4 canonical factors identified by Yamanaka (c-Myc, Klf-4, Oct3/4 e Sox2) through retroviral or lentiviral vectors. We performed 14 attempts and in 13 we generated cells that we defined as "partially reprogrammed" because they lost their typical fibroblasts morphology, but died after passaging. In the last experiment we used a commercial lentiviral vector with the same 4 pluripotency factors making a dup15 hiPSCs cell line positive to pluripotent expression markers; further characterizations are still ongoing. Once we have hiPSCs we will proceed with neuronal differentiantion. With our experiments we confirm that the hiPSCs genesis is a stochastic process with a successful rate of 0.1-2% and that no standard protocol exists. In the other hand this new challenging and arduous technology give to the scientists the possibility to understand the pathogenetic mechanisms in tissues that for obviously reasons are difficult to study.

P08.71-S
Maternal uniparental isodisomy of chromosome 4 in a subject with mild intellectual disability

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Uniparental Isodisomy (iUPD) is a rare condition characterized by the inheritance of two homologous chromosome from one parent and the absence of the homologous chromosome from the other parent. Several mechanisms of UPD formation have been previously decribed, including trisomy rescue, monosomy rescue and gamete complementation. Problems associated to

UPD are homozigosity of autosomal recessive inherited mutations and aberrant genomic imprinting. iUPD of chromosome 4 is a rare conditions. To date only few cases are reported, with heterogeneous phenotype, and all were maternal. We describe a patient with mild intellectual disability and slight speech delay, harboring maternal iUPD of chromosome 4 detected by high resolution SNP-array and confirmed by microsatellite analysis. Our patient did not show any dysmorphic feature and did not present any other anomaly. To the best of our knowledge, this is the second patient carrying maternal chromosome 4 iUPD that show a behavioral phenotype. Chromosome 4 maternal iUPD is a sporadic and rare event, that may present little or not distinguishable phenotype. Thus, it seems unlikely that important maternally imprinted genes are on this chromosome. Because iUPD is associate with a complete unmasking of recessive mutations, it is possible that such mutation could be responsible for the intellectual disability in our patient, and this hypothesis could justify the phenotypic variability among other reported cases. It will be useful to study further cases with chromosome 4 paternal iUPD, in order to compare clinical phenotype and to exclude or confirm the presence of paternally imprinted genes on chromosome 4.

P08.72-M
X- Chromosome imbalances by array-CGH - from single gene to chromosomal regions imbalances

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Array-Comparative Genomic Hybridization (array-CGH) has increased the diagnostic yield in patients with intellectual disability (ID), autism spectrum disorders and multiple congenital anomalies due to its improved resolution. X-chromosome has been focus of attention due to the bias in the affected male-to-female ratio and to the knowledge of X-linked genes associated with ID. With array-CGH we can either detect single gene imbalances, chromosomal region imbalances and even aneuploidies. In a cohort of 1000 patients studied by Agilent 180K oligonucleotide array-CGH we have detected several X-chromosome imbalances. Single gene deletions involving ZNF41 or IL1-RAPL1 genes were equitably observed in 8 patients; DMD imbalances in 3 females and SHOX gene duplications in 1 female and 9 males. We also detected an intragenic deletion in SLC9A6 gene associated with Christianson syndrome that segregated in the family. In 6 patients we identified Xp22.31 duplications, 3 females, 1 male with maternal inheritance and 2 males whose inheritance was not yet determined. We identified chromosome Xq27.1q28 interstitial duplications in 2 males, 1 maternally inherited and the other not yet determined. We also found other genomic imbalances but in single cases: a complex rearrangement with multiple imbalances at Xp22.33p22.2 in a male patient, maternally inherited; an Xp11.3p11.23 duplication in a female with ID whose mother is also affected and a case of triple X in an autistic female. The challenge with X-chromosome imbalances is to interpret their impact on the phenotype, due to the presence of some alterations in the normal population and to X-chromosome inactivation in females.

P08.73-S
Mutations in the P54NRB/NONO gene cause a novel syndromic XLID with a slender built-macrocephaly gestalt

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We report on two unrelated and sporadic patients presenting a novel syndromic XLID featuring macrocephaly, severe elocution disability with open bite, high arched palate, malar hypoplasia, a thin nasal bridge with deviated nasal septum, slender build, and scoliosis. Brain MRI showed a thick corpus callosum. High-throughput sequencing identified two distinct mutations (c.1131G>A and c.1394dup, p.Asn466Lysfs*13) in the *P54NRB/NONO* X-linked gene. P54NRB/NONO belongs to the DBHS (Drosophila Behaviour Human Splicing) protein family. In mammals three members belong to this family: P54NRB/NONO, PSPC1, and PSF/SFPQ. DBHS proteins are multifunctional nuclear proteins implicated in multiple aspects of RNA production and processing. Beyond RNA production, they also play a role in RNA surveillance (binding and retaining hyper edited RNA in the subnuclear bodies named paraspeckles), as well as in the control of mammalian circadian rhythms. Finally, PSF and P54NRB have also been implicated in dendritic RNA transport.
We demonstrated that both mutations lead to complete absence of the P54NRB/NONO protein and overexpression of the two other DBHS proteins. In patients' cells, whole transcriptome analysis revealed severe gene expression deregulation, and reporter assays showed reduced circadian clock amplitude. Finally, CT scans analysis of *P54nrb/Nono*-deficient mice demonstrate a dramatic flattened nose phenotype that may mimic the severe malar hypoplasia observed in patients.
These findings demonstrate the existence of a recognizable XLID syndrome due to mutations in *P54NRB/NONO,* and highlight the crucial role of DBHS proteins in brain development and function.

P08.74-M
The *KIAA2022* duplication found by high-resolution custom-designed array CGH in Polish patient with X-linked intellectual disability

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The X-linked intellectual disability (XLID) is a common, clinically complex and genetically heterogeneous disease arising from mutations in genes along the X chromosome. Approximately 5-10% of XLID cases in males are due to the presence of copy number variations (CNV). The array-based comparative genomic hybridization (aCGH) using NimbleGen custom assays (720K) dedicated for X chromosome was performed for 45 patients with the family history suggesting X-linked intellectual disability, excluded mutation in *FMR1* and *ARX* genes and normal results in MRX-MLPA test. In 7 out of 45 patients (15.5%) the molecular defect was identified. In one patient, the 363 kb microduplication (hg: 73,899,064-74,262,928) encompassing *KIAA2022* gene was found. According to DECIPHER CNVs database, reported microduplication *KIAA2022* gene, could be associated with XLID. The duplication was identified in a male patient with moderate intellectual disability that was also present in his four male relatives (brother, mother's 2 brothers and son of mother's sister). The female relatives of the patient were unaffected that supports the X-linked recessive inheritance. The KIAA2022 protein, also known as XPN, is related to neurite extension and regulation of the cell-cell, cell-matrix adhesion and migration. The copy number variations and point mutations in the *KIAA2022* gene have been recently found to be causative for intellectual disability. Therefore, we postulate that the found variant involving *KIAA2022* gene can be pathogenic and related to clinical features observed in our patient with XLID.

P08.75-S
Identification of intellectual disability genes in female patients with skewed X-inactivation

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Intellectual disability (ID) is a very heterogeneous disorder and the etiology remains unknown in about 50% of cases. Traditionally, X-linked ID (XLID) studies focused on males from XLID families due to the hemizygous state of the X chromosome. Females can be carrier of the mutation but are generally not affected due to inactivation of the mutant X chromosome in most of their cells (skewing). Previously we identified two female patients with RETT-like phenotypes caused by a de novo microduplication at Xq18, including *MECP2*. While carrier females of *MECP2* duplications are generally unaffected due to skewing of X-inactivation, these 2 females show complete inactivation of the apparently normal X chromosome. By exome sequencing, we identified a mutation in *ATRX* located on the other X, which might have caused the skewing in that patient forcing the *MECP2* duplication to be expressed. Since this two-hit mechanism could also occur in other female ID patients with skewed X-inactivation, we analyzed the X-inactivation pattern of 147 ID females, which revealed a higher percentage (6.8%) of extreme skewing (≥ 90% skewing) than reported in the general population (4.0%). X chromosome-array-CGH analysis of these skewed females revealed two de novo aberrations on Xp11 that could explain their ID phenotypes. Exome sequencing revealed a further two promising variants. Our data thus demonstrate that skewing can be used to screen for (novel) genetic mutations resulting in XLID in females.

P08.76-M
Microduplication of Xp22.31 region involving the STS gene in two males with intellectual disability

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Genomic instability is a feature of the human Xp22.31 region: deletions, duplications, triplications and other complex rearrangements were identified

at this locus. Submicroscopic duplication of Xp22.31 has been reported as either a possible cause of neurobehavioral phenotypes or a benign variant. Recently, two large cohorts of patients with the microduplication at Xp22.31 were reported. The size of the Xp22.31 duplication varied between 149 kb and 1.9 Mb and mostly included the steroid sulfatase (STS) gene. Patients with Xp22.31 recurrent duplications generally presented with a neurocognitive and behavioral phenotype, including developmental delay. The STS gene could be a candidate gene contributing to the abnormal phenotype in Xp22.31 duplication.

Here we report 2 boys with the microduplication of Xp22.31 found by MLPA analysis. The duplication was minimum 246,2 kb in size and included STS and HDHD1A genes. Both of them presented with mild to moderate intellectual disability/developmental delay mainly affecting speak ability, behavioural abnormalities and minor facial dysmorphisms. Proband B also had a hypotonia. The mother of proband A, carrying the same duplication, had a mild intellectual disability.

Our cases overlap with those previously described in most clinical features. Although there is no clear evidence to support pathogenicity of the Xp22.31 duplication, there is still enough evidence to consider the Xp22.31 duplication as a risk or modifier factor for intellectual disability and behavioural problems. We hope that the description of these two cases will contribute to the phenotype delineation and elucidation of the role of Xp22.31 duplication.

P08.77-S
Xq12 and Xq13 submicroscopic duplications in a single patient: confirming the existence of a new X-linked disorder and narrowing the critical region.

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In 2012 Kaya et al. reported three related patients with a recurrent duplication Xq12-q13.3 with mental retardation, autism, seizures and dysmorphic features. The duplicated region (9.4 Mb) contains numerous genes and the authors pointed out the possible role of an increased dosage of specific genes focusing on their presumed role in developmental delay and autism. Subsequent reports (Prontera et al. 2012; Wentz et al. 2013) described 4 more patients with a variable association of severe global developmental delay, microcephaly, dysmorphism and autism spectrum disorder, all carrying a duplication in the Xq12-q13.3 region, supporting the existence of a clinically recognizable X-linked recessive disorder. We describe a further patient affected by global developmental delay, epilepsy, autistic traits and carrying two non-contiguous duplications detected by array CGH, involving Xq12 and Xq13 regions. Detailed clinical description is provided and compared to previous reported cases. Due to the narrowed size of the duplicated regions (123 Kb and 509 Kb, respectively), this case helps to redefine the region of interest for this condition. The two duplications in our patients involve 13 genes, and 7 of them are mainly expressed in the nervous system (*OPHN1, SNX12, MED12, NLGN3, GJB1, ZMYM3, TAF1*) and all, except for *SNX12*, are involved in the pathogenesis of a known human disease. Our observation provides further evidence of the existence of a new genomic condition, helping to define the corresponding clinical phenotype, and to identify a more definite critical region.

P08.78-M
Xq28 duplication detected by SNP-array in two girls with microcephaly, intellectual disability and neurological features

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We report on two girls carrying a *de novo* Xq28 microduplication, distal to *MECP2*, overlapping for about 90Kb, including *RPL10*, *DNASE1L1*, *TAZ*, *AT-P6AP1*, *PLXNA3* and *GDI1* genes. X chromosomes resulted randomly inactivated in the first case while the test was not-informative in the second. Our first patient is the eldest daughter of an apparently healthy, non-consanguineous couple. Birth weight and length were around the 10th percentile and head circumference was 33cm (<<10th percentile). The girl had poor suction and anomalies of the face and extremities. At 19 months she had a generalized epileptic seizure followed by other episodes. Brain MRI showed dilatation of the ventricular system and evidence of small brain. Our second patient is the second daughter of an apparently healthy, non-consanguineous couple born at 39 weeks by cesarean section for small gestational age. The auxological parameters were all below the 3rd percentile. At birth, brain MRI showed mild hypoplasia of corpus callosum and cerebellum. She had generalized hypotonia, hyporeactivity, sucking deficit, seizures, and congenital heart disease. Physical examination showed microcephaly, dysmorphic features of the face and extremities. *GDI1* has been associated with ID and non-syndromic microcephaly in a high percentage of males. Our patients and the revision of cases from literature and DECIPHER database strengthen the *GDI1* involvement in intellectual disability and microcephaly not only in male but also in female subjects. Of note, neurological and neuroimaging abnormalities, such as ventricular system enlargements, cerebellar or corpus callosum hypoplasia are recurrent clinical features associated with this rare microduplication syndrome.

P08.79-S
Mutations in *YY1* cause intellectual disability and a distinct facial appearance

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Background - Trio-based exome sequencing of 10 patients with intellectual disability (ID) previously identified a *de novo* missense mutation in *YY1*, a transcription factor with an important role in various biological processes, such as proliferation, differentiation, embryogenesis, apoptosis, and tumor development. To ascertain whether the mutation in *YY1* was causative for the patient's phenotype, we set out to identify additional patients with *de novo* mutations in *YY1*.

Methods - Targeted resequencing of *YY1* and further exome sequencing studies in a cohort of patients with unexplained ID were performed. Detailed phenotype information of patients with mutations in *YY1* was compared.

Results - We identified three additional patients with *de novo* mutations in *YY1* (two missense and one nonsense mutation). The four mutations all occurred in the region containing the zinc finger domains of *YY1*, which is involved in DNA binding. All four patients had ID, intrauterine growth retardation and similar facial dysmorphism, including facial asymmetry, broad forehead, full upper eyelids, flat malar region, full nasal tip, thick alae nasi and a Gingko leaf-like shape of the upper lip.

Conclusions - We show that *de novo* mutations in the zinc finger domains of the transcription factor YY1 cause ID, low birth weight, and overlapping facial dysmorphism, including a distinctive shape of the upper lip.

P08.80-M
Mutations in ZBTB18 are responsible for intellectual disability

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Microdeletions of 1q43q44 were described in patients with intellectual disability (ID) with limited or no expressive speech, microcephaly, dysmorphic features, abnormalities of the corpus callosum, hand and foot abnormalities, and seizures. Different genes were predicted as candidate genes for the microcephaly (AKT3), abnormalities of the corpus callosum (ZBTB18), and seizure (FAM36A, C1orf199, HNRNPU). Recently, a mutation in the ZBTB18 gene was identified in a patient with ID and further signs of the 1q43q44 microdeletion syndrome. Knock-out mice with loss of Znf238 die at birth with neocortical defects but a brain-specific knock-out of this gene in mice causes microcephaly, reduced thickness of the cortex and agenesis of the corpus callosum. The ZBTB18 gene acts as a transcriptional repressor of key neurogenic genes such as NeuroG2 and NeuroD1 or ID2 and ID3. Here, we describe two patients carrying de novo mutations in ZBTB18, one patient with a missense mutation (p.R495G) and a further patient with a nonsense mutation (p.E286X). The first patient, a 23 years old woman, demonstrated a non-syndromic ID with mild facial dysmorphic signs. The other patient, a 7 years old boy, showed ID, facial abnormalities, significant speech and motor developmental delay. In both patients normal karyotypes (550 bands), array-CGH results and normal FMR1 gene analyses were shown. Our results indicated that ZBTB18 is responsible for neurogenic development and mutations within ZBTB18 cause ID with variable phenotypic abnormalities.

P08.81-S
Klinefelter syndrome: 48, XXXY aneuploidy in a patient with mild mental retardation and psychotic personality traits

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Klinefelter syndrome is the most common aneuploidy in males with a prevalence of 0.1-0.2% in the general population, which rises up to 3% in males with fertility issues, although only 35% of cases are diagnosed. The affected males tend to be tall, have narrow shoulders, wide hips, sparse body hair, gynecomastia and small testis; they present androgen deficiency and azoospermia. Besides the previous physical characteristics, there have been reports on the expression of behavioral and cognitive traits that tend to be very variable, possibly according to the type of aneuploidy, with an established association between the number of extra X chromosomes and cognitive deficit, and a not so clear association of different chromosomal variants of Klinefelter and psychotic behavior, with some authors proposing the origin of the extra chromosome as a determinant of different behavioral traits. Here is presented the case of a 13 years old male with karyotype 48,XXXY, who besides presenting the classical physical features, is under psychiatric treatment because of presenting trouble at home and at school for being aggressive and impulsive. A review on the literature is made, concluding on the importance of an opportune starting of hormonal therapy and a multidisciplinary approach including endocrinology, pediatrics, genetics, neuropsychology and psychiatry when needed.

P09.001-S
4H (Hypomyelination, Hypodontia and Hypogonadotropic Hypogonadism) syndrome caused by POLR3B mutations

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Leukodystrophies are a heterogeneous group of inherited neurodegenerative disorders characterised by alterations of Central Nervous System white matter. Several clinical forms have been described and genes involved in generating the hypomyelinating leukodystrophy, but recently, mutations in *POLR3A* and *POLR3B* genes have been reported to cause a specific syndrome named 4H characterized by the association of the hypomyelination with hypodontia and hypogonadotropic hypogonadism. We visited a 27-year-old female born from a non consanguineous mating with a leukodystrophy and an unlikely clinical / endocrinological diagnosis of Pelizaus - Merzbacher syndrome. Her phenotype was characterized by teeth alterations (neonatal tooth and permanence of deciduous dentition), thin hair, high myopia, hypogonadotropic hypogonadism, and minor dysmorphic features. Moreover, a gradual, but consistent motor and cognitive deterioration was noted. Putting together all the clinical, radiological and phenotipic characteristics of the proband a clinical diagnosis of 4H syndrome has been made. Subsequently, a Sanger sequence of the entire coding region and the flanking exon/intron boundaries of *POLR3A* and *POLR3B* has been performed. The analysis revealed the two mutations p.V523E and p.G695fs*5 in the *POLR3B* gene. This case further expands the knowledge about the role of the *POLR3B* gene in generating the 4H syndrome.

P09.002-M
A familial 9q22.1q22.31 deletion of 3.8 Mb, challenging interpretation of large, inherited copy-number variation

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Chromosomal microarray analysis are now the first-line diagnostic test for patients presented with intellectual disability, autism, or multiple congenital anomalies. An ongoing challenge for the clinician and biologist is the interpretation of copy number variation. Many tools have been proposed in order to facilitate their interpretation including linking data to several genome browsers, gene contains and prioritization, inheritance of the copy-number variation (CNV) and the size of the variant. Indeed, large CNV (>500 kb) are supposed to be strongly associated with morbid consequences. We report on a large 9q22.1q22.31 deletion of 3.8 Mb identified by whole genome SNP array (HumanCytoSNP-12, Illumina) in a 6 year old girl presented with growth retardation and Attention-Deficit/Hyperactivity Disorder (ADHD). Neuropsychological evaluation excluded a mental retardation. The deletion encompassed 24 genes, was inherited from her apparently normal father. Nevertheless, the familial history was marked by hyperactivity and dyslexia in the father's childhood. The older sister presented also with ADHD and carried the same deletion. Grandparents will be further analyzed. We discuss the implication of this CNV in the abnormal phenotype of the two sisters. Our observation highlights the difficulties of interpretation of CNV particularly in case of large CNV inherited from an unaffected parent. Moreover, regarding ongoing discussions about application of microarray in prenatal diagnosis, our case allowed us to postulate that the size is not sufficient to prejudge the pathogenicity of the CNV.

P09.003-S
aCGH for neurological disorders in paediatric population at Clinical Institute of Medical Genetics, UMC Ljubljana

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Array comparative genomic hybridization (aCGH) is a quick method to analyze the whole genome for imbalances at a higher resolution. In conventional diagnostic settings aCGH is used as a first-tier diagnostic test in the group of patients with unexplained developmental delay (DD) and/or idiopathic intellectual disability and/or dysmorphic features and/or multiple congenital anomalies.
AIM: Our aim was to evaluate the diagnostic yield/clinical utility of aCGH in the group of children with neurological disorders at Clinical institute of Medical Genetics Ljubljana, tested in 2012-2013.
PATIENTS AND METHODS: We included 244 children, 91 with DD and/or dysmorphic features, 44 with epilepsy, 34 with autism, 45 with developmental abnormalities of the CNS, and 30 with abnormal muscle tone. DNA was hybridized to Agilent 180K or 60K Human CGH microarrays. Discovered genomic imbalances were interpreted according to the data in Internet databases (ISCA, DECIPHER, ECARUCA) and scientific publications cited in PubMed.
RESULTS: Altogether, 37 pathogenic copy number variations (CNVs) were found that could explain the phenotype of patients (Table 1). In addition, 26 variants of unknown significance (VUS) were detected, most of them de novo. Eleven patients (4,5%) had complex CNVs.
CONCLUSION: We found out that pathogenic CNVs were causative in 15% of cases with neurological disorders with/or without dysmorphic features or CNS developmental abnormalities. Our data demonstrate that aCGH is an important diagnostic tool in neuropediatrics.

Table 1. CNVs in different phenotype groups

Phenotype	Number of tested patients	Pathogenic CNV	VUS	Diagnostic Yield (pathogenic CNVs)
DD with or without dysmorphic features	91	22	10	24.2%
Developmental abnormalities of the CNS	45	5	6	11.1%
Abnormal muscle tone	30	2	4	6.7%
Epilepsy	44	6	3	13.6%
Autism	34	2	3	5.9%

P09.004-M
Genetic analysis of amyotrophic lateral sclerosis in the Slovenian population

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Background: Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disease characterised by progressive degeneration and loss of upper and lower motor neurons in the cerebral cortex, brainstem and spinal cord leading to death due to respiratory failure within 2-5 years from onset. The most common genes involved in the disease process are *SOD1*, *FUS*, *TARDBP* and *C9ORF72*.
Patients and methods: Blood samples from 72 Slovenian ALS patients were collected and the exons of genes of interest were PCR amplified followed by Sanger sequencing on ABI310 Genetic Analyzer. In case of *C9ORF72* repeat-primed PCR was performed followed by fragment length analysis on ABI310 Genetic Analyzer. Results were analyzed using Gene Scan software.
Results: Genotyping of genes *SOD1*, *FUS*, *TARDBP* and *C9ORF72* revealed some changes in the DNA sequence. In *SOD1* gene two mutations (V15M and G94C) were detected. Sequencing of *FUS* and *TARDBP* genes did not reveal any amino acid changes. Although two substitutions were detected, R522R (G>A) in *FUS* and L330L (A>G) in *TARDBP*. The latter was not previously described. In 2 ALS patients we detected expansion of repeats in the first intron of *C9ORF72*.
Conclusions: This study represents first genetic analysis of Slovenian ALS patients. Our findings are in concordance with other studies provided on European ALS patients. Of these four analyzed genes repeat expansion in

C9ORF72 remains the most common cause of ALS although for the majority of cases the main causes are yet to be revealed.

P09.005-S
Aging-related genes modulate the early altered expression in young 3xTg-AD mice hippocampi: a whole transcriptome approach

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Alzheimer's disease (AD) is a multifactorial neurological condition associated with a genetic profile that is still not completely understood. In this study, using a whole gene microarray approach, we investigated age-dependent gene expression profile changes occurring in the hippocampus of young and old transgenic AD (3xTg-AD) and wild type (WT) mice. The aim of the study was to assess similarities between aging- and AD-related modifications of gene expression and investigate possible interactions between the two processes.
Global gene expression profiles of hippocampal tissue obtained from 3xTg-AD and WT mice at 3 and 12 months of age (m.o.a.) were analyzed by hierarchical clustering. Interaction among transcripts was then studied with the Ingenuity Pathway Analysis (IPA) software, a tool that discloses functional networks and/or pathways associated with sets of specific genes of interest.
Cluster analysis revealed the selective presence of hundreds of upregulated and downregulated transcripts. Functional analysis showed transcript involvement mainly in neuronal death and autophagy, mitochondrial functioning, intracellular calcium homeostasis, inflammatory response, dendritic spine formation, modulation of synaptic functioning, and cognitive decline.
Thus, over-expression of AD-related genes (such as mutant APP, PS1, and tau, the three genes that characterize our model) appears to favor modifications of additional genes that are involved in AD development and progression.
The study also showed overlapping changes in 3xTg-AD at 3 m.o.a. and WT mice at 12 m.o.a., thereby suggesting altered expression of aging-related genes that occurs earlier in 3xTg-AD mice.

P09.006-M
The role of p35/CDK5 regulation by miR-15/107 family in Alzheimer's disease

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Alzheimer's disease (AD) is characterized by the presence of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated Tau. CDK5 kinase has a key role in abnormal phosphorylation of Tau and β-Amyloid Precursor Protein (APP). CDK5 is activated by p35, encoded by CDK5R1, whose expression can be modulated by miR-103/107, members of the miR-15/107 group, a family of microRNAs involved in AD pathogenesis.
In our study, we observed a significant reduction of p35 levels in cells transfected with miR-103/107, -15a and -16 precursors compared to control, while the transfection with antisense LNA molecules for miR-15/107 group leads to increased CDK5R1 transcript and p35 levels, suggesting the action of the whole miR-15/107 family on CDK5R1 expression.
We thus hypothesize that reduced levels of miR-15/107 in AD can lead to Tau and APP hyperphosphorylation via upregulation of p35 levels and consequent enhanced CDK5 activity. In order to test this hypothesis, we are studying CDK5R1 and miR-15/107 expression and the CDK5 activity on Tau and APP in frozen brain tissues (hippocampus, temporal cortex and cerebellum) from 12 AD patients and 7 control individuals. In the temporal cortex and hippocampus most miRNAs are downregulated in AD compared to control samples, while in the cerebellum all miRNAs show a similar expression between AD patients and controls, with the exception of miR-107 and miR-15a which are more expressed in AD patients. Interestingly, CDK5R1 mRNA levels are higher in AD hippocampus, but not in temporal cortex and cerebellum tissues, compared to controls.
Project supported by FIRB2008 grant (RBFR0895DC_002).

P09.007-S
Human APP overexpression alters spontaneous quantal release at Drosophila larvae neuromuscular synapse

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While amyloid-beta-protein (Aβ) has been implicated in development of Alzheimer's disease, the exact functional role of amyloid precursor protein (APP) is still unclear. In our study, neuromuscular junction of transgenic *Drosophila melanogaster* lines was used as a model to analyze changes in presynaptic function caused by APP overexpression. Miniature excitatory junction potentials (mEJPs) were recorded intracellularly from muscles 6 and 7 in third instar larvae at room temperature in HL3 solution. Confocal microscopy with cytochemistry was also used. It was observed that the mEJP amplitude distribution in control was bimodal with peaks at 0.46 mV and 0.74 mV; only rare giant mEJPs were observed. Human *APP* gene expression in motor neurons decreased mean mEJPs frequency ($p<0.01$) from 2.5/s in control up to 1.6/s, while fraction of giant mEJPs (mean amplitude 1.41 mV) increased up to 29%. In addition, enhanced axon branching and decreased expression of synaptobrevin was observed. Co-expression of human *APP* and *β-secretase* genes (production of Aβ and decrease in APP level) recovered both mean mEJPs frequency and quantity of giant mEJPs to control values. The resting membrane potentials, time course of mEJPs as well as peaks of their amplitudes bimodal distribution never differs significantly from control line. Distributions of mEJPs latencies in all *Drosophila* lines were best fitted as a mono-exponential decay as predicted by the Poisson model. Our data suggests that APP overexpression disturbs molecular machinery of vesicular exocytosis without alteration in the random nature of spontaneous release. Supported by St.Petersburg State University grants #1.50.1621.2013 and #1.38.231.2014.

P09.008-M
An increase in X chromosome aneuploidy rates in the Alzhemer's disease brain can hallmark both neurodegeneration and aging

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Post-zygotic mosaic aneuploidy is common in the normal and diseased human brain. Somatic chromosomal mosaicism affecting the brain is, probably, the result of disturbances of adult neurogenesis/gliogenesis during the early ontogeny. It was proposed that aneuploidy of the brain is somehow involved in pathogenesis of neurodegenerative brain disorders including Alzheimer's disease (AD). Here, we have analyzed X chromosome aneuploidy (a feature of aged cell populations) in the brain tissues of females with/without AD. Molecular cytogenetic analyses were performed by multiprobe/quantitative FISH and interphase chromosome-specific multicolor banding (ICS-MCB) in 10 AD and 10 age/sex matched control samples, scoring 160,000 cells per each sample set. In AD, the mean rate of aneuploidy (2.79%, 95% CI 1.88-3.69) was two times higher than in control (1.32%, 95% CI 0.92- 1.71%); P =0.013 (Mann-Whitney U-test). An AD sample demonstrated mosaic aneuploidy (X chromosome loss) confined to the hippocampus in about 10% of cells. More than 75% of cells with X chromosome aneuploidy were NeuN-negative (non-neuronal cells; glia and, probably microglia). These preliminary data indicate that somatic (post-zygotic) chromosome instability causes large-scale genomic variation in a significant proportion of hippocampal cells in AD. In context of a causal relationship between brain aging and AD pathogenesis, our findings suggest that X chromosome aneuploidy can contribute to both brain aging and neurodegeneration in females with AD. We speculate that mosaic aneuploidy in the brain is a new non-heritable genetic factor predisposing to AD. Supported by BLR 11/002, the Russian Federation President Grant (MD-4401.2013.7), and RFBR 12-04-0021.

P09.009-S
Genetic analysis of Angiogenin gene in Italian patients with Alzheimer's disease

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Background. Despite enormous investigative efforts, the pathological basis for Alzheimer's disease (AD) remains unclear. It has been suggested that AD is mediated by pathological angiogenesis. Angiogenin is a angiogenic ribonuclease whose activity is related to its ability in regulating ribosomal RNA (rRNA) transcription [Cronin 2006]. Mutations in the coding region of ANG (Entrez Gene ID 283) have been detected in Amyotrophic Lateral Sclerosis

(ALS) and Parkinson's disease (PD). A decreased level of ANG protein was found in AD patients serum [Kim 2012]. Objectives. to investigate the role of the ANG gene in AD. Methods. Genetic analysis of ANG gene was done in a cohort of 509 AD patients and 417 healthy volunteers over 65 years of age using Sanger sequencing of the coding regions. Results. Genetic analysis showed the presence of a nonsynonymous mutation in heterozygosis in position g.2162012 causing the change of a lysine to a Stop codon (K73X). This new mutation was found in two AD patients (0.48% of the whole AD cohort): patient 1 is a man, 69 years old, with family history of dementia; patient 2 is a woman, 74 years old, with no familiarity for AD or dementia. No mutations were found in control subjects. Discussion and conclusion. This study suggests that ANG gene mutations may be associated with rare cases of AD. Interestingly, the K73X mutation may be specific for AD, since it has not been found in the numerous genetic studies on ALS and PD so far reported, proposing an important specificity mutation-disease.

P09.010-M

Dog as an animal model for idiopathic epilepsy: identification of common risk variants in the ADAM23 gene

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Idiopathic epilepsy (IE) is a common neurological disease in human and dog. Relatively few risk genes have been identified for common IE to date. We have used dog as an animal model for human focal and generalised epilepsy to identify disease risk genes. The seizure characteristics are similar between the two species and reduced genetic heterogeneity of purebred dogs is advantageous for genetic studies. Recently, we identified a risk locus for IE in the Belgian Shepherd breed on CFA37 in a genome-wide association study (GWAs). GWAs meta-analysis of 158 cases and 179 controls in three other breeds suggested association to the same locus (p=2.9e-07). To investigate this locus further, we performed targeted next-generation sequencing at the locus. Twelve Belgian Shepherd cases and twelve controls were selected for the sequencing based on homozygosity for the risk and non-risk haplotypes. Thirty-six variants unique for the cases were identified in the sequencing experiment. All variants located in the ADAM23 gene region. Twenty-seven variants were selected for validation in 235 cases and 320 controls from four dog breeds. Association analysis yielded a strong signal at the locus (p=5.3e-11). Haplotype analysis suggests a common risk haplotype in all studied breeds. This indicates that there is a common genetic risk factor for epilepsy in several dog breeds. ADAM23 interacts with LGI1 and LGI2, both known causative genes for Mendelian forms of epilepsy, and plays a role in synaptic transmission. Based on the genetic association and gene function, ADAM23 is a potential risk gene for epilepsy.

P09.011-S

High resolution array Comparative Genomic Hybridization (array-CGH) in syndromic microcephaly: clinical interpretation and genotype-phenotype correlation

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Introduction: Microcephaly can be either isolated with no additional anomalies, or it may coexist with other neurological entities and/or multiple congenital anomalies, known as syndromic microcephaly. Although many syndromic cases can be classified based on the characteristic phenotype, some others require further investigation. **Aim:** The present study describes the application of high resolution array-comparative genomic hybridization (array-CGH), as a diagnostic tool for the study of patients with syndromic microcephaly in order to identify clinically relevant copy number variations (CNVs). We evaluate the MRI findings with patients' clinical profile and the underlying molecular findings. Material and **Methods:** From a cohort of 210 unrelated patients, who were referred for genetic evaluation due to syndromic microcephaly of unknown etiology, accompanied by at least one further pathological manifestation, 50 undiagnosed cases underwent array-CGH analysis. In all cases previous standard karyotype as well as other genetic tests were negative. High resolution 4x180K and 1x244K Agilent arrays (>170.000 and >236.000 probes respectively, average resolution 8.9Kb) were used in this study. **Results:** In 32 out of 50 patients (64%) featuring microcephaly among other phenotypic anomalies, array-CGH revealed significant aberrations (microdeletions and/or microduplications) ranging in size from 0.015 to 31.6 Mb and encompassing important genes related with syndromic microcephaly. 25/50 patients (50%) had abnormal MRI findings and in 19 of 25, arrays-CGH revealed pathogenic CNVs. **Conclusion:** Array-CGH contributes to the elucidation of undefined syndromic microcephalic cases, by permitting the discovery of plausible novelmicrodeletion and/or microduplication syndromes and contributing to more precise genotype-phenotype correlations.

P09.012-M

Exome sequencing reveals a new CLN5 mutation in an adult form of cerebellar ataxia

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CLN5 mutations (13q21.1-q32) are reported in severe autosomal recessive forms of neuronal ceroid lipofuscinoses (vLINCL, OMIM*608102), characterized by rapid death of cortical neurons and intracellular accumulation of autofluorescent lipopigment material. The clinical course includes progressive intellectual and motor deterioration, seizures, and visual failure. Typical CLN5 forms have an onset between 4-7 yrs.

We describe two italian siblings affected by an adult form of ataxia, who presented with a homogeneous phenotype characterized by onset between 54-56 yrs, walk difficulties, dysarthria. Progressive cognitive decline associated to visual loss, ascribed to glaucoma, appeared only after few years of disease. Neurological evaluation at 61yrs. showed nystagmus and head/truncal tremor, and no sensory-motor neuropathy. MRI showed severe cerebellar atrophy and mild cortical atrophy of both hemispheres.

Exome sequencing identified a homozygous c.935G>A (p.S312N) transition in CLN5 gene, never reported before nor present in Exome variant Server (http://evs.gs.washington.edu/). Alignment of orthologous sequences and in silico softwares predict the substitution Ser>Asn to be disease causing.

We studied the subcellular localization of the mutated protein, transfecting the CLN5*S312N-pCMV plasmid in Hek293 cells: as reported for vLINCL mutations, p.S312N seemed to affect trafficking and maturation of the CLN5 protein, resulting in its accumulation in ER compartment and indicating that misfolding may be the major cause for its retention.

We speculate this mutation acts as mild or modifier genes/epigenetics factors may contribute to this late clinical onset.

Our results further demonstrated the power of exome sequencing in identifying genes disease causing, especially when linked to atypical phenotypes.

P09.013-S

Genetic and functional studies of autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)

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ARSACS is the second most frequent form of hereditary spastic ataxia after Friedreich's ataxia. ARSACS is caused by mutations in the *SACS* gene encoding for sacsin which is partially localized to the mitochondria. The function of sacsin is still poorly understood. Recently, the mitochondrial network abnormal was observed in immortalized fibroblasts of two Quebec's patients (Girard, *2012*). We searched for point mutations and gene dosage anomalies in *SACS* using direct sequencing and customized CGH array in 325 patients presenting with progressive spastic ataxia and age at onset < 45 years recruited through the SPATAX network. We obtained primary cultures from skin fibroblasts from ten ARSACS patients. On these fibroblasts we performed a labeling of the mitochondrion using the Mitotracker Green®. We identified 42 different mutations in a total of 32 patients carrying two mutations

in *SACS*. Customized CGH array on the two only patients carrying one heterozygous truncating mutation were revealed no anomalies. The diagnosis of ARSACS was confirmed in 13% of our cohort, the largest series of ARSACS patients reported. Mean age at onset was 4 years but late-onset > 25 years was observed. Although demyelinating neuropathy and cerebellar atrophy were frequently associated with spastic ataxia, the clinical spectrum appeared larger than previously. Analysis of the mitochondrial network in all patients' fibroblasts revealed altered mitochondrial shape associated with a significant decrease of the mitochondrial mass. These alterations observed in the mitochondrial network of ARSACS patients bring a real interest to study the role of sacsin regarding the mitochondrion.

P09.014-M
The 901bis pedigree of ataxia is linked to SCA37 locus

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Autosomal dominant spinocerebellar ataxias (SCAs) are a clinically, genetically and pathologically heterogeneous group of movement disorders defined by variable degrees of cerebellar ataxia and often accompanied by additional cerebellar and non-cerebellar symptoms. The clinical symptoms are triggered by neurodegeneration of the cerebellum and its relay connections. Recently we report the clinical and genetic findings from Spanish kindred (901 family) presenting a cerebellar phenotype characterised by ataxia, atypical early-altered vertical eye movements, variable severity, no evidence of anticipation, age at onset ranging from 35 to 64 years and Cranial CT or MRI showing cerebellar atrophy in several patients without brainstem involvement. We have found significant linkage with the highest two-point LOD score Z_{max} = 3.831 at theta = 0.00 (P < 0.001), between the locus trait and D1S2742, assuming an age-dependent penetrance model with twelve liability classes. Multipoint analysis and haplotype reconstruction traced this novel SCA locus to a 0.66-cM interval flanked by D1S200 and D1S2742 (multipoint Z_{max} = 6.052). The Human Genome Nomenclature Committee (HGNC) has assigned it as SCA37. We presented here another ataxia pedigree (901bis family) with 5 affected members over a total of 17 individuals, which show a multipoint lod score of 3.0 to respect the same genetic markers used in the 901 family. This family comes from the same region than the previous 901 and probably has a common founder ancestor. The linkage results from this pedigree reinforces the hypotheses of linkage from the same ataxia locus trait localized in 1p and named SCA37.

P09.015-S
Reduction of neurological symptoms in Ataxia Teleangiectasia patients by Intra-Erythrocyte infusion of Dexamethasone (EryDex)

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Ataxia Teleangiectasia (AT) is a neurodegenerative disorder characterized by early onset ataxia, oculocutaneous telangiectasias, immunodeficiency, recurrent infections, radiosensitivity and cancer proneness. No drug therapies are approved for treating AT; recent observational studies showed beneficial effect of steroid treatment.
To avoid side effects of long-term steroid administration we developed a method for encapsulation of dexamethasone sodium phosphate into autologous erythrocytes (EryDex) allowing the slow release of dexamethasone for up to one month.
The efficacy and safety of EryDex treatment was evaluated in an open-label, single-arm study on 22 AT patients (F:M=1) confirmed molecularly, with a monthly infusion for six consecutive months. Primary efficacy assessment was the International Cooperative Ataxia Rating Scale (ICARS) which best score is 0, while the worst 100. Significant improvements in ICARS were noted in the intention to treat (ITT) population (n=22; p=0.02) and in patients completing the study (PP, n=18; p=0.01), with a mean reduction of 4 points for ITT and 5.2 points for PP. EryDex was well tolerated, without steroid side effects.
At the end of the trial four patients continued Erydex treatment for 27 months (until now) and their ICARS variations were compared to those of four AT who discontinued. A mean ICARS score reduction of 11 points (range -4 to -19) was detected in the patients of the extended study, while a mean increase of 7 points (range + 1 to +14) was found in controls. This confirms the safety of infusion therapy suggesting that Erydex treatment could delay the progression of the disease.

P09.016-M
Eight new ATM gene alterations in Polish patients with ataxia-telangiectasia

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Ataxia-telangiectasia (AT, MIM#208900) is neurodegenerative and immunodeficiency disorder, is inherited in an autosomal recessive manner. AT results from mutations in the ataxia telangiectasia mutated (ATM) gene. We studied the entire ATM gene coding sequence for mutations using PCR-HD, PCR-SSCP and MLPA techniques. 38 changes in ATM sequence were detected in 25 AT families. The mutation types are diverse, including 18 nonsense (58,1%), 10 splicing (32.3%), 2 missense alterations (6,4%) and 1 large genomic deletion (3,2%). Only 2 mutations have been found in homozygous state ([c.4007_4008insA; c.4007_4008insA], [c.9021_9022insA; c.9021_9022insA]). Most frequent mutations among our AT patients are: c.6095G>A (7 times), c.7630-2A>C (6), c.5932G>T (5), c.7010_7011delGT (2). Several families had newly diagnosed DNA alterations in Polish population (8/38; 21,1%). New detected variants were: c.8441delC, c.6145T>G, c.434T>G, c.6754_6754delAfsX5, c.4007_4008insA, c.7606G>A, c.3402+30_3402+32delATC, deletion of 62 and 63 exons of gene. The effects of two missense changes were predicted to be pathogenic by in silico analysis. The c.3402+30_3402+32delATC was identified in 3 AT families (12%) and none of control samples. According to data from the NHLBI Exome Sequencing Project the ATC deletion allele has a frequency of 0.11% total alleles studied and is not observed in the homozygous state. In this study, we confirmed status of recurrent mutations, but also detected new changes in ATM sequence. Most of the Polish patients are compound heterozygotes and none of them having the same combination of mutations, what makes molecular diagnostic more difficult.
Supported by grant NN401098240 from the Ministry of Science and Higher Education in Poland.

P09.017-S
Finding patients with Parkinson's disease (PD) which are heterozygotes for Atp7b gene in Russia

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Significant part of PD patients are heterozygotes for Wilson's disease (WD). WD is a monogenic, autosomal recessive disease characterized by toxic copper accumulation in brain and liver, which results in hepatolenticular degeneration. The major biochemical manifestation of WD is reducing of serum copper status (CS) indexes: copper and ceruloplasmin concentration as well as oxidase activity. Atp7b gene encoding copper transporting ATPase P1 type that takes part in copper excretion and cuproenzimes metallation in Golgi complex is responsible for WD. In heterozygotes for WD, CS is ½ of wide type. Common mutations in ATP7B of patients with WD and PD are not the same.
The blood sera of 50 PD patients without regard to gender with severity indexes 1-4 (Hoehn and Yahr scale) were analyzed for copper (FAAS), ceruloplasmin (immunoelectrophoresis and immunoblotting), and SOD3 (ELISA) concentration as well as oxidase and SOD3 activities (p-phenylenediamine and quercetin assay, respectively). Multivariate statistical analysis showed that low levels of CS correlated with early onset of PD. Analysis of the primary structure of the exon 14, which encodes a nucleotide binding domain of the ATPase and contains the most common mutation associated with WD, showed that none of the 30 patients with PD (who are putative heterozygous for WD according to biochemical parameters) contain these mutations. However, C1079G was found in 11 patients of this group. This mutation in patients with WD has not been described previously.
Revealing heterozygous WD carriers among the PD patients may permit to treat these cases of PD more specifically.

P09.018-M
Genetic contribution to Attention Deficit Hyperactivity Disorder (ADHD) in 320 Spanish patients

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Introduction Attention-deficit hyperactivity disorder (ADHD) is one of the most common neurobehavioral disorders of childhood, characterized by age-inappropriate levels of inattention, hyperactivity and impulsivity. Despite its high heritability (76%), the results of association studies have been inconsistent and poor replicated. The aim of this study was to replicate in a Spanish cohort, the association of previously reported gene variants. **Patients and Methods** 324 children and adolescents (6-17 years old) diagnosed with ADHD according to DSM-IV-TR, and 344 controls were recruited. Patients and controls were genotyped for 23 genetic variants: *DAT1:* rs2550948, rs261759, rs2652511, VNTR-3UTR, VNTR-Intron8; *DRD2:* rs1800497; *DRD4*: rs3758653, VNTR-Exon-3, VNTR-Promotor, *SLC6A4*: VNTR-Promotor, VNTR-Intron-2; *HTR2A*: rs7322347; *NET1:* rs28386840, r5569; *ADRA2A*: rs553668, rs1800544; *LPHN3*: rs1397458, rs2305339, rs6551655; *COMT:* rs4680; *FADS2:* rs498793; *SNAP25:* rs3746544; *DDC* :rs6592961. Pearson's X^2 test, and Hardy-Weinberg equilibrium were used to assess genetic association. **Results** The most significant associations found in this study were: NET1-rs28386840 A/T genotype was significantly increased in both ADHD patients (OR:1.54, p: 0.027) and combined-ADHD subtype (OR:1.72, p:0.0043). DAT1-5/5 genotype resulted increased in inattentive-ADHD patients (OR:2.13, p:0.052) **Discussion and Conclusion** The difficulties found identifying risk ADHD associated gene variants could be explained by the heterogeneity and complexity of this disorder. Further studies designed to examine the contribution of gene-gene or gene-environment interaction are needed. In addition, the use of endophenotypes instead of DSM-IV diagnoses could improve the detection of genetic effects.

P09.019-S
Variation in the phenotype of Machado-Joseph disease (MJD/SCA3): the role of mitochondrial DNA (mtDNA) haplogroups

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Mitochondrial dysfunction has been implicated in the pathogenesis of several neurodegenerative disorders, such as Machado-Joseph disease (MJD), a late onset poly-Q ataxia that results from an unstable expansion of a CAG tract in the ATXN3 gene. The CAG expansion size is incompletely correlated with the age at onset, highlighting the existence of genetic modifiers. Although the way by which mitochondria is involved in the neurodegenerative cascade is still unknown, mitochondrial DNA (mtDNA) haplogroup-specific polymorphisms have been associated to other poly-Q disorders. To evaluate whether mtDNA variation contributes to MJD phenotype, namely to age at onset (AO), we determined the mtDNA haplogroups in 113 MJD patients with Azorean ancestry, by sequencing the mtDNA hypervariable region I. The frequency of mtDNA haplogroups in unrelated patients (n=68) was similar to the one previously described for the general Azorean population. MJD patients classified as haplogroup J present a significantly earlier onset (mean AO of 33 years). Haplogroup W seems to have a protective effect, causing a delay in onset (mean AO of 51 years). Although haplogroup J has already been implicated in other neurodegenerative disorders, there are no previous reports of an association between haplogroup W and disease. In conclusion, our results suggest that mitochondrial single nucleotide polymorphisms defining these haplogroups could modify AO in MJD. The complete mitochondrial genome of MJD patients classified as J and W haplogroup were further sequenced in order to better understand the impact of the haplogroup-defining variants on mitochondrial function and their interaction in MJD phenotype.

P09.020-M
Transcriptional profile of Machado-Joseph Disease (MJD): mTOR signaling pathway is dysregulated in blood cells of patients

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Machado-Joseph disease (MJD; MIM #109150; ORPHA98757), or spinocerebellar ataxia type 3 (SCA3) is a protein misfolding-associated disease, being the worldwide most prevalent autosomal dominant ataxia as well as the second more common polyglutamine (polyQ) disorder. Abnormal conformation of mutated ataxin-3, promotes a gain of a toxic function compromising several cellular mechanisms, namely transcription. Gene expression profiling arrays have the potential to contribute to advances in the understanding of mechanisms related with disease pathophysiology, often suggesting new potential targets for treatment. In MJD, however, the analysis of gene expression changes has been limited to animal and cellular models; thus far, transcriptional changes in MJD patients have not been investigated. In the present study we used microarrays to evaluate a global gene expression profile in blood samples of 12 MJD patients, compared with 12 normal controls. Ingenuity pathway analysis (IPA) software was used to analyze the most dysregulated pathways in MJD. Thirty pathways were found to be dysregulated (log p-value False Discovery Rate (FDR) adjusted > 3); amongst these, mammalian target of rapamycin (mTOR) signaling is observed, with significantly dysregulated expression levels in 211 genes (p-value FDR adjusted < 0.05). mTOR has been described as influencing a variety of molecular processes including protein synthesis and autophagy. Moreover, rapamycin, which inhibits the activity of mTOR, is pointed as having neuroprotective effects in several neurodegenerative disorders, mainly by induction of autophagy. The present results highlight the potential of mTOR inhibitors as potential therapeutic compounds that should be investigated for MJD.

P09.021-S
Three SNP haplotypes in Neuroligins may correlate to autism susceptibility

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Autism is a neurodevelopmental disorder showing a striking sex bias with a male:female ratio of 4:1. Despite some genetic variants are causative for autism in about 17% of cases, its etiology is still largely unknown. Increasing evidences highlight the possible role of environmental factors, such as infections, xenobiotics and drugs in enhancing autism genetic susceptibility. Among genetic variants, we focused on the X-linked neuroligins that are involved in synaptic plasticity, are mutated in a few number of autistic patients, and are hemizygous in males. Hence we analyzed NLGN-3 and NLGN-4X in 52 Italian autistic cases (male:female=4,6:1) and in 31 healthy siblings (male:female=1:1,1) by Sanger sequencing. Among the other variants, in NLGN-4X, we found 2 de novo SNPs and 3 SNPs in non-coding regions (1 intronic and 2 in the 3'UTR), giving 2 different haplotypes: one was new and one was already described in a non-specific mental retardation Chinese patients. In NLGN-3, we found 3 already described intronic SNPs in haplotype block. The 3 haplotypes have statistical significance in autistics comparing to the minor allele frequencies (MAF) from the 1000-Genomes Project CEU. Interestingly, healthy siblings, half of which were female, have a middle statistical significance between autistics and MAF-CEU. As these SNPs map to non-coding regions, they could be involved in the genetic susceptibility to trigging environmental factors (probably lacking in siblings), and, being located on X-chromosome, could explain the male prevalence of autism. ACKNOWLEDGEMENTS: Italian Ministry of Health "GR-2009-1570296" project, Italian Ministry of Education, University and Research "InterOmics" Flagship project.

P09.022-M
Diagnostic utility of microarray analysis to detect rare CNVs in autism spectrum disorders

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Background: The genetic causes of Autism Spectrum Disorders (ASDs) are heterogeneous and still unknown in the majority of cases. Copy number variant (CNV) detection is part of routine clinical workup. An interesting paradigm for clinical practice is that each rare CNV may account for only a small proportion of variance in ASD at population level but may have large effects in particular families. We aim to evaluate family and patient specific characteristics that may alter the clinical utility of autism risk variants. **Material and methods:** The study contains 466 clinically well characterized patients with ASD from 392 families. Individuals were genotyped by either Affymetrix SNP 6.0 array, OGT Oligo array or Illumina Omni2.5-8v1 SNP array. Genotype data of each platform were preprocessed and normalized by limiting the resolution to 200 Kb and developing customized analysis protocols per platform to detect smaller variants in targeted regions. A filtering pipeline specific for ASD was developed using standard and customized Cartagenia

Bench software applications. **Results:** We present a family based study on the validity of CNV detection in ASD using microarray analysis. The clinical utility of this screening is evaluated for different parameters (e.g. sporadic versus familial ASD, IQ, comorbidity). In some cases, we found a better diagnostic performance of the higher resolution platforms. **Conclusion:** We contribute to the diagnostic utility and indication of rare CNV detection in ASD families with respect to clinical presentation and family history.

P09.023-S
Genetic evidence that hyperactive Th2 and NK immune pathways contribute to the pathogenesis of Autism Spectrum Disorder

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Converging evidence suggests that abnormal immunity is involved in the pathophysiology of Autism Spectrum Disorder (ASD) both in children and adults. Altered immune processes include ongoing neuroinflammation in post-mortem brains, elevated pro-inflammatory cytokines in cerebrospinal fluid and blood, altered immune cell function, presence of brain-specific auto-antibodies, and dysregulated immune transcriptome. One strategy to assess whether dysimmunity contributes to ASD pathogenesis or represents a collateral by-standing effect is to investigate whether functional SNPs known to influence immune gene expression or function are associated with ASD. We genotyped 484 simplex and 18 multiplex families with an ASD proband at 34 known functional SNPs located in 26 immune genes involved in all major immune pathways. Statistically significant opposite transmission patterns between ASD and unaffected siblings were evident for rs2243250 (*IL4*) and rs231775 (*CTLA4*), with the combination of high *IL4* expression and low *CTLA4* function alleles associated with autism and the opposite alleles overtransmitted to unaffected siblings. Similarly, at rs361525 and rs2430561, functional SNPs located in the *TNFA* and *IFNG* genes, the combination of high expression alleles was associated with autism, while the opposite alleles were protective. These results indicate that common variants conferring hyper-responsive Th2 (*IL4* and *CTLA4*) and NK (*TNFA* and *IFNG*) activation also confer autism vulnerability. Hence previously-reported increases in plasma Th2 cytokine levels and NK cell activation are not mere by-standing effects, but are instead part of the pathophysiological cascade resulting in autistic disorder in a sizable subgroup of patients.

P09.024-M
Array-CGH contribution to Child & Adolescent Neuropsychiatry: update and prospectives

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Many behavioral disorders display prominent genetic underpinnings. We have thus introduced array-CGH analysis (Agilent, 180K) in our routine clinical assessment of all autistic and cognitively impaired children, as well as in the presence of high familial loading and/or dysmorphology. Blood samples were collected from 176 families and genetic counselling has already been provided to 65 of them. Eight families are multiplex, yielding a total of 81 patients. Proband diagnoses include: 52 autism, 9 cognitive disabilities, 4 developmental delay, 4 generalized dyspraxia, 3 learning disabilities and 9 other disorders. Patients were divided into 5 classes based on array-CGH results: [1] certainly causal CNV, N=12 (15%); [2] probably causal CNV, N=23 (28%); [3] rare variant of uncertain interpretation, N=19 (23%); [4] common variant without causal role, N=20 (25%); [5] negative result, N=7 (9%). Array-driven medical diagnostic work-up was prescribed to 10 (12,3%) patients and 3 parents, while drug therapies or supplements were prescribed to 4 (7,5%) patients. The diagnostic work-up was positive in 3 patients and 1 parent, while therapies were ineffective in 1 case and still pending evaluation in the other three. Array-CGH is thus a powerful clinical tool, provided its focus includes the functional role of duplicated or deleted genes, their presence in neurodevelopmental disorder databases and possible imprinting. Currently the main limitation is the translation of this knowledge into effective drug therapies. Array-CGH data will be especially useful when drugs currently undergoing phase II-III trials and potentially beneficial to specific subgroups of patients, will become available.

P09.025-S
Band-like brain calcification (BLC) or pseudoTORCH syndrome: A report of six Egyptian families with expansion of the phenotypic and mutational spectrum

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Band-like brain calcification (BLC) or pseudo TORCH syndrome is a rare autosomal recessive with distinctive clinical and neuroimaging. Severe microcephaly, early onset seizures, profound developmental delay together with band-like calcification in brain, simplified gyral pattern and polymicorgyria are the hallmark of the syndrome. In 2010 O'Driscoll et al. attributed BLC to homozygous mutation in occludin (OCLN) gene through a description of 5 families from a worldwide series including an Egyptian one. Since then, a single family was reported associated with extracranial phenotype namely renal involvement due to cortical calcification. Herein we describe eight patients derived from six Egyptian Families with BLC. All presented at early life with severe microcephaly, failure to acquired developmental skills, growth arrest and mixed seizures types with predominance of the myoclonic. Patients showed a unique calcification pattern; subcortical band, basal ganglia, dentate nucleus and pons. Molecular analysis revealed that two families were linked to known genetic mutations while four families had novel mutations in OCLN gene. Three of our patients deceased at the end of the first year of life. Interestingly, the single patient with novel mutation in exon 5 had non-neurological manifestations including high imperforate anus and genital anomalies. Further, he had the best life performance with fairly controlled seizures and achieved few skills although no comparable neuroimaging findings were present. This study is considered the largest series with BLC from same ethnic group. Assigning more families will allow delineation of the phenotype-genotype spectrum of BLC.

P09.026-M
Association study of 240,000 rare coding variants in bipolar patients and controls from Germany and Norway

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Genome-wide association studies (GWAS) of bipolar disorder (BD), a highly heritable disorder of mood with a lifetime prevalence of approximately 0,5-1% in all populations world-wide, have identified several common genetic risk factors. It is currently unclear, to what extent low-frequency and rare variants contribute to disease development. A valid hypothesis seems to be that low-frequency and rare variants in coding gene regions have a higher probability to have a functional (deleterious) effect. This subset of human genetic variation might therefore be enriched for disease-relevant variants. The Illumina HumanExome arrays make particularly this window of genetic variation accessible for association studies. This array contains 240'000 rare markers derived from exome sequencing. That array was used to test 1314 bipolar patients (895 from Germany, 419 from Norway) and 2700 controls (2366 / 339). Genotypes were exproted from a single GenomeStudio project to minimize clustering artifacts. Statistical analysis was performed using Cochrane-Mantel-Hansel test statistics. Clusters for all associated variants were checked by zCall and manually. The two variants with the strongest association to bipolar disorder were found in the gene SYNE2. Interestingly, common variants in another member of the same gene family (SYNE1) had shown genome-wide significant association in the discovery step of the first mega-analysis of bipolar disorder performed by the international Psychiatric Genomics Consortium (PGC; Sklar et al. 2011). We are currently aiming to follow-up this and other strong findings of our analysis in independent samples of bipolar disorder genotyped on the HumanExome array.

P09.027-S
Clinical profile of paediatric Brown-Vialetto-VanLaere syndrome

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Brown-Vialetto-Van Laere Syndrome (BVVLS) is an autosomal recessive neurological disorder presenting with progressive sensorineural hearing loss and bulbar neuronopathy, followed by a mixed picture of upper and lower motor neuron palsies. Until recently there has been no treatment available for this progressive neurodegenerative disorder. However, the discovery that it is caused by mutations in the riboflavin transporter genes, SLC52A2 and SLC52A3, have led to the use of riboflavin supplementation and there is growing evidence to suggest that this may be an effective treatment. This makes the identification of this disorder particularly important and led us to review clinical information about the phenotype of children

with BVVLS. Our cohort consisted of patients diagnosed at the Molecular Diagnostic Laboratory at Guy's and St. Thomas Hospital and by our collaborators in Germany and Iceland, as well as molecularly-confirmed reports in the literature. After excluding patients with insufficient clinical data available, we identified a total of 28 SLC52A2 and 15 SLC52A3 patients for analysis. We identified differences in the clinical presentation and progress of these two patient groups. In particular, while hearing loss, muscle weakness and bulbar palsy were common clinical features in both patient groups, amongst SLC52A2 mutation carriers, ataxia and optic atrophy were also surprisingly common. These findings suggest that BVVLS may have a wider clinical phenotype than presently appreciated. This is an important observation given emerging evidence that it can be ameliorated using riboflavin supplementation.

P09.028-M
Effects of sapropterin on endothelium-dependent vasodilation in patients with CADASIL: a randomized controlled trial

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Background and Purpose - CADASIL, a rare autosomal dominant disorder caused by NOTCH3 mutations, is characterized by vascular smooth muscle and endothelial cells abnormalities, altered vasoreactivity and recurrent lacunar infarcts. Vasomotor function may represent a key factor for disease progression. Tetrahydrobiopterin (BH4), essential cofactor for nitric oxide synthesis in endothelial cells, ameliorates endothelial function. We assessed whether supplementation with sapropterin, a synthetic BH4 analogue, improves endothelium-dependent vasodilation in CADASIL.
Methods -- In a 24-month, multicenter randomized, double-blind, placebo-controlled trial, CADASIL patients aged 30-65 years were randomly assigned to receive placebo or sapropterin 200-400 mg b.i.d. The primary endpoint was change in the reactive hyperemia index by peripheral arterial tonometry (RH-PAT) at 24 months. We also assessed the safety and tolerability of sapropterin. Analysis was done by intention-to-treat (ITT).
Results -- The ITT population included 61 patients. We found no significant difference between sapropterin (n=32) and placebo (n=29) in the primary endpoint (mean difference in RH-PAT changes 0.19 (95% CI -0.18;0.56)). RH-PAT increased after 24 months in 37% of patients on sapropterin and 28% on placebo; however, after adjustment for age, sex and clinical characteristics, improvement was not associated with treatment arm. The proportion of patients with adverse events was similar on sapropterin and on placebo (50% vs 48.3%); serious adverse events occurred in 6.3% vs 13.8%, respectively.
Conclusions -- Sapropterin was safe and well-tolerated at the average dose of 5 mg/kg/day, but did not affect endothelium-dependent vasodilation in CADASIL patients.
Registered at URL: https://www.clinicaltrialsregister.eu Unique Identifier 2007-004370-55.

P09.029-S
Comparison of NOTCH3 expression in fibroblasts from CADASIL patients versus normal controls

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CADASIL is an inherited cerebrovascular disease caused by mutations in the NOTCH3 gene, encoding a protein belonging to the Notch receptor family. Notch signalling is involved in a broad spectrum of function, from the cell proliferation to apoptosis. Recently it has been shown that it is expressed in adult human tissues such as peripheral blood lymphocytes (PBLs) and fibroblasts. Our aim was to evaluate Notch3 protein expression in human fibroblasts from CADASIL patients.
We perform the investigation on the fibroblasts from four CADASIL patients and a normal subject. The patients were genetically characterized, and carried a C174Y, a R332C, a c.341-24_26delAAC, a R61W mutations, respectively. Fibroblasts were grown in DMEM with 10% FBS, Glutamine, Streptomycin-Penicillin. At 80% of confluence, cells were washed in TBS and fixed on a slide with paraformaldehyde.
The slides were treated with a mouse anti Human Notch3 antibody that recognizes the extracellular domain ECD and a rabbit antibody that recognizes the intracellular domain NICD. Anti-mouse and anti-rabbit secondary antibodies revealed the primary antibodies.
Different patterns of localization were detected. In cells with mutation R61W an immunoreactive predominance of the intranuclear NOTCH3 NICD, and in cells from patients with R332C mutation an immunoreactive predominance of the N3ECD extracellular NOTCH3 domain was observed when compared both with cells from normal subject, and with the remaining CADASIL cells.
The obtained results show that in CADASIL fibroblasts the mutation type is associated with a relative differential localization of Notch3. This likely might be due to alterations in the protein processing.

P09.030-M
aCGH reevaluation reveals clinically relevant CNVs, in patients with previously unexplained developmental delay / ASD, candidate for exome sequencing. Presentation of selected cases.

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Array comparative genomic hybridization (aCGH) constitutes a first-tier diagnostic test for individuals with unexplained developmental delay (DD) or autism spectrum disorder (ASD) with an estimated diagnostic yield of about 15% to 20%. The advent of exome sequencing has revolutionized the diagnostic process in the remainder of undiagnosed patients, allowing the identification of causal mutations in an additional 25% to 55% of such patients, depending on the different studies' criteria.
Over the last few years, copy number variation databases have accumulated significant amounts of data, often facilitating the task of distinguishing between benign and pathogenic copy number variants and the delineation of novel, recurrent, microdeletion/microduplication syndromes.
We present selected cases of patients, candidate for exome sequencing analysis. Reevaluation of aCGH data allowed re-classification of variants of previous unknown clinical significance, to the category of clinically relevant CNVs overlapping newly recognized DD/ASD genes such as *CAMTA1*, *RB1-CC1*, *CNTNAP2* and others.
Our examples demonstrate that aCGH remains a valuable diagnostic tool for the investigation of such patients, and underscore the need to reassess the pathogenicity of previously detected aCGH variants before consideration of exome sequencing.

P09.031-S
CNVs analysis in patients with Cerebellar and Brainstem Congenital Defects (CBCD)

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Cerebellar and brainstem congenital defects (CBCD) affect about 1 in 3000-4000 live births. Typical clinical signs include hypotonia, ataxia, abnormal ocular movements and psychomotor delay/intellectual disability, but phenotypic variability is wide with possible multiorgan involvement.
With the exception of few autosomal recessive or X-linked conditions, CBCD are frequently sporadic, suggesting that *de novo* mutations or genomic rearrangements may represent common pathogenetic mechanisms.
We analyzed by genomic microarray platforms 83 sporadic CBCD patients, enrolled in a multicentric project focused on studying the genetic causes of CBCD. Eight pathogenic or potentially pathogenic CNVs were detected in 7 patients (8.4%).
Further 11 patients, investigated in our Cytogenetic diagnostic lab for intellectual disability and/or congenital anomalies, have been subsequently included in the CBCD project, since they showed CNVs overlapping to deletions/duplications detected in CBCD patients and/or were affected by CBCD.
Of note, we detected eight deletions that defined three recurrent imbalances. Three deletions were partially overlapping in 3q22.3q26.1, three in 6q25.1q27 and two in 10q26.2q26.3. These three chromosomal regions have been already associated to CBCD, although literature and DECIPHER database revision suggest incomplete penetrance. In addition we report six patients with non-recurrent CNVs: del(2q36.3), dup(6p22.3), inv/dupdel(8p), del(8p21.3p21.2), dup(13q34), dup(Xq28).
These data confirm the crucial role of microarray analysis as a tool to identify

pathogenic CNVs in patients with congenital defects. Our data confirm that CBCD are characterized by high genetic heterogeneity and incomplete penetrance, suggesting the involvement of further mechanisms (modifier genes, environmental factors) in cerebellar and brainstem mal-development.

P09.032-M
The structure and functions of the transcription factor PHOX2B: new insights in the molecular pathogenesis of Congenital Central Hypoventilation Syndrome

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Congenital Central Hypoventilation Syndrome (CCHS) is a very rare neonatal neurological disorder characterized by abnormal ventilatory response to hypoxia and hypercapnia, owing to failure of autonomic respiratory control: affected children hypoventilate during sleep, with possible very severe neurological damages.
Frameshift mutations (5%) and poly-alanine triplet expansions (95%) have been detected in the coding region of the homeobox gene PHOX2B in about 90% of CCHS patients. A correlation between length of the expansion and severity of the respiratory phenotype has been reported. Since some of the mutations alter the sub-cellular localisation, the DNA-binding affinity and the transcriptional activity of the protein, and that mutated PHOX2B proteins can interfere with the activity of the wild-type protein by sequestering it into aggregates, one crucial question concerns the identification of the functional domains of the protein, the role of the poly-alanine tract and the effects of its expansion on the general architecture and function of the protein. We have performed a deletion analysis of PHOX2B and identified two nuclear localization signals in the homeodomain, both required for the complete import of the protein in the nucleus, corresponding to residues necessary for the binding to DNA, and partially blocked by the expanded poly-alanine tract. By using mammalian two-hybrid system we have also demonstrated that PHOX2B can form homo-dimers and we are currently investigating if the mutations alter the dimerization properties of the protein and the potential contribution to the range of phenotypes and pathogenesis in CCHS patients.

P09.033-S
Transcriptional dysregulation and impairment of PHOX2B auto-regulatory mechanism in the pathogenesis of Congenital Central Hypoventilation Syndrome

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The PHOX2B transcription factor plays a crucial role in autonomic nervous system development. In humans, heterozygous mutations of the *PHOX2B* gene lead to Congenital Central Hypoventilation Syndrome (CCHS), a rare disorder characterized by a broad variety of symptoms of autonomic nervous system dysfunction including inadequate control of breathing. The vast majority of patients with CCHS are heterozygous for a poly-alanine repeat expansion mutation of a twenty residues poly-alanine tract in the C-terminus of PHOX2B. Although several lines of evidence support a dominant-negative mechanism for *PHOX2B* mutations in CCHS, the molecular effects of PHOX2B mutant proteins on the transcriptional activity of the wild-type protein have not yet been elucidated. One of the targets of PHOX2B is the *PHOX2B* gene itself, and we have recently demonstrated that mutated PHOX2B variants can actually negatively interfere with the expression of the normal allele. Since in vitro the poly-alanine expanded proteins alter the regulation of other three PHOX2B target genes (*PHOX2A*, *DBH*, *TLX2*) in a promoter-specific manner and that, in CCHS patients, different PHOX2B mutations may affect different target genes, this highlights the importance of identifying PHOX2B target genes in order to get new insights into the molecular pathogenesis of the disease. To this purpose, we carried out Chromatin Immunoprecipitation experiments from IMR32 neuroblastoma cell line, followed by massively parallel sequencing of the co-immunoprecipitated genomic DNA fragments (ChIP-Seq). We are currently validating the most promising candidate target genes by biochemical and functional approaches.

P09.034-M
Molecular investigation of CDKL5 gene in patients with Rett like infantile spasms in Greece-Preliminary results

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A subset of atypical Rett syndrome with infantile spasms or early seizures starting in the first postnatal months is caused by mutations in the Cyclin-Dependent Kinase-Like 5 gene (*CDKL5*) located in the Xp22 region. Mutation screening of *CDKL5* was performed in 20 female and 5 male patients, referred for infantile spasms, who were previously tested negative for methyl CpG-binding protein 2 gene (*MECP2*) mutations. All coding exons (2-21) and intron-exon boundaries of *CDKL5* gene were screened for mutations by Enzymatic Cleavage Mismatched Analysis (ECMA) followed by direct sequencing. A novel frameshift mutation (c.2530delC; p.H844IfsX19) was detected and, due to its deleterious nature, predicted to affect the carboxy-terminal region of the protein. The female patient, aged 10- years-old, presented psychomotor delay and epileptic spasms which began at the second month. Subsequent studies in both parents and in 50 chromosomes from normal subjects failed to detect the c.2530delC mutation indicating that it is probably *de novo* and disease-causative. The novel mutation was directly submitted in the RettBASE (IRSF MECP2 Variation Database) and LOVD databases. Our studies of *CDKL5* gene were carried out in patients with Rett-like phenotypes and epileptic spasms with early onset. The incidence of *CDKL5* mutations in female patients with Rett-like features who were negative for *MECP2* mutations was 5% (1/20), while in female patients with spasms it was 17% (1/6), rates both lower than those reported by other studies (7.8% and 28%, respectively) probably because of their larger sample sizes.

P09.035-S
No evidence for a contribution of CHRNA7 rare variants in autism susceptibility

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Recurrent microdeletions of chromosome 15q13.3 are causally associated with a wide range of phenotypic features, including autism spectrum disorder (ASD), seizures, intellectual disability, and other psychiatric conditions. The pathogenicity of the reciprocal microduplications is more uncertain.
Even if the recurrent deletion contains several genes, CHRNA7, encoding for the alpha7 subunit of the neuronal nicotinic acetylcholine receptor, is considered the culprit gene in mediating the neurological phenotypes in patients with the 15q13.3 deletion.
In order to evaluate the role of CHRNA7 rare variants in ASD susceptibility, we have performed copy number variant (CNV) analysis and mutation screening of the coding sequence of CHRNA7 in a sample of 135 ASD individuals from Italy. Rare sequence variation in this gene remains largely unexplored, given the existence of a fusion gene, CHRFAM7A, which includes a partial duplication of exons 5-10 of CHRNA7. Hence, any attempts at sequencing to detect mutations must distinguish between CHRNA7 and CHRFAM7A, making next-generation sequencing approaches unreliable for this purpose.
CNV analysis led to the identification of a CHRNA7 microduplication in a subject with autism and moderate cognitive impaiment. No pathogenic mutations were identified in CHRNA7 coding regions. However, we detected rare variants in the proximal promoter region, previously described to functionally reduce transcription. In conclusion, rare sequence variants in CHRNA7 do not significantly contribute to ASD susceptibility, at least in our clinical sample characterized by low frequency of associated medical comorbidity. This study represents the first sequence variant analysis of the CHRNA7 gene in a sample of idiopathic autism.

P09.036-M
Phenotypic and genetic heterogeneity of CLCN2-related leukodystrophy

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Mutations in the CLCN2 gene encoding the brain chloride channel ClC-2 have been recently associated with a rare autosomal recessive leukoencephalopathy, characterized by variable age at onset and clinical features, with specific brain MRI findings caused by chronic white matter edema. We sequenced CLCN2 in 6 patients from 5 families presenting characteristic MRI white matter alterations involving middle cerebellar peduncles, cerebral peduncles, and posterior limb of the internal capsules. We identified

3 homozygous CLCN2 mutations in 5 patients (4F/1M) from 4 families, while one male patient tested negative. One novel homozygous splice-site mutation was identified in a young asymptomatic woman who underwent MRI because of headache. A previously described nonsense mutation was identified in 3 women from 2 apparently unrelated families from Southern Italy. One patient presented at 58y with dystonic posture of the neck, mild postural tremor of the hands, and head tremor. Of the two sisters, one presented at 26y with migraine without aura followed, at 46y, by progressive postural imbalance, hypoacusia and tinnitus, and degenerative retinopathy; her sister, aged 60y, suffers from cluster headhache since 30y with negative neurological examination. Repeated MRI in both sisters show stability of the leukodystrophic pattern. Finally, a novel homozygous missense mutation was found in a male patient presenting asymptomatic leukoencephalopathy discovered during assessment for infertility and azoospermia. This study shows that CLCN2 mutations can be associated with a wide, but relatively mild, phenotypic spectrum and suggests that a second, yet unidentified, gene can be associated with this peculiar white matter disease.

P09.037-S

A mutation in *SBF2* deleting the plekstrin homology domain affects carriers

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We searched for the molecular basis of Charcot Marie-Tooth neuropathy type 4B (CMT4B) in a consanguineous Bedouin family consisting of the parents and their 4 children. Two of the children were affected, another child presented with slow motor conduction velocity similar to the father who also had mild pes cavus and in-toeing feet. Assuming homozygotization of a founder mutation we used linkage analysis for the known genes affected in CMT to identify the chromosomal locus of the mutation. The genes *PRX, FGD4, SH3TC2, EGR2, NEFL, GDAP1, MPZ,* and *MFN2* were negated by the linkage analyses. Homozygosity was identified for the *SBF2* locus. Sequencing the patients' DNA for *SBF2* identified a homozygous deletion of 12 bases including the acceptor splice site at the 5' of an exon of the gene. The transcript presented a deletion of 19 bases in this exon causing a frameshift that deletes the plekstrin homology domain. Quantitative RT-PCR determined that the stability of this transcript was comparable to that of the normal transcript suggesting that the truncated protein is produced. The parents and the child with slow motor conduction velocity were heterozygotes for the mutation. The mutation causing elimination of the plekstrin homology domain may impair motor conduction velocity in some carriers. This is the first documentation of an effect of a mutation in *SBF2* in the heterozygote state that may act in a dominant negative manner.

P09.038-M

Rare Copy Number Variants underlying Genetic Epilepsy: a regional study

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Background: Epilepsy is a common neurological condition affecting 0.5-1% of the population. Increasingly, it is being recognised that genetic predisposition underlies most epilepsy. The use of new technologies such as whole exome/genome sequencing and microarrays have led to the identification of several single genes and copy number variants (CNVs) as the cause of epilepsy.
Objective: To identify and discuss causal CNVs in patients with suspected genetic epilepsy
Methods: We studied all 213 patients with epilepsy referred to the Oxford University Hospitals Genetics Laboratory for arrayCGH as part of their clinical diagnostic work up between 2006 and 2013. We classified the abnormal CNVs into "definitely", „probably", "uncertain" and "benign" pathogenicity groups. We studied the epilepsy phenotype of the patients, especially those in the „definitely" and "probably" groups, wherever possible. .
Results: Abnormal CNVs were identified in 69/213 patients (32.4%); single CNVs in 50/69 (72.5%) and multiple CNVs in 19/69 (27.5%) of patients. We classified rare CNVs as „definitely pathogenic" in 18/213 (8.5%), „probably pathogenic" in 12/213 (5.6%) and "uncertain" or "benign" in 39/213 (18.3%). Our most frequent rare CNV was 2p16.3 microdeletion (49,294,769-51,251,557) disrupting the *NRXN1* gene, present in 7/69 (10.1%) patients. Other regions of interest included 2q22, 6q26, 15q11.2, 16p11.2 and 22q11.2. Potential candidate genes will be discussed.
Conclusions: Our study highlights the importance of the use of microarrays in the clinical diagnosis of patients with epilepsy. It discusses 'definitely pathogenic' variants which have been previously reported and potential candidate genes within 'probably pathogenic' CNVs which need further research.

P09.039-S

Genome-wide search implicates a potassium channel gene in cognitive performance in the elderly

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Normal aging of the brain is characterized by changes of its structure and function resulting in decreasing cognitive abilities. However, the age-dependent decline of the cognitive performance (CP) can vary greatly in subjects of the same age. Here we assessed CP-profiles (attention, executive functions, language, and memory) of 482 elderly subjects from a population-based cohort from Germany (1000BRAINS) revealing 323 high-performers (HPs) that cognitively performed better than 159 low-performers (LPs). To detect genetic variants that contribute to these CP-differences, we compared both groups in a genome-wide association study (GWAS).
No SNP reached genome-wide significance (P<5E-8). However, 28 SNPs showed strong-to-moderate evidence for association with CP-differences (P<5E-5). The most significant finding was a SNP located 200 kb upstream of the *KCNH8* gene. The minor allele was significantly over-represented in LPs compared to HPs (38% vs. 26%, OR=1.77, P=1.94E-6) suggesting that it contributes to reduced CP.
Neither the top SNP nor a SNP in strong linkage disequilibrium have received evidence for association with a cognitive endophenotype through a previous GWAS. KCNH8 is expressed in important brain regions and belongs to a family of voltage-gated potassium channels that likely are involved in modulating the overall excitability of neurons (Zou et al., 2003). Our study proposes a promising new candidate gene for CP. However, replication in independent samples is necessary to confirm our result.

P09.040-M

Two novel missense COL4A1 mutations and genetics heterogeneity in porencephaly

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COL4A1/COL4A2 mutations have been reported in porencephaly and in small vessels cerebral vascular diseases (CVD), often associated with ocular, renal and muscular features. We screened three families with a broad spectrum of porencephaly and vascular leukoencephalopathy for COL4A1/COL4A2 mutations. In family LEU-1-TO, three patients on two generation presented with leukoencephalopathy variably associated with retinal, deep and periventricular cerebral haemorrages and aneurysms. One subject presented a small periventricular porencephalic lesion. The proband in family LEU-2-TO had a history of congenital glaucoma and cataract, presented a sudden and reversible motor impairment at 14 yrs, followed by progressive gait imbalance in her 30thies. She delivered two newborn severely affected by porencephaly. In the post partum she had an haemorragic stroke. MRI showed parietal haemorrage, diffuse microbleeds, severe leukoencephalo-

pathy and aneurisms of intracranial vessels. In family LEU-3-TO two male brothers presented an overlapping clinical picture with history of recurrent haemorragic strokes in the first two decades and a severe leukoencephalopathy, brain microbleeds and ischemic lacunae at the neuroimages. We identified two novel missense mutations in COL4A1 (c.1249 G>C, p.G417R in LEU-1-TO; c.2662 G>A, p.G888R in LEU-2-TO) by sequence analysis. Both are predicted pathogenic and hit highly conserved Gly residues of the Gly-X-Y repeat in the collagen triple helical domain. As reported for COL4A1 missense changes in the first third of the gene, mutation p.G417R seems associated with a variant phenotype in which porencephalic lesions are not the major characteristic. Patients in family LEU-3-TO, negative for COL4A1/COL4A2, suggest a genetic heterogeneity for this disease.

P09.041-S

The role copy number variations (CNVs) in cryptogenic Cerebral Palsy

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Background: Cerebral palsy (CP) is an „umbrella term" for congenital, non-progressive motor disability, reflecting multifactorial insults to the developing brain. Etiologic factors include perinatal asphyxia, infection, inflammation, and stroke. However, in many individuals with CP the pathogenesis is unknown. Copy number variations (CNVs) cause various neurodevelopmental conditions. We investigated the prevalence and characteristics of CNVs in individuals with cryptogenic CP.
Methods: 52 participants with non-progressive pyramidal or extra-pyramidal signs since infancy and no identified etiology were enrolled. Individuals with acquired cause (i.e. stroke) were excluded. Analysis was performed using the Affymetrix HD array. CNVs were classified as: pathogenic CNVs, likely pathogenic, or likely benign. Main outcome measures were: clinically significant (pathogenic and likely pathogenic CNVs) or not-significant (likely benign and no CNVs) findings.
Results: 40 CNVs were found in 26/52 (51%) participants. Most CNVs were considered clinically significant (11/26 pathogenic and 5/26 likely pathogenic vs. 10/26 likely benign) and were not previously reported to cause motor disability (12/16). Most CNVs were de-novo. Compared to individuals without clinically insignificant CNVs, individuals with clinically significant CNVs were more likely to have multiple CNVs ($p<0.001$), dysmorphic features ($p=.01$) and non- motor comorbidities ($p=0.03$). In 2/16 participants with clinically significant CNVs the phenotype was characteristic for the genomic findings (SPAT and KANK1 deletions).
Conclusions: Clinically significant CNVs were found in 16/52 (31%) of individuals with cryptogenic CP and were often multiple and de-novo. We recommend that this useful test be performed in individuals with cryptogenic CP *especially in individuals with dysmorphic features and comorbidities.*

P09.042-M

Behavioral phenotype in Costello Syndrome with atypical mutation: a case report

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Costello syndrome (CS) is a rare genetic disorder caused, in the majority of cases, by germline missense HRAS mutations affecting Gly12 promoting enhanced signaling through the MAPK and PI3K-AKT signaling cascades. In general, the neuropsychiatric phenotype in CS is fairly homogeneous with mild or moderate intellectual disability, and a behavior usually characterized by irritability and shyness at younger ages, and sociable personality and good empathic skills after 4-5 years. We report on a 7-year-old boy a heterozygous for a rare duplication of codon 37 (p.E37dup) in HRAS, manifesting an impairment in his social interaction and non-verbal communication with circumscribed interests. The neuropsychiatric evaluation meets the DSM-IV criteria for Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). He showed marked impairment in the use of multiple nonverbal behaviors (eye-to-eye gaze, facial expression, body posture, and gestures to regulate social interaction). Also a neuropsychological battery of tests was used to evaluated the subject. A behavioral treatment was prescribed to reduce aberrant behaviors and compulsions. Regarding communicative aspects, we recommended a speech therapy intervention. After one year the patient and his parents have benefited from these treatments. In conclusion we have investigated a CS patient with an uncommon HRAS lesion showing an atypical profile in which a careful neuropsychological and behavioral evaluation showed the indication to immediately start a behavioral therapy and a speech therapy intervention. These features improve the delineation of the phenotype of individuals with rare HRAS mutations.

P09.043-S

Anticipation of age at death and possible bias in Creutzfeldt-Jacob Disease

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Mutations in PRNP that encodes the prion protein are causative for inherited Creutzfeldt-Jakob disease (CJD). The E200K substitution is the most frequent mutation. Earlier age at death in successive generations has been reported in two clusters, from Israel (Rosenmann et al. Neurology 1999;53(6):1328-9) and Italy (Pocchiari et al. PLoS One 2013;8(4):e60376). No molecular or environmental explanations have been found. The aim was to analyze a possible observational bias in E200K CJD patients from families living in France. Ages at death from 42 parent-offspring pairs from 19 families were collected and compared: parents 63.5 years ±13.9 (range 43 to 90) offspring 59.8 years ±10.4 (25 to 80), indicating a significantly earlier age at death in offspring ($p=.015$ paired t-test). Including ages at last follow-up or at death of an unaffected but obligate carrier-parent, the difference increased to - 9.8 years anticipation $p=.004$. Considering the large range of ages at death and the intra-sib-ship variability, the most obvious bias lies in the fact that sibs could develop disease later in life. When analyzing pairs with at least 50% affected with known ages at death, the anticipation was lost: parents 68.1 ± 19.9 years and offspring 62.8 ± 7.9 years $p=.957$, $n=12$. This result could indicate a possible observation bias. Another hypothesis could be the loss of modifying genes outside inbred clusters. The search for genetic modifiers in familial CJD is of great importance.

P09.044-M

A new autosomal recessive syndrome: Severe developmental delay and dysmorphic features causing by a missence mutation in *FTO* gene

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Fat mass and obesity-associated gene (*FTO*) associated with variation in body weight and metabolic disorders. Recently, Boissel et al. reported a homozygous loss-of-function mutation in a family with nine affected members who had a severe development delay and multiple congenital anomaly. We performed whole-exome sequencing analysis and identified a novel homozygous missense mutation (c.812A>C) within the *FTO* gene in a 9.5 months -old girl with dysmorphic facies and developmental delay. She was the first child from the consanguineous parents. She had microcephaly, prominent metopic ridge, coarse face, long philtrum, microretrognathia, prominent alveolar ridge, antevert nostil, and brachydactyly. She follow up until 4 years and 9 months of age and she gained head control at first year of life, sitting 2 years, walking with help at 4.5 years old of age. She could say only three words at 4 years and 9 months. Denver testing at 4 years old of age confirmed severe developmental delay (DQ:23). Cranial CT imaging showed premature fusion of metopic suture. She also had splenomegaly on abdominal examination and grade-1 esophageal varices on endoscopy. Hearing assessment by auditory brainstem response showed conductive and sensorineural hearing loss. Eye examination revelaed optic atrophy, strabismus, nystagmus and abnormal electroretinogram. The clinical findings of the patient were very similar to first described family. In addition, she had high levels of creatine kinase was persisted since birth. As a result, we report second patient with novel homozygous missense mutation in *FTO* and discuss phenotypic extension of this gene mutations.

P09.045-S

Diagnosing dystonia using a Next-Generation-Sequencing panel

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Dystonias are a heterogeneous group of movement disorders which a strong inherited basis. Overlapping, non-specific features of many dystonias hamper a clear clinical diagnosis and make targeting a specific gene difficult or impossible. We developed a panel of 42 genes for Next Generation Sequencing containing the most relevant dystonia genes and covering the most relevant dystonia phenotypes known so far in order to analyze a cohort of unselected dystonia patients
We have established a selector-based enrichment method (HaloPlex, Agilent)

targeting 42 dystonia genes. A total of 310kb is enriched and sequenced by Illumina MiSeq (2x 150 bp paired-end). A first batch analysis in 27 dystonia patients showed that HaloPlex enrichment provided enrichment efficiency superior to standard whole exome procedures (>95% covered >20 reads; 90-95% of reads on target; mean coverage >500 reads per base).
We identified presumed disease-causing mutations in 2 out of 27 patients including one patient with a DYT6 dystonia and one patient carrying a mutation in SLC2A1. In 7 patients, we identifed so far unknown probable disease-causing variants including mutations in ATP7B, PLA2G6, PARK2, FBCO7, VPS13A and GCDH. This technology enabled the identification of a genetic cause in approximately 7 % of patients in an unselected cohort of dystonia patients in whom the most common genetic form (DYT1) was excluded. Targeted NGS may be a useful and cost-effective method to screen for mutations in multiple genes associated with dystonia.

P09.047-S
Using exome-sequencing for the diagnosis of rare disorders: two siblings affected by a congenital encephalopathy with microcephalia, polymicrogyria and dystonia

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Exome sequencing was performed on two siblings of a Sardinian family affected by a severe, congenital encephalopathy characterized by microcephalia, polymicrogyria and dystonia. MPS was achieved with the platform HiSeq2000 (Illumina). Quality filtered reads were aligned to the human reference hg19. SNPs and in/dels were detected using Samtools and BWA softwares. To obtain a list of candidate genes, the variants were filtered against a set of polymorphisms available in public databases (dbSNP138, 1000Genomes, ESP6500) and prioritized with Ingenuity Variant Analysis software. This analysis led us to identify in both siblings an homozygous variant in a gene of the MBT (Malignant Brain Tumor) family, expressed in mammalian brain. This variation causes a Ser-Asn substitution in one of the MBT domains. The MBT domain is a "chromatin reader", a protein module that recognizes mono/di-methylated lysines on histones tails. The MBT proteins interact with the enzymes that catalyze the histones methylation. This interaction plays a role in the repression of target genes. Although the pathway of the candidate gene is not well characterized, several studies show that its ortholog downregulates genes expressed in the early stages of neuronal development in Drosophila.
In a preliminary study in vitro we have found that the protein binds the dimethyltransferases SUV4-20H demonstrating its involvement in the process of histone methylation.
We speculate that the variant protein in our patients could destabilize the interaction between the MBT domain and the dimethyltransferases, altering the affinity for methylated histones and presumably its repressive properties.

P09.048-M
A new mutation in SYNGAP1 expanding the phenotypic spectrum: a case report

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We report a 15 year-old girl presenting with generalized epilepsy and intellectual disability. After normal development in the first two and a half years of life, delayed language development became apparent. At age 3 drop attacks occurred. Later on the patient developed clonic and clonic-tonic seizures, predominantly at night time. Most remarkably, photosensitivity and a special EEG phenomenon occured. Under the treatment with antiepileptic drugs the patient remained nearly seizure-free. Anti-convulsive treatment was stopped at the age of 13, leading to aggravation of the EEG only. Astonishingly, two years later the EEG showed normalization of the paroxysomal activity triggered by eye opening. We performed next generation sequencing (NGS) for a panel of genes known or suspected to be causative in the pathogenesis of seizures. We identified a heterozygous stop mutation (c.348C>A, p.Y116*) in *SYNGAP1* which was validated using Sanger sequencing. Analysis of the parents suggests that the mutation arose *de novo*. The gene *SYNGAP1* encodes an excitatory synapse-specific Ras GTPase and is a major component of the postsynaptic density. It is thought to regulate synaptic strength e.g. by suppressing signaling pathways linked to NMDAR-mediated synaptic plasticity. Mutations in *SYNGAP1* are known to cause autosomal dominant mental retardation type 5 (MRD5). More recently, the publication of *SYNGAP1* mutations in patients with seizures suggested a role in idiopathic generalized epilepsy combined with a variable degree of intellectual disability including severe speech impairment. Our patient shows additional symptoms, expanding the spectrum of phenotypes for patients with *SYNGAP1* mutations.

P09.049-S
Linkage and subsequent exome sequencing analyses in a consanguineous family reveal two novel genes associated with pattern-sensitive idiopathic generalized epilepsy

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Epilepsy is a complex neurological disorder affecting 1% of the world's population. Among different forms of epilepsies, idiopathic generalized epilepsies (IGEs) are characterized by generalized seizures in the absence of detectable brain lesions or metabolic abnormalities. Thus, the primary etiology for this disorder is believed to be genetic. This study includes a consanguineous family diagnosed with pattern-sensitive IGE and aimed to identify novel epilepsy gene(s) to delineate the molecular basis of this intriguing form of IGEs. Physical, neurological, neuroimaging and electroencephalography (EEG) examinations were performed on the subjects recruited with information on family history. There were two affected siblings having pattern sensitivity in form of absence, myoclonic and generalized tonic-clonic seizures. Illumina HumanCytoSNP-12 BeadChip was used in genotyping of affected and five unaffected family members. Genotype data was utilized using easyLinkage Plus interface, where multipoint LOD scores were calculated under the assumption of autosomal recessive inheritance and haplotypes were constructed through GeneHunter. Linkage peak with a LOD score of 3.16 was revealed on Chromosome 22 comprising 113 genes within a 3.72Mb region. To analyze the candidate linkage region and identify any novel epilepsy genes residing outside this region, Illumina HiSeq2000 was used to perform whole exome sequencing on one of the affected sibs. Two genes with homozygous variants were located within the linkage region that segregated with the condition. Our study indicated homozygous variations in a gene encoding brain specific, spliceosome component and a gene with an unknown protein function possibly responsible in a family with pattern sensitive IGE.

P09.050-M
Whole genome SNP genotyping confirms segregation of Unverricht-Lundborg Disease (ULD) with a repeat expansion in CSTB on 21q in a large consanguineous family followed by a novel haplotype based approach identifying the parent of origin and carrier status in the child with Trisomy 21 under the age of onset for ULD

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Unverricht-Lundborg disease (ULD) is an autosomal recessive progressive myoclonus epilepsy characterized by generalized myoclonic jerks and tonic-clonic seizures. In this study, we analyzed a large consanguineous family with 10 children from Turkey afflicted with an undiagnosed form of syndromic epilepsy. All 6 affected family members along with 3 unaffected members and the youngest child with trisomy 21 have been genotyped using Illumina 300K SNP array. Linkage analysis was performed utilizing easyLinkagePlus interface under the assumption of autosomal recessive inheritance. Linkage analysis pinpointed a locus on chromosome 21q22.3 with a LOD score of 4.33. CSTB gene which resides within this region was screened for mutations in this family via DNA sequencing and long PCR, as mutations in CSTB have been implicated in ULD. This analysis revealed a dodecamer repeat expansion of almost 40 copies in the promoter region of CSTB in all affected children. We have also analyzed the genotyping data of the youngest child with Trisomy 21, as CSTB resides on this chromosome. It is not possible to detect the carrier status with conventional PCR techniques, as there are 3 copies of the gene. We have first managed to detect parent of origin for trisomy via analyzing the chromosome 21 genotyping data as a trio. Additional haplotype analysis of three copies of chromosome 21 revealed that this individual, who was under the age of onset at the time of the study, has only one copy of the defective allele and probably will not develop ULD.

P09.051-S

Exome sequencing reveals mutations of a solute carrier gene in an autosomal recessive form of epileptic encephalopathy of the first days of life

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Epileptic encephalopathy (EE) refers to a clinically and genetically heterogeneous group of devastating disorders characterized by seizures combined with abnormal inter-critic encephalography Age of onset can be a key diagnostic feature for epileptic syndrome definition, treatment and prognosis. We ascertained two multiplex families (including one consanguineous family) consistent with an autosomal recessive inheritance pattern of EE. All seven affected individuals developed seizures in the first day of life, resistant to treatment and leading to repetitive "Etat de mal". Evolution was marked by severe EE with major delay in motor acquisitions and one patient died at 11 years of age. No facial dysmorphism was noted, but oligodontia. Given the similarity in clinical presentation in the two families, we hypothesized that the observed phenotype was due to mutations in the same gene, and performed exome sequencing in three affected individuals. Analysis of rare variants in genes consistent with an autosomal recessive mode of inheritance led to identification of mutations in a solute carrier gene. Causality was confirmed by co-segregation analysis in additional family members. To assess the frequency of alterations of this gene in early onset EE, coding exons were screened for mutations in a cohort of 70 unrelated affected individuals by targeted sequencing on a MiSeq instrument (Illumina). This experiment led to identification of compound heterozygous pathogenic variants in a simplex case with a similar clinical presentation as the other affected patients. These results highlight the value of careful clinical characterization for genetic studies in heterogeneous diseases such as EE.

P09.052-M

Exome sequencing identifies novel SCN8A mutation associated with neonatal epileptic encephalopathy, multiple congenital anomalies and movement disorder

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Epileptic encephalopathies represent clinically and genetically heterogeneous group of disorders of which the majority are of unknown aetiology. It is hypothesized that novel variants may contribute to most of these devastating group of epilepsies. Within recent years the role of *SCN8A* in human disease has become apparent from next generation sequencing studies. Whole-exome sequencing of a parent-offspring trio was used to identify the genetic cause of severe early infantile epileptic encephalopathy in a boy, where previous chromosomal, gene and metabolic investigations revealed no abnormalities. The patient had neonatal seizures, movement disorder, multiple congenital anomalies. He died at the age of 17 months due to respiratory illness. We identified a *de novo* heterozygous missense mutation (c.3979A>G; p.Ile1327Val) in *SCN8A* (voltage-gated sodium-channel type VIII alpha subunit) gene. The variant was confirmed in the proband with Sanger sequencing. Since the clinical phenotype associated with *SCN8A* mutations have previously identified only in a number of cases, these data together with our results suggest that mutations in *SCN8A* can lead to early infantile epileptic encephalopathy or intellectual disability with broad phenotypic spectrum. Additional investigations will be worthwhile to determine the prevalence and contribution of *SCN8A* mutations to epileptic encephalopathy and intellectual disability, and provide insight into the mechanisms of pathogenesis in neurologic diseases. This study was supported by the EuroEPINOMICS grant SARLA 11091E.

P09.053-S

Ambroxol and Bromhexine derivatives act promoting on mutant alpha-galactosidase A forms in cell culture systems of Fabry disease

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Fabry disease (FD) is a rare hereditary disease caused by the absence or deficiency of lysosomal enzyme activity of the glycosylase α-galactosidase A (GLA, EC 3.2.1.22, α-gal A). This enzyme breaks down macromolecular structures of neutral glycosphingolipids. A lack of particular hydrolase activity leads to lysosomal glycosphingolipid storage and subsequently multisubcellular dysfunction. Many mutations in the GLA gene have been identified compromising the enzyme's stability causing a premature proteasomal degradation. Therefore, treatment strategies apply the use of small molecule enzyme inhibitors having the ability to promote enzyme folding and transport intracellularly, the so-called pharmacological chaperones (PC). It was demonstrated that those molecules specifically bind to the (mutated) target enzyme which leads to a thermodynamically favoured conformational change and in turn further cellular transport and an increase of the required activity in the lysosomes. One such compound is 1-Deoxygalactonojirimycine (DGJ). We recently described an auxiliary function of Ambroxol (ABX), a PC described for mutant glucocerebrosidase in Gaucher disease, for the observed DGJ activity in over-expression based cell culture systems of FD. Here, we report the results of a derivatisation project of Ambroxol and the structural analogue Bromhexine that led to the discovery of compounds that preserved the ability to enhance mutant α-gal A activity in combination with DGJ. Structure-function analysis may give indications on the underlying functional mechanism we aim to elucidate. Moreover, our findings did not support the occurrence of a direct compound:enzyme interaction.

P09.054-M

Functional Analysis of *DEPDC5* variants identified in familial focal epilepsy with variable foci (FFEVF) and in cancer.

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The target of rapamycin (TOR) complex 1 (TORC1) is an essential regulator of cell growth. Stimulation of TORC1 kinase activity promotes anabolic metabolism, including protein, lipid and nucleotide synthesis. Multiple upstream signals, including growth factors and the availability of nutrients and energy, act through two GTPase activating protein (GAP) complexes to control TORC1 activity. The TSC complex, consisting of TSC1, TSC2 and TBC1D7, is required for growth factor and energy dependent inhibition of TORC1, while the GATOR-1 complex, consisting of DEPDC5, NPRL2 and NPRL3, is required for amino acid-dependent regulation of TORC1. In humans, inactivating mutations in *TSC1* or *TSC2* cause tuberous sclerosis complex, a disease characterised by seizures and benign hamartoma-like lesions. Mutations in *DEPDC5* have been identified in human tumours and were recently described in association with familial focal epilepsy with variable foci (FFEVF). We have investigated the effects of *DEPDC5* variants identified in tumours or in individuals with FFEVF on TORC1 signaling and GATOR-1 complex formation using simple *in vitro* assays. Our functional data demonstrate that some *DEPDC5* mutations result in increased TORC1 activity, similar to pathogenic mutations in *TSC1* or *TSC2*. Functional analysis of *DEPDC5* variants of uncertain clinical significance will help define the role of the GATOR-1 complex in FFEVF and tumour pathogenesis.

P09.055-S

A female with clinical features of FOXG1 syndrome and 1.5-Mb size 14q12 microdeletion located more proximal to *FOXG1*

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A severe Rett-like neurodevelopmental disorder is associated with de novo

FOXG1 point mutations or submicroscopic 14q12 deletion, which involve *FOXG1* gene. It is now known as *FOXG1* syndrome which clinically causes postnatal microcephaly, severe mental retardation, absent language, dyskinesia, and dysgenesis of the corpus callosum. More than 30 cases of FOXG1 syndrome have been published. Cases of a submicroscopic 14q12 deletion, involving regulatory elements of *FOXG1*, with the coding region of *FOXG1* being unaffected, are described very seldom. A *cis*-acting regulatory sequence, acting as a silencer, is deleted more than 0.6 Mb distally from *FOXG1* in these cases. We report a new case with clinical features of FOXG1 syndrome and 14q12microdeletion. She was born at term with birth weight 3636g, length 51 cm and head circumference 33.5 cm. Since the birth developmental delay was noticed. At 11 months she has only weak head control, microcephaly (-3 SD), focal epilepsy, deep set and almond shape eyes, protruding tongue, increased muscle tonus and brisk tendon reflexes. Brain MRI showed hypogenesis of the corpus callosum, hypomyelinisation, arachnoid cyst in the left temporal region and subtle pachygyria. Chromosomal microarray analysis revealed 1.5-Mb size 14q12 microdeletion (arr[hg19] 14q12(27,584,943-29,170,974)x1) appeared to be *de novo*. The end of this deletion is 65-kb proximal from *FOXG1* leaving the gene itself intact. There are no known protein-coding genes located in the deleted area. Therefore, we can hypothesize that there is some regulatory element of *FOXG1* located proximal to the gene which deletions can also cause FOXG1 syndrome.

P09.056-M
Cy5 Analysis System in molecular diagnosis of Fragile-X Syndrome and Huntington disease

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Expansion of DNA repeats causes hereditary disorders in humans; our genetic diagnosis center is studying $(CGG)_n$ repeat in the FMR1 gene in Fragile-X Syndrome and $(CAG)_n$ repeat in the HTT gene in Huntington's disease (HD) by a high performing systems, Cy5-labeled **Fragile-X Syndrome** The critical issues in FMR1 analysis usually are:
- discriminate between fully mutated females from normal homozygotes.
- determine the proper CGG repeat size between 110 and 200 repeats
- determine methylation status

We routinely use a combination of two PCR-systems (CE-IVD) to determine the proper CGG repeat size until 200 repeats and alternative methods to Southern Blot (MS-MLPA, High Resolution Melting) to determine methylation status.
Huntington Disease In HTT gene the critical issue is define the exact CAG repeat size considering the small range that exists between premutation (36 repeats) and mutation status (40 repeats). The PCR-system (CE-IVD) we introduced in 2013 in our laboratory includes a positive control that helps to estimate the proper CAG repeat size in sample.
Our results refer to 400 Fragile-X Syndrome suspected cases analyzed from 2010 to 2013 and to 30 Huntington's Disease suspected cases analyzed in 2013. Use of these systems (CE-IVD), high performing, fast and easy, has allowed us to reach good diagnostic results using instruments already supplied in our department.

P09.057-S
Comparison of Friedreich Ataxia Patients with Trinucleotide Repeat Expansions and Point Mutations in FXN-Encoded Frataxin

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We retrospectively reviewed records of 40 patients with Friedreich Ataxia (FRDA). The majority of patients, 88% had bi-allelic GAA expansions (Group 1, n=35) while 12% were heterozygous for an expansion and point mutation (Group 2, n=5). Group 1 had a mean age of onset of 12.26 years compared to Group 2 with a mean of 5 years. The average age at diagnosis in Group 1 was 17.86 years and 15.2 years in Group 2. Neurological manifestations were the presenting symptom in the majority of both groups at 94% and 80% respectively. Within Group 1, 53% had lost their ability to ambulate at a mean of 10 years after disease onset compared to Group 2 where 50% had lost their ability to ambulate at a mean of 16 years after disease onset. In Group 1, all patients had evidence of cardiac involvement, including 43% having left ventricular hypertrophy (LVH) on echo. In Group 2, all patients had evidence of cardiac involvement with 60% having LVH. Frataxin enzyme levels had a mean of 6 ng/mL (N=2) in Group 1 while the mean for Group 2 was 2.67 ng/mL (N=3).
Genotype-phenotype correlation within FRDA patients is currently not well characterized. A greater percentage (Group 2) of patients with FRDA evaluated at Mayo Clinic do not have the classic bi-allelic expansion as compared to available literature. Patients heterozygous for a GAA expansion and point mutation had an earlier age at onset, but were able to ambulate longer compared to patients with bi-allelic GAA expansions.

P09.058-M
Mitochondrial genome encoding tRNAs sequence in frontotemporal lobar degeneration

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Frontotemporal lobar degeneration (FTLD) is the second most common type of degenerative dementia, characterized by progressive changes in behaviour, executive dysfunction and/or language impairment. Some patients present clinical and neuropathological overlap with Alzheimer's disease, suggesting similarities in pathophysiology, including mitochondrial DNA (mtDNA) involvement. Mutations in mtDNA, particularly in mt-tRNAs, have been described as an important cause of human disease.
The aim of present work was sequencing the 22 mitochondrial tRNAs genes, ascertaining their involvement in FTLD.
A sample of 70 patients, diagnosed with probable FTLD, was studied (39 females and 31 males; age range: 38-82 years, mean±SD: 63±11 years. Total DNA was extracted from peripheral blood. The 22 tRNA genes sequences were sequenced and variants were submitted to in silico analysis. A total of 28 different sequence variations were identified in 32 patients (46%). According to in silico analysis, 6 variations are probably pathogenic, all causing structure and binding minimum free energy changes. The most frequent variation found is m.12308A>G, in the variable region of mt-tRNALeu2, and it is totally conserved in all mammals tested. The m.15946C>T variation is located in the acceptor stem and it is highly conserved.
Further investigation is needed to better understand the relationship between mtDNA alterations found and FTLD, considering also the involvement of nuclear genes in this disorder. However, this is the first study of complete sequencing of the mt-tRNA genes in FTLD.
Supported by "Fundação para a Ciência e a Tecnologia" (PTDC/SAU-EPI/121811/2010 and PEst-C/SAU/LA0001/2013-2014).

P09.059-S
Assessment of Plasma Glucosylsphingosine as a biomarker for Gaucher Disease

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Biomarkers play an essential role in the early detection, and monitoring of metabolic diseases, this also holds true for Lysosomal Storage Disorders (LSDs). The ideal biomarker facilitates the initial diagnosis, evalutates the disease severity and progress and may be assist in determining proper treatment. Here, we analyzed Glucoyslsphingosine for the primary diagnosis and monitoring of Gaucher disease (GD), where a defect in the beta-Glucosidase (GBA) gene leads to the accumulation of glucocsyleramide. Overall, we evaluated the sensitivity and specificity of Glucosylsphingosine by comparing healthy controls, Gaucher patients, Gaucher carriers and patients with other LSDs. The determined cut-off of 12ng/ml yielded a 100% sensitivity and specificity for Glucosylsphingosine. In addition the biomarker was compared to Chitotriosidase and CCL18/PARC, which both are highly elevated in a number of LSDs and reflect the burden of disease on macrophages due to accumulation of macromolecules but are not specific for GD. In addition, Chitotriosidase levels may be normal even in GD patients due to a common 24-bp duplication in the CHIT1 gene. Glucosylsphingosine proved to be more specific and sensitive than Chitotriosidase (p=0.027) and CCL18/PARC (p<0.001). We also assessed long-term data of 19 GD patients before and after onset of enzyme replacement therapy. Overall, Glucosylsphingosine is a reliable biomarker for the primary diagnosis and follow-up of Gaucher Disease. We will proceed with determining the correlation of disease severity, progress of the disease and treatment with varying levels of Glucosylsphingosine.

P09.060-M
Saposin C deficiency: an inherited lysosomal disease caused by rapidly degraded mutant proteins

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Saposin (Sap) C is a 80 residues-long glycoprotein functioning as essential cofactor for the lysosomal degradation of glucosylceramide (GC) by gluco-

sylceramidase (GCase). It promotes rearrangement of the organization of lipids in lysosomal membranes and provides GCase greater accessibility to GC substrate. Rare functional deficiency in Sap C results in a rare variant form of Gaucher disease (GD). Sap C has six conserved cysteine residues involved in three disulphide bonds making the protein structure remarkably stable to acid environment and degradation. Five different mutations (*i.e.,* p.C315S, p.342_348FDKMCSKdel, p.L349P, p.C382G and p.C382F), four of them involving a cysteine residue, are known. Here we report on the functional and biological behaviour of disease-associated Sap C proteins. Lipid-binding properties and activating efficiency on GCase of Sap C mutants, analyzed by surface plasmon resonance and biochemical assays, were comparable to those of wild type Sap C. On the contrary, mass spectrometry analyses of protease-treated mutants revealed a rapid degradation of those carrying a mutation involving a cysteine residue. Our data provide evidence that mutant activator protein instability is the underlying pathogenetic mechanism of Sap C deficiency.

P09.061-S
Genetic analysis of glucocerebrosidase (GBA) gene in Italian patients with Parkinson disease and Lewy Body Dementia

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Gaucher's disease (GD), the most common lysosomal storage disorder, is associated with recessive mutations in the glucocerebrosidase gene (GBA). It is known that GD patients and their heterozygous relatives more commonly develop parkinsonism, and that GBA heterozygous mutations increase of at least 5 times the risk to develop Parkinson's disease (PD) and Lewy bodies dementia (LBD). In this study, we evaluated the frequency of GBA mutations in Italian patients with PD or LBD. To this aim, we used long-template PCR to screen the entire GBA coding region for mutations in 216 patients with PD and 84 with LBD. GBA mutations were identified in 24 of 216 PD (11.1%) and in 4 of 84 LBD (4.7%) cases. Fourteen different GBA heterozygous mutations were detected, including two previously unreported mutations (c.1095G>C [p.Glu365Asp]) and a frameshift mutation (c.1197_1198insCTGTA [p.Met400Leufs*2]).
In conclusion, we report a high frequency of GBA mutations in Italian patients with either PD or LBD, indicating that GBA mutations represent a significant risk factor for these disorders in Italy.

P09.062-M
Genome sequencing identifies a novel mutation in ATP1A3 in a family with a seemingly atypical phenotype

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Mutations in ATP1A3 have been reported in rapid-onset dystonia-parkinsonism (RDP). Dystonia in RDP has a characteristic sudden onset, typically in adolescence in response to physical or mental stress. Dystonic symptoms usually involve the bulbar region and are accompanied by symptoms of parkinsonism. More recently, mutations in ATP1A3 have been linked to alternating hemiplegia of childhood (AHC) and CAPOS syndrome, respectively.
We investigated a family with dystonia from New Zealand with ten affected members. Interestingly, only females were affected. After exclusion of mutations in TOR1A and THAP1, we performed genome sequencing in two affected cousins. For filtering, we used the KnomeDiscovery Data Filtering Software. Since re-sequencing of 20 candidate variants did not elucidate the genetic cause, we performed exome sequencing in another affected individual. Analysis of the raw data using an in-house bioinformatics pipeline revealed a previously unreported three base-pair deletion (c.443_445delGAG, p.148_149delSer) in ATP1A3 in all three genome/exome sequenced patients. Segregation analysis showed the mutation in all patients and in one unaffected male. The mutation was not found in 200 controls. Subsequent clinical re-examination, revealed sudden onset of dystonic symptoms after stressful events. None of the patients reported any history of AHC or CAPOS syndrome.
In conclusion, our study identifies a novel mutation in ATP1A3 as cause of RDP and highlights two important challenges when using next generation sequencing: 1) The importance of detailed clinical information. In our family, we have initially missed the characteristic sudden onset. 2) The difficulties with the annotation of deletions/insertions in the used software package.

P09.063-S
Very late onset Friedreich ataxia: a case report

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Friedreich ataxia (FRDA) is a rare autosomal recessive hereditary disorder caused by expansion of a GAA repeats in the first intron of the X25 gene. It typically begins before the age of 25, but up to 25% of patients may be considered atypical with respect to the established diagnostic criteria, showing delayed age at onset with mild clinical impairment, slower progression of disease, and fewer secondary complications. These patients are arbitrarily subdivided into late-onset FRDA [LOFA; 25-39 years] and very late-onset FRDA [VLOFA; ≥40 years], show retained deep tendon reflexes and unusually gradual disease progression. Here we describe a 63-year-old white woman showing lower limb spasticity with ataxia and preserved ankle and knee jerks. The progression was slowly during the past 6 years, family history was negative for neurological diseases and no consanguinity was reported. Cerebellar dysartria, axonal neuropathy and reduced glucose tolerance were also present. Brain NMR showed cerebellar and cortical atrophy. Molecular analysis revealed a pathologic GAA expansion in the gene encoding frataxin, carrying expanded alleles in the low-range size, corresponding to 110 and 180 GAA repeats. VLOFA is an infrequent subtype of the disease with onset ranging between 40 and 67 years. Nevertheless, considering the frequency of FRDA mutations carriers in the general population (1/60), and the associated risk of recurrence, this condition deserve to be considered in atypical ataxic patients, even when the full clinical criteria for a classical form are not satisfied.

P09.064-M
Correlation between genotype, phenotype, and effect of mutant protein in REEP1-associated motoneuron degeneration

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Mutations in REEP1 have classically been associated with a pure form of the neurodegenerative condition hereditary spastic paraplegia (HSP type SPG31), i.e. a phenotype resulting from exclusive involvement of upper motoneurons. We recently reported on a unique REEP1 alteration which results in complete skipping of the in-frame exon 5 and is associated with distal hereditary motor neuropathy (dHMN type V), i.e. an exclusive lower motoneuron phenotype. Using minigene constructs we found that additional exon 5 mutations, apparently representing non-sense or silent variants, confer partial missplicing of this exon. Interestingly, these mutations are associated with complicated forms of HSP in which both upper and lower motoneurons seem to be involved. A meta-analysis of published reports reveals that lower motoneuron involvement in SPG31 is significantly associated with mutations that truncate the protein after the first ~100 residues. This border co-incites with the C-terminus of the REEP homology domain which mediates hetero- and homomeric interactions. By overexpression we show that such truncated polypeptides resemble the previously reported exon 5 deletion variant in localizing to large peri-nuclear "clumps". Moreover, we observed that these structures indeed depend on presence of the REEP homology domain, and that their number and size increases over time. This indicates that they represent aggregates rather than intact cellular structures. We thus suggest that N-terminal mutations act via haploinsufficiency which causes pure HSP, while for more C-terminal mutations there is an additional, aggregate-mediated toxic gain-of-function effect which results in complex phenotypes due to lower motoneuron involvement.

P09.065-S
Identification of the first Spastic Paraplegia 11 families in Sudan and report of novel SPG11 mutations

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Hereditary spastic paraplegia (HSP) constitutes a heterogeneous group of syndromes with core features of progressive lower limb spasticity and weakness, without or with other manifestations (pure / complex HSP). All modes of inheritance have been described.
HSP with thin corpus callosum (TCC) represents a distinct entity for which mutations in SPG11 are the most frequent causes.
As a part of a larger study to elucidate the molecular basis and the phenotypic patterns of HSP in Sudan, we report 2 extended Sudanese families with complex HSP and multiple consanguinity loops. Ten patients were clinically phenotyped and DNA was obtained from blood and saliva samples.
The age at onset in the first family ranged from 12 to 17 years. Patients presented with ataxia, mental impairment and skeletal deformities in addition to spasticity. Exome sequencing performed in 3 patients identified a homozygous deletion (c.6709del/p.Ala2237Gln*7) in exon 36 of SPG11.
In the second family, the age at onset extended from 11 to 24. The phenotype was more complex, with psychiatric symptoms, dysphagia and peripheral motor involvement with distal muscle atrophy of both limbs. Direct sequencing of SPG11 identified a stop mutation (c.6349G>T/p.Glu2117*) in exon 34 that segregated with the disease within the family.
MRI of 3 cases from both families revealed TCC, cerebellar and cortical atrophy and WMLs.
Both mutations are novel and lead to premature truncation of spatacsin protein. These families are the first described Sudanese families carrying SPG11 mutations. This study illustrates the wide range of clinical presentations associated with SPG11.

P09.066-M

Assessing an anaplerotic therapy on the brain metabolic profile in Huntington disease

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Objective: To obtain a proof-of-concept for an anaplerotic therapy in Huntington disease (HD) using a validated functional biomarker of brain energy metabolism.
Background: Energy deficit has been greatly implicated in the pathophysiology of HD. Our previous work has indicated a need to refill the Krebs cycle which can be achieved using anaplerotic therapy.
Methods: 31P brain magnetic resonance spectroscopy (MRS) was coupled with the activation of the occipital cortex to measure the levels of PCr and Pi before (rest), during (activation) and after (recovery) a visual stimulus. At visit 1, we performed 31P brain MRS in 10 patients at the early stage of HD and 10 controls. HD patients were then treated at home for one month with triheptanoin and came back for a second visit during which we performed 31P brain MRS.
Results: At visit 1, we confirmed a significant increase in Pi/PCr ratio ($p=0.022$) during brain activation in controls - reflecting increased ATP synthesis - followed by a significant return to baseline levels during recovery ($p=0.008$). In HD patients, we confirmed an abnormal brain energy profile with decreased Pi/PCr ratio before treatment. After one month of triheptanoin therapy, the MRS profile was greatly improved in HD patients with increased Pi/PCr ratio during visual stimulation ($p=0.004$).
Conclusion: This study suggests that triheptanoin is able to correct the bioenergetic profile in HD patients' brain at an early stage of the disease. The administration of triheptanoin over a longer period of time is now required to assess its clinical benefit.

P09.067-S

PMCA, SERCA and VEGF as potential patogenetic factors in Huntington's disease and as biomarkers of onset and progression

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To study calcium homeostasis deregulation in Huntington's disease (HD) ethiology we investigated calcium pumps expression level in HD cellular models and in peripheral blood of pre-symptomatic and symptomatic HD patients. We compared the steady-state level of cellular membrane associated PMCA1-4 and endoplasmic reticulum associated SERCA2,3 pumps in wild-type STHdh(Q7)/Hdh(Q7) and mutant STHdh(Q111)/Hdh(Q111) cell lines derived from the murine embryonic striatum. We observed a significant reduction of PMCA1 protein in glial differentiated mutant cells ($p<0.05$) and SERCA2 protein in undifferentiated mutant cells ($p<0.001$). Using doxycicline-inducible rat striatal cell lines (Hd19 and HD43 Sipione et al. 2002) we confirmed the reduction of SERCA2 as a mutant huntingtin expression dependent event.
To evaluate the role of PMCA1 and SERCA2 gene expression as biomarkers of disease onset or/and progression we compared their mRNA levels in the peripheral blood mononuclear cells (PBMC) of 20 pre-symptomatic and 90 symptomatic HD subjects and in sex and age matched healthy subjects. We observed a reduction of SERCA2 mRNA both in pre-symptomatic and symptomatic HD patients compared with healthy subjects. Our data highlight SERCA2 gene down-regulation as a biomarker of HD having a role in early cellular dysfunction.
Finally, in PBMC we studied the neuroprotective angiogenic factor vascular endothelial growth factor (VEGF) mRNA level. VEGF transcript level resulted significantly lower in pre-symptomatic HD compared to healthy subjects ($p<0.01$) and even lower in late-symptomatic compared to pre-symptomatic group ($p<0.05$). This finding suggests VEGF transcript as a peripheral biomarker useful for monitoring disease onset and progression.

P09.068-M

Mutations in *B9D1* and *MKS1* cause mild Joubert syndrome: expanding the genetic overlap with the lethal Meckel ciliopathy

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Joubert syndrome (JS) is a congenital disorder diagnosed by the presence of a peculiar mid-hindbrain malformation (the "molar tooth sign"), that consists of cerebellar vermian hypodysplasia, thickened mal-oriented superior cerebellar peduncles and a deepened interpeduncular fossa. The typical neurological features of pure JS include hypotonia, ataxia, psychomotor delay, abnormal ocular movements and intellectual impairment. This phenotype may be complicated by defects of the kidneys, eyes, liver, skeleton and orofacial defects, resulting in wide clinical variability. JS is recessively inherited and genetically heterogeneous, with 24 known genes. All genes encode for proteins of the primary cilium, and indeed there is clinical and genetic overlap with other ciliopathies. In particular, JS shares 13 genes with Meckel syndrome (MS), a lethal condition characterized by cystic kidneys, bile duct proliferation of the liver, encephalocele and polydactyly. As part of a large screening of ciliopathy genes in 260 JS patients, we identified novel pathogenic mutations in two genes not previously implicated in this condition. Two patients carried mutations in the *MKS1* gene, a 44-year-old man with JS and retinal dystrophy, and a two-year-old child with a pure JS phenotype. Mutations in the *B9D1* gene were identified in two other patients, a 9-year-old boy and a 6-year-old girl both presenting with pure JS. All identified mutations were inherited from heterozygous healthy parents, were not reported in public databases, and affected highly conserved residues. Missense mutations were predicted as pathogenic by prediction web tools.

P09.069-S

Pseudo-dominant inheritance of a novel CTSF mutation associated with type B Kufs disease

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Kufs disease (KD) is the rare adult form of neuronal ceroid lipofuscinosis (NCL). Mutations in the cathepsin F gene (CTSF) (MIM 603539) have recently been discovered in autosomal recessive Type B KD families characterized by movement and behavioral abnormalities and dementia. We present a family in which pseudo-dominant transmission of Type B KD was explained by a novel homozygous splice site mutation in CTSF leading to the lost of exon 1.
Three affected individuals showed similar neurological pictures characterized by generalized seizures, cerebellar dysarthria and cognitive decline, evolving into frank dementia. The presence of three additional relatives with a neurological syndrome compatible with KD and two instances of parent-

to-child transmission proposed an initial hypothesis of a dominant form of dementia representing a confounding factor for genetic testing. A more detailed clinical assessment of the patients, meticulous collection of family history and recognition of the high degree of inbreeding in the isolated community where this family lives, were crucial in disclosing an autosomal recessive pattern of inheritance, prompting investigation of CTSF.
In vitro experiments in cultured skin cells from the proposita and her aunt demonstrated a dysregulated autophagy and aggresome-like structures, confirmed by ultrastructural studies in skin biopsies. These data put forward the hypothesis of a cytoplasmic toxicity of pathological cathepsin F in KD type B.

P09.070-M
Lamin B1 expression is affected by EBV infection in lymphoblasts of patients with Autosomal Dominant Leukodystrophy through mir-23 deregulation

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Lymphoblastoid cell lines (LCLs), obtained through the immortalization of B lymphocytes by Epstein-Barr virus, can provide a virtually unlimited source of DNA, mRNA and protein for research studies and have been used as a replacement for molecular and functional analyses of diseases including those affecting central nervous system (CNS). Autosomal dominant adult-onset leukodystrophy (ADLD) is a demyelinating disease of the CNS associated with duplication of the lamin B1 gene (LMNB1). Studying LCLs compared to fibroblasts from a survey of patients affected by ADLD, we showed a wide variability in LMNB1 levels both at mRNA and protein levels. Lamin B1 expression was inversely proportional to miR-23, a known LMNB1 regulator. Linear regression analysis showed that there was a significant inverse correlation between the mean miR-23 levels and lamin B1 expression in patients' LCLs, whereas no correlation was observed in fibroblasts. We speculate that the immortalization of LCLs randomly deregulates miR-23 expression that, in turn, alters lamin B1 levels. This work further demonstrates the importance of miR-23 in the regulation of lamin B1 expression, and suggests caution when using LCLs as the only source of mRNA in the contest of in vitro research and of lamin B1 as Western blots loading control for nuclear extracts.

P09.071-S
Molecular analysis of EIF2B genes in adult-onset leukodystrophy with vanishing white matter

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Leukodystrophy with vanishing white matter (LVWM) is a rare autosomal recessive white matter disease due to mutations in one of the five eukaryotic initiation factor 2B genes (EIF2B1-5). Onset is typically in late infancy or early childhood, but later onset is also reported. The adult-onset form is usually associated with EIF2B5 Arg113His mutation while, thus far, there are only 3 reports of mutations in EIF2B3. Genetic analysis of EIF2B5 and EIF2B3 genes was performed in 6 adult-onset Italian patients from 5 families, for whom LVWM was suggested by MRI pattern. We identified 5 different EIF2B5 missense mutation (2 novel), in 4 patients (2M, 2F) and 2 different EIF2B3 missense mutations (1 novel) in 2 female patients. The clinical presentation of mutated patients is heterogeneous and nonspecific, ranging from nearly-isolated neurogenic bladder or ovarian failure to stroke-like episodes or various psychiatric disturbances. Overall, the clinical course was slowly progressing over years or decades, although rapid motor or cognitive deterioration was observed in one case. In all cases, brain MRI was characterized by diffuse leukoencephalopathy associated with a varying burden of areas of rarefaction/cavitation. This study provides further evidence that not only EIF2B5, but also EIF2B3 mutations can be associated with a wide clinical spectrum of adult-onset LVWM, whereas the neuroradiological pattern - characterized by areas of white matter rarefaction/cavitation - unequivocally guides the clinician to request the proper diagnostic molecular test. [Partly supported by Italian Ministry of Health grant RF-2009-1539841 to FT]

P09.072-M
Combined analysis of linkage and exome sequencing identifies a novel SYNE1 mutation in a consanguineous family from Turkey with a rare form of recessive cerebellar ataxia

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Cerebellar ataxias are characterized by incoordination and unsteadiness of movement due to cerebellar dysfunction. In this study, we analyzed a consanguineous family with 4 affected individuals presenting very slowly progressive cerebellar symptoms including dysartria, dysmetria and gait ataxia. All 4 affected and 3 unaffected members from this family were genotyped using Illumina Human HumanCytoSNP-12 BeadChip kit. The genotyping data obtained were further analyzed in terms of copy number variation (CNV) and linkage using Illumina proprietary software cnvPartition and easyLinkagePlus interface, respectively. The patients were negative for a common CNV, while a single linkage peak on chromosome 6q25 was obtained with a maximum LOD score of 3.42.
Whole exome sequencing (WES) was performed in two affected sibs from the family in parallel to linkage analysis. Genetic variants of the affected individuals within the linkage interval were filtered against novel variants with harmful effect. This approach has led to identification of a novel mutation in gene SYNE1 (c.13086delC; p.His4362Glnfs*2) segregating with the condition in the family as confirmed by Sanger sequencing. SYNE1 has 147 exons encoding 8797 amino acids. This mutation is predicted to truncate almost half of the protein.
SYNE1 encodes a nuclear envelope protein, which is expressed in various tissues, particularly in the cerebellum. Mutations in SYNE1 have previously been implicated in a rare form of recessive spinocerebellar ataxia observed especially in French-Canadian population.
Our study presents the combined analysis of linkage and WES as a powerful and precise tool to diagnose a clinically unknown condition at the molecular level.

P09.073-S
Screening the LRRK2-3'UTR region on a Spanish cohort of PD-patients and controls and mRNA relative quantification in brain

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Mutations in LRRK2 are recognized as the most common genetic determinant of sporadic and familial Parkinson's disease (PD). Recently, several studies of exonic variants in large populations were published; however, none of them was focused in the 3'UTR region. Our aim was to screening the 3'UTR of LRRK2 looking for risk variants, modifying factors, disrupted targets for microRNA binding and a possible effect of this region in mRNA expression.
Our cohort consisted of 743 PD patients (67±10 years; 52% male) and 523 healthy controls (67±12 years; 50% male). The human post-mortem tissues were obtained from three different brain regions of 9 PD and 5 healthy donors.
Patients were genotyped for the LRRK2 mutations G2019S and R1441G/C/H resulting in 16 G2019S carriers and 15 R1441G carriers. None of the tissue donors was mutation carrier. We identified a total of 12 variants; 2 of them new (c.*130_131del and c.*1382C>A) in the 3'UTR region. We found rs66737902 T>C as a possible variant related with PD risk being the C allele overrepresented in patients; p= 0.01 OR=1.37 CI=1.07-1.74. To tested if rs66737902 had an effect in the gene expression through the binding of miRNAs, we study miR-138-2* as candidate miRNA (TargetScan, microRNA.org) and miR-205 as control miRNA. We did not find any effect of miR-138-2*. In the mRNA expression study we found significant differences between TT and TC rs66737902 genotypes in the SN of PDs, with a minor expression level in the TC group (p=0.011). No differences between patients and control were found.

P09.074-M
Variation in the promoter of the autophagic beclin-1 gene (BECN1) and its impact on expression levels: a study in Machado-Joseph disease (MJD/SCA3) patients

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Background and Objectives: Autophagy, as a process of intracellular components degradation, is especially important in disorders where accumulation of the mutant protein is a hallmark, such as MJD, a late onset polyglutamine ataxia. We documented the variation in the promoter of the BECN1 gene whose overexpression has been reported to exert neuroprotective effects in MJD. The relation between BECN1 promoter variation and expression levels were studied, as well as its impact on disease onset.
Methods: The BECN1 promoter was sequenced in 95 MJD patients and 120 controls. In silico analysis (PROMO) were performed to detect differences in putative transcription factor binding sites (TFBS). BECN1 expression level was quantified by Real-time PCR in 29 MJD patients and 27 controls, for which cDNA from peripheral blood was available.
Results: Two previously described variants (rs60221525 and rs116943570) were found in MJD patients and controls. In silico analysis predicted the existence of less putative TFBS for rs60221525 and of more TFBS for rs116943570. BECN1 expression levels were in agreement with the in silico predictions, showing decreased and increased expression for rs60221525 and rs116943570, respectively. CAG number explained 60.5% of the variance in onset; when rs60221525 and rs116943570 were added to the multiple regression model, there was a tendency for the increase in the explanation.
Conclusions: Variation found in the BECN1 promoter modulates expression and has a potential to modify onset of MJD. The analysis of further patients should increase the power of the study and confirm the role of BCN1 as modifier of MJD.

P09.075-S

Study of the genetic architecture behind mood disorders by whole exome sequencing on a large Italian pedigree

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Major depressive disorders (MDD) and bipolar disorder (BD) are mood disorders with a lifetime prevalence in the adult population of approximately 16% and 4%, respectively. Estimated heritability is about 37% for MDD and 75% for BD and the two disorders have a genetic correlation of about 43%. As of today their genetic architecture remains unclear and no major genetic risk factors or causative genes have been identified yet. With the aim of identifying susceptibility loci responsible for the MDD/BD phenotype, we studied a IV generation Italian pedigree composed of 20 subjects, 7 of whom are affected by mood disorders (5 MDD, 2 BD). Linkage analysis identified 5 regions that were inherited by all the affected members, extending for 222 Mb overall. Exome sequencing performed on the 7 affected subjects and 2 unaffected relatives identified ~1930 genetic variants within linkage regions, 222 of which are functional variants (missense, LoF, splicing) shared by all affected subjects. These latter variants affect several genes already associated to MDD/BD and other relevant biological pathways, such as synaptic development and neurodevelopment. We also identified 28 rare variants (MAF<1 % in dbSNP, 1000G and ESP6500), 8 in the linkage regions, present only in the affected subjects. Among the genes affected by these variants, we selected 4 candidates that could differentiate affected from unaffected subjects in the family. In conclusion, our data suggest that MDD/BD arise from the combination of a shared mutational burden on risk genes together with a few rare variants triggering the observed phenotype.

P09.076-M

Spectrum of Mutations in a New Cohort of Pakistani Families with Primary Microcephaly

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Autosomal Recessive Primary Microcephaly (MCPH) is a neurodevelopmental disorder resulting in diminution of brain growth in utero. The noticeable features are reduced head circumference at birth, and varying degree of intellectual impairment without other neurologic findings. To date, twelve genes have been reported to be associated with MCPH, including *MCPH1, WDR62, CDK5RAP2, CASC5, ASPM, CENPJ, STIL, CEP135, CEP152, ZNF335, PHC1, and CDK6.* Among these, *ASPM* has been found to be most frequently mutated in the Pakistani population. In the current study, 30 new families were ascertained from different regions of Pakistan. We performed SNP array-based homozygosity mapping and revealed linkage to at least one of the known MCPH gene loci in 23 families. In seven families all known MCPH loci could be excluded. Subsequently, we analyzed DNA samples from members of the "linked" families by Sanger sequencing of the corresponding known MCPH gene. We found 10 mutations in *ASPM*, five of them are novel and 9 families carry the already reported founder mutation p.W1326*. Among the remaining five families, two showed novel overlapping microdeletions of 580.8 kb and 164.2kb in *MCPH1*, two other showed novel mutations in *CDK5PAP2*, and the last one showed a previously reported mutation in *WDR62.* This study adds to the mutational spectra of known MCPH-associated genes. The observed high frequency of the *ASPM* mutation p.W1326* underscores its prominent role as a founder mutation in the Pakistani population.

P09.077-S

The metabolism of GABA and glutamate is affected in the brain of the Mecp2-deficient mouse, a model for Rett syndrome.

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Rett syndrome (RTT) is a severe neurological disorder affecting females. Most RTT cases are caused by mutations in the X-linked methyl-CpG binding protein 2 (MECP2) gene. RTT patients develop normally until 6-18 months of age, before the onset of deficits in autonomic, cognitive and motor functions. Studies on Mecp2-deficient mouse models have revealed severe neurotransmission dysregulations, including deficits in bioamine levels and dysfunction of the GABAergic and glutamatergic systems leading to an imbalance between excitation and inhibition in the brain of the mutant animals. However, published results are divergent due to differences in age, model used and/or brain areas studied. Here, we have used real-time PCR, western blotting and HPLC dosage to compare the GABA and glutamate metabolism in eight different brain areas of the Mecp2-deficient mouse brain at two developmental stages (early and late symptomatic). Several key enzymes of GABA and glutamate metabolism have been studied (Kcc2, Nkcc1, Vglut1/2, Gad1/2). Our results show : 1- a progressive reduction of the GABA and glutamate contents; 2- a spatial and temporal deregulation of the GABAergic and glutamatergic key enzymes. We have used that information to assess a pharmacological stimulation of the GABAergic system in vivo and we showed that such a treatment increases the lifespan of Mecp2-deficient mice.

P09.078-M

MEF2C haploinsufficiency is a recurrent finding in patients with autism spectrum disorders

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Since the first description of MEF2C haploinsufficiency syndrome in 2009, 42 patients have been reported either with deletion or point mutation of this gene. Intellectual disability (ID) is the core disorder of this syndrome but autistic features such as stereotypic movements and lack of social communications are commonly reported. To further assess the role of MEF2C in autism spectrum disorder (ASD), we looked for MEF2C point mutations by Sanger sequencing and for MEF2C copy number variations by SNP-array in a cohort of 195 patients with ASD and mild to severe ID.
We identified a de novo frameshift mutation leading to a premature stop codon in 2 siblings with ASD and severe ID. A maternal germinal mosaicism was confirmed based on the haplotype. A de novo MEF2C deletion was also found in a patient with ASD and a mild ID. In this study, we observed MEF2C haploinsufficiency in 1,5% (95% confidence interval: 0-3.2%) of patients with ASD. This is the first study to look specifically at MEF2C in ASD. Of note, a mutation in MEF2C was also found once by whole-exome sequencing in another cohort of 175 trios with ASD. According to this notable MEF2C haploinsufficiency frequency in ASD, we strongly recommend to pay specific attention to this gene when performing non-targeted genetic screening in ASD with a comorbid ID even for patients without severe ID.

P09.080-M

Importance of genotype-phenotype correlation in genetic diagnosis of microcephaly

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New genetic tools for the investigation of cases with neurodevelopmental delay increased continuously the knowledge concerning the underline causes of genetic syndromes.
Array CGH is a highly sensitive method for diagnosis of pathological CNVs responsible for malformative syndromes.
We report a case of a 5y.o girl, who was referred to Medical Genetics Department for evaluation due to microcephaly, mild dysmorphic features and developmental delay. From her medical history, severe and frequent urinary infections, in the absence of any malformation present in the urinary system, are noticeable.
Conventional karyotype from peripheral blood revealed a 12p terminal deletion. Discrepancies between clinical findings and karyotype results required additional investigation using comparative genomic hybridization method. Array CGH revealed a 5Mb terminal deletion on the long arm of chromosome 10, result that matches with the clinical findings of our patient. The molecular result was verified by FISH analysis .This finding is consistent with a complex chromosomal rearrangement involving insertion of genetic material from chromosome 12p to chromosome 10 and consequent deletion of terminal region on the long arm of chromosome 10.
Parental karyotypes are normal, suggestive of a "de novo" rearrangement and consequence low recurrence risk for other siblings.
This case report illustrates the importance of considering genotype-phenotype correlation for each patient and the advantages and the limits of genetic diagnostic techniques in our practice.
In conclusion, clinical judgment complementary to genetic tests provides an accurate diagnosis, prognosis and recurrence risk evaluation.

P09.082-M
New deletion in exon 32 and 33 in MLL2 gene causes Kabuki Syndrome in a spanish patient

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Kabuki syndrome (KS; OMIM# 147920) is a rare congenital disorder, characterized by typical facial features including: long palpebral fissures, eversion of the lateral third of lower eyelids, arched and broad eyebrows with lateral sparseness, short columella and prominent ears. Point mutations and large intragenic deletions and duplications of the histone methyl transferase *MLL2* gene are the main causes of KS. *MLL2* encodes a large protein that belongs to the SET1 family of human SET-domain protein methyltransferase superfamily. Recently, *de novo* partial or complete deletions and point mutations of *KDM6A gene*, have been identified as additional causes of KS. Case report: We report the molecular and phenotype studies of a Spanish patient who have typical features of KS. The patient is a 9 y.o. girl with mental retardation, a peculiar facies characterized by long palpebral fissures, a broad and depressed nasal tip, large prominent earlobes, a cleft palate, scoliosis, radiographic abnormalities of the vertebrae, hands, and hip joints, and recurrent otitis media. Genomic DNA was extracted from the patient and parents and mutation screening of coding exons and intron-exon junctions of the *MLL2* gene was performed by direct sequence analysis using a 3130XL Genetic Analyzer. All found changes were *in silico* analyzed (Polyphen 2.0) to estimate a possible damaging effect in the protein. We identified a deletion in exon 32 that expands to the first portion of exon 33 (c.20711-c.21014 NG_027827). This deletion is absent in both parents and it has not been previously described.

P09.083-S
SGK223, a novel candidate gene for an autosomal recessive form of dHMN

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Distal hereditary motor neuropathies (dHMN) are a subgroup of Hereditary Motor Sensory Neuropathies characterized by a predominant motor involvement of the peripheral nervous system. In the present study a family affected by an autosomal recessive form of dHMN was examined by using a combined strategy of linkage analysis and whole-exome sequencing (WES) in two patients. WES variants in the known disease-related genes associated with similar phenotypes, and copy-number variants shared by the affected subjects were excluded. Considering the presence of two consanguineous marriages in the family, the homozygosity mapping approach was performed and a candidate autozygous region shared exclusively by the affected subjects was highlighted on chromosome 8p23.1-p22. The candidate region contains 120 genes and is characterized by a high number of pseudogenes and paralogs which led to many false positive calls due to multiple-mapping reads. No evident mutations were identified in the also poorly-covered exons of the linkage region but the prioritization analysis of the about 230 WES variants within the candidate region pinpointed a novel missense substitution in the SGK223 gene (c.1529T>C). In silico predictions strongly suggested an alteration of a cryptic splice site in presence of this variant. Interestingly, SGK223 codes for the pragmin protein involved in the reorganization of cytoskeleton, which is a pathway already involved in the dHMN pathogenesis. Despite this functional consistency, further studies are warranted to confirm these findings.

P09.084-M
In search of a new vascular dementia: An exome sequencing approach to a Swedish multi-infarct family

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In 1977, Sourander and Wålinder described a case of hereditary multi-infarct dementia (MID) in a Swedish family. Later their disease was suggested to be cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). The clinical picture of recurrent strokes resembles CADASIL, but we did not detect any pathogenic mutations in the entire 8091 bp reading frame of *NOTCH3* nor found evidence for the *NOTCH3* gene linkage. Neither did we find any evidence of the CADASIL-typical granular osmiophilic material (GOM) in skin biopsies. These multiple approaches suggest that in this Swedish family the hereditary MID suspected to be CADASIL is a different disorder with dissimilar pathological features, and belongs to the growing group of genetically uncharacterized familial small vessel diseases (SVDs).
In order to search for the pathogenic mutation behind the disease, we did a whole-exome sequencing for two healthy and three affected family members. The exon targeting was done with NimbleGen's sequence capture (Roche Inc. USA) and the sequencing with Illumina's sequencing platform (Illumina Inc. USA).
After sequencing the results were filtered against public SNP databases in order to identify the sequence variants that have not been published previously as polymorphisms. Subsequently, we concentrated on the variants that are shared by all the three patients and excluded the variants that are also shared by the healthy controls. Since the disease is dominant we narrowed our search by excluding the variants that are homozygous in all patients. Detailed results of our studies will be presented at the congress.

P09.085-S
Multiple sclerosis risk variant at the ch12q14.1b locus affect the expression of the CYP27B1 gene in activated monocytes

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Background: The region ch12q14.1 was shown to be associated with several autoimmune diseases in different Genome Wide Association Studies. We have determined that a variant which alters the enhancer activity of a regulatory element explains the association of the locus with multiple sclerosis (MS). This region contains the *CYP27B1* gene encoding the enzyme 1-alpha-hydroxylase which catalyses the conversion of 25(OH)D to 1,25$(OH)_2$D, the active form of vitamin D. Since the vitamin D is a key regulator of the immune response and plays a role in MS susceptibility, we want to determine the effect of the variant on the expression of the *CYP27B1* gene in specific population of immune cells. **Methods :** The monocytes CD14 cells were purified from 111 healthy individuals. They were activated with Interferon-gamma (IFNγ) and lipopolysaccharide (LPS). The *CYP27B1* expression was then measured by quantitative real time PCR and correlated with the genotype of the variant. **Results:** We observed that the MS risk allele of the polymorphism rs10877013 was significantly associated with a low expression of the

CYP27B1 gene (p=0,007) in activated monocytes. **Conclusion:** In this work we demonstrate that the causal variant of MS association at the ch12q14.1 locus, alters the transcription of *CYP27B1* gene suggesting that the intracellular vitamin D levels could be affected. Our data point to lower production of $1,25(OH)_2D$ is the functional cause of the genetic association with MS.

P09.086-M
Alterations of gene expression at the peak level in the experimental allergic encephalomyelitis

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Experimental allergic encephalomyelitis (EAE) is the widely used model for studying multiple sclerosis (MS). MS is a chronic disease where the inflammation throughout the brain and spinal cord cause demyelinated plaques of gliotic scar tissue. We have studied the alterations in gene transcription in the EAE induced in the C57/BL6 mouse using real-time PCR. We have studied the genes mediating MS progression, such as cytokines and chemokines, inflammatory and immune response genes, as well as genes involved in cell adhesion, cellular stress and apoptosis whose expressions correlated across multiple analyses. For this purpose female C57/BL6 mice weighing between 18-20 gr were immunized s.c. with 250 µg of MOG peptide (the 35-55 sequence) emulsified in complete Freund's adjuvant (CFA) supplemented with 4 mg/ml killed *M. tuberculosis*. Pertussis toxin (PT, 500 ng/mouse) was injected *i.p.* immediately and 48 hours later. Control mice were received only CFA and further PT. Clinical assessment was performed on the general clinical scales. The average score of EAE group was 3.5. Expression of amyloid beta precursor protein, complement component 1s, chemokine (CC motif) ligand 5, CXC ligand 9, CXC ligand 10, CD4 antigen, G protein alpha inhibiting 2, histocompatibility 2 class II antigen Eβ, IL6, IL13, MBP, NFκB light polypeptide gene, proteolipid protein 1, and TNF are found to be significantly different in the brain at about the peak of disease than control animals. Our data revealed strong transcriptional regulation of genes involved in inflammatory processes, such as antigen presentation and processing, complement activation and chemotaxis.

P09.087-S
Target resequencing of regions associated with Multiple Sclerosis in the Italian population

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Multiple Sclerosis (MS) is a multifactorial autoimmune demyelinating disease of the CNS. Several large international association studies detected over 100 MS loci. However, for the majority of them the causal variant is not yet identified and, generally, only common variants have been studied. The aim of this study was to follow-up the MS loci in the Italian population, searching for the primarily associated variants, focusing also on rare variants. After an association study in 1750 Italian MS cases and 2272 matched controls (Illumina 660-Q, and Immunochip), we selected for target resequencing 32 MS associated regions showing a significant association in the Italian population. The selected regions (1.9 Mb), either including the whole genomic segment (N=17 regions, 45 genes) or only the coding sequences (further 48 genes), were captured (Agilent SureSelect) and sequenced (GAIIx Illumina) in 600 Italian MS patients and 400 matched controls pooled in groups of 12 individuals.We used the variant caller CRISP to call the variants, ANNOVAR to annotate them, and custom R-scripts to calculate allele frequency. Validation of a subset of variants by individual genotyping demonstrate a high correlation with allele frequency in the pools.After QC, results in the MS patients showed that among the 23065 detected variants, 67% were absent in public databases, including 1061 variants with a predicted functional consequence on the gene product (missense, nonsense, splice variants), thus potentially directly involved in the MS susceptibility. The comparison with the data of the controls and the replication in independent cohorts is ongoing

P09.088-M
Modulation of Protein Kinase C Alpha (PRKCA) mRNA expression and alternative splicing by functional polymorphisms contribute to multiple sclerosis susceptibility

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The Protein Kinase C Alpha (PRKCA) gene, encoding the Th17-cell-selective PKCα kinase, was repeatedly associated with multiple sclerosis (MS), but the underlying biological mechanism remains unknown.
We replicated this genetic association in an Italian population (409 cases, 723 controls), identifying a protective signal, corresponding to a GCC microsatellite in the PRKCA promoter (P=0.033, OR=0.12, 95%CI=0.015-0.94), and a risk haplotype in intron 3 (P=7.7*10-4; OR=1.57, 95%CI=1.24-1.99, meta-analysis P=1.1*10-12). Expression experiments demonstrated that the protective signal is associated with PRKCA promoter variants conferring higher expression levels of the gene. Minigene-based transfection experiments proved that the risk signal is driven by an ins/del polymorphism (rs35476409/rs61762387) influencing, with an hnRNP-H-dependent mechanism, the skipping of a PRKCA alternative exon.
Studies performed on RNA extracted from different cell lines and human tissues evidenced a complex pattern of alternatively-spliced (AS) PRKCA isoforms, which are modulated by the nonsense-mediated mRNA decay (NMD) and display a preferential association with the use of alternative polyadenilation sites.
MS patients showed significantly decreased PRKCA levels (49 cases, 61 controls; P<1*10-4) in peripheral blood mononuclear cells, with a concomitant unbalance in the relative abundance of its mRNA variants. One of these alternative transcripts is predicted to encode a truncated PKCα that, when overexpressed, aberrantly localizes to the plasma membrane and/or in cytoplasmic clusters.
Our data suggest that AS-NMD coupling, in the presence of specific genotypes, modulates PRKCA expression and isoform diversity, and point to a dysregulation of PRKCA splicing in MS, with possible implications in Th17-cell-mediated immune responses.

P09.089-S
A novel mutation in SCN4A gene, in a patient with an unusual clinical presentation of Myotonia Permanents: Expanding the clinical and molecular spectrum of SCN4A mutations

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Congenital myotonias are a group of hereditary muscle disorders that present in infancy and childhood, characterized by impaired muscle relaxation following a voluntary forceful contraction. The core symptoms either relate to myotonia or to periodic weakness. These disorders are caused by mutations in genes encoding the skeletal muscle chlorides, sodium and calcium channels.
We report a 7-years old boy with a severe early onset unique phenotype of myotonia permanents. He had neonatal respiratory failure and as well as severe recurrent episodes of laryngospasm during infancy. Massive general muscle hypertrophy, eye lid myotonia and prolonged hand grip relaxation, were present since early childhood. An unusual complication of tongue hypertrophy was noted during infancy and reoccurred again after surgical resection.
A novel V717G mutation in SCN4A was identified in the proband, but not in his parents.
This is the first case of myotonia permanens reporting recurrent tongue hypertrophy as a major clinical feature in this case. Tongue hypertrophy resulted most probably from continued tongue contraction caused by his permanent myotonia .
The novel mutation found in SCN4A in this boy, has not been found in his parents. It has been suggested to be pathological by a number of in silico prediction programs. It is located in a well conserved area of the gene in the transmembrane helical part of the Sodium channel protein subunit. This mutation has not been describes previously in SCN4A related disorders.
This case further expands the genetic and clinical spectrum of myotonia permanents.

P09.090-M
PANK2 and C19orf12 mutations in neurodegeneration with brain iron accumulation (NBIA)

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Neurodegeneration with brain iron accumulation (NBIA) defines a group of neurodegeneration diseases that share prominent extrapyramidal features, dementia and radiographic evidence of iron deposition in the basal ganglia. Different forms of early onset NBIA with autosomal recessive transmission are associated with mutations in specific genes as (1) pantothenate kinase-associated neurodegeneration (PKAN); (2) phospholipase-associated neurodegeneration (PLAN); and (3) fatty acid hydroxylase-associated neurodegeneration (FAHN). Mutations in PANK2 are the most common cause of these disorders. C19orf12 was recently reported as another causative gene. In this study we recruited 20 subjects with NBIA that include clinical data and associated DNA samples. DNA and clinical information were collected and used after partecipants gave written informed consent. The 8 exons of PANK2 and 3 exons of C19orf12 were amplified by PCR and sequenced on an ABI PRISM 3130 XL-AVANT Genetic Analyzer. Phenotypic data were obtained by neurologic examination and magnetic resonance imaging. Mutation screening of PANK2 and C19orf12 were found in 3 and 5 patients, respectively. In conclusion mutations in both PANK2 and C19orf12 contributed significantly to NBIA in the our patients.

P09.091-S
Identification of the genetic factors involved in neural tube development and defects

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Neural tube is the embryonic precursor of brain and spinal cord, and forms as a consequence of closure of the developing neuroepithelium. If closure events fail, the embryo will manifest a neural tube defect (NTD: spina bifida or exencephaly). Despite significative advances in the field, the elucidation of genetic factors associated to NTD has remained elusive and NTDs are the second most common congenital defects affecting human pregnancies. This project aims at investigating two different aspect of NTDs: on one side the analysis of the transcription factor Sax-1, that was found to be down-regulated in a microarray screening in murine NTDs model (Zic2Ku). Sax-1 expression correlates remarkably closely with the progression of posterior closure of the neuroepithelium, overlapping with Zic2 expression. Functional studies of Sax1 and Zic2 in zebrafish will be flanked to mouse embryos and in vitro analyses. On the other side, this project investigates the role of miRNAs in neural tube development on human samples. From a database of 534 fetal autopsies, we selected 9 fetuses with NTDs (7 myelomeningocele, 2 anencephaly). Using a combination of bioinformatics tools (MirWalk, CO-ME-TA, DAVID), we have identified 4 candidate miRNAs whose predicted targets are significantly enriched for functional pathways related to neurulation, that are now under functional evaluation.

P09.092-M
Mutations in RNA kinase CLP1 cause neurodegeneration

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Cleavage and polyadenylation factor I subunit (*Clp1*) is an important kinase in RNA metabolism. It is involved in processes as tRNA maturation and mRNA 3' end processing. Recently, a missense mutation in the *CLP1* gene has been identified in patients with atrophy of the cerebellum, pons and corpus callosum. We have isolated an ENU induced zebrafish mutant, harbouring a p.R44X nonsense mutation in the *Clp1* gene. Using *in situ* hybridization and morpholino knockdown of p53 we examined the phenotype of homozygous p.R44X zebrafish embryos. We show that *Clp1* knockout zebrafish do not survive beyond 4 days post fertilisation (dpf), have a reduced head size and show an S-curved body. At 2dpf, *Clp1* knockout fish show reduced expression of midbrain marker *otx2*, increased cell death in the brain and a disturbed organisation of motor neurons. The phenotype can be partly rescued by injecting human wild type, but not by mutant p.R140H *CLP1* mRNA. Morpholino knock down of p53 partially rescues the phenotype as well. We show that *Clp1* is an essential gene in both the central and peripheral nervous system. Similar to the human situation, *clp1* mutations cause neurodegeneration and motor neuron problems in zebrafish. We show that the neurodegeneration is mediated by the p53 apoptosis pathway. Our data supports the hypothesis that amongst other RNA processing genes, *CLP1* plays a crucial role in neurodevelopment.

P09.093-S
Familial case of NBIA

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Neurodegenerative brain iron accumulation (NBIA) is a group of inherited disorders with various symptoms and genetic aetiology. Common feature of all NBIA forms is accumulation of iron in basal ganglia and progressive movement disorder.
NBIA belongs to rare genetic conditions affecting 1-3 per million individuals which makes it insufficiently studied and understood. Nevertheless, new mutations and genes implicated in different NBIA types are being discovered. One of them is C19ORF12 mutation, which causes MPAN - *mitochondrial membrane protein-associated neurodegeneration*, characterized by childhood/early adulthood onset, dystonia, spacity, optic atrophy and neuropsychiatric changes. The disorder is progressive and symptomatic therapy is the only currently available option.
Parents of affected child came to our institution for genetic counselling and testing for previously detected mutation on exon 3 (C19ORF12) of MMIN gene, c.204-214del11bp homo, in the index patient (daughter, aged 17). Family members (mother, father, daughter-proband and son-aged 5, asymptomatic) were tested using specifically designed amplification primers. Agilent 2100 bioanalyzer was used for analysis of PCR products.
Deletion in sample of affected patient was confirmed as basis for linkage analysis. Both parents and son are heterozygous carriers for described mutation. Since MPAN is autosomal-recessive, risk of affecting future offspring is 25 % in this case. Risk for asymptomatic carriers is 50 %, so genetic counselling is highly recommended.
Discovering new mutations associated with NBIA is significant process as it could elucidate mechanisms underlying this group of disorders and create new possibilites in terms of gene therapy and effective therapeutic options in the future.

P09.094-M
A cell reprogramming-based approach to study 7q11.23 gene dosage imbalances in Williams Beuren syndrome and autism spectrum disorder

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Symmetrical gene dosage imbalances at 7q11.23 cause two neurodevelopmental diseases, Williams Beuren Syndrome (WBS) and the 7q11.23 microduplication associated to autistic spectrum disorder (7dup-ASD). Besides intellectual disability and craniofacial dysmorphisms, WBS patients display hypersociality and comparatively well-preserved language skills while 7dup-ASD is associated with impairments of varying severity across the autistic spectrum. The striking symmetry in genotype and phenotype between the two conditions points to the 7q11.23 cluster as a surprisingly small subset of dosage-sensitive genes affecting social behavior and cognition. The molecular phenotypes of these syndromes in disease-relevant cell-types remain to be elucidated however, due to scarce availability of primary diseased tissues. Convergent evidence both from human studies and mouse models, points to transcriptional dysregulation as a critical aspect of both conditions, consistent with the presence of several transcription factors in the 7q11.23 interval.
Here we present the first analysis of transcriptional dysregulation in human physiopathologically relevant cell types carrying 7q11.23 dosage imbalances. We selected a large and unique panel of WBS and 7dup-ASD patients and derived induced pluripotent stem cells (iPSC) with the most advanced reprogramming technology based on synthetic mRNAs. These were then differentiated into relevant lineages to establish experimentally tractable models of these conditions. Next, we integrated high-throughput sequencing for transcriptional and chromatin analysis and present here a functional dissection of these symmetric conditions that uncovers the principles of dosage-dependent transcriptional dysregulation in disease-relevant human cells types.

P09.095-S
Next-generation sequencing in the diagnosis of neurodevelopmental disorders

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Introduction

Recently, genetic bases of neurodevelopmental diseases including CNS anomalies, intellectual disability (ID), and epilepsy have been significantly elucidated, but are still largely unknown. We have developed a target and whole exome sequencing (WES) based mutation screening strategy. The target sequencing system enabled us to screen 284 genes associated with neurodevelopmental disorders. Unresolved patients were studied by proband-parent trio approach using WES.

Materials and Methods

Forty seven patients were included in the study. Fourteen patients were studied by WES. They were analyzed under the approval by our institutional ethics committee. To capture the exonic DNAs, we used SureSelectXT Custom capture library for neuronal genes capture. We performed sequencing using Illumina HiSeq 2000 sequencer and obtained paired-end sequence reads. We excluded known variants found in dbSNP, 1000 Genomes Project, ESP6500 and our control samples, and narrowed the candidates to missense, nonsense SNVs and frameshift indels.

Results

Fourteen mutations including *FOXG1, AHI1, PTPN11, DYRK1A, ACTB, CASK, GABRD* were identified by target screening and conventional sequencing. WES revealed de novo mutations in *CREBBP, KIF1A, GRIN2A* and *ALDH18A1. HNRNPH2* and *AMMECR1* mutations were noted in X-linked pattern. None of these mutations were recorded in database. Some other mutations have unknown pathogenic significance.

Discussion

NGS method could clarify a consistent percentage of patients with neurodevelopmental disorders. NGS method would be an alternative to current technologies for identifying the multiple genetic causes of neurodevelopmental disorders.

P09.096-M

Seven novel neurofibromatosis 1 (NF1) gene mutations identified in Spanish pediatric patients

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Introduction: Neurofibromatosis type 1 (NF1) is one of the most common human autosomal dominant disorder with an estimated incidence of 1:3500. This is a neurodermal dysplasia characterized by café-au-lait spots, axillary freckling, dermal neurofibromas, and Lisch nodules of the iris. The condition is fully penetrant and has a highly variable expression. It is caused by mutations in the neurofibromin gene (*NF1*) located on chromosome 17q11.2. **Subjects and Methods:** Seven children suspected of having NF1 were tested for mutations in the *NF1* gene. Some of them only have café-au-lait spots and two presented other clinical features. Single and multiexon deletion/duplication were tested by MLPA assay. PCR and sequencing analysis in RNA was performed. Segregation of the mutations was checked in 3 cases. **Results:** Seven pathogenic not previously reported mutations in NF1 gene were identified in heterozygous (Table 1). Only 1 sample showed a deletion of the exon 23 by MLPA. In one case the mutation was considered to be *de novo.* **Discussion:** Mutational NF1 analysis is considered to be a difficult task owing to the size of the gene, the absence of clear mutational hot spots and the presence of pseudogenes. RNA and MLPA-based techniques should be used in conjunction to provided reliable and accurate molecular genetic testing.

Table 1

Case	Mutation	Familiar study
1	c.4009C>T, p.R1337W	yes
2	c.2894T>G, p.I965R	yes
3	c.3639_3640insA	yes *(de novo)*
4	c. 3935C>T, p.S1312F	ND
5	c.23505_3508insAAGT	ND
6	c. 36086_6087insG	ND
7	c.3611G>T, p.R1204L	ND

ND: not determined

P09.097-S

Genetic diagnosis of neurological diseases using NGS: first year of experience

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The diagnosis of neurological diseases is not straightforward, mainly due to the presence of nonspecific overlapping clinical symptoms and of genetic heterogeneity. A global approach using high throughput technologies such as NGS could be of great help to improve their diagnosis.

We present our experience in the analysis of Neurological diseases using a 200-gene NGS targeted re-sequencing panel. Samples from 48 patients were submitted to our laboratory for genetic testing. Clinical diagnosis included Spastic Paraplegia (17), Motor/Sensitive Hereditary Neuropathy (12), Ataxia (6), Parkinson (4), Miastenia (3), Joubert Syndrome (2), Frontotemporal Dementia (2), Hyperkalemic Periodic Paralysis (1) and SMA (1). Coding exons and splice-site regions of the genes associated with each pathology were analysed. Enrichment and sequencing were carried out using SureSelect Enrichment System (Agilent) and SOLiD 5500/MiSeq (Life Technologies/Illumina). Mean depth was established at 200x.

In 7 out of 48 samples (14%), a genetic cause that justifies the clinical diagnosis was found. Taking into account all the samples, 63 variants (confirmed by Sanger sequencing) were identified and classified into pathogenic (7), probably pathogenic (2), Unknown (53) and probably benign (1). According to their effect, missense (29), synonymous (21), splice-site (8), frameshift (3) and nonsense (2) mutations were found. In 21 out of 48 samples, no variants (pathogenic/unknown) were identified. Despite being moderate, 14% of genetically diagnosed patients suggests that NGS is effective for the diagnosis of neurological diseases. A better understanding of unknown variants, that allow classifying them into SNPs or pathogenic mutations, could significantly increase the diagnosis rate.

P09.098-M

Individual cMRI based genetic testing algorithms for neuronal migration disorders increase mutation detection rates

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Background

Neuronal migration disorders are an important cause of severe psychomotor retardation and seizures, frequently resistant to antiepileptic medication. Based on cerebral MR imaging (cMRI) neuronal migration disorders (NMD) can be subdivided into various radiological forms including classic and cobblestone lissencephaly.

Method

Genetic testing was performed for 1034 index patients either (1) individually after in-house cMRI reevaluation (n=214) or (2) as assigned by the referring doctor (n=820). Individual testing strategies included MLPA analysis, Sanger sequencing and massive parallel sequencing. In an ongoing study we assess long-term development and genotype dependent response to antiepileptic and supportive therapies.

Results

119 NMD patients of study arm 1 were genetically analyzed leading to the identification of the underlying genetic alterations in 35.3% of the analyzed samples incl. classic lissencephaly 65.4%, subcortical band heterotopia 83.3%, polymicrogyria 10.0%, cobblestone lissencephaly 14.3%, periventricular nodular heterotopia 37.5% and complex cortical malformations 20.0%. In comparison, the overall mutation detection rate in study arm 2 without in-house cMRI reevaluation was only 18.5% (p<0.0005).

Overall, pathogenic mutations were identified in 18.9% of independent patients incl. *LIS1* (34), *DCX* (47), *ARX* (10), *TUBA1A* (4), *TUBB2B* (4), *GPR56* (10), *FLNA* (30), *POMT1* (22), *POMGnT1* (15), *FKTN* (3), *FKRP* (9), *ISPD* (1), *LARGE* (2) and *DAG* (1). Our preliminary data suggest a genotype dependant antiepileptic response in *LIS1* associated classic lissencephaly.

Conclusion

Genome wide genetic testing strategies will further improve the number of identified genes and genetic alterations, but continue to require their critical interpretation in the context of clinical findings and cerebral imaging.

P09.099-S

In a consanguineous family with two patients showing a novel autosomal recessive inherited syndrome another patient with an unlinked autosomal nonsyndromic form of mental retardation was identified

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We previously characterized in a large family of Turkish origin a male and a female patient showing a new syndrome, that includes following symptoms: severe mental retardation, corpus callosum agenesis, ataxia, moderate microcephaly, square face, hypertelorism, bilateral ptosis, arched eyebrows, epicanthal folds, downslanting palpebral fissures, strabismus, amblyopia, broad nasal bridge, low set ears, short philtrum and downturned corners of the mouth. Since mothers of both patients are first degree cousins and both fathers are second degree cousins, pedigree analysis makes an autosomal recessive mode of inheritance of this particular syndromic disorder very likely. Homozygosity mapping by 250 k Affymetrixs SNP array analysis allowed reducing homozygous genomic regions shared by both patients to only 4 segments ranging from 3.05 Mb to 8.84 Mb in size. Recently a brother of the male patient was diagnosed with nonsyndromic mental retardation (NSMR). Affymetrix CytoScan ® HD analysis of his DNA was performed, indicating his NSMR is not linked to the new syndrome. Because the number of potential candidate genes in these genomic regions according to obtained RS-loci on chromosome 1 (1p36.22), 2 (2q22.3), 3 (3p14.1) and 9 (9q21.13) is considerable, whole exome next generation sequencing will be applied to search for homozygous mutations in both patients especially in those segments. Although the phenotype of our patients shows similarities with Toriello-Carey syndrome and Charlevoix disease, it clearly does not resemble these syndromes, so we think that our patients exhibit a new syndromic disorder and another NSMR form could be mapped in this family as well.<br

P09.100-M

Next-generation analysis of the amyotrophic lateral sclerosis / Parkinsonism-dementia complex of Guam

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The amyotrophic lateral sclerosis (ALS)/ Parkinsonism-dementia complex (PDC) is a progressive, age-associated neurodegenerative disorder described in Guam, Western Papua and the Kii peninsula of Japan. Despite decades of research the excess incidence and pathogenesis of ALS-PDC on Guam remains enigmatic and mutations underlying disease have yet to be elucidated by genome-wide linkage analysis of multi-incident pedigrees. In this study we have used next-generation targeted resequencing to evaluate the contribution of genetic variability in the pathogenesis of Guamanian neurodegeneration. A target-capture panel covering the exonic regions of 116 major genes previously linked and/or associated with parkinsonism, dementia, ALS and related syndromes has been developed. Ninety-three indigenous Chamorro Islanders, including patients with ALS, parkinsonism and/or dementia, as well as neurologically normal subjects, were longitudinally examined and comprehensively assessed. We report putatively pathogenic variants in HTT, PINK1, DCTN1, CHMP2B, DNAJC13, FUS, GRN and ALS2, identified in patients with parkinsonism and/or dementia, that explain multi-incident disease in several pedigrees.

P09.101-S

Multiple sclerosis and NF1: it's time to think about it

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Neurofibromatosis type 1 (NF1) is a rare genetic condition, with a frequency of 1/2500-1/3000, caused by mutations in the NF1 gene (OMIM * 613 113). Since the first description in the literature, knowledge about the clinical picture and the possible associated conditions is gradually increased. In the past, cases of multiple sclerosis (MS) in patients with NF1 have been sporadically described . The association between these two diseases is rare, but probably not accidental, because intron 27b of the the NF1 gene contains OMGP gene (OMIM * 164 345), which acts in myelination processes and seems to be involved in immunopathogenesis of MS. Moreover, NF-1 and MS may share a common influence from genes on chromosome 17 affecting cell proliferation and inflammation processes. We report our 12 years experience in the follow up of NF1 adult patients. The cohort is composed by 301 patients, with an age range of 18-72 years, and Males/Females ratio of 111/190. A multidisciplinary equipe evaluates patients yearly, thus allowing a precise knowledge about the natural history of the condition. Over the years, MS has been diagnosed in four of our patients (3 women and 1 man). This observational study enables us to define for the first time MS prevalence in a large cohort of NF1 adult patients (1.3%). Detailed clinical features of each MS-NF1 patient, together with review of literature and current aetiopathogenetic hypothesis will be provided.

P09.102-M

An NGS gene panel for the genetic diagnosis of rare autosomal recessive cerebellar ataxias

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Autosomal recessive cerebellar ataxias (ARCA) are a genetically highly heterogeneous group of neurological disorders involving both central and peripheral nervous system. One of the goal of the E-Rare EUROSCAR project is to establish an NGS-based targeted genotyping for the diagnosis of known ARCA genes. One-hundred-forty-two ataxic patients referred from different European partners were analyzed using a HaloPlex-based gene panel targeting the coding regions of 127 genes involved in recessive and dominant ataxia. Patient inclusion criteria for analysis were: progressive ataxia, exclusion of nongenetic causes, family history suggestive of autosomal recessive ataxia (AR) or sporadic (S) patients with onset before age 40. We contributed with 34 Italian patients (14AR and 20S) previoulsy tested for FRDA, AVED, or APTX, as appropriate. This approach allowed us to identify homozygous or compound heterozygous pathogenic mutations in ADCK3 and 2 challenging genes (SYNE1 and SACS) in 6 patients (4S and 2AR). Moreover, different missense variants of uncertain pathogenicity were identified in genes responsible for dominant ataxia (PDYN, SPTBN2, CACNA1A). Finally, several heterozygous mutations were identified in recessive genes. For 6 patients, analysis revealed no candidate variants in screened genes. All high-quality variants were confirmed by Sanger sequencing indicating reliability of this approach. Further analyses are required for the validation of uncertain variants and the evaluation of large in/del mutations in recessive genes in which heterozygous mutations have been identified. In conclusion, NGS gene panel represents a necessary tool for genetic diagnosis of highly heterogeneous diseases such as ataxia. (E-Rare grant to MK, PB, and FT)

P09.103-S

Complete recovery from psychosis upon miglustat treatment in a juvenile Niemann-Pick C patient

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Niemann-Pick disease type C (NPC) is a rare recessive lipid trafficking disorder characterized by the accumulation of unesterified cholesterol and glycosphingolipids in the brain and viscera. In the juvenile form, patients in their early years are symptom free, but present with neurodegeneration later in their lives. These include clumsiness, seizures, ataxia, and motor and intellectual decline. Psychiatric manifestations (schizophrenia, presenile dementia, psychosis or depression) may occur at any stage of the disease. Recently, miglustat was approved for the therapy of NPC. We present a case of a patient with juvenile NPC disease whose psychosis was reversed completely by miglustat treatment. At age 14 of the patient, slurred speech and coordination problems, frequent falls and loss of balance were observed. Later, dyslexia and dysgraphia developed. Fine motor skills started to decline more rapidly at age 17, and dysphagia developed. Vertical supranuclear gaze palsy when looking downwards, a hallmark of NPC was observed. Laboratory diagnosis of NPC was established by sequencing of the NPC1 gene. Genotype of the patient was [c.3019C>G] + [c.3182T>C] . Both pathogenic mutations were described previously in the literature. Brain MRI was performed at age 17, 20.5, and 23. At age 17 no signs of atrophy could be detected. Moderate degree atrophy developed between ages 17 and 20.5 years. Miglustat therapy was introduced at age 20.5. Between ages 20.5 and 23, atrophy showed no progress. In conclusion, the introduction of miglustat in NPC patients even in the most advanced cases, with respect to psychiatric

illness might be beneficial.

P09.104-M
VEGF-mediated sphingosine kinase activity decreases in Niemann-Pick Type C neurons and contributes to pathology in NP-C mice

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Sphingosine is a major storage compound in Niemann-Pick type C disease (NP-C), although the pathological role(s) of this accumulation have not been fully characterized. Here we show that sphingosine kinase (SphK) activity is reduced in NP-C patient fibroblasts and NP-C mouse Purkinje neurons (PNs) due to defective VEGF levels related to deficiency of NPC1. VEGF released from bone marrow mesenchymal stem cells also activated SphK by binding to VEGFR2, resulting in decreased sphingosine storage as well as improved PN survival and clinical outcomes in NP-C cells and mice. Similar effects were noted after genetic and pharmacologic replenishment of VEGF in NP-C mice. Further, iPSC-derived human NP-C neurons were generated for the first time and the sphingosine accumulation caused by SphK inactivity in these cells was corrected by replenishment of VEGF. Overall, these results reveal a novel pathogenic mechanism in NP-C PNs where defective SphK activity is due to impaired VEGF.

P09.105-S
The role of the NR2A and NR2B subunits of the NMDA receptor in epileptogenesis

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NMDA receptors are tetrameric ligand-gated ion channels composed of two glycine-binding NR1 subunits and two glutamate-binding NR2 subunits (NR2A, NR2B, NR2C, NR2D) regulating synaptic plasticity. Mutations in the NR2A and NR2B subunits encoded by the genes *GRIN2A* and *GRIN2B* have been associated with different phenotypes of intellectual disability (ID). Mutations in *GRIN2A* were known to cause unspecific ID and epilepsy as well as other neurodevelopmental disorders, whereas mutations in *GRIN2B* have mainly been associated with autism spectrum disorders (ASD) but not seizures. We show for the first time that mutations of NR2 subunits of the NMDA receptor cause different and specific epilepsy phenotypes. NR2 mutations are involved in benign Rolandic epilepsy, the most frequent childhood epilepsy as well as in a variety of rare infantile epileptic encephalopathies, such as Landau-Kleffner and West syndrome. Furthermore, we demonstrate distinct genotype-phenotype correlations. Severe encephalopathic phenotypes are significantly more often caused by truncating mutations in *GRIN2A*, whereas missense mutations are by far more common in benign Rolandic epilepsy patients. For *GRIN2B*, the majority of ASD individuals present with truncating mutations, whereas all epilepsy cases appear to have gain-of-function mutations. The severity of phenotypes depends on the affected domain and the extent of receptor activation. Our observations highlight the so far underestimated role of dysregulated NMDA signalling in both frequent and rare epilepsy disorders and reveal promising pharmacologic targets for novel therapeutic approaches.

P09.106-M
No evidence for a role of NOL3 gene in Italian families with familial adult myoclonic epilepsy or essential tremor

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Cortical tremor (CT) is characterized by fast, small-amplitude, focal or multifocal jerks. It is commonly induced by movement or somatosensory stimulation. CT may have different etiologies: metabolic abnormalities, various neurodegenerative disorders and posthypoxic myoclonus. CT may also be present in familial adult myoclonic epilepsy (FAME) defined by an autosomal dominant inheritance, but despite the locus has been mapped to chromosomes 8, 2 and 5, no causative mutations have been identified to date. A recent study, however, illustrated that FAME may be caused by mutation in NOL3 gene.
Here we wished to screen families with FAME for mutations of NOL3. Since there is growing evidence of an overlap between FAME and essential tremor (ET), we also included families with ET to screen for mutations of NOL3.
We analyzed 10 probands of families that originated from Southern of Italy. Four families had FAME, and six ET. After obtaining the informed consent, the DNA was extracted according to standard procedures and analyzed by direct sequencing with the Sanger method.
No causative mutations have been identified in all patients analyzed for NOL3 gene. However, three polymorphic rare variants have been identified: one in the encoded region, one in 5' UTR region and one in 3' UTR region of NOL3 gene in three different patients.
Exclusion of mutations in NOL3 gene in our families further illustrates the great genetic heterogeneity of FAME/ET and suggests the involvement of other genes. Role of the polymorphic variants in our population remains to be clarified.

P09.107-S
Analysis of RIT1, a novel gene for Noonan syndrome, in patients with suspected Noonan syndrome and negative for other known Noonan syndrome gene mutations

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Noonan syndrome (NS) is an autosomal dominant multisystem disorder (1/2,000 live births) caused by mutations in genes dysregulating the RAS-MAPK pathway. The syndrome belongs to the RASopathies that includes Costello syndrome (CS), Cardio-facio-cutaneous syndrome (CFC), Neurofibromatosis type 1 (NF1) and other syndromes sharing a common pattern of congenital anomalies. Recently the RIT1 gene encoding for a new member of the RAS subfamily was functionally characterized. Moreover, gain-of-function mutations in RIT1 gene were found in 9% of the individuals with Noonan syndrome or a related condition without detectable mutations in known Noonan-related genes. In order to confirm these findings, we examined 11 patients suspicious of Noonan syndrome/RASopathies including one abortion with Hydrops fetalis and one prenatal case presenting with nuchal translucency > 97th pct and a normal karyotype. In an initial study, all 11 patients were screened for mutations in the known genes for Noonan syndrome or RASopathies using a panel of 12 genes (PTPN11, SOS1, RAF1, KRAS, BRAF, NRAS, MAP2K1, CBL, SHOC2, MAP2K2, HRAS, NF1). DNA sequence analysis was carried out on the Illumina MiSeq Next-Generation Sequencing platform. Data analysis was performed using the CLCbio workbench (v6.5) and custom developed Perl scripts. Target regions with a coverage of less than 20X were reanalyzed by Sanger sequencing in order to ensure complete coverage of all coding regions and adjacent splice sites (-20/+10). No causal mutations could be identified using the 12 gene panel. In a second step all patients were screened for mutations in RIT1 gene using Sanger sequencing.

P09.108-M
Novel sequence variations in the human NPC1 gene

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The NPC1 gene spans more than 47 kb, located at locus 18q11, and encodes for an 1278-amino-acid protein located primarily into late endosomes. Mutations in this gene are associated to Niemann-Pick type C (NP-C) disease, which is a fatal autosomal recessive lysosomal disorder characterized by storage of unesterified glycolipids and cholesterol that leads to progressive neurodegeneration. The aim of this work was to define pathogenic variations in patients with clinical suspicion of NP-C. In total, 244 DNA samples were included. DNA was isolated from peripheral blood by standard methods. The whole coding region of the gene was amplified by PCR and sequenced by Sanger sequencing followed by electrophoresis in the genetic analyzer ABI3130xl. Sequence variations were compared to data in the NP-C database (http://npc.fzk.de/) and in silico analyses were performed when necessary. Fourteen new variations were identified in 20 DNA samples that were classified as pathogenic. From those, 4 (28,57%) mutations (p.P434S, p.S667L, p.G911S, p.V1115F) are responsible for change in amino acid polarity, and two of those are located within transmembrane regions. Additional changes were observed in other functional domains, such as in disulfide bond (p.C238R), and in cholesterol binding and transfer (p.A183T) regions. Same of them (p.C238R, p.P434S, p.R615H, p.S667L) were shown to be loca-

ted in conserved regions based on gene alignment among different species. We can predict that alterations reported here can affect protein conformation structure and/or protein function. These findings can contribute to the understanding of important functional domains in the human NPC1 gene.

P09.109-S
PARK2 deletions in patients with Autism Spectrum Disorder (ASD) and other neurodevelopmental pathologies

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The PARK2 gene encodes Parkin, a component of a multiprotein E3 ubiquitin ligase complex that targets misfolded proteins for proteasomal degradation, such as dopamine transporters, synaptotagmin and ?-synuclein. PARK2 genetic mutations are associated with Parkinson Disease, while Copy Number Variants (CNV) have been found in patients with developmental delay (DD), Attention Deficit Hyperactivity Disorder (ADHD) and ASD.
We identified 5 male patients with inherited PARK2 deletions in 342 ASD individuals, screened for CNVs using Illumina 1M SNP arrays (frequency=1.5%). Three patients had a 45Kb deletion of intron 9, and two patients had a deletion of either 29Kb in intron 6 or 266Kb encompassing exons 5 and 6. Clinical presentation was heterogeneous in these ASD patients. A literature search showed recurrent deletions of introns 2 and 3 (N=15 ADHD, N=7 ASD), as well as exons 1 (N=1 ASD) and 2 (N=2 ASD and DD), while DECIPHER reported one ASD individual with an exon 5-6 deletion. In available control databases, deletions of segments from exons 1 to intron 4 as well as intron 9 were recurrent, but only 1/4139 controls had a CNV overlapping exon 6, suggesting a pathogenic role of deletions in this region. The smaller exon 6 CNVs deleted a functional domain SYT11 binding site, compromising the attachment of synaptotagmin and ubiquitination.
These results support a role of PARK2 structural variants in ASD. However, certain PARK2 regions are also frequently deleted in control subjects, and therefore rigorous analysis is mandatory before assuming a pathogenic role for identified CNVs.

P09.110-M
GBA mutations are a major genetic risk factor for Parkinson Disease and Dementia with Lewy Bodies in the Italian population.

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Mutations in the glucocerebrosidase (GBA) gene increase risk of Parkinson Disease (PD). We determined the frequency and relative risk of the two most frequent GBA mutations in a large series of Italian patients with PD or other parkinsonisms. We studied 2,766 unrelated consecutive patients with diagnosis of primary degenerative parkinsonism (including 2,350 PD), and 1,111 controls, who contributed to the Parkinson Institute Biobank (www.parkinsonbiobank.com). The entire cohort was screened for mutations in GBA exons 9 (containing p.N370S) and 10 (containing p.L444P), covering approximately 50% of GBA mutations. Five mutations were identified in the heterozygous state: 4 missense (p.N370S, p.L444P, p.E388K, p.D443N), and the splicing mutation IVS10+1G>T, which we showed to result in the in-frame exon-10 skipping. GBA mutations were significantly more frequent in PD (4.5%, RR=7.2) and Dementia with Lewy Bodies (DLB) (13.8%, RR= 21.9), compared to controls (0.63%), but not in the other form of parkinsonisms, such as Progressive Supranuclear Palsy (PSP)(2%), Corticobasal Degeneration (CBD)(0%), and Multiple System Atrophy (MSA)(0.85%). Considering only the PD group (2,350), GBA carriers had an earlier onset (51±10 vs. 56±10, p<0.0001) and were more likely to have a positive family history for PD (34% vs. 20%, p<0.001). GBA mutations in the Italian population resulted one of the major genetic factor predisposing to synucleinopathies (parkinsonisms due to alpha-synuclein deposition: PD and DLB), but not to tauopathies (parkinsonisms due to tau deposition, PSP and CBD), with the exception of MSA, another synucleinopathy. This suggests a different pathogenic mechanism for MSA compared to PD.

P09.111-S
Polymorphisms and mutations in a specific population of Central Europe (South-Eastern Moravia, Czech Republic) with autosomal-dominant Parkinsonism

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Background: In an epidemiological study carried out in an isolated population of South-Eastern Moravia in the Czech Republic, a surprisingly high prevalence of parkinsonism was found, differing from the published prevalence rates in other European countries. Objective: To determine the type of mutation in families with autosomal-dominant parkinsonism with dementia. Methods: On the basis of a detailed genealogical examination of all the individuals with confirmed parkinsonism, the pedigrees were compiled and a DNA analysis of probands from each pedigree was subsequently initiated. A massive parallel sequencing method using Ion Torrent technology was used; the DNA sequence analysis was focused on the gene loci in which the causal mutations related to Parkinson's disease (PD) have been described. Results: Three large pedigrees with an autosomal-dominant inheritance pattern with reduced penetration of parkinsonism were identified. None of the previously described pathogenic mutations associated with PD were found; rare variants or yet-undescribed mutations were also found. In 5 of 10 examined probands, a novel missense Q230H mutation of the MAPT gene was detected. Polyphene and SIFT in silico predictors indicate this mutation as "probably damaging". Conclusion: Confirmation sequencing using an independent method is underway to examine the targeted MAPT mutation in other individuals from all three pedigrees and to compare it with healthy controls. Supported by grants: IGA MZ ČR NT - 14407-3/2013 and IGA LFUP 2013-024

P09.112-M
HOMER1 promoter analysis in Parkinson's disease: association with levo-dopa dosage

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AIMS: HOMER1 gene expression has been linked to abnormal movements in animals receiving chronic administration of antipsychotics. The continuing neurodegeneration of Parkinson's disease (PD) and the prolonged use of L-dopa are associated with motor complications, such as dyskinesia, and psychotic side effects, including hallucinations and paranoid delusions. Approximately 25-40% of patients with idiopathic PD experience hallucinations. Genetic variability within different candidate genes has been implicated in the clinical severity of sporadic PD in many populations.
METHODS: We investigated 3 polymorphisms located in the 5' flanking region of the HOMER1 gene within a sample of 131 sporadic PD patients from southern Italy, using a 3-SNP genotype and haplotype combination (rs4704559, rs10942891, rs4704560). RESULTS: Our study implicates the effects of allele A of the rs4704559 marker in high dosage levo-dopa treatment in PD (p <0.05). CONCLUSION: Even though our results are preliminary, this HOMER1 gene variant may represent a genetic variant for personalized treatment in PD patients.

P09.114-M
PCDH19 mutations in girls with infantil epilepsy: four novel mutations and prediction of their functional impact

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The *PCDH19* gene encodes for protocadherin 19, a transmembrane protein with six extracellular cadherin (EC) domains containing adhesive interfaces involved in neuronal connection. Over a hundred mutations in PCDH19 gene have been identified in girls with epilepsy, with or without cognitive impairment. While some mutations are recurrent, many new variants remain private. Furthermore, transmitting hemizygous males are devoid of seizures or cognitive impairment, making it difficult to establish the pathogenic nature of newly identified variants.
Here, we propose an approach to evaluate the pathogenicity of four novel PCDH19 mutations found in girls with epilepsy and variable degree of de-

velopmental delay. Segregation analysis is complemented with an *in silico* analysis of mutation effects on the protein structure and function. Using sequence information, we compared different computational prediction methods. We also used homology modelling to build structural models of two PCDH19 EC-domains containing the novel missense mutations. Wild-type and mutant models were compared to identify the differences in the presence and type of residue interactions or in the biochemical properties (conservation, electrostatic charge, hydrophobicity) of the model surfaces. This analysis revealed that these novel mutations exert their pathogenic role using different molecular mechanisms. Two of them interfere with or alter functional residues predicted to mediate ligand or protein binding, another alters the EC-domain folding stability, and the frame-shift mutation produces a truncated protein lacking the intracellular domain.
We also observed that the two girls with severe neurological impairment carry mutations predicted to have the worst effects on the protein structure.

P09.115-S
Molecular genetic background of patients with PEHO-like features

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PEHO syndrome (Progressive encephalopathy with Edema, Hypsarrhythmia and Optic atrophy; MIM 260565) is an autosomal recessive inherited progressive infantile encephalopathy. The main features of PEHO syndrome are hypotonia, infantile spasms and/or hypsarrhythmia, psychomotor retardation, absence or early loss of visual fixation, edema of the face and limbs, and typical dysmorphic features. Brain atrophy is progressive and most prominent in the cerebellum, where the molecular layer is strongly reduced, Purkinje cells are deformed and misaligned, and the cells of the granule cell layer are significantly reduced in number. In addition, the optic nerves show varying degrees of loss of myelinated axons and gliosis, and the retinal nerve fiber and ganglion cell layers are atrophic. A number of patients present with many of these clinical features, but lack typical neuroradiological and neuropathological findings or progression of brain atrophy and do not have the PEHO founder mutation. These patients are classified as having PEHO-like syndrome. This group is clinically heterogeneous and therefore it is likely that there are multiple underlying genes. To characterize the genetic background of patients showing PEHO-like features, we performed exome sequencing of 33 Finnish patients, and parents of six of them. We identified likely pathogenic mutations in known disease genes (CDKL5, ABAT, SPTAN1, SCN2A, MT-CYB, WDR45 and KCNQ2) in nine individuals. Analysis of mutations in novel disease genes is ongoing. Our preliminary findings imply that patients with PEHO-like features are genetically highly heterogeneous and that the entity overlaps with early-infantile epileptic encephalopathies.

P09.116-M
De novo mutations in periventricular heterotopia: an exome sequencing study

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Disorders involving neuron mispositioning encompass a heterogeneous group of phenotypes affecting neuronal development. Periventricular heterotopia (PH) is one such disorder characterised by failure of neurons to migrate to the outer cortex of the brain, resulting in ectopic positioning along their sites of origin – the lateral ventricles. Although mutations in *FLNA* account for an estimated 80% of familial PH cases, mutations at this locus account for only 25% of sporadic cases, leaving the vast majority with no genetic diagnosis. Here we describe a project intended to characterise the mutational heterogeneity underlying PH and gain insight into the molecular networks that when defective underpin this disorder. We describe an approach involving careful phenotyping of each proband and employ a trio design to exome sequence characterise causative mutations. This study will encompass both non-syndromic and syndromic cases of PH and seek to map candidate causative variants into gene interaction networks. Similar approaches have proved successful for other neurological disorders such as autism. Illustrating the legitimacy of our approach we describe an identified *de novo* mutation in a gene encoding a calmodulin isoform. The mutation resides in one of four highly conserved calcium binding pockets of this protein where it is predicted to alter function. Substantial evidence demonstrates direct binding between calmodulin and filaminA. Although the exact pathogenic status of this variant has yet to be corroborated by a second individual, this illustrative case demonstrates that functional genetic relationships can contribute to the implication of extremely heterogeneous disorders such as PH.

P09.117-S
PLA2G6-associated neurodegeneration (PLAN): long-term follow-up, genotype-phenotype correlates and identification of a founder mutation in a large North-African cohort

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Mutations in the *PLA2G6* gene are causative of PLAN, a spectrum of neurodegenerative conditions including infantile, childhood and adult onset forms. Seventeen North African patients with a clinical suspicion of infantile-onset PLAN underwent clinical and instrumental examinations and *PLA2G6* sequencing. Haplotype analysis was performed to date the identified founder mutation. All patients carried pathogenic biallelic mutations in *PLA2G6*. Sixteen children had the commonest form of infantile-onset PLAN, with early onset of psychomotor regression, hypotonia, cerebellar signs and abnormal ocular movements. The phenotype was highly homogeneous, with rapid development of severe spastic tetraparesis, cognitive impairment, optic atrophy, and cerebellar atrophy at brain MRI. Motor neuropathy and EEG fast rhythms were also frequent. Nine patients from six families shared the same founder mutation (p.V691del) which likely arose in the late seventeenth century. Only one patient fits the diagnosis of the much rarer childhood-onset PLAN. Despite the early onset (18 months), progression of cerebellar and pyramidal signs was slower, with behavioral disturbances and dystonia. Typical features of infantile-onset PLAN such as hypotonia, nystagmus/strabismus, optic atrophy, EEG fast rhythms and motor neuropathy were absent. Besides cerebellar atrophy, MRI showed iron deposition in the basal ganglia. This patient carried a missense mutation predicted to be less deleterious.

P09.118-M
Identification and functional characterisation of a new KCNJ2 mutation

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Autism is a genetic disorder, with an estimated heritability greater than 90%. Over the past years, the convergence of genomic technologies has led to the identification of several susceptibility loci by means of linkage studies. Autism is associated with epilepsy in early childhood and epilepsy occurs in 10-30% of autism patients. Recently, a role of potassium inward rectifying channels in the pathogenesis of autism disease has been postulated due to the identification in patients affected by autism and epilepsy of two mutations in the KCNJ10 gene. The gene encodes the Kir4.1 channel which is expressed in astrocytes and neurons.We then searched for mutation in the KNCJ10 gene, as well as in two genes (KCNJ2 and KCNJ16) coding for additional Kir channels with reported expression in astrocytes and neurons, in two unrelated patients. We detected a new mutation in the KCNJ2 gene in a boy affected by autism. The mutation has been detected also in the healthy father and in the paternal grandmother who reported a case of SIDS (Sudden Infant death Syndrome) in her family. A functional analysis of the mutation effects was performed using an electrophysiological approach based on whole cell patch clamp.

P09.119-S
A novel 2p21 deletion isolated to the *PREPL* gene

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Hypotonia-cystinuria syndrome (HCS) is an autosomal recessive disease characterized by hypotonia at birth, failure to thrive, growth retardation, cystinuria type I and, in some cases, hypergonadotropic hypogonadism. These symptomes are attributed to a homozygous deletion on chromosome 2p21, encompassing the genes *SLC3A1* and *PREPL*. Since loss-of-function *SLC3A1* mutations cause isolated cystinuria, the other HCS symptoms have been considered to be caused by PREPL deficiency. Using a high-density whole genome array, we identified an 11 kb homozygous deletion in the *PREPL*

gene in a patient presenting with symptoms characteristic of HCS, but without cystinuria.
Immediately after birth, the patient presented with severe muscular hypotonia and feeding problems, followed by growth hormone deficiency and hypergonadotropic hypogonadism. Initial molecular analyses excluded a number of syndromes, including Prader-Willi (PWS). The homozygous deletion in *PREPL* identified by array analysis corresponded to heterozygous deletions in each of the nonconsanguineous parents. qPCR analysis confirmed these findings and pinpointed the extent of the deletion to exons 5-10. An isolated homozygous microdeletion involving only *PREPL* has not been previously described, however, Régal *et al*., (in press) recently identified a patient carrying a nonsense mutation in *PREPL* combined with a microdeletion involving *SLC3A1* and *PREPL*, resulting in a similar phenotype to the case presented here. These findings may contribute to further delineate the normal function of PREPL, as well as support the notion that homozygous *PREPL* inactivation should be considered in the diagnosis of patients with a PWS phenotype, but no PWS genotype (Maartens *et al*., Biol Chem 387;879-883,2006).

P09.121-S
Compound heterozygous mutations in two known MCPH genes in autosomal recssesive primary microcephaly

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Autosomal recessive microcephaly with severe mental retardation with no dysmorphism or other anomalies was diagnosed in seven individuals of an Arab Israeli family. Brain CT scan of affected individuals showed no structural anomalies. Whole exome sequencing of an affected individual identified four mutations in two known MCPH genes: compound heterozygous mutations both in STIL and in ASPM. Of 100 ethnically matched controls, none had more than one of the 4 mutations, and only in a heterozygous state. Segregation analysis and functional assays are underway to unravel which of the above variations are the causative mutations for microcephaly in the affected individuals within this family.

P09.122-M
VPS53 compound novel mutations cause progressive cerebello-cerebral atrophy type 2 (PCCA2)

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Progressive cerebello-cerebral atrophy (PCCA) leading to profound mental retardation, progressive microcephaly, spasticity and early onset epilepsy, was diagnosed in four non-consanguineous apparently unrelated families of Jewish Moroccan ancestry. None of the known SepSecs (MIM613009) mutations were found in the investigated families. Common founder mutation(s) were assumed. Genome wide linkage analysis and whole exome sequencing were done, followed by realtime PCR and immunofluorescent microscopy. Genome wide linkage analysis mapped the disease-associated gene to 0.5Mb on chromosome 17p13.3. Whole exome sequencing identified two mutations within this locus, which were common to the affected individuals: compound heterozygous mutations in VPS53, segregating as expected for autosomal recessive heredity within all four families, and common in Moroccan Jews (~1:37 carrier rate). The Golgi-associated retrograde protein (GARP) complex is involved in the retrograde pathway recycling endocytic vesicles to Golgi. Both c.2084A>G and c.1556+5G>A VPS53 founder mutations are predicted to affect the C-terminal domain of VPS53, known to be critical to its role as part of the GARP complex. In fact, mRNA studies showed detrimental effects of the mutations on VPS53 transcripts, and immunofluorescent microscopy demonstrated swollen and abnormally numerous CD63 positive vesicular bodies, likely intermediate recycling endosomes, in fibroblasts of affected individuals. Thus, VPS53 mutations cause autosomal recessive PCCA type 2.

P09.123-S
Clinical and molecular characterization of progressive encephalopathies in children

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Progressive encephalopathies (PE) are defined as progressive diseases of the Central Nervous System often accompanied by decline of cognitive and/or motor functions and include a number of different disorders. PE can primarily be divided into neurodegenerative and metabolic, the latter defined by defects in subcellular organelles or in the intermediary metabolism of macromolecules. In the neurodegenerative disorders, progressive loss of neural tissue is often detected by cerebral MRI or markers in the cerebrospinal fluid. PE is associated with high morbidity and mortality rate, but treatment options are available for some PE disorders. Genetic explanations lack for about 20% of PE disorders, which are classified according to MRI findings and phenotype.
Our aim is to characterize novel mutations causing PE. We have collected more than 60 PE-patients in 45 families. In all patients, we ruled out CNS infection, trauma, vascular accidents and sequelae after prematurity. Evaluation of all patients by biochemical examinations and neuroimaging revealed cortical atrophy, cerebellar degeneration or basal ganglia abnormalities in the majority of the patients. Congenital anomalies, also those outside the nervous system, were evaluated using the London Medical Database for syndrome identification.
Karyotyping, aCGH and analysis of candidate genes by MLPA and/or sequencing revealed no relevant findings. We have initiated Whole Exome Sequencing (WES) on DNA from 43 family trios. Among the 16 trios finalized through the bioinformatics pipeline, a putative disease causing mutation has been identified in eight. Functional studies are currently being performed to characterize their clinical implications.

P09.124-M
OTX2, PRRX1 and PRRX2 gene: molecular characterization of two cases of agnathia-otocephaly foetus

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Agnathia-otocephaly is a rare, sporadic and lethal malformation with an estimated incidence of less than 1 in 70000 births. Otocephaly is considered a defect of blastogenesis and it can further result in the mal-development of the craniofacial midline, extracephalic and axial body structures. This malformation spectrum is usually lethal, because is associated severe respiratory distress.
We screened 2 agnathia cases for orthodenticle homeobox 2 (OTX2), paired related homeobox 1 (PRRX1) genes and for the homeobox 2 (PRRX2) gene by sequencing in a ABIPRISM 3130xl genetic analyzer.
In the first case, a full autopsy in the fetus proband of 12+6 weeks, revealed agnathia, holoprosencephaly, cyclopia, microstomia, malformed pinnae (misplaced toward the angle of the mandible), omphalocele and shortening of the long bones. The cytogenetic analysis showed a partial trisomy 13 and partial monosomy 18. Screening for the OTX2, PRRX1 and PRRX2 gene in foetal tissues, identified a heterozygous S144N in the exon 2 of PRRX2 gene. An analysis *in silico* with Polyphen v2.0 and Shift Mutation showed this mutation as a high probability of altering protein function.
In the second case, the proband was a foetus of 14+3 weeks. The autopsy revealed agnathia and omphalocele. Screening for these three genes identified a heterozygous P181L in the exon 3 of PRRX1 gene, mutation with a high probability of altering the protein function as analysis *in silico* shows.
In summary, we report novel mutations in PRRX1 and PRRX2 gene implicated in two case of agnathia.

P09.125-S
Study of *FOXG1* gene in Spanish patients: review of clinical presentation

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Introduction: Since ,mutations in *MECP2* gene were described as causing Rett syndrome (RTT, OMIM#312750) in 1999, other genes have also appeared to be responsible of RTT: X-linked cyclin-dependent kinase-like 5 (*CDKL5*) lying in Xp22 and forkhead box G1 (*FOXG1*) in 14q12. We present our experience in clinical and molecular diagnosis of congenital form of RTT.
Materials and methods: We have studied the *FOXG1* gene by direct sequencing and MLPA (Probemix-P075, MC-Holland) in patients RTT-without

MECP2 mutation and in patients with mental retardation and Rett-like clinical features.
Results: We analyzed 211 patients with clinical presentation likely to have mutations in *FOXG1* gene: 143 RTT (classic and congenital form *MECP2*-negative), 39 male patients with severe mental retardation and hypotonia and 29 patients with RTT like features. We detected 9 mutations: 5/23 girl patients with congenital form and 4/39 male patients with severe mental retardation.
None of classical form or girls with RTT-like clinical features carried mutation in *FOXG1.*
Conclusions: Genetic etiologies of variant Rett syndrome are heterogeneous; screening the *FOXG1* gene should be done not only in females, but also in male patients with severe hypotonia and acquired microcephaly since the first months of life.
FOXG1 is not an X-linked gene and therefore there can be a higher incidence of mutation detection in RTT-like males than in *MECP2* and *CDKL5* genes.

P09.126-M
Modifiers of age at onset in spinocerebellar ataxia type 2: a preliminary study in a Brazilian population

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The spinocerebellar ataxia type 2 (SCA2) is caused by CAGn expansion at ATXN2 gene, which account for 50% of the variability in age at onset (AO). Previous reports pointed to CAGn variations and polymorphisms at other genes as responsible for the remaining AO variance. Aims: to address a confirmation study of other polyglutamine tracts and of a mitochondrial DNA polymorphism as modifiers of AO in SCA2 patients. Methods: symptomatic individuals with a molecular diagnosis of SCA2 were recruited from Brazil. Capillary electrophoresis was performed to detect CAG lengths at SCA1, SCA2, SCA3/MJD, SCA6, SCA7 and RAI1 associated genes; the mitochondrial complex I gene polymorphism (10398G) was determined by PCR followed by restriction endonuclease analysis. Pearson correlations with AO were tested against each CAGn for each individual; the 10398G polymorphism of one person per maternal lineage was analysed by t test; all followed by a step-wise linear regression. Results: 57 individuals (33 families and 42 maternal lineages) were studied. Mean (range) AO and CAGn at normal and expanded ATXN2 alleles were 32.9 (3-76) years and 23 (22-33) and 42 (34-67) repeats. At first, AO correlated with the large alleles at ATXN2 and ATXN3 genes, and with small allele at RAI1. 10398G was not associated with AO. On step-wise regression, the unique correlation maintained was with ATXN2 expanded allele ($r = -0.78$; $r2 = 0.61$; $p < 0.0001$). Discussion: our preliminary data did not support previous published results; they should be confirmed with an outlier sampling strategy, in the future.

P09.127-S
Heterozygous deletion of KLHL1/ATXN8OS at the SCA8 locus are likely not associated with cerebellar impairment in humans

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Spinocerebellar ataxia type 8 is a dominantly inherited ataxia, mainly occurring in adulthood, caused by a CTG.CAG tract expansion in the ATXN8OS gene, an untranslated antisense RNA partially overlapping the KLHL1 gene, and the complementary CAG repeat in the ATXN8 gene.
We report a father (59 years) and his daughter (10 years) carrying a genomic deletion (800 kb) completely overlapping ATXN8OS gene and part of KLHL1 (from 70,345,271 to 71,196,665 - NCBI Build 37/hg19 - Array-CGH 60K) and a 4 Mb duplication in 8q13.33q21.11 (72,268,801-76,311,849) segregating in an unaffected brother. The girl was evaluated for dysarthria, difficulties in sentence structuring, short attention span and mild intellectual deficit. No other neurological signs were reported. Brain MRI was normal. Neurological examination of the father revealed short attention span; coordination, sensitivity, reflexes and cranial nerves were normal; no abnormal gait or dysarthria. Brain MRI revealed several areas of gliosis in the frontal white matter.
Databases mining showed a partially overlapping 357 kb deletion involving only KLHL1 and ATXN8OS (chr 13:70,486,026-70,843,210) in a patient with mild speech delay (Decipher 278559), inherited from a healthy mother.
In contrast with the KLHL1/ATX8OS knockout mouse model, our data suggest that heterozygous deletion of KLHL1/ATX8OS is not associated with ataxia/cerebellar involvement in humans.

P09.128-M
Genetics of Schizophrenia: preliminary results of an Italian multicenter study

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Schizophrenia (SCZD) is a major psychiatric disease causing severe disability and with a prevalence of 1% worldwide. Genetic factors play a key role in the etiology of schizophrenia but its genetic bases are complex and not yet clarified. In the last few years, innovative technologies have been widely applied to the genetic study of schizophrenia. This multicenter study aims to apply aCGH and NGS technologies to investigate genetic risk factors for specific SCZD-related endophenotypes that greatly affect real-life functioning of people with schizophrenia or variables strongly associated with it. To investigate the contribution of *de novo* CNVs to schizophrenia vulnerability, we have used the Enhancer Chip (*PLoS One 2012;7(12):e52264*), a customized aCGH recently developed by our group, which is able to examine CNVs in the whole human genome as well as testing over 1,250 enhancers for their potential pathogenic role. Clinical research units have provided us DNAs from 60 sporadic SCZD patients and their parents (family trios). To date, we have identified two *de novo* partially-overlapping deletions at 7q31.2 in unrelated SCZD patients. Interestingly, both CNVs include *MET*. This proto-oncogene has been previously associated to schizophrenia (*Am J Psychiatry* 2010;167(4):436). Another SCZD patient presented a *de novo* duplication at 19p13.3. A similar rearrangement is reported in Decipher, possibly associated to intellectual disability. *De novo* protein-altering mutations will be also investigated by NGS-based exome sequencing of all family trios recruited (now in progress). These preliminary data may help to strengthen the role of *de novo* variants in the pathogenesis of schizophrenia.

P09.129-S
Genome-wide methylation profiling of schizophrenia

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Background: Schizophrenia is one of the major psychiatric disorders. It is a disorder of complex inheritance, involving both heritable and environmental factors. DNA methylation is a fundamental inheritable epigenetic modification that stably alters the gene expression levels. We reasoned that genetic modifications, resulting from environmental stimuli could also make a contribution to the disease development.
Materials and Methods: We have performed 26 high-resolution genome-wide methylation array analyses to determine the methylation status of 27,627 CpG islands and compared the data between patients and healthy controls. Methylation profiles of DNAs were analyzed in six pools (220 schizophrenia patients; 220 age-matched healthy controls; 110 female schizophrenia patients; 110 age-matched healthy females; 110 male schizophrenia patients; 110 age-matched healthy males) and 20 individual patient DNA samples (7 females and 13 males).
Results: We find significant differences in the methylation profile between schizophrenia and control DNA pools. New candidate genes that principally

participate in apoptosis, synaptic transmission and nervous system development (*GABRA2, LIN7B, CASP3*) were discovered. Methylation profiles differed between the genders. Among females the most important genes participate in apoptosis and synaptic transmission (*XIAP, GABRD, OXT, KRT7*). Among males *DHX37, MAP2K2, FNDC4, GIPC1* genes represent the most significant candidate-genes.
Conclusion: Our data revealed substantial differences in methylation profiles between schizophrenia patients and controls and between male and female patients. Dysregulated activity of these candidate genes could play a role in schizophrenia pathogenesis.

P09.130-M
De novo mutations identified in sporadic cases of Childhood Onset Schizophrenia

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Childhood Onset Schizophrenia (COS) is a severe neurodevelopmental disorder, for which the genetic etiology remains largely unknown. Recently, whole exome sequencing studies have identified severe rare *de novo* variants in sporadic cases of adult onset schizophrenia and autism. In this study, we performed exome sequencing of 17 COS trios with the aim of identifying *de novo* variants in the COS affected children. We identified 20 *de novo* mutations in 17 probands, which is consistent with the *de novo* mutation rate thus far identified in adult form of schizophrenia. Our findings also suggest that *de novo* missense variants identified in our study have a high likelihood for pathogenicity. In fact, the pool of genes we found to present *de novo* variants appears to be enriched for genes that are less likely to tolerate such alterations. Among the genes found to be disrupted in our study, there is evidence suggesting that some of them are involved in neuronal functions. Moreover some of these genes were previously reported to be associated with neurodevelopmental disorders. Hence this list of genes should be considered to represent genuine COS candidate genes. We believe that insights from the identification of COS genes may help to develop diagnostic tools, which in turn may help to intervene before the onset of symptoms. Eventually, knowing the genetic etiology may lead to the identification of therapeutic avenues for better treatment.

P09.131-S
Systematic association analysis of human microRNAs with schizophrenia

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Schizophrenia (SCZ) is a severe neuropsychiatric disorder. SCZ genome-wide association studies (GWAS) have identified common single nucleotide polymorphisms (SNPs) and a large polygenic contribution to illness risk, but biological mechanisms remain unclear. The known role of microRNAs (miRNAs) as potent disease modifiers in neuropsychiatric disorders raises the question of whether genetic variation in miRNAs plays a critical role in SCZ etiology.
Therefore, we implemented a systematic set-based test for all miRNAs defined in the miRBase based on test statistics from GWAS data. Results from the largest SCZ meta-analyses to date [Ripke et al., 2013] provided the basis for this analysis. Alike the popular gene set testing tool VEGAS [Liu et al., 2010], SNPs in within miRNAs were grouped together, corrected for linkage disequilibrium and controlled for the number of SNPs within each miRNA to calculate a miRNA test statistic.
From all analyzed miRNAs, 2.76% were significantly associated with SCZ after correction for multiple testing; further 18.90% were nominal significant. As expected from the GWAS results, the strongest association was found for hsa-mir-137. Moreover, miRNAs involved in neural and synapse development such as mir-9 and let-7 as well as in miRNAs with yet unknown function were identified. Further evaluation of targets from significantly associated miRNAs as well as their presence in brain QTLs will be presented.
Overall, our results give the first unbiased screening of miRNA association based on large SCZ GWAS data and might lead to the discovery of key players suitable for further functional studies.

P09.132-M
Whole exome sequencing of schizophrenia patients with high level of autozygosity

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Schizophrenia (SZ) has a high heritability (about 80%), but the genetic architecture of the disease is still unclear. The numerous genome wide association studies and the recent next generation sequencing analyses indicated that about 32% of the genetic variability of SZ is due to the additive effect of a high number of susceptibility alleles with a modest effect size and/or to the presence of rare deleterious copy number variants (CNVs) or coding single nucleotide variants (SNVs). To evaluate the contribution of rare recessive homozygous SNVs in SZ, we initially identified, with SNP-array analysis of 180 unrelated SZ patients, the offspring of mates that are closely related (inbreeding) and therefore have an increased prior probability to carry potentially deleterious recessive mutations; subsequently, we performed whole exome sequencing analysis of these patients.
An average of 269 (min-max: 196-384) functional (frameshift, missense and nonsense) homozygous SNVs has been observed in the autozygous regions of the 7 sequenced patients. About 8 SNVs per individual are novel (not in dbSNP, 1000G and dbESP6500) and have a high phyloP conservation score. Among these, we identified, in three patients, three different SNVs representing good candidate SZ mutations, since they are predicted to be damaging and map in genes mainly expressed in brain and previously associated with SZ.
Our data suggest that rare homozygous SNVs could be involved in the clinical phenotype of SZ, at least in patients with high level of autozygosity.

P09.133-S
Copy number variants analysis and targeted resequencing of the schizophrenia candidate gene RB1CC1

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Schizophrenia is a severe neuropsychiatric disorder with heritability estimates of ~80%. Xu et al. (2011) published the first exome-sequencing study focusing on de novo mutations in patients with schizophrenia. To provide additional genetic evidence for any of the genes suggested by the exome-sequencing study, we performed a follow-up study focusing on copy number variants (CNVs). We screened 1,637 patients and 1,627 controls for CNVs in any of the genes suggested by the exome-sequencing study. Duplications in RB1CC1 on chromosome 8 were overrepresented in patients. The duplications were followed-up in independent European samples. In the combined analysis, comprising of 8,461 patients and 112,871 controls, duplications in RB1CC1 were associated with schizophrenia (P = 1.29 x 10-5; odds ratio = 8.58). The aim of the present study was to further explore RB1CC1 as a candidate gene for schizophrenia. The gene consists of 24 exons. We focused our targeted resequencing on exon 15: (i) it contains >30% of the gene's total protein-coding sequence, and (ii) Xu et al. (2011) identified a de novo frameshift deletion in this exon. After quality control, the data from 1740 patients were available. Among 22 patients, a total of 17 different variants were identified and verified by sequencing the complementary strand. Of these, 10 were neither detected in the 1000 Genomes Project nor the Exome Variant Server. Currently, we are analyzing whether these variants co-segregate with a psychiatric diagnosis within the families of the affected probands. Furthermore, detailed phenotypic description of the mutation carriers are being assembled.

P09.134-M
Combination of whole-genome and whole-exome sequencing, to identify rare and de-novo variation in cases of schizophrenia and bipolar disorder from the Faroe Islands

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Isolated populations represent an advantage to identify rare disease variants that may appear at higher frequencies compared to outbred populations. In this work, we use the Faroese population to test this hypothesis, and combine low-depth (6X) whole-genome (WGS) and high-depth (35X) whole exome (WES) sequencing approaches to describe genomic variation, de-novo mutations and perform association mapping in patients with schizophrenia (SZ) and bipolar disorder (BP).
Our sample consists of 106 SZ cases, 28 BP and 214 controls (344 total): together with unrelated individuals, it includes 54 complete trios.
A total of 17,345,307 and 259,904 variants have been called in WGS and WES respectively. We discovered 9,130 de-novo mutations in WGS and 417 in WES. Clear differences emerge between WGS and WES in identifying different types of variants. A specificity of this work is the combination of WGS and WES in the discovery of de-novo mutations: both approaches discover the same number of de-novo loss-of-function mutations but high-depth WES is clearly more powerful in calling coding variants, mostly because of depth filtering criteria. In the association mapping we identified 6 genome-wide significant loci, which are currently being replicated in 3,300 BP and controls from UCL. The results of this analysis, the concordance between WES and WGS, and their perspectives will be presented and discussed.

		Whole Exome (35X)		Whole Genome (6X)	
		count	percent	count	percent
All Variants	Loss of Function	3,140	1.21%	5,753	0.03%
	Missense	65,785	25.31%	80,087	0.46%
	Synonymous	41,904	16.12%	42,989	0.25%
	Non coding	149,075	57.36%	17,216,478	99.26%
	Total	259,904		17,345,307	
De Novo Variants	Loss of Function	6	1.44%	6	0.07%
	Missense	89	21.34%	12	0.13%
	Synonymous	32	7.67%	6	0.07%
	Non coding	290	69.54%	9,106	99.74%
	Total	417		9,130	

P09.135-S
Identification of a de novo 15q13.1-13.3 deletion in a reading and language impaired cohort

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Dyslexia and specific language impairment (SLI) are developmental disorders presenting deficits of written or spoken language respectively in individuals with normal intelligence and education, and without overt neurological abnormalities. Copy number variations (CNVs) have been implicated in neurodevelopmental and psychiatric conditions, such as autism and schizophrenia, but it is not clear to what extent they might contribute to reading and language abilities. Using data from a longitudinal study investigating the development of children between 3 and 7 years of age, we performed CNV analysis (n=94 children; 65% family risk of dyslexia, 23% language impaired, 12% typically developing) and identified a large deletion on chromosome 15q13.1-13.3 in an individual with an early age SLI diagnosis. This single copy deletion spanning BP3-BP5 (~3.2Mb) was validated by qPCR, and shown to be *de novo*. Both parents and a sibling are non-carriers of this deletion and do not display any reading or language deficits, suggesting this deletion may be directly involved in the phenotype of the proband. This is the first report of a BP3-BP5 deletion to be identified in an SLI cohort, in the absence of intellectual disability. The identification of a *de novo* chromosome 15q CNV in this cohort provides further support for the implication of this locus in contributing to a wide range of neurodevelopmental phenotypes.

P09.136-M
Gain-of-function $Na_v1.7$ and $Na_v1.8$ mutations in patients with idiopathic small fiber neuropathy

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Small fiber neuropathy (SFN) is a relatively common disorder of thinly myelinated and unmyelinated nerve fibers and is clinically characterized by burning pain and autonomic complaints. We have recently described the presence of gain-of-function variants in $Na_v1.7$ and $Na_v1.8$ (encoded by *SCN9A* and *SCN10A*) sodium channels in a small cohort of patients (n=92) meeting strict criteria for idiopathic small fiber neuropathy (I-SFN). In this study a cohort of 393 patients with I-SFN was tested for the presence of variants in *SCN9A*. Patients that did not harbor a *SCN9A* variant subsequently underwent *SCN10A* analyses. Electrophysiology was used to test functional effects of variant channels. In *SCN9A*, 17 different heterozygous variants classified as class 3 (unknown significance) or 4 (likely to be pathogenic) based on in silico prediction were found in 34 patients (~9%). For two $Na_v1.7$ variants, electrophysiology did not provide evidence for pathogenicity. In *SCN10A*, ten different heterozygous $Na_v1.8$ variants classified as class 3 (unknown significance) or 4 (likely to be pathogenic) based on in silico prediction were found in 15 patients (~4%). For one Nav1.8 variant no evidence for pathogenicity was provided by electrophysiology. In conclusion, heterozygous *SCN9A* or *SCN10A* variants are present in a substantial proportion (~12%; 49 of 393) of our cohort of I-SFN patients. For many variants electrophysiological analysis revealed gain-of-function attributes in mutant channels. This implies that functional variants in *SCN9A* and *SCN10A* may predispose carriers to the development of channelopathy-associated SFN. Analysis of *SCN9A* and *SCN10A* should be considered for patients with I-SFN.

P09.137-S
The CD45+ blood cells alpha-synuclein level in Parkinson's disease

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Impaired metabolism of alpha-synuclein and its aggregation are implicated in the pathogenesis of Parkinson's disease (PD). It was shown that L-DOPA treatment during the clinical course of the disease induces the alpha-synuclein oligomerization. Several studies estimated alpha-synuclein level in blood cells of PD patients. However the alpha-synuclein level in uncontaminated fraction of the peripheral blood lymphocytes remains unknown. Here we examined alterations in alpha-synuclein level in magneticaly separated CD45+ cells from the whole blood in 18 drug-naïve patients with sporadic PD and in 23 controls without neurological disorders by ELISA method (Human alpha-synuclein ELISA kit, Invitrogen, USA). CD45+ MicroBeads and MACS Columns (Miltenyi Biotec, USA) are used for positive selection lymphocytes after Ficoll-Paque PLUS peripheral blood separation. All subjects were residents of the North-Western region of Russia and ethnically matched. The level of a total alpha-synuclein in peripheral blood CD45+ cells was higher in patients with PD (median 9,59 (min - 2,23; max - 36,80), ng/ml) than in the control subjects (median 4,81 (min - 1,21; max - 28,1), ng/ml) (p=0,04). Taken together our findings suppose that the increased level of alpha-synuclein in blood CD45+ cells of PD patients can be used as diagnostic marker for PD.

P09.138-M
Linking amyotrophic lateral sclerosis and spinal muscular atrophy: analysis of the C9ORF72 gene in 162 SMA patients

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Spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) are both motor neuron disorders. Several studies have tried to establish a link between the two diseases but the subject is still under debate. It has been reported that large expansions of the hexanucleotide GGGGCC in intron 1 of the *C9ORF72* gene are often responsible for familial and sporadic ALS cases. On the other side, mutations in the SMN1 gene cause SMA and its highly homologous copy, *SMN2*, is a phenotypic modifier. **Objective:** We investigated whether the number of the hexanucleotide repeats in *C9ORF72* was associated with the phenotype and the number of *SMN2* copies in a group of 162 SMA patients. **Methods:** Conventional PCR was used to determine values within the normal range (less than 30 repeats), while Repeat Primed-PCR (RP-PCR) and Southern blot methods were used to exclude the existence of large expansions that are out of the range to be amplified by conventional methodology. **Results:** No pathological (> 30 repeats) or premutated alleles (20-30 repeats) were found. The allelic distribution of the *C9ORF72* gene in SMA patients overlapped with the data obtained in our control population discarding putative repeats that may be associated with the disease. No association was either observed with the SMA phenotype or the number of *SMN2* copies. **Conclusion:** The involvement of *C9ORF72* as a genetic marker in SMA is unlikely. Current investigation of modifier genes in SMA should consider other possible candidates (Supported by FIS 11-2606).

P09.139-S
Spinocerenellar ataxia type 6(SCA6): clinical pilot trial with medicinal herbs

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Spinocerebellar ataxia 6 (SCA6) is an autosomal dominant cerebellar ataxia associated with small polyglutamine-dependent expansions in the alpha 1A-voltage calcium channel. At present, we have no effective therapeutic tools. We report here six cases of spinocerebellar ataxia 6(SCA6)with typical symptoms. Genetic tests revealed expanded allele of 22~25 CAG repeats at the spinocerebellar ataxia type 6 locus. Head MRI revealed a typical atrophic image in cerebellum. For the systems therapy with medicinal herbs, the differential diagnosis by traditional herbal medicine was made according to the guideline. Mixtures of 18~26 medicinal herbs were given according to the differential diagnosis in each patient. The remedies used for the cases consist of several different ingredients, which have well-established histories of use for treatment of vertigo, tremor, or ataxia and are expected to exert their specific effects. In 5 of 7 patients, ataxia of gait and stance was significantly improved in 30~ 60 days of the herbal treatment. 34~85% reductuion were obtained on the 100-point semiquantitative International Cooperative Ataxia Rating Scales (ICARS) scores. The results imply the therapeutic potential of herbal medicine for spinocerebellar ataxia 6. Further extensive investigations are required to clarify the mechanisms by which the remission induction of this genetic disease of CAG repeat expansion mutation has been attained with the medicinal herbs.

P09.140-M
Mutant Ataxin-2 Induces Reactive Oxygen Species and Autophagy in transformed lymphoblastoid cells from patients with Spinocerebellar Ataxia Type 2

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Spinocerebellar ataxia type 2 (SCA2), an autosomal dominant neurodegenerative disease, is caused by the expansion of a CAG triplet repeat located in the N-terminal coding region of the ATXN2 gene. Alleles of the ATXN2 gene that carry 13-31 CAG-trinucleotide repeats are present in normal individuals. Contrariwise, alleles with a CAG triplet repeat number of >31 and up to approximately 200 are present in patients with SCA2. Although the detail mechanism of pathogenesis is yet to be defined, neurotoxin, especially reactive oxygen species (ROS), released from aggregated mutant proteins, may play a role in the pathogenic process. In this study, the lymphoblastoid cell lines (LCLs) isolated from SCA2 patients were utilized to compare with the wild-type lymphoblastoid cells. We investigated the crucial relationship between the expression of p-ERK1/2 and autophagy. Results found the endogenous p-ERK1/2 and autophagy marker protein, Atg8 (LC3 class II) were higher in SCA2 patients. Electron micrographs showed that only the cells expressing expanded Ataxin-2 contained aggregated protein and autophagic vacuoles. Based on the above observations we hypothesized that the aggregated mutant Ataxin-2 proteins may generate ROS in mitochondria, which subsequently up-regulate Atg8 expression levels and ultimately lead to autophagy and cell death.

P09.142-M
Intragenic deletions affecting two alternative *IMMP2L* transcripts in patients with Tourette syndrome

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Tourette syndrome (TS) is a childhood onset neurodevelopmental disorder characterized by involuntary movements and vocalizations, known as tics. The etiology of TS is complex and largely unknown, but has a strong genetic component.
IMMP2L (inner mitochondrial membrane peptidase, subunit 2) is one of the few genes that have been suggested to increase susceptibility to TS, after identification of chromosomal rearrangements affecting *IMMP2L* in several families with TS or tics. However, to date only a single study has investigated the role of structural copy number variations (CNVs) of *IMMP2L* in a small cohort of TS patients without finding any deletions/duplications.
Through CNV screening of a cohort of 188 unrelated TS patients and 316 controls from Denmark, we identified seven patients (3.7%) and 3 controls (0.9%) with intragenic *IMMP2L* deletions, thus, the frequency of *IMMP2L* deletions was significantly higher in patients than in controls (P= 0.0447).
Four of the deletions identified in the patients did not include any known exons of *IMMP2L*, but were within intron 3. These deletions were found to affect a shorter *IMMP2L* mRNA species with two alternative 5'-exons, one of which included the ATG start codon. We showed that this short transcript and the previously published long transcript were expressed in several brain regions, with particularly high expression in cerebellum and hippocampus.
The current findings give further evidence for the role of *IMMP2L* as a susceptibility factor in TS and suggest that intronic changes in disease susceptibility genes should be investigated further for presence of alternatively spliced exons.

P09.143-S
Molecular diagnosis of TTR gene mutations in Italy: the experience of the Molecular Genetics Laboratory of Ferrara

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Transthyretin (TTR), a plasma and cerebrospinal fluid protein secreted by the liver and choroid plexus, is mainly known as the physiological carrier of thyroxine (T(4)) and retinol. Under pathological conditions, various TTR missense mutations are known to destabilize the tetramer composed of mutant and wild type subunits, causing misfolding of the protein and fibril aggregation. This condition is associated with the amyloid diseases: senile systemic amyloidosis, familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC). Direct sequencing of TTR gene detects more than 99% of disease-causing mutations.
In twelve years we analyzed 562 subjects, referred to our service by neurologists and cardiologists. About 47% of the exams were required for polyneuropathy, 33% for cardiac impairment and 20% for familiarity for TTR mutations.
We found the causative mutation (index cases) in 80/428 subjects with no familiarity for TTR-related diseases and in 78/134 subjects with a family history of the disorder.
The most frequent mutation in our cohort is Ile68Leu (31%), typically present in cases with cardiac involvement, followed by Phe64Leu (22%), Val30Met (15%) , Glu89Gln (14%) and Thr49Ala (5%). A novel mutation has been identified in exon 2: Val14Leu. The subject was affected by cardiac amyloidosis.
The Detection Rate achieved is good, keeping in mind the complexity of clinical diagnosis. The choice of the complete sequencing of TTR gene has proved successful, given the allelic heterogeneity of the disease. The molecular confirmation of the pathology is essential, also in order to offer an appropriate genetic counselling to the patient and his family.

P09.144-M
Tuberous sclerosis complex phenotypes suggestive of TSC1/TSC2 mosaicism

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Tuberous sclerosis complex (TSC) is due to mutations in TSC1 or TSC2 but 15% of patients have no mutation identified (NMI). A recent study suggests that NMI patients are mosaic TSC. A TSC1/TSC2 mutation in a neural crest progenitor would explain NMI patients without tubers and subependymal nodules (SENs), because neural crest is the origin of the majority of extracranial lesions in TSC. We performed 2 studies aiming to support this hypothesis. First, a review of the MRIs of 220 TSC patients was performed to assess the phenotype-genotype of patients with tubers or SENs but not both lesions. In our cohort, 6/220 (2.7%) patients, all with NMI, had tubers without SENs, and five of the 6 patients had 3 or fewer tubers. Patients with SENs without tubers were not identified. This suggests that patients with NMI and absence of SENs had a first postzygotic mutation in neuroectoderm, which gives rise to neural crest progenitors harbouring the mutation. Second, we studied 19 patients with possible TSC who had brain MRI. Five of the 19 patients had TSC1/TSC2 mutational analysis, which was NMI. None of the 19 had tubers or SENs. None of the patients with NMI in our studies had any family member with TSC. These data suggest that patients with NMI

have a first postzygotic mutation in neuroectoderm or neural crest, explaining the negative mutational studies in leukocytes. They may present with 3 clinical recognizable phenotypes: a)possible TSC, b)TSC without tubers and SENs and c)TSC with tubers and without SENS.

P09.145-S

Novel compound heterozygous *UBE3B* mutations in two sisters with a craniofacial-intellectual disability syndrome

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Ubiquitination is a fundamental post-translational modification pathway involved in a wide range of cellular activities. The substrate specificity of the Ubiquitination is mostly dependent on the E3 ubiquitin-protein ligase family. Homozygous and compound heterozygous mutations in the E3 ubiquitin-protein ligase UBE3B were found to cause Kaufman Oculocerebrofacial Syndrome (OMIM 244450) or Blepharophimosis-Ptosis-Intellectual Disability Syndrome (OMIM 615057) in six patients from five families.
We describe two affected sisters, carrying a compound heterozygous mutation *in trans* in *UBE3B*. They exhibited cranio-facial anomalies including microcephaly, microphthalmia, blepharophimosis, upslanting palpebral fissures, high-arched and interrupted eyebrows, and myopia. In addition, they had laryngomalacia, abnormal genitalia, severe intellectual disability, delayed motor development, hypotonia, failure to thrive, and growth delay. The younger sister had small kidneys and impaired hearing, and the older sister had corpus callosum hypoplasia. The facial dysmorphisms including arched and interrupted eyebrows and long eyelashes overlap with Blepharophimosis Ptosis and Epicanthus inversus Syndrome and Kabuki Syndrome.
By exome sequencing, followed by Sanger sequencing, we found in both sisters a *UBE3B* missense mutation in exon 1 (p.Met1Val) and a 1bp frameshift deletion (p.Phe591fs) in exon 17. The mutations were predicted to result in a truncated UBE3B protein and cause the disease.
We compared the findings in our patients with the previously described patients. Loss of *UBE3B* seems to result in a relatively homogeneous phenotype, recognizable by the distinct facial dysmorphisms of the ocular region in particular, and the severe psychomotor developmental delay.

P09.146-M

Exome Sequencing reveals a novel WDR45 Frameshift mutation and POLR3A compound heterozygous variants in a female with complex phenotype and mixed brain MRI findings

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WDR45 and *POLR3A* are newly recognized genes; each is associated with a distinct neurodegenerative disease. *WDR45* is an X-linked gene associated with a dominant form of Neurodegeneration with Brain Iron Accumulation (NBIA), manifested by progressive disabilities, dystonia, cognitive decline, spastic paraplegia, neuropsychiatric abnormalities and iron deposition in the basal ganglia on brain imaging. *POLR3A* on the other hand is an autosomal gene and its mutations cause a recessive form of a hypomyelination with Leukodystrophy disease, also known as 4H syndrome, characterized by congenital Hypomyelination with thinning of corpus callosum, Hypodontia and Hypogonadotropic Hypogonadism. We report a female child with severe intellectual disability, aphasia, short stature, ataxia, failure to thrive and structural brain abnormalities. MRI of the brain obtained in late infancy showed hypomylelation involving the central periventricular white matter and corpus callosum with no evidence of iron accumulation. MRIs of the brain obtained in childhood showed stable hypomyelination, with progressive iron accumulation in the basal ganglia in particular in the globus pallidus and substania nigra. Whole Exome Sequencing (WES) identified a novel *WDR45* frameshift deleterious mutation in Exon 9 (c.587-588del). WES also revealed *POLR3A* 3 missence heterozygous variants. The first is a novel missence variant in exon 4 which is maternally inherited (c.346A>G). Exon13 carried 2 heterozygous missence variants; a maternally inherited variant (c.1724A>T) and a paternally inherited variant (1745G>A). These variants are considered likely damaging. The patient's complex clinical phenotype and mixed brain MRI findings might be attributed to the confounding effects of the expression of these 2 genes.

P09.147-S

Severe presentation of *WDR62* mutation: is there a role for modifying genetic factors?

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Mutations in *WDR62* are associated with primary microcephaly; however they have been reported with wide phenotypic variability. We report six individuals with novel *WDR62* mutations who illustrate this variability and describe three in greater detail. Of the three one lacks neuromotor development and has severe pachygyria on MRI, another has only delayed speech and motor development and moderate polymicrogyria, and the third has an intermediate phenotype. We observed a rare copy number change of unknown significance, a 17q25qter duplication, in the first severely affected individual. The 17q25 duplication included an interesting candidate gene, tubulin cofactor D (*TBCD*), crucial in microtubule assembly and disassembly. Sequencing of the non-duplicated allele showed a *TBCD* missense mutation, predicted to cause a deleterious p.Phe1121Val substitution. Sequencing of a cohort of five individuals with *WDR62* mutations, including one with an identical mutation and different phenotype, plus twelve individuals with diagnosis of microlissencephaly and another individual with mild intellectual disability (ID) and a 17q25 duplication, did not reveal *TBCD* mutations. However, immunostaining with tubulin antibodies of cells from patients with both WDR62 and TBCD mutation showed abnormal tubulin network when compared to controls and cells with only the WDR62 mutation. Therefore we propose that genetic factors contribute to modify the severity of the WDR62 phenotype and, although based on suggestive evidence , TBCD could function as one of such factors.

P09.148-M

Serotonergic signaling as modulator of Machado-Joseph disease pathogenesis

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Introduction: Machado-Joseph disease (MJD) is a neurodegenerative disorder caused by the expansion of a polyglutamine (polyQ) tract within the C-terminal of the ataxin-3 (ATXN3) protein. The lack of therapeutic strategies that effectively prevent neurodegeneration in MJD patients prompted us to search for compounds that modulate mutant ATXN3-related pathogenesis. Recent data from our lab have shown that many aspects of MJD can be properly modeled in the round worm *Caenorhabditis elegans*. This study is based on the idea that our *C. elegans* MJD model is amenable for large-scale drug screenings, in which the identification of effective drugs can be accomplished by looking simultaneously at protein aggregation in the live neuronal cells, and on its impact on neuron-regulated behavior of the whole-animal**. Methods:** We used our C. elegans MJD model to screen a library of ~1200 mainly FDA-approved compounds for their ability to prevent or delay the formation of mutant ATXN3 aggregates and neurological dysfunction. Results: We excluded the small molecules that were found to be toxic or cause developmental delay to the C. elegans at the concentrations tested. Of the remaining, ten percent of the compounds significantly reduced the locomotion deficits of the animals. **Discussion:** We found that one group of the newly identified compounds exert their function in *C. elegans* by increasing serotonin signaling. Rescue of MJD pathogenesis seems to be dependent on the serotonin receptors. We should be able to identify efficacious compounds that can be tested in higher organisms, including our transgenic mouse model, and eventually enter clinical development.

P09.149-S

In vitro neurogenesis of human OPHN1 mutated iPS cells: morphological and biochemical analysis and phenotypic rescue with a ROCK inhibitor

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The X-linked gene Oligophrenin-1 (OPHN1) encodes a RhoGTPase activating

protein (Rho-GAP) which is mutated in a syndromic form of intellectual disability associated with cerebellar hypoplasia. In vitro and in vivo studies on hippocampal neurons of ophn1 deficient mice have shown defects in synaptic morphology and function. The administration of Y-27632, an inhibitor of the ROCK signaling pathway, specifically hyperactivated in ophn1 loss of function, fully rescues the cellular and biochemical phenotype. We have developed a cellular model based on human induced pluripotent stem cells (iPSCs) technology and in vitro neurogenesis to analyze the morphological and biochemical properties of OPHN1 loss of function in patients cells. The neurogenic potential of the OPHN1-defective iPS cells has been assessed and compared with those of control iPS cells following the protocols for differentiation into different neuronal cell lineages. The results obtained show that human OPHN1-defective neurons have altered morphology, with shorter neurites and decreased branching level, when compared with control neurons. We also confirmed the hyperactivation of the ROCK signaling pathway in OPHN1-defective iPSCs through Western blot and immunofluorescence assays of the Myosin Phosphatase Target Subunit 1 (MYPT1) and its phosphorylated state on Thr-853. We treated control iPS cells with ROCK inhibitors and observed a phenotypic rescue in terms of ROCK activity and neuronal morphology. We are currently exploring the molecular mechanisms underlying these processes and analyzing in detail the phenotype of OPHN1 defective cells at different stages of neuronal differentiation, before and after treatment.

P09.150-M
Rett syndrom:current situation in Algéria

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Rett syndrome is a progressive neurological disease that affects mainly girls. It is characterized by severe developmental disorder of the central nervous system. It is caused by mutations in the MECP2 (methyl CpG-binding protein 2) in Xq28, comprising four exons, the first of which is non-coding.
In this study, we analyzed the entire coding sequence of the MECP2 gene in 55 patients Algerians. 8 differents mutations were identified in exon 4, 2 missens mutations (p.E 394 K, p.K 135 E), 3 nonsense mutations (R255X, R294X), and 4 frameshift mutations (a deletion of one base pair: c.Del 806 G, c.Del 750 C).

P09.151-S
The association analysis of CDH10 gene (rs4307059 and rs4327572) with autism in a South African population

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Autism spectrum disorders encompass a group of childhood neurodevelopmental and neuropsychiatric disorders characterized by deficits in verbal communication and impairment of social interaction. Numerous researches have pointed out that strong genetics components are involved in susceptibility to autism. Genome wide association studies have revealed out strong association signal for *CDH10* gene with SNP rs4307059 and rs4327572. This gene had not been investigated for its association with Autism in South African population. Aim: In this study we aimed to investigate the association of two SNPs (rs4307059 and rs4327572) of *CDH10* gene of autism in the South African (SA) population. For SNPS rs4307059, the present study group was comprised of typed cases (188, unrelated autistic children) and typed controls (212, unrelated healthy children) where the respective figures for rs4327572 were 72 and 209 respectively. The Taqman ®Real-Time PCR and genotyping assay was utilized to determine the genotypes. Results: There was no significant association of SNP rs4307059 and4327572 with autism in the South African (SA) population. Conclusion: There might be a possible role of CDH10 in autism but we need to validate it with a larger sample number. The present study represents the first report on study of genetic association of *CDH10* gene in SA population.

P09.152-M
The Haplotype analysis to test for the association of three SNPs with autism in a South African Population

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Autism spectrum disorder is a neuropsychiatric developmental disorder characterized by communication difficulties and impaired social interaction. The anhydrolase domain containing 11 gene (*ABHD11*) is located on 7q11.23 which is a hotspot region for autism and *ABCA13* maps to chromosome 7p12.3 in which multiple rare coding variants are observed. The aim of this study was to investigate the association of SNPs from genes *ABHD11* (rs2293484 and rs10279013) and *ABHD13* (rs17060) with haplotype analysis in South African autistic population. A total of 435 individuals were recruited including 197 autistic and 213 control subjects. The Taqman ®Real-Time PCR and genotyping assay was used to determine the genotypes. A significant association of SNP rs10279013 with autism in the South African population is observed. Haplotype analysis with three SNPs revealed that the frequency of CGC, TGT and TCT haplotypes is significantly higher in controls than cases ($p<0.05$). The haplotypes TGC and TCC presented a significantly higher frequency in cases and the odds documented nearly 3-3.47 fold higher risk to the disease in carriers of the haplotypes [OR=3.0 (1.16-7.79), OR=3.47 (0.95-12.63), respectively]. In conclusion there is a role of various haplotypes of these SNPs in increasing susceptibility to autism for South African populations. The present study represents the first report on haplotype analysis on these SNPs in a SA population.

P09.153-S
Mutations in *XRCC4* associated with a complex ataxic syndrome

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We studied two monozygotic twins (II-1 and II-2), born to first cousin parents (I-1 and I-2), affected by a multisystem disease. Since birth they presented with cryptorchidism, dysmorphism (short arms, hypotelorism) and short stature. In the adulthood they suffered of an ataxic syndrome with cognitive decline, pyramidal signs and depression; dilatative cardiomyopathy was also observed. We performed an exome-sequencing analysis on one proband (II-1) and his unaffected sister (II-3). First we filtered out common variants (>0.1% in public databases); then we selected non-synonymous variants that were homozygous only in II-1, based on a hypothesized recessive trait and on the known consanguinity of the parents. The remaining 28 gene variants were prioritized according to the predicted deleterious outcome of the corresponding amino acid substitutions. The most severe variant was the nucleotide change c.673C>T in the gene *XRCC4*, predicted to create a stop codon (p.R225*) causing the synthesis of a truncated protein; this variant segregated within the family. Quantitative PCR on cDNA extracted from patients' fibroblasts revealed that the *XRCC4* transcript level was strongly reduced, probably due to mRNA decay. Functional assays after gamma-ray irradiation demonstrated a reduction in DNA repair efficiency in mutant cells. These are the first patients reported with *XRCC4* mutations. XRCC4 protein has a role in double-strand DNA repair processes; mutations affecting other proteins with a role on DNA repair (SCAN1, AOA1, ATM) have been associated with peculiar forms of ataxia, suggesting an important role for DNA repair in the nervous system, in particular in the cerebellum.

P09.154-M
Screening for C9ORF72 repeat expansion in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by a degeneration of upper and lower motor neurons, leading to a progressive muscle weakness, wasting and paralysis that results in death within a few years from disease onset. Moreover, evidences of ALS as a multisystem disorder also compromising frontotemporal functions in up to 50% of patients are reported. Recently, a hexanucleotide repeat expansion (GGGGCC) in the first intron of C9ORF72 gene was identified as the most common genetic cause of familial ALS, frontotemporal dementia and ALS-FTD. Aim of the present study was to determine the prevalence of C9ORF72 repeat expansion in our cohort of 753 ALS patients. C9ORF72 expansion was analyzed by using the repeat primed PCR: this method can detect repeat numbers of approximately maximum 60 and it is able to discriminate the repeat range detected in the normal population (0-20) from the higher mutated range (≥30). The expansion, in the pathogenic range (≥30), was identified in 34 ALS patients (4.5%); moreover, 4 cases (0.5%) resulted to be carriers of an intermediate number of reapets (20-30). Interestingly, 2 of the C9ORF72 expansion carriers also carried a causative mutations in one of other known causative genes of ALS (TARDBP and FUS). Our findings, in agreement with literature, suggest that C9ORF72 repeat expansion is the major genetic cause of ALS. Further efforts are needed to implement, in terms of intra- and inter-laboratory reproducibility, the method used for C9ORF72 analysis for using it in diagnostics.

P10.01-S
Analysis of the c9orf72 GC- rich low complexity sequence in ALS cases carrying GGGGCC expansion

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Large expansions of a noncoding hexanucleotide G4C2 repeat in the C9orf72 gene have been identified as the main cause of Amyotrophic Lateral Sclerosis (ALS) accounting for about 40% of the familial and 7% of the sporadic cases. The c9orf72 G4C2 repeat is contiguous with another GC-rich region with imperfect repeats. A recent study observed heterozygous deletions of variable size (from 5 to 23 bp) in patients carrying a G4C2 expansion (about 37% in a Belgian cohort and 24% in an European cohort). These deletions are contiguous to the G4C2 repeat and create one long imperfect G4C2 repeat that could make this sequence prone to replication slippage. To confirm the role of these deletions, as a possible trigger event of the instability of G4C2 repeats, we sequenced the adjacent GC-rich region in 28 Italian ALS patients carrying c9orf72 expansion, , as described by the original study. We did not observe any deletion of the GC-rich region adjacent to G4C2 repeat in the Italian c9orf72 expanded cohort, although we expected at least 7 deleted cases since the deletion frequency in the European cohort was 24% (Fisher's exact test, two-tailed P value = 0.0102). We cannot exclude that our data could be population specific or due to technical artefacts. Further studies will be necessary to confirm the role of these deletions in c9orf72 genomic instability.

P10.02-M
ANO5 mutations in Spanish LGMD patients

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Recessive mutations in *ANO5* gene have been reported in patients suffering from limb girdle muscular dystrophy (LGMD) 2L, distal myopathy resembling Miyoshi myopathy and patients manifesting with mialgia and high CK levels.
ANO5 gene, located on chromosome 11p14, is composed of 22 exons. To date, forty different mutations have been described in the literature, being c.191dupA the most frequent mutation. Interestingly, previous studies show considerable geographical differences regarding the prevalence of *ANO5* mutations. LGMD2L is the second most common form of LGMD in Northern Europe, in contrast to the low prevalence (2%) that has been reported in the Italian cohort of LGMD patients.
We present data from an *ANO5* gene molecular study in 27 Spanish patients manifesting the three different phenotypes: 1) LGMD 2L, 2) distal myopathy resembling Miyoshi myopathy, 3) mialgia and high CK levels. We identified six different mutations, two of them not previously described, in ten individuals (8 males and 2 females). Six patients presented 2 mutations and 4 only 1, suggesting symptomatic carrier status.
Among patients presenting two mutations, six of them carried the c.191dupA: three in homozygous state, 2 in compound heterozygous state and one as a single mutation. In our cohort of patients, c.191dupA is, as in the majority of populations, the most frequent *ANO5* mutation.
Our mutational screening reveals a higher prevalence of *ANO5* mutations in Spanish population compared to the Italy cohort of patients previously reported.

P10.03-S
Deletions involving the 17-22 repeats in the rod domain of dystrophin gene result in a late-onset Becker Dystrophinopathy

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Dystrophin, the protein product of DMD gene is a rod-shaped protein consisting of 3684 amino acids composed by four domains: actin binding domain; central rod domain; cysteine-rich domain and carboxy-terminal domain.
Mutations in DMD gene cause phenotypes ranging from severe (DMD) to mild (BMD) myopathic form or X-linked cardiomyopathy (XLCM). Mutations causing premature translation termination result in a total absence of the protein that leads to DMD, while mutations derived from in-frame deletions result in a reduced protein expression leading to BMD or XLCM.
We investigated the percentage of patients sharing mutations in the rod domain in a cohort of 160 BMD patients with gene deletions, followed at the Cardiomyology and Medical Genetics of Naples Second University. We found that 20 of them (12.5%) shared a deletion involving exons from 45 to 57, at the 17-22 repeats level.
From a clinical point of view, based on muscular, cardiac and respiratory assessment, 8 (40%) were asymptomatic, 11 (55%) had a mild phenotype and only 1 (5%) had dilated cardiomyopathy, as the first manifestation.
Compared with data reported by Kaspar et al. (2009), the percentage of asymptomatic patients in our cohort was higher (40 vs 8%) while that of patients with mild phenotype was lower (89 vs 11%). The difference probably lies in a younger mean age of our patients in both groups (30.5 vs 20.2 and 24.3 vs 30.5, respectively).
Data here reported confirm that mutations involving repeats 17-22 of the rod domain result in a late-onset mild Becker Dystrophinopathy.

P10.04-M
Mutations in the Charcot-Marie-Tooth disease–associated GDAP1 gene leads to calcium homeostasis dysfunction and endoplasmic reticulum stress

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Charcot-Marie-Tooth (CMT) disease is the most common inherited neuromuscular disorder and is characterized by large locus heterogeneity. Mutations in the *GDAP1* gene show phenotypic and Mendelian heterogeneity in CMT patients. GDAP1 is a mitochondrial outer membrane protein related to mitochondrial dynamics network and different effects on the fission and fusion pathways have been reported for recessive and dominant mutations, respectively. Recently we have described the role of GDAP1 in the regulation of store-operated calcium entry (SOCE), a complex process needed to fill endoplasmic reticulum (ER) after Ca^{2+} release. Dysregulation of calcium processes and ER stress represent a common pathway in neurodegenerative diseases. Here we present how missense mutations of *GDAP1* expressed in the human neuroblastoma SH-SY5Y cells also affect calcium homeostasis depending of their mode of inheritance and its relative position in the protein. Recessive mutations within the protein interaction domain of GDAP1 blocks Ca^{2+} influx during SOCE avoiding ER-Ca^{2+} refilling, while dominant mutations show an exacerbated Ca^{2+} influx with high resting Ca^{2+} levels. These mechanisms may affect the proper function of ER, which produce ER stress that eventually could lead to neurodegeneration.

P10.05-S
Diagnostic time trend and genetic analysis of Charcot-Marie-Tooth Disease in Denmark

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Aim: To classify Danish patients diagnosed with CMT in the period 1977-2012 according to genetic analysis, sex, and age at diagnosis.
Background: Charcot-Marie-Tooth Disease (CMT) is the most common inherited neurological disease. More than 70 genes associate with a CMT phenotype, but mutations in only four genes accounts for the vast majority of cases. Little is known about the epidemiology of CMT in Denmark.
Material and Methods: Records with diagnostic codes ICD8 33009 (atrophia mm. neuropathica, Charcot-Marie-Tooth) and ICD10 G60.0 (hereditary motor sensory neuropathy) from 1977 to 2012 were retrieved from The Danish National Patient Register (DNPR). These data were linked with data on genetic analysis for CMT between 1990 and 2012 at Department of Clinical Genetics, Aarhus University Hospital and Copenhagen University Hospital.
Results: A total of 2084 patients with a CMT diagnosis were identified in DNPR. Of these 712 patients (34%) had genetic testing, a genetically confirmed CMT diagnosis was obtained in 50%. In total, 17% of the patients clinically diagnosed with CMT since 1977 had a genetically confirmed CMT diagnosis. This percentage was largest in the 0-9 years age group (35%). In the 2008-12 cohort, 573 patients received a CMT diagnosis, 246(43%) had a genetic analysis for CMT, of which 124 (50%) confirmed the diagnosis. The majority of confirmed CMT cases where caused by duplication of the PMP22 gene (58%).
Conclusion: Only half of the newly clinically diagnosed CMT patients have been genetically tested. Fifty percent of the tested patients had a genetically confirmed diagnosis.

P10.06-M
Combined 12q24.3del and 17p12dup in a patient with truncal hypotonia and psychomotor delay

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The use of substantially improves the diagnosis of chromosomal abnormalities that are not evident by conventional karyotype. We report the clinical and molecular findings of a 2 year old boy presented with truncal hypotonia, absent deep tendon reflexes and psychomotor delay, but no major malformations or dysmorphic features. Cytogenetic analysis was normal and DNA analysis for SMA revealed a triplication of *SMN1*. Subsequently an array CGH analysis with high resolution 4X180K Agilent arrays (> 236.000 probes, average resolution of 8.9 Kb) showed a combination of the following: 1) 12q24.33 deletion [del12q24.33, 1.5 Mb; 132,302,325-133,733,528; hg19] containing the genes *FBRSL1, P2RX2, POLE, PXMP2, PGAM5, ANKLE2, GOLGA3, CHFR, ZNF605, ZNF26, ZNF84, ZNF140, ZNF10, ZNF268* 2) 17p12 duplication[dup 17p12,1.3Mb; 14,111,772-15,379,208; hg19] involving the Charcot Marie Tooth syndrome type 1A (CMT1A) critical region within which *PMP22* gene is contained. To our knowledge this is the first report of combined 12q24.3 deletion/ 17p12 duplication in a patient whose phenotype is not typical of either the described 12q24.3 deletion or CMT1A, perhaps the combination of dosage sensitive OMIM genes in the aberrant regions contribute to the specific phenotype. Niyazov et al., 2007; Kehrer et al., 2013; Choi et al., 2011;

P10.07-S
Whole exome sequencing in patients with congenital myopathies

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Congenital myopathies (CM) are a group of disorders presenting at birth or early infancy, characterised by muscle weakness and specific changes in the muscle biopsy. During the recent decade a number of genes have been discovered, however, additional novel genes are yet to be identify as genetic diagnosis cannot be currently established in many CM patients. With the aim to reveal the genetic defect in 30 CM patients, in whom mutations in suspected genes have been previously excluded, we carried out whole exome sequencing. Recently, a homozygous missense mutation in STAC3 gene was identified in patients with Native American myopathy. Analysis of WES data in our cases identified a homozygous STAC3 mutation in a patient with King-Denborough syndrome and core-like changes on muscle biopsy. A second patient with a severe phenotype and muscle biopsy changes suggestive of nemaline myopathy, carried a homozygous missense mutation in KLHL40. Mutations in KLHL40 have been very recently identified as a frequent cause of severe nemaline myopathy. Two heterozygous truncating TTN mutations were detected in a patient with severe cardiomyopathy and muscle biopsy suggestive of a centronuclear myopathy (CNM), supporting the emerging data that TTN mutations should be investigated as causative in cases with unresolved CNM. The remaining cases carried mutations in potentially novel genes which are under investigation.

P10.08-M
Desmin mutations associated with myofibrillar myopathy in the Polish population.

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Desmin is a muscle-specific intermediate filament protein, which forms a network connecting sarcomere, T tubules, sarcolemma, nuclear membrane, mitochondria and other organelles. DES mutations cause myofibrillar myopathies, cardiomyopathies and related phenotypes. Molecular mechanisms of the changes leading to the disease remain ambiguous. Here we describe DES mutations found in the Polish population.
The study group comprised 22 individuals representing 7 families with clinical diagnosis of desminopathy. DNA was extracted from peripheral blood using standard methods. Sanger sequencing identified one novel mutation (Q348P) and one previously described (A357_E359del) in the Polish population. Both mutations were predicted pathogenic using *PolyPhen-2*, *SIFT* and *PROVEAN* software. A common ancestry of A357_E359del found in the novel and previously reported families was confirmed. To test whether the mutations affect DES expression and intracellular distribution *in vitro* analyses of quadriceps muscle biopsies were performed. Western Blotting revealed elevated desmin level in the patients' muscles. The increased expression was confirmed using immunostaining. Abnormal localization of desmin with aggregates within the fibers was also observed. Additional staining for M-cadherin, α-actinin and MHCs confirmed severe disruption of myofibrillar organization. Abnormalities were more prominent for the Q348P muscle which additionally displays numerous centrally localized nuclei in the aberrant muscle fibers.
Based on the *in silico* and *ex vivo* analyses, Q348P and A357_E359del mutations may be assumed pathogenic. It could be speculated that abnormal structure of mutated desmin results in aberrant folding and aggregation, triggering myofibril disruption.

P10.09-S
Haplotype analysis and age of mutation in DMPK gene in Yakutia.

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We studied the genetic variability DMPK locus in patients with MD and healthy population of Yakutia. The objectives of the study included the estimate of age mutation in the Yakuts. In this work we used six SNP-markers in DMPK gene and six STR-markers which flanking DMPK locus.It was shown significant differences from patients MD and Yakut's population. The frequencies of alleles in three loci: rs572634, rs527221, rs915915 were significantly different. 29 haplotypes were found in patients with MD, and 37 haplotypes were found in Yakut's population. Major haplotype TTTCTC had 40% patients. Haplotype GTCCTT was typical only for patients Yakuts. The haplotype analysis by microsatellite markers of MD patients was identified 14 alleles and 114 haplotype (frequencies from 0.6 to 8.6%). It was found the founder haplotype. Average number of generations were found 158.95 ± 192.51. The age of mutation was estimated 3179 years. This period before the formation of ethnic Yakuts.

P10.10-M
Mutational analysis of DMD gene reveals two novel small deletions in patients bearing no large deletions or duplications

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Dystrinopathies are inherited muscular dystrophies with mutations in DMD gene which contains 79 exons and is the largest human gene. DMD encodes Dystrophin protein and is the only gene responsible for the spectrum of dystrinopathies.Genotype analysis has indicated that deletions of one or more exon account for ~65% of all cases, while 5-10% are in-frame or out-frame duplications and the remaining 30% of affected individuals may have point mutations, small deletions or insertions within the gene. In the present study, two patients diagnosed with Duchenne muscular dystrophy (DMD) are investigated. Multiple ligation-dependent probe amplification (MLPA) analysis which detects up to 98% of all deletions and duplications in DMD gene did not reveal any large rearrangement in these patients. Further investigation with application of two different techniques led to identification of two novel small deletions in DMD gene. In the first patient, all coding as well as flanking intronic regions of the dystrophin gene were PCR-amplified followed by sequencing and a hemizygous novel deletion, c.650-16_653del20 (p.D217V fs11X), was detected. Next, whole exome sequencing was applied to investigate the causative variant in the second patient that revealed another novel homozygous deletion defined as c.8297delT (p.Leu2766Arg fsX17). The identified variant was confirmed by Sanger sequencing in the proband as well as other family members. This result extends the mutational spectrum of the disease by introducing two novel mutations in DMD gene and is another indication that the advent of recent technologies such as whole exome sequencing has made the diagnostic approaches much easier.

P10.11-S
Next Generation Sequencing in facioscapulohumeral muscular dystrophy patients supports the idea that FSHD is a complex genetic disease

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Facioscapulohumeral muscular dystrophy has been associated with reduction of the number of D4Z4 repetitive elements at 4q35. FSHD is characterized by great clinical variability within families in which D4Z4 reduced allele (DRA) segregates. An increasing number of cases are sporadic with no other affected relatives and several findings suggest that additional factors (genetic modifiers) might modulate FSHD expression. Thus molecular diagnosis, prognosis and genetic counseling have become more challenging. To gain additional information on the complexity of FSHD, we tested 40 FSHD patients belonging to families with reduced penetrance by testing, a broad core panel of 93 genes involved in myopathies (Motorplex). This Next Generation Sequencing-based workflow permit the analysis of 2,544 exons. We studied 40 samples from FSHD patients belonging to families in which other DRA carries are healthy. In all subjects we found putative pathogenic variations in genes causing different myopathies. In addition to DRA, all patients carried at least one damaging variations in other disease genes. These variants, if they had been detected alone in the context of a single gene testing, would have been considered as causative. The high number of damaging mutations identified in each sample support the hypothesis of "multiple factors" leading to the FSHD phenotype. In conclusion, the use of a reliable, sensitive and specific method has been able to identify putative pathogenic mutations that can explain the variable penetrance of DRA. Importantly these large set of mutations observed in FSHD patients highlight the genetic complexity that might contribute to the disease expression.

P10.12-M
Clinical and molecular characterization of FSHD cases with 1-3 DRA :data from the Italian National Registry of FSHD

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Facioscapulohumeral muscular dystrophy has been genetically linked to reduced numbers (≤8) of D4Z4 repeats at 4q35. Typically a rough inverse correlation between the number of repetitive elements and disease severity is accepted. Particularly severe FSHD cases have been associated with D4Z4 reduced alleles with 1-3 repeats (1-3 DRA) with some presenting ancillary features such as Coats-like retinopathy, facial diplegia, hypoacusia, epilepsy and cognitive impairment. Out of 110 probands from the INRF carrying 1-3 DRA, we identified 40 index cases carrying a de novo DRA and 26 with at least one relative carried the same DRA. In order to investigate the earliest signs of disease and access the information about addition clinical condition, we designed an Anamnestic Infantile Form (AIF). Thirty-six probands (54.6%) showed an early disease onset (within 10 years): among them, 26 cases (72.2%) were de novo and 10 (27.8%) were familial. Collectively our study reveals that congenital form is not present in the group of 1-3 DRA carriers and infantile disease onset is more common among de novo cases. Importantly we observed that earliest onset is not always associated with a more severe clinical progression of disease. Interestingly, out of 76 carriers of 1-3 DRA we detected complex phenotype with extramuscular features in only 14 subjects, 10 de novo and 4 familial cases.

P10.13-S
HPSE2, mutated in human urofacial syndrome (UFS), directs neuromuscular differentiation

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Background: Homozygous mutations in HPSE2, encoding heparanase 2, cause UFS, characterised by congenital bladder dysfunction, constipation, and inability to smile. We investigated the developmental roles of this gene in an animal model to further clarify UFS pathogenesis. **Methods:** Hpse2 transcripts were measured by qPCR during X. tropicalis development. Single cell embryos were injected with morpholinos to knockdown hpse2. Immunostaining was performed using an antibody raised to Xenopus heparanase 2 and imaged by laser confocal micrsoscopy, and protein levels were assessed by western blotting. **Results:** Frog embryos showed increased hpse2 soon after gastrulation, with expression maintained through organogenesis. Using hspe2 morpholinos caused a phenotype comprising a bent tail, proctodeal/cloacal protrusion and impaired swim response. In wildtypes heparanase 2 localised in nascent skeletal muscle, and morphants had impaired muscularisation. Heparanase 2 was also expressed in the neural tube and hindbrain, and morphants showed compromised neural outgrowths from these structures. Intruigingly heparanase 2 was found to co-localise with activated ERK (pERK) in the embryonic neural tube, and analysis of the morphants by western blot showed increased levels of pERK, suggesting altered regulation of key signalling pathways. **Conclusion:** Heparanase 2 plays a key role in vertebrate neuromuscular development. A role has been elucidated in ERK signalling, suggesting potential targets for experimental therapies.

P10.14-M
Exome sequencing as a highly efficient diagnostic approach in muscular weakness in newborns and early infancy

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Muscular weakness in newborns and early infancy is a challenging clinical feature which may reflect either a neuromuscular disorder or only a secondary feature associated with a primary disorder for example of the central nervous system. Although the first signs may be present in first days of life, the full clinical picture might not be evident in newborns. An earlier and accurate diagnosis in those cases currently depends on a careful clinical assessment followed by the appropriate investigations. Recently developed research tools as next generation sequencing provide a distinct advantage over candidate gene sequencing to discover the underlying genetic defect in a timely manner. In this study, a clinical exome sequencing approach using the Illumina TruSight Rapid Capture Kit was used to investigate the disease-related mutations in 11 infants with unknown conditions presenting with muscle involvement. With a mean average coverage of 394 and at least 20-fold coverage in 98% of the targeted region, we have identified genetic defects in 3 of 7 patients analyzed so far (43%): a previously reported homozygous splice site mutation in the IGHMBP2 gene known to be altered in spinal muscular atrophy with respiratory distress 1, a novel compound heterozygous mutations in the MUSK gene recently associated with congenital myasthenic syndrome, and novel de novo missense mutation in the TPM3 gene involved in congenital fibre-type disproportion myopathy.
Our data suggest that clinical exome sequencing is an efficient tool for timely and accurate diagnosis of clinically and genetically heterogeneous disorders in infants with undiagnosed muscular weakness.

P10.15-S
Interaction among folat/homocysteine metabolism genes and endothelial nitric oxide synthase gene polymorphisms predicts the severity of Duchenne muscular dystrophy in Moldavian patients

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Introduction. We try to modeling gene-gene interaction between 5 mutations in 4 genes, involved in folat/homocysteine metabolism (FHMG) and eNOS- play a pivotal role in vascular homeostasis and endothelial function, that may be alter the severity (in our case on the age at wheelchair dependency- 9 or 12 years) of this genetically simple disease.
Methods: A retrospective single institution long-term follow-up study was carried out in 148 corticosteroids-free DMD patients. The genotyping of

dystrophin gene were performed by the MPCR to detect the deletion in DMD gene and the PCR-RFLP to identify the MTHFRC677Tand A1298C, MTRA2756G, MTRRA66G, eNOS polymorphisms. Gene-gene interactions were analyzed using entropy-based multifactor dimensionality reduction (MDR).
Results: We evaluated single-site allelic and genotypic associations, genotype equilibrium and multilocus genotype associations, using MDR, which failed to show a genetic model of severity of myopathic process. Have been adopted the MDR method to explore the synergistic effects of the studied polymorphisms on modifying to myopathic process. We selected the best model, which included the. MTHFRC677T and A1298C, MTRA2756G, eNOS polymorphisms, cross-validation consistency is 9/10 ($\chi 2$ =54.22, p<0.0001) for case of wheelchair up to 12yrs). Since the selected polymorphisms were not associated with DMD there is evidence for the existence of epistasis between the two polymorphisms MTHFRC677T and eNOS (CVC10/10, $\chi 2$=5.3, p=0.02) in case of wheelchair up to 9yrs.
Conclusion: Our results indicate that the MTHFR, MTR and eNOS genes are modifying loci and presence of the high-risk alleles may associate with an increase in the severity of DMD.

P10.16-M
Limb girdle muscular dystrophy in the Czech Republic

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Limb-girdle muscular dystrophy (LGMD) is defined as a muscular dystrophy with predominantly proximal distribution of muscle weakness. It includes a number of disorders with heterogeneous etiology. We determined the frequency of recessive LGMD subtypes (LGMD2A, LGMD2D, LGMD2I and LGMD2L) within a cohort of Czech LGMD2 patients using mutation analysis of the calpain3 (*CAPN3*), fukutin-related protein (*FKRP*), α-sarcoglycan (*SGCA*), and anoctamin5 (*ANO5*) genes. Last year we introduced next generation sequencing to accelerate patient diagnosis and to widen spectrum of analysed genes. We designed capture library to target the coding exons of genes responsible for all known types of LGMD and genes responsible for muscular dystrophy with similar phenotype to LGMD. We observed that mutations of the *CAPN3* gene are the most common cause of LGMD2. The frequency of particular forms of LGMD2 was 32.6% for LGMD2A, 4.1% for LGMD2I, 2.8% for LGMD2D, and 1.4% for LGMD2L. Using next-generation sequencing, we identified two patients with mutations in the gene encoding dysferlin (*DYSF*) - LGMD2B and a patient with mutations in the gene encoding β-sarcoglycan (*SGCB*) - LGMD2E. In total, we determined mutations in 41 % of Czech LGMD2 patients.
This work was supported by research grants CEITEC-CZ.1.05/1.1.00/02.0068, SuPReMMe - CZ.1.07/2.3.00/20.0045

P10.17-S
Missense variations in ACADVL catalytic domain identified by the next generation sequencing of nonspecific LGMD patients

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About one third of autosomal recessive limb-girdle muscular dystrophy patients show no mutations in the ARLGMD genes so far identified. The possible explanations are that several genetic cause of LGMD cannot be discovered by traditional DNA sequencing tests, or that the genetic heterogeneity is greater than expected with many other genes that should be analyzed. However, the present genetic testing is long, expensive and ineffective to cope with this second possibility. New powerful approaches for DNA analysis, like next-generation sequencing (NGS) and array CGH are revolutionizing the field, with the entire human genome that can be properly analyzed.
We combined SNP array-based linkage analysis and NGS technology to discover "orphan" LGMD mutations.
The patients to be studied were selected according to the following criteria: a) clinical diagnosis of LGMD; b) inconclusive molecular testing after the complete study of the known LGMD genes c) severity of disease.
A number of mutations were identified. In particular in a LGMD family with an autosomal recessive inheritance, we have recently identified a new homozygous mutation in the ACADVL gene, Acyl-CoA Dehydrogenase- Very Long Chain, that is shared by all the affected family members and by another patient from the same town. In other two family a heterozygous compound mutations and homozygous mutation in the *ACADVL* gene was also found.
This demonstrates that specific amino acid changes in the catalytic domain of the *ACADVL* Very gene may be associated with an LGMD-like phenotype and this should be considered in the differential diagnosis.

P10.18-M
Exome sequencing identifies mutations in a gene coding for the LIM-domain protein N-RAP in a BAG3 myofibrillar myopathy-affected patient

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Myofibrillar myopathies are neuromuscular disorders with disorganized myofibril structures at the Z-disk and accumulation of protein aggregates. BAG3-related myopathy represents a subgroup of myopathies caused by a mutation in the Bcl-2-associated athanogene 3 (BAG3), a co-chaperone with no direct role in muscle function. In order to investigate the possible involvement of other genes in BAG3 myopathy, we performed whole exome sequencing in an Italian family: a BAG3 myopathy proband affected by muscle weakness, respiratory insufficiency, cardiac arrhythmia, rigid spine, her unaffected parents and brother. Genomic DNAs from peripheral blood were exome enriched using Agilent SureSelectXT Human All Exon 50Mb kit. All samples were sequenced in paired-end with 72 bp length reads on Illumina Genome Analyser IIX. In accordance to a compound heterozygous model of inheritance, we identified in the proband three non-synonymous heterozygous variants in N-RAP gene: one predicted extremely damaging was inherited from her father and the other two less damaging from her mother. The mutation inherited from her father is very rare (MAF=0.0005) and causes an aminoacidic substitution in a domain essential for organizing actin filaments during myofibrillar assembly. The mutations from her mother cause aminoacidic substitutions in a domain critical for the Z-line assembly. Immunohistochemistry showed reduced N-RAP expression in the proband's muscle biopsy compared to a control. The muscle specific N-RAP protein scaffolds I-Z-I assembly during myofibrillogenesis and has a LIM-domain. This is the first exome approach to a BAG3 myopathy and suggests a contribution of a LIM-domain protein to the complex phenotype of the proband.

P10.19-S
A large NGS screening of myopathic patients reveals a great genetic heterogeneity and supports the hypothesis of "multiple troubles"

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In myopathic patients molecular diagnosis is hampered by the genetic heterogeneity and the clinical interpretation of molecular findings are further hindered by the presence of low penetrant variations and by the effect of modifier genes. To gain a comprehensive view of all sequence variants in patients, we developed a broad core panel of 93 genes involved in myopathies. To analyze their 2,544 exons, we have developed a Next Generation Sequencing-based workflow, based on a Haloplex custom enrichment strategy (Motorplex). We studied 200 samples from myopathic patients with prevalent limb-girdle muscle involvement and 150 samples with congenital myopathies. In about 20% of patients we found typical causative mutations, while in an additional 30% other putative pathogenic variations were detected. In addition to the causes of monogenic disorders, we also discovered more than 35% of patients showing damaging variations in other disease genes. These variants, if they had been detected alone in the context of a single gene testing, would have been considered as causative. The high number of damaging mutations identified in each sample support the hypothesis of "multiple troubles" leading to a such complex phenotype. In conclusion, in a large cohort of patients with non-specific symptoms, our reliable, sensitive and specific method has been able to identify pathogenic mutations, despite of the heterogeneous conditions, improving significantly the diagnostic yield. Finally, intrafamilial and interfamilial phenotypic variability of patients sharing the same pathogenic mutations must be reevaluated considering the comprehensive view of all sequence variants.

P10.20-M

Childhood onset tubular aggregate myopathy associated with *de novo STIM1* mutations.

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We investigated on three unrelated patients with tubular-aggregate myopathy and slowly progressive muscle weakness manifesting in the first years of life. All patients showed type 1 muscle fiber predominance and hypotrophy of type 2 fibers. Tubular aggregates of the sarcoplasmatic reticulum were abundant. In all three patients, *de novo* heterozygous mutations were identified in the Stromal Interaction Molecule 1 gene, *STIM1.* In one of the patients, the mutation was identified by exome analysis, coupled to a hypothesis-driven filtering strategy. Two patients harbored the previously described c.326A>G p.His109Arg change while the third patient carried a previously unreported mutation (c.343A>T, p.Ile115Phe). All mutations affected the EF-hand motif, which is required for Ca2+ ions binding. Besides, the tubular aggregates, autophagic debris and myophosphorylase deficiency were documented in patients' muscle cells. Consistent with previous findings, the c.326A>G change represent a recurrent mutation specifically related to early onset muscle weakness.

P10.21-S

Myotonia congenital type Becker in an endemic Bulgarian region

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Myotonia congenita type Becker is an autosomal recessive nondystrophic skeletal muscle disorder primarily affecting lower limb muscles and later progressing to the arms, neck, and facial muscles. The disease is characterized by muscle stiffness and an inability of the muscle to relax after voluntary contraction. Sometimes muscle weakness may be transient. The autosomal recessive Becker type is caused by mutations in the CLCN1 gene, localized on chromosome 7q34 and encoding skeletal muscle chloride channel-1.
Here we report 5 Bulgarian families genetically proved to carry mutations in the CLCN1 gene. The following mutations were detected: nonsense (p.Arg894*), splice-site (c.1471+1G>A), missense (p.Val273Met; p.Tyr524Cys). Two additional nucleotide changes were detected in an asymptomatic individual (c.2284+5C>T possible splice-site change and p.Phe167Leu). It was not possible to clarify, whether these changes are located on a single allele or affect both alleles. Two of the detected mutations are interesting from population point of view. The missense substitution p.Val273Met was detected in a large Roma family with a high proportion of endogamous marriages. Secondly, the novel missense substitution p.Tyr524Cys was detected in a large Bulgarian family with a number of affected individuals both in vertical and horizontal pedigree direction. The later family originates from a small Bulgarian village and the citizens traditionally marry within the village, which is very surprising for the Bulgarian habits. Most probably there is an endemic region for myotonia congenital type Becker in Bulgaria, caused by the missense mutation p.Tyr524Cys.

P10.22-M

Targeted second generation resequencing reveals a homozygous CLCN1 mutation in a patient with an ambiguous myotonic dystrophy type 2 grey zone allele

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In our previous study focused on the analysis of variability of the repetitive CCTG tract in the *CNBP* gene in patients with myotonic dystrophy symptoms and healthy population, we reported a patient harbouring an uninterrupted allele containing 34 CCTG repeats. Although there are several indications that it is not a causative allele in that patient, it was not possible to exclude its causality, until other candidate genes were screened for causative mutations. Therefore the patient's DNA was sequenced by targeted resequencing approach using a custom panel and Ion Torrent PGM. After alignment to the GRCh37/hg19 reference sequence three homozygous single nucleotide variations (SNVs) were identified. A missense variation p.S524G in the *SCN4A* gene (mutations of the gene are associated with paramyotonia congenita) and a missense variation p.G118W in the *CLCN1* gene (mutations of the gene are associated with myotonia congenita) were found to be mistakenly identified as variations, since according to the HapMap and 1000 Genomes Project data, the alleles harboured by our patient are in both cases the most common alleles and the reference sequence contains the rare variants (MAF=0,059 and MAF=0,009, respectively). The third SNV was a homozygous nonsense mutation p.P894* which is a known pathogenic mutation leading to myotonia congenita (Thomsen's disease). Identification of a known homozygous *CLCN1* mutation found by targeted resequencing led to conclusion that the previously identified possible DM2 grey zone allele is not a disease causing allele in the case of the reported patient with consequence in the genetic testing in this family.

P10.23-S

Rapid High-Sensitivity Single-Step Screen for CTG Repeat Expansion Mutations in Myotonic Dystrophy Type I

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Myotonic dystrophy type 1 (DM1), the most common adult muscular dystrophy, is caused by expansion of a CTG repeat in the 3'UTR of the *DMPK* gene. Repeat size is correlated positively with phenotypic severity and negatively with age-of-onset, with affected individuals classified as mild (50-150 CTGs), classic (100-1000 CTGs), or congenital (>1000 CTGs) DM1. Southern analysis reliably detects all expansions, but requires micrograms of DNA, yields approximate allele sizing, and is labor-intensive, time-consuming, and costly. PCR across the repeat accurately sizes all normal and also expanded alleles, although the upper limit of detection is unknown. Newer triplet-primed PCR (TP-PCR) assays will detect all normal and expanded alleles, but product analysis by capillary electrophoresis (CE) is still costly when applied in high throughput screening situations where a majority of tested samples may be screen-negative. We have now adapted the 5' and 3' TP-PCR assays for single-step detection of DM1 expansions by melting curve analysis (MCA), wherein melt peak profiles from normal samples were distinctly different from samples carrying an expansion. In a blinded validation of 60 clinical samples enriched for affected individuals, a 48-repeat plasmid clone was used to establish a cut-off temperature separating normal from affected samples, and 100% concordance with their known genotypes was achieved. We conclude that TP-PCR MCA is an accurate yet simple, rapid and inexpensive screen useful for identifying DM1 expansion mutations. Follow-up confirmation and sizing can be accomplished by capillary electrophoresis following a quick 5-cycle extension-labeling of the positive TP-PCR product.

P10.24-M

Elevated miRNA levels in serum of myotonic dystrophy patients relate to disease progress

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Myotonic Dystrophy type 1 (DM1) is the most common form of adult-onset muscular dystrophy. Clinically, DM1 is a highly variable multisystemic disorder that primarily affects skeletal muscle and is characterized by progressive skeletal muscle weakness, wasting and myotonia. DM1 patients are diagnosed using genetic tests and their muscle wasting progression is currently monitored through electromyography and regular physical examinations. Many scientific reports emphasize the importance for the discovery of non-invasive serum-based biomarkers for the diagnosis and therapy of diseases, since they are easily accessible and convenient. The identification of blood-based biomarkers for DM1 would provide added value for the monitoring of the progressive muscle wasting. Scientific reports showed that circulating miRNAs are stably present within blood circulation and have the potential to be used as clinical biomarkers for many diseases. The aim of this pilot study was to detect and evaluate the usefulness of miRNAs as biomarkers for DM1. DM1 patients and healthy participants were recruited and miRNA analysis was performed on RNA isolated from serum samples. Results show that the levels of particular miRNAs are elevated in the serum from DM1 patients compared to controls, presumably, as a consequence of the degradation of muscle tissue during muscle wasting. Moreover, the miRNA serum levels correlate with the progression of muscle wasting observed in the patients. Based on these results, we propose that miRNAs can be used as potential serum-based molecular biomarkers for monitoring the progress of muscle wasting in DM1 patients.

P10.25-S
Large copy number variations in *NEB* are frequent in nemaline myopathy patients

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Recently, new large mutations have been identified in the nebulin gene (*NEB*) causing nemaline myopathy (NM). NM constitutes a heterogeneous group of disorders among the congenital myopathies, and mutations in *NEB* are a main cause of the recessively inherited form. *NEB* consists of 183 exons and it includes a 32 kb triplicate region (TRI) where eight exons are repeated three times. We have designed a custom NM-CGH microarray to detect copy number variations in the currently known nine NM genes and one unpublished gene. To date, 230 samples from 170 families have been run with the NM-CGH microarray and we have identified *NEB* TRI variation in approximately 14% of the NM families in this study cohort. The results suggest that the adjacent intronic repeat elements may predispose to the recurrent TRI variations. The possible pathogenicity of the TRI variations warrants further studies and elucidation of the exact breakpoints in each family. Moreover, we have identified seven different, novel, large disease-causing aberrations in *NEB* in seven different families. The size of the aberrations varies greatly, covering from only a part of one exon (0.9 kb) to more than half of the gene (133 kb). The NM-CGH microarray method is currently available for mutation analysis in our laboratory. Additionally we have analyzed ten samples with exome sequencing and identified causative mutations for half of the families. We believe that the combination of the NM-CGH microarray followed by exome sequencing will accelerate mutation detection and improve the diagnostics of NM and related disorders.

P10.26-M
A novel homozygous deletion in SGCB identified by a targeted next generation sequencing approach and the Motor Chip

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Different forms of muscular dystrophy are caused by mutation in genes coding for proteins of sarcoglycan complex, resulting in severe childhood autosomal recessive muscular dystrophies (SCARMD). Point mutations but also copy number variations (CNVs) have been found in sarcoglycan genes. Here we report one patient with SCARMD phenotype resulting from a new homozygous deletion of the last 12 codons in the exon 6 and 3'UTR of beta-sarcoglycan gene (SGCB). For the genetic diagnosis of this heterogeneous condition, we have performed the next generation sequencing. In particular, we have used the Motor Haloplex, a customized target enrichment of regions related to muscular disease, recently developed by our group. We targeted all the exons and the ten flanking bases of 89 genes involved in muscular dystrophies. At first, no point mutations or ins/del have been reported by bioinformatics analysis of the data, but a homozygous deletion in the exon 6 of SGCB has been identified by IGV analysis of sarcoglycan genes. To confirm the result and map the breakpoint, we have used the Motor Chip (Clinical Chemistry, 2011 Nov;57(11):1584-96), a customized aCGH developed by our group, which is able to investigate CNVs in 425 muscular genes. This data suggests that Motor Haloplex may be appropriate for routine diagnoses. It is able to investigate point mutations but also deletions and duplications so that is easier, less time-consuming than traditional gene by gene approach. Finally, the combination between Motor Haloplex and Motor Chip is a strong strategy for the diagnosis of muscular dystrophies.

P10.27-S
Trio-based study of neuromuscular dysfunction using next-generation sequencing under a diagnostic setting

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We evaluated the use of next-generation sequencing to provide genetic diagnoses using a family trio consisting of healthy parents and an affected fetus which died intrauterine. The phenotype of the fetus was consistent with early onset neuromuscular disease. Suspected seizures, arthrogryposis multiplex congenita and suspected intrauterine epilepsy were detected in a sonography. We compared clinical gene panel analysis vs. whole exome sequencing (WES) for the family trio in order to identify candidate variants that account for the disease phenotype of the fetus. We used the TruSight™ One Sequencing Panel, which targets 4813 genes associated with known clinical phenotypes, and the Nextera Exome Enrichment Kit (62Mb). Data was analyzed using an in-house bioinformatics pipeline. We analyzed the 4813 disease-associated genes in both sequencing approaches and found two compound heterozygous non-synonymous substitutions in a gene that is involved in neuromuscular functioning. Extending the analysis to the whole exome did not provide any new candidate variants. Gene panel sequencing yielded higher quality and coverage results compared to the WES, which showed a higher number of uncovered nucleotides within the clinical genes. In addition, WES revealed many variants in genes which cannot be directed towards answering questions related to the medical conditions. As a diagnostic test, clinical gene panel sequencing including all relevant disease-associated genes was more efficient than WES by means of quality, costs, time and reporting. Although WES might not always lead to a direct diagnosis test, it is relevant for research to uncover new gene-disease associations.

P10.28-M
A pilot study using a targeted next-generation sequencing panel for diagnostic use in primary myopathies

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Myopathies are a clinically and genetically heterogeneous group of disorders, most of which are genetic and many cause progressive weakness and atrophy of muscles. Mutations in more than 200 different genes are known to cause the disorders and several genes may have to be sequenced in order to identify the molecular defect in a patient. The large number of possible candidate genes, overlapping phenotypes as well as an enormous size of some of the genes e.g. *DMD, TTN* and *NEB* bring difficult challenges to diagnostics. Molecular characterization is nevertheless important for an accurate diagnosis and management of the diseases. Targeted next-generation sequencing (NGS) is an efficient and cost-effective method to sequence several genes simultaneously. Our first targeted NGS custom panel was designed for the coding exons and UTRs of 180 myopathy related genes with a total size of 1.3 Mb. For DNA capture a custom NimbleGen SeqCap EZ Choice Library was designed. Sequencing was performed at Institute for Molecular Medicine Finland (FIMM) using Illumina MiSeq with sequencing depth of 100X, and files were processed with their Variant Calling Pipeline. In the pilot study DNA samples of 20 patients were sequenced, of which four served as mutation controls with previously reported mutations. We were able to detect all the mutations in the control samples, except for one heterozygous 11-bp insertion/deletion mutation which was missed in the variant calling. Disease-causing mutations were detected in seven of the 16 patients, with three of them having previously reported mutations and four showing novel mutations.

P10.29-S
Highly efficient genetic diagnostic testing in patients with neuromuscular disorders using Panel-based Next Generation Sequencing

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Neuromuscular disorders (NMD) represent a genetically heterogeneous group of diseases. Deciphering the causative molecular defect can be valuable for diagnosis and in consequence for estimation of prognosis and recurrence risk. Towards this aim we have developed a comprehensive diagnostic panel comprising genes known to cause spinal muscular atrophies, neuropathies, muscular dystrophies, myopathies, myasthenia and myotonia to study their underlying genetic heterogeneity. 187 relevant genes associated with NMDs were subdivided according to their clinical phenotypes. MLPA was performed where appropriate, following customized target enrichment and NGS. Identified mutations were validated using Sanger sequencing. All genes associated with the primary diagnosis were analyzed first. In unsolved cases our approach allows to expand the analysis to all remaining genes of the panel. In our study over 200 patients with NMD were analyzed. In 29% the underlying cause could be identified and in 20% likely pathogenic but unclear variants were detected. 52% of the cases remained unsolved. We observed clearly pathogenic mutations in 34 different genes, with *CAPN3, MFN2* and *TTN* being the most frequently mutated genes, and unclear variants in additional 27 genes. Interestingly, in 7% of solved cases we could identify pathogenic mutations in genes not associated with the original diagnosis. We have developed a comprehensive diagnostic NGS panel to analyze the genetic basis of NMDs. As suspected, high genetic heterogeneity among patients could be shown in our cohort. Additionally, in a significant amount of cases we found evidence for new genotype-phenotype correlations, emphasizing the usefulness of NGS panels for diagnostic purposes.

P10.30-M
Genetic testing of inherited muscular dystrophies and myopathies using Sequence capture and targeted resequencing

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The inherited muscular dystrophies and myopathies comprise a heterogeneous group of muscle diseases that share similar clinical features as progressive skeletal muscle weakness and wasting. Mutations in several genes that encode proteins of extracellular matrix, endoplasmic reticulum, nuclear envelope, sarcolemmal proteins and glycosyltransferases are known to be responsible for muscular dystrophies and myopathies. The large overlap of phenotypic manifestations resulting from different gene mutations poses a challenge for determination exact type muscular disease. Targeted resequencing using next-generation sequencing technology is a cost-effective strategy to accelerate patient diagnosis. We designed capture library to target the coding and all flanking intron regions of 42 genes associated with group of disease as muscular dystrophies, congenital muscular dystrophies, congenital myopathies, distal myopathies and other myopathies. We performed targeted capture combined with next-generation sequencing using NimbleGen SeqCap EZ Choice library and Roche 454 sequencing platform in 33 Czech probands. Mutations associated with muscular dystrophies or myopathies were identified in 16 of them. Mutations were detected in *ACTA1, CAPN3, COL6A1, COL6A3, DNM2, DYSF, LAMA2, RYR1, SGCB* and *SEPN1* gene. This work was supported by grants IGA MZ CR NT14574-3.

P10.31-S
Swiss Cheese localization and function in the nervous system of Drosophila melanogaster third instar larvae

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Neuropathy target esterase (NTE) is the 6th member of 9-proteins of patatin-like phospholipase domain-containing proteins, PNPLA1-9. Mutation in the catalytic domen of NTE (PNPLA6) can lead to the slowly developed diseases, known as organophosphorus compound-induced delayed neuropathy (OPIDN) and hereditary spastic paraplegia called NTE-related motor neuron disorder (NTE-MND). The Swiss Cheese (SWS) protein is an ortholog of NTE in *Drosophila* and shares 39% sequence identify with human NTE. The *Drosophila sws* mutants are characterized by progressive degeneration of adult nervous system, glial hyperwrapping, and neuronal apoptosis. *Drosophila sws* mutant thus provides an experimental model for functional studies of the SWS/NTE role in maintaining neural integrity. There is an evidence of SWS role in the age-dependent brain changes. However, possible disturbance caused by point mutations in *sws* gene in *Drosophila* larvae remained unknown.
The *sws*-point mutants were used to investigate SWS localization and function in the nervous system of *D.melanogaster* larvae. We were shown SWS localization mainly in glial tissue of larvae brain as well as in glial cells, wrapped axons in all investigated lines. Presence of SWS was also observed in the postsynaptic membranes of the larval NMJ. SWS protein in small amount was also detected in larvae neurons. Quantitative and qualitative parameters of NMJ in all investigated *sws*-mutants also were changed comparing with wild type flies.
Thus, SWS dysfunction in earlier stages of *Drosophila* ontogenesis can be important for nervous system development and aging in *D.melanogaster* adults.

P10.32-M
Central bradypnoe and hypotonia as diagnostic feature for male Rett syndrome

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Mutations in the MECP2 gene causing the X-linked dominant Rett syndrome are a relatively common cause of intellectual disability in girls. Although MECP2 mutations in males are usually considered lethal, leading to the prenatal loss of the fetuses, few observations of boys with severe congenital encephalopathy were reported. We present here a novel case of a newborn boy with Rett syndrome caused by the de novo MECP2 frameshift mutation c.806delG. The clinical suspicion of male Rett syndrome was triggered by the rare combination of neonatal hypotonia and central bradypnoe.
At the age of four days the newborn was transferred to the neonatal unit of a tertiary children's hospital because of poor suck, dysregulation of muscle tone and a pathological breathing pattern. On admission, the neonate was lethargic and the clinical examination showed multiple abnormalities, such as a severe brady-/hypopnea with apneas of up to 30 seconds, followed by an arousal reaction, frequent yawning, axial hypotonia and hypertonia of the extremities. The cerebral magnetic resonance (MR) image revealed bilateral T2 signal hyper intensities of the white matter, especially in the temporal and parietal areas, which raised the question of perinatal asphyxia. In the spectroscopy the lactate was elevated and the n-acetylaspartate level was low. The electroencephalogram (EEG) showed an immature activity with too many multifocal sharp transients. At discharge from the hospital, at the age of 1.5 months, the patient was more alert and was no longer dependent on nasogastric feeding.

P10.33-S
Evaluation of AR genetic polymorphisms influencing spinal and bulbar muscular atrophy phenotype

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Spinal and bulbar muscular atrophy (SBMA) is caused by a pathological expansion over 38 of a CAG repeat in the first exon of the androgen receptor (*AR*) gene on chromosome X, coding for a polyQ tract (La Spada et al, 1991). SBMA is an androgen-dependent disorder, with males with full disease manifestations, and females showing only mild symptoms even if homozygous for the mutation. While a correlation between expansion size of polyQ tract and disease severity has been reported, patients with the same number of CAG repeats have different age at onset and disease progression even if relatives. Human *AR* exon 1 encodes further short aminoacidic stretches. The effect of these sequences on SBMA phenotype has not been studied yet. In order to further characterize the effect of *AR* coding repeated sequences on SBMA phenotype, we genotyped *AR* exon1 polymorphisms in 132 molecularly defined SBMA patients, referring to the Motor Neuron Clinic of the University of Padua and to Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan. Among AR exon-1 trinucleotide stretches, a polymorphic GGN sequence coding polyG showed a tendency to be higher in patients with an earlier onset. Our study confirms that the length of SBMA-causing polyQ tract does not fully explain the disease phenotype and point to a polyG strech within *AR* exon 1 that is a potential disease modifier in SBMA:
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La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature. 1991;352:77-9.

P10.34-M
Mutation analysis of SCN1A gene in Slovak patients with various types of childhood epilepsy

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Mutations in SCN1A gene are by far the most frequent cause of Dravet syndrome (DS) worldwide, with mutation prevalence approximately between 70 and 80%. One of the main features of DS is the fact that many patients do not respond well to the treatment with anticonvulsive drugs. Furthermore, there have been observations that some drugs, which function as sodium channel blockers, may induce aggravation of seizures in patient, thus worsening their symptoms. As most of the DS SCN1A mutation result in truncation of the protein, the use of antiepileptic drugs would further decrease the inhibitory function in central nervous system. The aim of this study was to identify mutations in SCN1A gene in a broad testing group of patients with phenotypes raging from DS to milder phenotypes such as Genetic Epilepsy with Febrile Seizures Plus (GEFS+). Completing mutation screening in 37 unrelated patients, 4 causative mutations located in the coding region were identified. All mutations were found in DS patients and in accordance with previously identified disease variants, found mutations, of which three are novel, result in creation of a premature stop codon, either by substitution or an insertion and thus to a possible loss of function of the altered protein. In summary, our findings contribute to the broadening of the mutation spectrum of the SCN1A gene and enrich the clinical data of DS. Genetic testing may prevent the need for unnecessary clinical examinations and provide genetic counseling for families.

P10.35-S
De-novo deletion or uniparental disomy as SMA determining cause: a case report

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We report a 3-months old female with type I spinal muscular atrophy (SMA) born to a young and non-consanguineous couple. Molecular analysis confirmed the diagnosis of SMA with an homozygous deletion of SMN1. Parents analysis revealed that only the mother was a carrier of the SMA causative deletion. It is important to find out if the father, that carries two SMN1 copies, has a CIS-duplication of the gene on one chromosome bearing to a 2/0 genotype or the deletion occurred de-novo during gametogenesis. Indeed, the risk for future pregnancies could be reduced from 25%, usually estimated for SMA couple, to residual risk arising from recurrent de-novo mutation (2% of the affected individual). Alternatively, the uniparental disomy (UPD) of the maternal-deleted-chromosome, with an estimated incidence of about 1:3500 live birth, must be taken into account. Gene dosage analysis of the father's relatives does not support a paternal 2/0 genotype. To discriminate between a paternal de-novo mutation and a maternal isodisomy we analyzed in the affected infant and parents a total of nine microsatellite markers in a region spanning 3.0 Mb at the SMN locus. Five microsatellites at 5'/3'-ends of that region demonstrate a maternal and paternal inheritance but could not definitively exclude the maternal segmental UPD. Finally, using a NGS approach we identify three paternal and one maternal-inherited variants in the SMN2 genes of the infant. All together our data support for a de-novo mutation of paternal chromosome 5 as could result by unequal crossing-over during gametogenesis.

P10.37-S
Prevalence of SMN1 gene duplication in different ethnic groups: implication for carrier testing

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder, characterized by symmetrical muscular weakness and atrophy. The incidence is variable from 1 in 6000 to 1 in 10000 live births in different geographic areas. A homozygous deletion involving exon 7 in SMN1 gene is present in more than 95% of the cases. Carrier testing in parents and relatives of SMA patients is complicated by the occurrence of SMN1 gene duplication (4-5 %).
Here we report on the SMN1 genetic test results obtained in our lab in the last two years. The most frequent clinical indication is represented by positive family history for SMN1 gene deletion or SMA, partner heterozygous for the deletion and consanguinity with the partner.
We calculated the frequency of different SMN1 genotypes in 326 tested subjects. We found a percentage of SMN1 gene duplications (subjects with 3 copies of SMN1) a bit higher than expected, ranging from 6 to 6.5%. The 55% of the subjects with the duplication is represented by North and West Africans that have been tested because of consanguinity with the partner, the remaining are Italians, 25% with SMA positive family history and 20% with partners heterozygous for the deletion.
Our results highlight once again the importance of taking into account the occurrence of SMN1 gene duplication when performing carrier testing and suggest that the prevalence varies in different ethnic groups, therefore affecting the recurrence risk assessment .

P10.38-M
Methylation level of SLC23A2, NCOR2 and CDK2AP1 genes' regulatory regions correlates with spinal muscular atrophy severity

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Spinal muscular atrophy (SMA) is a monogenic neurogenerative disorder that is subdivided into four different types and caused by SMN1 gene deletion. Whole genome methylation analysis revealed about 40 CpG sites associated with genes which were significantly different methylated between SMA patients and healthy individuals of the same age. To investigate the relevance of discovered methylation changes for SMA severity, we compared the methylation level of target and nearest CpG sites in a larger group of SMA patients. The methylation level of CpG sites situated in regulatory regions of ARHGAP22, CDK2AP1, CHML, NCOR2, SLC23A2, RPL9 genes were analyzed in 24 I type, 43 II type and 29 III-IV SMA patients using bisulfite sequencing. The methylation level of target CpG site and one nearby CpG site belonged to 5'UTR of SLC23A2 was significantly lower by 11-15% in III-IV type comparing to I type SMA patients. Moreover, a significantly increased allele A frequency of polymorphism rs1279683 (g.G>A) situated in target CpG site was found in III-IV type SMA patients. III-IV type comparing to I type SMA patients demonstrated decreased methylation level by 9-16% of target CpG site and nearest CpG site belonged to 5'UTR of NCOR2. Significant difference in the methylation level between different types SMA patients was revealed for three CpG sites located in the region 1735-1398 bp of TSS of CDK2AP1. Thus this study confirms that DNA methylation changes of SLC23A2, NCOR2, and CDK2AP1 might be associated with spinal muscular atrophy severity.

P10.39-S
Molecular analysis of common mutation associated with SMA în Romanian population

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Spinal muscular atrophy (SMA) is characterized by degeneration of motor neurons that cause progressive muscle weakness and muscle atrophy. SMN1 homogizot gene deletion is present in most patients with SMA (~95%). The objective of this study was to evaluate the SMN1, SMN2 and NAIP gene mutational status in SMA Romanian patients. In this study, we analyzed by PCR-RFLP 90 patients with suspected SMA and 52 relatives. We also performed three prenatal test fetal with DNA obtained by amniocentesis from mother, who had prior history of another child diagnosed with SMA. We identified homozygous deletion of exons 7 and 8 of SMN1 and SMN2 genes in 35 patients, and only deletion of exon 7 at 2 patients. Homozygous deletion of exon 7 and 8 of SMN2 gene was identified in 2 patients and one relative of a patient. NAIP gene deletion was identified in 8 patients and 3 relatives. For prenatal testing, we identified homozygous deletion of exon 7 and 8 of SMN1 gene. Molecular diagnosis of SMA by identifying of SMN1 gene deletion is a useful tool for the diagnosis of SMA in Romania, being necessary deermination of SMN copies for a better matching phenotype - genotype.
This work was supported by CNCSIS - UEFISCDI, project number PN II - IDEI 2152/2008 and Program 8, the project „Intervention for diagnosis and management of spinal amiotrophy and muscular dystrophy type Duchenne and Baker, and the prevention of their hereditary transmission" 2011.

P10.40-M
Exon skipping mutation in collagen VI detected in a patient from Lithuania

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We report on a patient who presented with clinical features of Ullrich congenital muscular dystrophy. The patient is a girl, four years of age, the first child of healthy non-consanguineous Lithuanian parents. The proband was born at 40 weeks gestation in normal delivery. Hip dysplasia was noted from birth. The girl has chronic constipation from 2 months of age. Her intelligence is in normal range, but motor development is delayed. She could sit at age of 6 months, stand up with support at age of 2 years, can walk only with support. Clinical examination at the age of 4 years revealed the contractures in elbows and knees but striking distal phalanx hyperlaxity. The consentration of CK was mildly elevated (315 U/L). Spine CT and MRT investigations, cardioechoskopy were normal. The clinical diagnosis of Ullrich congenital muscular dystrophy was suspected and molecular genetic testing of *COL6A1*, *COL6A2* and *COL6A3* genes was performed. The mutation c.6210+5G>A, IVS16+5G>A, in intron 16 of the *COL6A3* gene in a heterozygous state was detected. The analysis of parents confirmed *de novo* origin of the mutation. The protein immunohistochemistry analysis of the muscular biopsy concluded that the finding is compatible with dominant in frame deletion in exon 16.
The mutation c.6210+5G>A, IVS16+5G>A, has already been described as causative for a dominant form of Ulrich congenital muscular dystrophy. The molecular and clinical characterization of our patient provides additional information for genotype-phenotype correlation and confirms dominant acting mutation in collagen VI gene as a course of Ullrich disease.

P10.41-S
Paternal germline mosaicism in COLVI related myopathies: a case report

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Mutations in the COL6A1, COL6A2 and COL6A3 genes cause collagen VI related disorders, characterized by muscle wasting, weakness, joint contractures, distal laxity, serious respiratory dysfunction and cutaneous alterations. The severe Ullrich congenital muscular dystrophy (UCMD) can be due to autosomal recessive mutations in one of the three genes of collagen VI with a related 25% recurrence risk. In the majority of UCMD cases nevertheless, the underlying mutation is thought to arise *de novo* and the recurrence risk is considered as low.
Here we report a family with recurrence of UCMD in two sibs only by father's site. In both, the molecular analysis revealed heterozygosity for the c.896G>A mutation in COL6A1 exon 10 (Gly299Glu) and for the COL6A1 c.1823-8G>A variation within COL6A1 intron 29. The Gly299Glu mutation, despite not previously reported, is likely to be pathogenic, leading to the disruption of the Gly-Xaa-Yaa motif in the triple-helix domain of collagen VI alfa(1)-chain. The intronic variation was inherited from the father and RNA analysis in skin fibroblasts allowed to exclude its role in affecting COL6A1 transcript processing. The Gly299Glu mutation occurred apparently *de novo* in the two sibs.
The described mutational segregation strongly suggests the occurrence of paternal germline mosaicism , to be confirmed by mutational analysis of the sperm. The reported family represents the first observation of gonadal mosaicism in collagen-VI related myopathies and, similarly to other collagen related diseases as osteogenesis imperfecta, this possibility deserve to be considered in genetic counseling and recurrence risk estimation.

P10.42-M
Diagnosis of spinal muscular atrophy in Algeria

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The spinal muscular atrophy is neuromuscular diseases characterized by degeneration of motor neurons and paralysis associated with muscular atrophy.
Depending on the age of onset and progression of the disease , there are 3 types of spinal muscular atrophy : the severe form or type 1 (Werdnig - Hoffman disease), intermediate or standard form II, and the least severe form or type III (Kugelberg - Welander disease of) . The frequency of these ASI is 1/10000 new borns.
Infantile spinal amyotrophy resulting in over 95 % of cases , a homozygous deletion of exon 7 of SMN1 gene located in 5q13 .
This deletion can be detected by a PCR - Digestion, by creating an artificial restriction site for the enzyme Dra I (ACRS : artificial created restriction site). This technique is now validated in our laboratory , which allowed us to analyze 150 patients (128 childrens and 22 adults).
The deletion was found in the homozygous state in 95 of them , representing a value of 53%.
While our series of patients tested from specialized services but it does not explain all the high rate of positive cases, this shows that the disease is not as rare in our population.
Our vision for the future is to establish a genetic counseling for families.

P11.001-S
14q32.2 Microdeletion and Thyroid carcinoma

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We described a young man, the third son of healthy and non consanguineous parents. He was born small for gestational age. He had delayed height growth and psychomotor development. Karyotype was 46,XY. At the age of 6, he developed precocious puberty At the age of 9 years, brain MRI showed partially empty saddle. At the age of 15, we evaluated him for microsomia, delayed psycomotor development, dysmorphisms. On physical examination, height and weight were below 3° centile, there was microcephaly, triangular face, frizzy hair, laterally sparse eyebrows, strabismus, hypotelorims, hypoplasia of midface, wide nasal bridge, short broad nose, large and low set ears, pointed chin, micrognatia, arched palate, scoliosis, kyphosis, long hands with tapering fingers, hallux valgus bilaterally, clubfeet. An aCGH showed 14q32.2□14q32.31 de novo microdeletion of 1.106 Mbp. At the age of 20, he had enlargment of the thyroid gland, exophtalmos, weight loss, tachycardia and diarrhea. Blood test showed an hyperthyroidism An ultrasound scan of the thyroid showed the presence of a dishomogeneous pseudonodular area, bilateral laterocervical lymphnodes The patient underwent thyroid fine-needle aspiration. The histology was compatible with a papillary carcinoma. Thyroidectomy was performed. In 2013, Buldrini et al reported the first patient with 14q32.2 microdeletion and thyroid carcinoma. We suggest that these patients have an increased cancer risk. We hypothesize that it is caused by haplo-insufficiency for one or more imprinted or dosage sensitive genes. The DLK1 gene plays a critical role in tumorigenic growth in 14q32 deletions.

P11.002-M
15qter deletion/duplication and growth effects

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We describe two cases with opposite rearrangements (deletion and duplication) in 15q region including Insulin-like Growth factor I receptor (IGF1R) gene. The IGF1R is involved in growth, insulin-related phenotypes, and longevity and is expressed equally from the maternal and paternal alleles.
Case 1: Girl of 11 years with unexplained intrauterine growth retardation (IUGR), birth weight <-1.5 SD and persistent short stature (<-2.0 SD). She displayed dysmorphic features suggestive of Silver-Russell Syndrome, with unspecific multiple hyperpigmented lesions on the body. She had a normal methylation study of the 11p15.5 region. Significant increases in IGF-I levels were observe during two IGF-I generation tests. SNP-array analysis identified a heterozygous de novo 4,92 Mb deletion in15q26.2 including IGF1R gene.
Case 2: proband was first evaluated at the age of 4 months for overgrowth, hypotonia and some dysmorphic features (a prominent nasal bridge, deep set eyes, epicanthic folds and micrognathia). He delivered by C-section at 39 weeks of gestation for breech presentation, with a birth weight of 3600 g (50th centile), a birth length of 53 cm (75th centile) and an OFC of 35 cm (+1.7 SD). He had presented craniosynostosis, corrected at 3 months of age. Array-CGH analysis showed a 17,3 Mb de novo duplication from 15q25.2 to 26.3 encompassing the IGF1R gene.
Our two cases again demonstrate that overgrowth, craniosynostosis, macrocephaly, and mild developmental delay in patients with trisomy 15q26.1 result from dosage increase of IGF1R, whereas gene dosage decrease of IGF1R can cause IGF1-resistance and underline IUGR with subsequent short stature.

P11.003-S
Two children with triplication of 16p11.2 associated with global developmental delay and dysmorphic features

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We report two patients with de novo triplication of 16p11.2. This region is particularly prone to genomic disorders due to the large number of low copy repeats on the p-arm of chromosome 16. Patient 1. The proband is a 4 year old male and a product of an uncomplicated pregnancy delivered at 35+2 weeks to a non-consanguineous couple. A moderate ASD, peri-membranous defect of the tricuspid valve, and coarctation of the aorta was repaired at 6 weeks of age. He also had bilateral cryptorchidism, chronic constipation, and developmental delay. Patient 2. The proband is a 12 year old female delivered at 36 weeks gestational age to a non-consanguineous couple. Hydronephrosis was diagnosed antenatally with grade III reflux confirmed postnatally. Conjugated hyper-bilirubinaemia was found in early infancy and a liver biopsy demonstrated a paucity of bile ducts; genetic testing for Alagille syndrome was negative. There is a history of failure to thrive with delayed bone age and moderate developmental delay. Both children had microcephaly and dysmorphic facial features. Deletions and duplications of 16p11.2 are relatively well-characterized, and associated with global developmental delay, behavioural problems, dysmorphic facial features, seizures, abnormal head size, and variable congenital anomalies. This is the first report of 16p11.2 triplications and the associated phenotypes.

P11.004-M
Familial cases of 22q11.2 Deletion syndrome in Belarus: phenotype's variability, genetic counseling, prenatal diagnostics

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The 22q11.2 Deletion syndrome (Del22S: #188400; #192430) manifested by distinct phenotype is characterized high (~10%) incidence of inherited cases (InhC). Disorder is displayed variability of phenotype's expression for heart defect (HD) and mental delay (MD). Adult's del22q11.2 carriers may demonstrate mild signs.We present InhC del22q11.2 (4 families, 9 patients) confirmed by FISH and the scheme of genetic counseling in Belarus. InhC incidence composes 10,5% (4/38). Two families (F1, F2) underwent through genetic counseling due to affected children aged 1,5 and 3,5 months with prenatal hypoplasia, dysmorphic signs, retardation, hypocalcemia, thymus agenesia, immunodeficiency, HD (F1: multiple VSD+ASD; F2: VSD+ASD+PDA). Birth weight was 1720g (F1) and 2100g (F2) at 36 and 37 week's gestation accordingly. Affected parents showed borderline intellect, facial dysmorphisms; HD: ASD (F1); hearing impairment, unilateral renal agenesis (F2). In F3, F4 Del22S was firstly revealed at pregnants counseled due to fetus's heart malformation. F3 presented 3 affected persons: pregnant with dysmorphisms, cleft palate, hypertension; fetus with HD (VSD+aortic coarctation) and normal thymus was born at 35 weeks gestation (weigh 2400g), died in 2 days; 11 years old child with MD, VSD. F4: pregnant operated for tetralogy Fallot (TF) with dysmorphisms, myopia, scoliosis, renal hypoplasia; fetus with TF. Both couples refused pregnancy's termination. Patient's management: cardiac surgery; follow-up for heart, mentality, immunological status, age-dependent assessment. Counseling del22q11.2 carriers: discussion for etiology, diagnostics, variability, impossibility to predict phenotype impairment→genetic risk (50%)→prenatal del22q11.2 testing (FISH)→discussion for pregnancy course. Our data illustrate wide phenotype's variability and importance of early Del22S carriers detection.

P11.005-S
Phenotypic characterization of a recurrent 22q11.2 deletion spanning low copy repeats (LCR)s C-E

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The well characterized 22q11.2 deletion syndrome is associated with a wide range of clinical phenotypes including the velo-cardio-facial syndrome (MIM 1924300) or DiGeorge syndrome (MIM 188400). About 90% of patients have a common 3Mb deletion (typical deletion), between LCR22-A and LCR22-D, while a recurrent 2.1Mb deletion is found spanning LCR22-D and LCR22-H (distal deletion). We describe 2 patients (ages 4 and 2 years) with a 1,3Mb 22q11 deletion (19362239 bp-20788469 bp, Hg18) detected by a-CGH, that spans the distal end of the typical deletion and the proximal end of the distal deletion (between LCR22-C and LCR22-E).
We compare their clinical findings with those described in patients with similar deletions in an attempt to further delineate the associated phenotype. Patients present a quite homogeneous phenotype which seems to overlap with those described in Russel-Silver and Goldenhar syndromes. It is characterized by prematurity, pre and postnatal growth retardation, microcephaly, cranio-facial dysmorphic features, heart defects, ear and renal anomalies, and mild developmental delay. Cleft palate, skeletal defects and behavioural problems were also present. Genotype-phenotype correlation supports the hypothesis that haploinsufficiency of the *CRKL* (Crk-like) gene is the major candidate gene for the cardiac anomalies associated with the 22q11.2 deletion involving LCRs C-E.

P11.006-M
MLPA reveals broader spectrum of abnormalities in patients with 22q11.2DS phenotype

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MLPA analysis is considered to be a reliable, accurate and cost-effective method with high sensitivity and specificity for detecting 22q11.2 microdeletion, both typical and distal. MLPA analysis with SALSA MLPA P250-B1 DiGeorge (MRC-Holland) kit was performed on the group of 78 probands with phenotype suggestive of 22q11.2 deletion syndrome (22q11.2DS) (CHD, facial anomalies, cleft palate, hypocalcemia). Different-sized deletion 22q11.2 was revealed in 28 cases. Most patients had common recurrent 3 Mb 22q11.2 deletion (79%). In our study MLPA test didn't reveal deletions in 49 patients. One patient was found to have deletions of probes within 4q34.2 region included in the probe set. The phenotype of patient presented with double outlet right ventricle, hypotrophy, brachycephaly, narrow fontanelles, dysplastic ears, anteverted nares, broad face, small lower jaw, double-sided cryptorchidism, chiasm of hand digits, seizures, cleft of soft palate. The patient's karyotype showed derivate 4q terminal deletion of maternal origin (46,XY,der(4)t(4;8)(q35;q22)mat).

Previously we evaluated a group of 140 probands with phenotype typical for 22q11.2DS. Forty three patients were diagnosed with 22q11.2 deletion by FISH and MLPA techniques (31%). Three patients with highly suggestive 22q11.2DS phenotype were found to have chromosomal abnormalities and three patients in non-deleted group presented with other syndromes detected afterwards. Thus patients with negative 22q11.2 deletion test results showed quite a vast etiological heterogeneity and may have been underdiagnosed for some genomic disorders. Array CGH should be advised in cases where discernibly suggestive phenotypes aren't confirmed by FISH and MLPA methods.

P11.007-S
3p deletion syndrome: clinical presentation and molecular description of array CGH

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Deletion 3p syndrome is a rare contiguous-gene disorder involving the loss of 3p25-p26 delineated by array CGH and associated with characteristic dysmorphic features, microcephaly, developmental delay (DD) and growth retardation. It was suggested that a 1,5 Mb minimal terminal deletion causes the syndrome, but there is a strong connection between the severity of the disease and the size of the deletion (Gunnarsson and Foyn Brunn 2010, Peltekova et al 2012, Reiss et al 2012). We present three patients with normal karyotype and DD, various congenital anomalies and dysmorphic features. For array CGH analysis Agilent arrays 4x180k and 1x244k (>170.000 and > 236.000 probes respectively, average resolution 8.9kb) were used. An interstitial 3p25-p26 microdeletion was detected in all patients, ranging in size from 1.9Mb to 11.3Mb and comprising the genes *CRBN, CNTN4, CHL1, LRRN1 and SRGAP3* (Ellery PM et al 2014). Additional pathogenetic aberrations which were also observed in two of our patients (dup 8q24.23-q24.3, dup 22q11.21) might contribute to the severe phenotype, presumably acting as modifiers of their clinical manifestations. Further studies of patients with application of array-CGH, will probably expand our knowledge on the phenotypic and genotypic spectrum of 3p deletion syndrome.

P11.008-M
3q27.1-q27.3 microdeletion syndrome: description of a pediatric case with previously undescribed features

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Here we describe a case of a 5 year old boy who came to our genetic service due to small stature in association with hypospadias and psychomotor retardation. Convenctional karyotype was normal, while a microarray-CGH detected a 2.8Mb deletion spanning from 3q27.1 to 3q27.3: 46,XY.arr 3q27.1q27.3(185852379-188697676)x1[Hg18]. Recently a new 3q27.3 microdeletion syndrome has been described, overlapping molecular features with our case, but only partially concordant regarding clinical phenotype. Our patient exhibit a genitourinary malformation previously unreported and a borderline IQ, opposed to reported severe mental impairment. IUGR and subsequent small stature and peculiar dysmorphisms are also seen. It is remarkable that all cases described before are adults, and as we concern this is the first case described at young age. This case expands the phenotype and clinical features of the 3q27.3 microdeletion syndrome and offers new insights about genotype-phenotype correlation.

P11.009-S
A New Syndrome? Severe growth and develop mental delay associated with Bilateral Polycystic kidney Disease.

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In this report, we describe two girls with severe growth and developmental delay with polycystic kidney disease. Long arm of chromosome 4was consistently deleted in almost same posisions. The common locus in the chromosome is 4q21-22. One patient had a simple 4q contiguous gene deletion, whereas the other patient had a complicated chromosomal rearrangement. In the first patient, a smaller part of the 4q was inserted to 3p. In both patients, abdominal ultrasonography revealed renal cysts. In the deletions PKD2, which causes autosomal dominant polycystic kidney disease (MIM 613095) was mapped. , The common 52 genes deleted in both patients. Typical phenotypes, were severe growth and developmental retardations, and a characteristic facial appearance consisting of frontal bossing, thin broad

eyebrows, epicanthus fold, missing, missing teeth. And mild hand and foot anomalies. Although these patients carry different chromosomal aberration, the common deletion causes the quite similar phenotype, suggesting a new syndrome due to 4q21-q22 deletion.

P11.010-M
9p deletion syndrome-like in a girl with a 9p insertion on chromosome 2 without 9p deletion

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Partial deletions of 9p have been reported as a clinically recognizable syndrome characterised by the variable association of intellectual disability, speech delay, hypotonia, cardiac anomalies and dysmorphisms.
We report a 3 years old girl with midface hypoplasia, downslanting palpebral fissures, teeth agenesis and teeth fusions. Language was not acquired. At birth, severe neonatal hypotonia and patent ductus arteriosus were present. Phenotype was consistent with the clinical diagnosis of "9p deletion syndrome" (OMIM#158170), with uncommon features such as bilateral cataract (diagnosed at 5 months), chronic constipation, high pain threshold and poor temperature control.
Karyotype analysis showed an apparent 9p deletion. Array-CGH (60K Agilent) demonstrated a 2q microdeletion (600 Kb, from 166,993,501 to 167,602,904, NCBI Build 37/hg19) with normal dosage of chromosome 9. Subsequent FISH analysis revealed a 9p22.1p24.3 insertion in chromosome 2q [46,XX,ins(2;9)(q24.3;p22.1p24.3)dn]. The 2q microdeletion involved only two genes: *SCN9A* and *SCN7A*.
SCN9A recessive mutations cause congenital insensitivity to pain. *SCN7A* is not associated with human disease, and in mouse is likely to be a sodium-level sensor of body fluids in the brain. *SCN9A* haploinsufficiency may support the clinical observation of the patient high pain threshold and the poor temperature control. We are verifying the presence of a second mutation on the remaining allele. Assuming that the 2q deletion cannot account for the whole phenotype, we speculate that a critical gene/regulatory region key for the "9p deletion syndrome" is located in the 9p breakpoint(s). This patient deserves future studies that may help to refine 9p critical region.

P11.011-S
Identification of de novo 2,62Mb deletion in chromosome 15q26.1 in a boy with stigmata dysplastica, developmental delay, short stature, microcrania, nasal polyposis, hypermetropia

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We report a 14 years old boy with developmental delay, stigmata dysplastica and multiple organ involvement. History: no familiar congenital anomalies or consanguinity; born on 38th gestational week. IUGR. Developmental delay: sitting at the age of 8 months, walking at 16 months; at the age of three years speech with only some words. Short stature, microcrania, stigmata dysplastica, nasal polyposis and hypermetropia. Head MR showed suspect hypophyseal calcinations. Normal feeding and voiding. Dry skin. Normal hearing. No cerebral paroxysms. Postnatal microarray analysis (Agilent, Human CGH Microarray Kit 8x60k): de novo interstitial microdeletion of 2620±70kb in chromosome 15, region 15q26.1 (arr[hg19] 15q26.1(90,971,047-93-,586,680)x1 dn)1. The deleted region contained 18 RefSeq genes, encompassing CHD2, ST8SIA2 and RGMA, among others. Background: So far, there has been no case with similar 15q26.1 deletion submitted to the publicly available databases1. CHD2 gene belongs to the CHD family of proteins, characterized by the presence of chromo (chromatin organization modifier) domains and SNF2-related helicase/ATPase domains. Moreover, ST8SIA2 and RGMA genes play an important role in developing and adult central nervous system. Conclusion: Taking into consideration the de novo origin of the 15q26.1 deletion, its size and functional characteristics of the encompassed genes, the deletion is most likely associated with the developmental delay, dysplastic signs and multiple organ involvement. We believe phenotype is predominantly the consequence of CHD2 haploinsufficiency. Further studies are required to determine the role of CHD2 gene in the characteristic phenotype. 1Data generated by the ECARUCA, DECIPHER, ISCA

P11.012-M
A novel mutation in the AAAS-gene in a Bulgarian patient with Triple-A syndrome

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Triple A (Achalasia-Addisonianism-Alacrima, Allgrove) syndrome is an inherited condition characterized by three specific features: achalasia, Addison disease, and alacrima. Allgrove syndrome is inherited in an autosomal recessive pattern and is caused by mutations in the AAAS gene localized at 12q13.13. The gene encodes a protein called ALADIN. It localizes to nuclear pore complexes, large multiprotein assemblies that are the sole sites of nucleocytoplasmic transport. Achalasia-Addisonianism-Alacrima syndrome is a rare condition, incidence is unknown, and only scattered family and case reports are noted in the literature. Here we report a female patient with Triple A syndrome born to non-consanguineous parents from Bulgarian origin. She was diagnosed as Triple A at the age of 17. The patient express a typical phenotype: achalasia, Addison disease, and alacrima. The affected patient also has muscle weakness, peripheral neuropathy, skin hyperpigmentation, optic atrophy and papillae atrophy. Many of the features of triple A syndrome are caused by dysfunction of the autonomic nervous system and the neurological symptoms have worsen over time. Molecular genetic testing of the patient showed 2 heterozygous mutations in the AAAS-gene: an already reported nonsense mutation c.1024C>T, p.(Arg342*) and a novel splice site mutation c.1331+2dupT. The one basepair duplication, located 2 basepairs upstream exon 14, disrupts the donor splice site of intron 14 of the AAAS-gene. Based on Alamut software prediction, it is very likely that the mutation leads to skipping of exon 14.

P11.013-S
Acrocallosal syndrome caused by novel mutations in the KIF7 gene_a report on a Polish family and review on KIF7 syndromology

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Acrocallosal syndrome (ALCS) is a multiple congenital anomaly disorder characterized by postaxial and/or preaxial polydactyly, cutaneous syndactyly, macrocephaly, widely spaced eyes, absence of the corpus callosum, and intellectual disability. It was first described by Albert Schinzel in 1979, but the diagnosis of this syndrome still remains challenging. This may be due to both non-specific clinical symptoms, as well as to a heterogeneous and difficult to clearly define molecular background. In 2011 mutations in the *KIF7* gene were identified for the first time in patients with acrocallosal syndrome. The *KIF7* gene encodes a cilia-associated protein belonging to the kinesin family that plays a role in the hedgehog signaling (SHH) pathway through the regulation of GLI transcription factors; it also regulates acetylation and stabilization of microtubules.
We report on a family with 2 children (a girl and a boy) affected with acrocallosal syndrome caused by novel mutations in *KIF7*. The presented sibs met the clinical criteria of acrocallosal syndrome, i.e.: polydactyly, corpus callosum anomalies, facial dysmorphism (frontal bossing and hypertelorism) and hypotonia/developmental delay. Despite the same spectrum of congenital malformations noted in the presented patients, their psychomotoric development is quite different. The boy is much more delayed, with poor contact, cannot sit unsupported. He has hyperventilation and, as his sister, sleep disorders (frequent awakening). On the basis of this family, we discuss the heterogeneity of *KIF7*-associated disorders.
The study was partially financed by NCN Project Harmonia 4 no. UMO-2013-/08/M/NZ5/00978.

P11.014-M
A new locus for syndromic acroosteolysis with intellectual disability, sensory neuropathy and enhanced osteoclast function

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We describe an inbred family with three individuals affected by syndromic acroosteolysis, inherited as an autosomal recessive trait. This condition is characterized by intellectual disability, hypogonadism, sensory neuropathy and recurrent infections. Furthermore, decreased bone mineral density and low levels of 25-hydroxyvitamin D_3 were detected. Mutations in genes previously associated to acroosteolysis (*NOTCH2*, *MMP2*, *WNK1*, *FAM134B*) were excluded by Whole Genome Sequencing. This same technique, in combination with SNP haplotyping of the pedigree, allowed the identification of two homozygous missense mutations in neighbouring genes *TMEM41B* and *DENND5A* on 11p15.4. These genes have not been implicated in human disease yet and encode distinct Golgi proteins. Different bioinformatic analyses suggest that both mutations in the neighbouring genes may underlie the pathogenesis. Messenger RNA levels of both genes are upregulated in osteoclast cell cultures obtained from normal controls. Functional studies show that significantly more osteoclasts are derived from patients' blood mononuclear cells compared to heterozygous individuals and normal controls; finally, patients' osteoclasts exhibit an enhanced bone resorption activity compared to controls. Further genetic studies in animal or cellular models are needed to sort out the role of each gene in the new locus in causing this rare recessive disorder.

P11.015-S

Acromegaloid Facial Appearance Syndrome should not be forgotten in the differencial diagnosis of pseudoacromegaly

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Acromegaloid facial appearance (AFA) syndrome is a very rare inherited cause of pseudoacromegaly characterized by progressive coarsening of facial features, overgrowth of oral mucosa and large, doughy hands. Six case reports have been described. Herein we describe a further family with AFA syndrome.

Probands were a 31 years female and his 37 years brother who were referred to our department due to suspected acromegaly. Both siblings had a previous history of oral surgery 13 and 20 years before due to hypertrophic intraoral tissue. Additionally, the male proband had mild learning difficulties, uncontrolled hypertension, left ventricular hypertrophy and a paroxystic atrial fluter. He was medicated with lisinopril, amiodarone and clobazam. Family history was remarkable for their mother, who was remembered by both siblings as having "rough facial features". At physical examination both patients were hypertensive, obese and had a raised cephalic perimeter. Their facial appearance was coarse with thickened lips and superior eyelids, enlarged nose and gingival hypertrophy. Biochemical surveys were normal. At follow-up both patients underwent repeated oral surgeries due to their evolving extensive gingival hypertrophy. AFA syndrome was considered the final diagnosis of this somatic overgrowth disease.

We highlight the importance of a careful family history and physical examination of a kindred with AFA syndrome to collect the striking features of each disorder associated with an acromegaloid phenotype and achieve an accurate diagnosis. The phenothype includes a progressively coarse acromegaloid-like facial appearance with thickened lips/gums, with learning difficulties in two consecutive generations, suggesting AD inheritance.

P11.016-M

A novel splice-site mutation in *NOTCH2* in a Norwegian family with Alagille syndrome

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The index patient, a boy, presented neonatally with conjugated hyperbilirubinemia which resolved in 2-3 months. He was born five days post term (birthweight 2840 g, length 50 cm, occipitofrontal head circumference 34 cm) after an uneventful pregnancy. His healthy non-consanguineous parents are of Norwegian descent. Of note is that his father was icteric for the first 2-4 months of life. In the non-dysmorphic index patient, hepatic ultrasound and scintigraphy were normal neonatally. Bile duct paucity, giant cell transformation and intracanalicular cholestasis was evident histologically. Serum cholesterol was <5mmol/L. His proteinuria improved over the first 12 months. Additional findings included posterior embryotoxon and bilateral mild peripheral pulmonary artery stenosis. Butterfly vertebrae were not demonstrated. No sequence or copy number variants were detected in *JAG1*. Sequencing of *ABCB4* and *CFTR* revealed no pathogenic variant. Sequencing of *NOTCH2* established heterozygosity for the novel synonymous variant c.2817G>A (p.P939P) (NM_024408.2) both in the child and his father. *In silico* analysis predicted introduction of a novel splice-site. cDNA sequencing confirmed the presence of a splice-site causing skipping of the 5'-end of exon 18 (r.2753_2818del). The deletion maintained the reading frame (p.Asn918-Gly940delinsSer), resulting in a protein lacking most of the 24th EGF-like domain (p.911-947). Amplification of the correctly spliced mRNA revealed monoallelic expression of the normal allele (c.2817G), confirming that c.2817G>A in *NOTCH2* is likely the cause of the variable Alagille syndrome phenotype in this family. To our knowledge, this is the first reported case of a synonymous variant in *NOTCH2* causing Alagille syndrome.

P11.017-S

A new mutation in the COL4A3 gene associated to autosomal dominant alport syndrome with hearing disorders as sole clinical manifestation

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The autosomal dominant Alport syndrome accounts for 5% of cases of Alport syndrome and, unlike the recesive and sex-linked forms, clinical expression is benign and appears in the later stages of life. Autosomal dominant Alport syndrome generates a form of late-onset mild sensorineural hipoacusia. However, as in the other forms of Alport syndrome, this is always accompanied by renal failure, the rate of hearing loss being directly linked to the progression of renal failure, and its progression suggests a poor prognosis of kidney disease. We have studied the COL4A3 and COL4A4 genes by PCR, CSGE and automatic sequencing of the full coding region and the exon-intron boundaries in a Spanish family with autosomal dominant Alport syndrome, in which most of its members are suffering from hearing loss only, one member suffers lenticonus, and only two suffer renal disease. We found a heterozygous c.345DelG/p.G115GFSX37 mutation in the COL4A3 gene, which generates a truncated protein. This mutation was found in all family members with symptoms but was absent in the healthy members of the family. Thus, we describe a new mutation in COL4A3 gene mainly associated to hearing loss and for the first time we describe a case of autosomal dominant Alport syndrome that presents lenticonus. Furthermore, we report that deafness and renal impairment could not be associated in the same individual, this being the first case of a family in which deafness occurs amongst several members who do not show any alteration in the kidney.

P11.018-M

Prenatal diagnosis using array CGH in 163 cases

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Karyotype analysis has been the standard procedure for prenatal cytogenetic diagnosis since the 1970s. However, the major limitation remains requirement for cell culture, resulting in a delay of as much as 14 days to get the test results. FISH and QF-PCR do not provide a genome screen for unexpected imbalances. CGH array technology has proven to be useful in postnatal diagnosis of mental retardation, development delay, and congenital malformation syndrome. We evaluated the use of array comparative genomic hybridization (aCGH) for prenatal diagnosis including assessment of variants of uncertain significance, and the ability to detect abnormalities not detected by karyotype. Women undergoing amniocentesis or chorionic villus sampling (CVS) for karyotype were offered aCGH analysis using a targeted cytochip ISCA 4x44K v1.0 oligonucleotide array. Parental samples were obtained at the same time to exclude maternal cell contamination and determine if copy number variants (CNVs) were de novo, or inherited.We analyzed 163 samples, most were CVS (75.5%) and amniotic fluid (19.6%). The most common indication were advanced maternal age (N=74), abnormal ultrasound finding (N=46) and a previous child with multiple congenital abnormalities (N=40).We detected 27 CNVs (16.7%). Of these 16 (9.8%) were interpreted as likely benign, 9(5.5%) were of defined pathological significance, while 2(1.2%) were of uncertain significance. We concluded that array CGH identified clinically significant abnormalities in approximately 5.5% of fetuses with ultrasonographic abnormalities and normal conventional karyotype. This study demonstrate the potential for array CGH to replace conventional cytogenetic in the great majority of prenatal diagnosis cases.

P11.019-S
Deletion of 9q22.3-33.1 in a child with corpus callosum hypoplasia and thymus asymmetry

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Interstitial deletion of chromosome 9q is reported in context of many types of cancer but congenital cases are rarely published. The authors report on a child presenting with multiplex congenital anomalies, muscle hypotonia and characteristic facial features. Because of the symptoms suggesting primarily chromosomal disease, high resolution array CGH was indicated among a series of examinations, which detected a *de novo* 15,8 Mb interstitial deletion of 9q (ch9:102,589,027-118,400,548x1). The genotype-phenotype analysis made it clear that there are many symptoms in the clinical picture which are not included in the description of similar cases as heart anomalies (patent foramen ovale and vitium), brain malformation (corpus callosum hypoplasia), and many minor anomalies. An excessive sweating and frequent falling asleep are also characteristics not reported at known 9q deletion patients. The analysis of the possible role of the genes affected by deletion drew our attention to *NR4A3* and *CTR1* which may be related to brain malformation. Two genes (*CTNNAL1* and *RAD23*) involved in DNA repair and cell cycle regulation may have also impact on the growth retardation and dysmorphic facial features observed in 9q deletion. The *TNC* may be a candidate in development of the heart defects, and in the background of muscle hypotonia, the *MUSK* gene may be significant. Probably the haploinsufficiency of *KLF4* might be associated with excessive sweating being present in our patient. The authors provide a detailed clinical report, and the possible pathogenic role of individual genes involved in the deletion will also be discussed.

P11.020-M
Novel mutations in GNAI3 in patients with Auriculocondylar Syndrome suggest a dominant negative effect with disruption of GTP/GDP binding

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Auriculocondylar syndrome is a rare craniofacial disorder comprising core features of micrognathia, condyle dysplasia and question mark ear. Causative mutations have been identified in PLCB4, GNAI3 and EDN1, which are predicted to function within the EDN1-EDNRA pathway during early pharyngeal arch patterning. To date, two GNAI3 mutations in three families have been reported. Here we report an Australian patient with ACS with a novel de novo GNAI3 mutation. We also present two other novel GNAI3 mutations, one segregating with affected members in a family previously linked to 1p21.1-q23.3 and a de novo mutation in an unrelated simplex case. The mutations occur in known functional motifs common to G proteins and RAS family members. Structural modeling shows that all five mutated GNAI3 residues cluster in a region involved in GDP/GTP binding. Two of the five residues lead to dominant negative proteins when mutated in related proteins. We hypothesize that all GNAI3 mutations lead to dominant negative effects.

P11.021-S
A case of a microscopically balanced familial translocation t(5;14)(p13;q22) and phenotype of lacrimo-auriculo-dento-digital (LADD) syndrome

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We report a 15 years old male patient with clinical presentation of MCA syndrome including facial dysmorphism with micrognatia and small dysplastic ears, absent lacrimal glands and limb anomalies (muscle hypotrophy and contractures of upper limbs, bilateral pes varus deformity). The boy's intellectual and growth development was in normal age ranges. The child was born from a second pregnancy of young and healthy couple. The first pregnancy in the family ended with birth of a baby with diaphragmatic hernia which died after three days. The performed cytogenetic analysis of the patient at one month revealed a microscopically balanced chromosomal translocation between chromosomes 5p and 14q: 46, XY, t(5;14)(p13;q22). The father's karyotype was normal 46,XY while the mother's karyotype was 46,XX,t(5;14)(p13;q22) identical with her son's rearrangement. The performed array-CGH of the patient identified a heterozygous deletion in 5p12 chromosome with 489 kb size, including only one mapped gene: FGF10 (fibroblast growth factor 10) gene. Mutations in FGF10 gene may underlie autosomal dominant conditions such as lacrimo-auriculo-dento-digital (LADD) syndrome and aplasia of lacrimal and salivary glands (ALSG). The main features of lacrimo-auriculo-dento-digital (LADD) syndrome are abnormal tear production, malformed ears with hearing loss, decreased saliva production, small teeth, and hand deformities. The haploinsufficiency of FGF10 gene caused by heterozygous loss of on gene copy may explain the phenotype in our patient. This rare case of a cytogenetically balanced familial translocation causing abnormal phenotype demonstrates the diagnostic importance of genome analysis in patients with chromosomal rearrangements detected on routine karyotype.

P11.022-M
Whole exome sequencing identifies a vertically transmitted novel ACTB mutation in a mother-son pair presenting with atypical Baraitser-Winter syndrome

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Baraitser-Winter syndrome is a rare, congenital disorder characterized by distinctive facial features, brain malformations and intellectual disability. It is caused by *de novo* dominant mutations in the *ACTB* or *ACTG1* genes. We present the first case of a mother-son pair concordant for Baraitser-Winter syndrome. Both had the facial gestalt of Baraitser-Winter syndrome and were found to carry a novel *ACTB* mutation by whole exome sequencing. The mutation, c.593A>C (p.S199R), occurs in a conserved amino acid and is situated near a recurrent substitution (p.R196H) commonly seen in other cases of Baraitser-Winter syndrome. The observation of transmission from parent to child broadens the clinical spectrum of Baraitser-Winter syndrome to include a relatively milder form that includes additional features such as a severe, early onset glaucoma.

P11.023-S
A new compound heterozygous mutation in the *BBS7* gene in a Korean family with Bardet-Biedl syndrome

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Background: Bardet-Biedl syndrome (BBS) (OMIM 209900) is a rare autosomal recessive, pleiotropic ciliopathy. To date, at least fifteen genes causing BBS have been reported.
Case: A 26-year-old Korean male from non-consanguineous Korean parents presented with rod-cone dystrophy, truncal obesity, mental retardation and end stage of renal disease. A 28-year old his brother showed rod-cone dystrophy, truncal obesity, mental retardation and deep vein thrombosis. To rule out the BBS, genomic DNAs were obtained from the peripheral blood leukocytes of all the family members. All the exons and intron flanking regions of the fifteen *BBS* genes were analyzed by direct sequencing. A compound heterozygous mutation of *BBS7* gene (NM_176824) was identified in both brothers; one was a novel acceptor splice site c.103-1G>A mutation (IVS2-1G>A) altering the splicing recognition site at the intron 2 and exon 3 boundary of *BBS7* gene. The other was a novel missense mutation of c.728G>A changing codon 243 from cysteine to tyrosine. The father was a heterozygous carrier for c.103-1G>A, and the mother was a heterozygous carrier for c.728G>A. Array comparative genomic hybridization analysis revealed a normal hybridization pattern with no evidence of significant chromosome imbalance.
Conclusion: This is the first BBS case in Korean family genetically confirmed that had a compound heterozygous mutation in *BBS 7* gene. Considering that the BBS is genetically heterogeneous and the prevalence differs among ethnic group, our clinical experience would be helpful to diagnose these patients accurately and understand the genetic events in BBS.

P11.024-M
Genotype-Phenotype correlation for BBS1 gene in Bardet-Biedl Syndrome patients

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Bardet-Biedl syndrome (BBS, #209900) is a rare genetic disorder characterized by highly variable phenotype and genetic heterogeneity. BBS belongs to a group of diseases known as ciliopathies, which show partial overlapping phenotypes that further complicates the molecular diagnosis. *BBS1* gene accounts for 25% of total mutations described in BBS, of which p.M390R is the most frequent mutation in European population. Many efforts have been done to establish a genotype-phenotype correlation for *BBS* genes in order to facilitate diagnosis confirmation. In this sense, the objective of this study was to correlate different *BBS1* mutations with some clinical features. We selected 36 patients from 22 families for whom a *BBS1* mutation had been identified. Clinical features of these patients were analysed by SPSS v.19. p.M390R mutation was the predominant mutation identified in 33 patients (92%), 20 of them harboured this mutation in homozygous state (56%) and 13 were compound heterozygous (36%). Only 3 patients (8%) had two mutated alleles with *BBS1* mutations different from p.M390R. By comparing all clinical features of these three groups of patients, we found statistically significant differences between these groups: while homozygous p.M390R showed more frequently high blood pressure, compound heterozygous manifested more secondary features, especially psychomotor impairment/retardation, hearing loss and worse visual defects. In conclusion, we confirm the important role of p.M390R mutation in the diagnosis of BBS, and it seems that homozygous patients have a milder phenotype. This will be important for genetic counselling purposes in these families.

P11.025-S
Potential impact on splicing of *BBS12* variations found in Bardet-Biedl syndrome Spanish patients

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Bardet-Biedl syndrome (BBS, #209900) is a multisystem rare disease which belongs to the emerging group of diseases called ciliopathies, and is inherited within an autosomal recessive pattern. However, little is known about other molecular mechanisms which may be involved in BBS development. For example, it is well established that synonymous (sSNPs) and non-synonymous (nsSNPs) changes can affect the conformation and stability of mRNA, the splicing process, the accuracy of translation and the protein structure. The former would lead to the disruption of the highly conserved network of cellular pathways for maintaining proteostasis. In addition, those exonic mutations predicted as pathogenic could affect splicing process via the creation and/or elimination of Exonic Splicing Enhancer/Silencer sequences (ESEs/ESSs).
In this regard, we have sequenced the coding region and intron boundaries of *BBS12* gene in fifty BBS patients. Then, we have used specific software tools to predict the potential effect at protein level (PolyPhen, PMut and SIFT) or on the splicing process (NetGene2, NNsplice, SpliceView, Human Splicing Finder and Rescue ESE) of all variants identified.
We have found two pathogenic mutations in six patients and found at least one sequence variation in twenty-nine of them. Eight out of twenty changes were selected as putative to affect the splicing process since at least two software tools predicted this effect.
As these results are only computational predictions, further functional studies will have to confirm this effect on mRNA processing. Thus, minigenes and RNA assays will be performed in order to elucidate the role of these changes.

P11.026-M
Cognitive, behavioural, and adaptive functioning of patients affected with Bardet-Biedl syndrome (BBS)

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Bardet-Biedl syndrome is a rare genetically heterogeneous multisystem disorder characterized by retinal degeneration, genital and kidney malformation/function, and other features. Variability in cognitive, social, and emotional impairment is reported; however, studies were small and completed prior to availability of molecular characterization. Our aim is to define neuropsychological function in a group of patients with molecular diagnosis of BBS to better understand phenotypic variability.
Methods: Eighteen patients (12 females; mean age: 20.95 years; range 6.4-38 years) completed standardized measures of cognition. Magnification was available. Standardized informant-proxy questionnaires were completed to assess behavioural and adaptive functioning. Results were compared to normative data (norms).
Results: Significant weaknesses ($p<.001$) in verbal and perceptual intellectual reasoning emerged compared to norms (mean= 8th and 3rd percentiles respectively; range < 2nd-53rd percentiles). More individuals displayed Average ability (25th-74th percentile) on verbal (33.3%) than visual (15.4%) domains. More individuals displayed Extremely-Low ability (<2nd percentile) on visual (53.9%) than verbal (11.1%) domains. Significant weaknesses in auditory attention span ($p\leq.01$), and auditory and visual working memory ($p<.001$) emerged. Informant ratings indicated significant patient challenges, relative to norms, in depression ($p=.004$), withdrawal ($p<.001$), atypical-behaviour ($p=.029$), social-communication ($p=.001$), unusual-behaviours ($p<.001$), aspects of executive functions ($p\leq.01$), and adaptive independence ($p<.001$); most patients (81.3%) fell below the 2nd percentile in functional independence; the remainder fell from the 2nd-5th percentiles. Verbal reasoning was correlated with independence ($p=.001$).
Conclusion: BBS impacts cognitive, behavioral, and adaptive functioning to various degrees. Assessment of these patients on a larger scale will allow further definition of the neurocognitive phenotype.

P11.027-S
Beckwith Wiedemann Syndrome in 9 Tunisian patients : From typical sporadic presentation to unusual familial isolated adrenocortical tumor

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Beckwith-Wiedemann syndrome (BWS) is a pediatric overgrowth disorder expressed through a highly variable clinical presentation involving a predisposition to tumor development, exomphalos, macroglossia, and gigantism. In addition to this clinical heterogeneity, BWS also exhibits etiologic molecular heterogeneity involving a variety of genetic and/or epigenetic alterations in growth regulatory genes on chromosome 11p15. The clinical heterogeneity turns out to be insufficient in diagnosis and prognosis of BWS, thus the need of genetic analysis of 11p15 region.
We conducted a study of 9 patients who underwent molecular analysis of chromosome 11p15 region. Reasons of referral vary from sporadic typical presentation encompassing abdominal wall defects, macroglossia and gigantism to atypical mild familial BWS revealed by isolated benign adrenocortical tumor. Molecular analysis using methylation specific multiplex ligation-dependent probe amplification revealed different methylation patterns along the BWS critical region.
6 patients showed complete KvDMR hypomethylation, one paternal uniparental disomy at 11p15 case with both H19DMR hypermethylation/ KvDMR hypomethylation confirmed by STR analysis, one patient ; the youngest; with mosaic paternal isodisomie and one patient; the eldest; with KvDMR methylation mosaic pattern. This mosaicism was associated in adrenocortical tumoral tissue to a complete KvDMR loss of methylation, however skin tissue analysis showed a normal methylation profile.
These results have allowed us to offer our patients adapted management and genetic counseling. BWS illustrates the complexity of the mechanisms involved and the need for collaboration between geneticists, pediatricians, neonatologists and pediatric surgeons.

P11.028-M
A novel IGF2/H19 domain triplication in the 11p15.5 imprinting region - new insights into the pathogenesis of Beckwith-Wiedemann and Silver-Russell syndromes

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The imprinted 11p15.5 region contains two domains (*IGF2/H19* and *KCNQ1OT1/CDKN1C*), each of them under control of its own imprinting control region. Defects of 11p15 imprinting result in two growth disorders with opposite phenotypes: Beckwith-Wiedemann (BWS) and Silver-Russell (SRS) syndromes. Various 11p15.5 genetic and epigenetic aberrations have been revealed, among them microduplications and microdeletions.

We report a novel *IGF2/H19* domain triplication in the 11p15.5 region identified in a girl with BWS and her father with symptoms of SRS. Methylation specific multiplex ligation-dependent probe amplification (MS-MLPA) performed in the patient's DNA revealed triplication of *IGF2/H19* domain as well as increased methylation of *H19* region. The same genetic change in the father's DNA was associated with reduced methylation of *H19* region. The presence of triplication was confirmed by aCGH.
This is the first report of *IGF2/H19* domain triplication associated with BWS or SRS. A few BWS patients with *IGF2/H19* domain duplication of paternal copy have been described so far. Duplications of maternal copy of this domain have been reported in three individuals and were not associated with an abnormal phenotype (i.e. Silver-Russell syndrome). The clinical outcome of 11p15.5 copy number variations depends on their size, localization and the parental inheritance. These aberrations may influence chromatin organization affecting the regulation of imprinted genes. Our findings bring new insights into the regulation of genomic imprinting at 11p5.5 region and underline difficulties of genetic counseling in patients with 11p15 defects.
The study was financed by National Science Centre, project 1149/B/P01/2011/40 (NN407114940) and EU Structural Funds, POIG.02.01.00-14-059/09.

P11.029-S
Uniparental disomy in Beckwith-Wiedemann syndrome: new insights from genotype-phenotype correlations

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Beckwith-Wiedemann syndrome (BWS) is the most common overgrowth syndrome, with a prevalence of about 1:10,000 live birth. Clinical findings include macrosomia, macroglossia, abdominal wall defects and other less frequent features, the most severe of which is predisposition to embryonal tumors.
The molecular etiology of BWS is complex, involving alterations in the expression of multiple imprinted growth regulatory genes on chromosome 11p15.5. Genes that are imprinted are expressed predominantly from one allele in a parent of origin-specific manner. Epigenetic and/or genetic alterations in the 11p15.5 imprinted gene cluster have been associated with BWS in approximately 80% of patients. One of the major categories of BWS molecular alteration (20% of cases) is represented by mosaic paternal uniparental disomy (pUPD), namely patients with two paternally derived copies of chromosome 11p15 and no maternal contribution. pUPD is also the molecular alteration associated with the most severe BWS phenotype because of the highest tumor risk.
Here we report a fine analysis of patients with BWS and pUPD from our cohort. By SNP array and microsatellite analysis we could distinguish three different categories of pUPD: whole genome, whole chromosome 11 and chromosome 11short arm.The three UPD subgroups show differences in some clinical features (hemihyperplasia, hypoglycemia, umbilical hernia, hepato/splenomegalia) and similarities in others (macrosomia, polyhydramnios). Our results highlight the importance of a fine molecular analysis of the UPD cases for a more accurate prognostic prediction to facilitate management and surveillance of patients.

P11.030-M
KCNJ8 mutation in a spanish family with Cantú syndrome including a case of fetal demise

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Cantú syndrome, (MIM 239850) is an autosomal dominant disorder causing severe hypertrichosis, congenital and progressive coarseness of the facial features, thick skin with deep palmar and plantar creases, cardiomypathy and lymphedema. Polyhydramnios and macrosomia can be present in the affected fetus. ABCC9, a multimeric subunit of a ATP sensitive potassium channel K(ATP) has been the causal gene in most cases, but not all. A second gene KCNJ8, also a subunit of the same K(ATP) channel, was proposed to be causal after a de novo mutation (p.V65M) was found in a sporadic case with unusual cardiovascular findings. Another mutation in KCNJ8 (p.S422L) has been reported in at least 9 individuals with Brugada sudden death syndrome. A 30 week old fetus was followed due to macrosomia and polyhydramnios, unexplained fetal demise occurred at 34 weeks. Evaluation of the family identified the fetus, the father, the paternal grandmother and the aunt of the fetus had clinical features of Cantú syndrome. No cardiomyopathy or other potential causes for the fetal demise were identified. Sequencing and MLPA for ABCC9 did not detect any pathogenic changes. Subsequently, an inherited missense change in KCNJ8 (NM_004982.3: c.34T>C (p.Tyr12His) was identified. In silico prediction and segregation (4 affected, 1 unaffected) indicated the mutation is most likely pathogenic. The phenotype in the family is indistinguishable from typical Cantu syndrome caused by mutation in ABCC9, although fetal demise was never reported before. Mutation in KCNJ8 in families with Cantu syndrome may represent increased risk for sudden death.

P11.031-S
De novo mutations associated with sporadic cases of Caudal regresion syndrome

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Aim: The identification of de novo disease causing mutations in three Caucasian patients with sporadic Caudal Regression Syndrome (CRS). CRS is a rare and diverse congenital disorder which is characterised by different degrees of agenesis of the caudal spine. Known genetic mutations are only able to explain a fraction of cases and are not accounting for sporadic occurrences or the diversity of the disorder. Methods: Exome sequencing assay was conducted of the three sporadic cases and their biological parents. We targeted rare genetic variants as the underlying cause of CRS as well as de novo mutations. Further we investigated de novo indels, copy number variations (CNV) and compound heterozygosity. Identified mutations were ranked and filtered based on genomic, genetic and statistical features. Results: Sanger sequencing confirmed two different de novo mutations in two cases (detailed results will be presented). In addition, our analysis revealed several potentially causal compound heterozygous mutations which are also under investigation. Conclusion: CRS may be caused by de novo or compound heterozy mutations thus, i) the diversity of the disorder is mirrored in the underlying genetic architecture and its mutations; ii) ranking of compound heterozygous mutations enables identification of candidate genes.

P11.032-M
A de novo microdeletion of chromosome 18q11.2q12.2 causes a new distinct clinical phenotype with coarctation of aorta and intellectual disability.

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Cardiac abnomalities are diagnosed in 25-30% of the patients with 18q syndrome and the most common heart abnormalities are reported in patients with terminal 18q deletion (18q22): atrial septal defect, ventricular septal defect, polmonary valve anomalies and total anomalous polmonary venus return. We report on one girl with coarctation of the aorta, moderate development delay, behavioral problems, with an interstitial de novo deletion within chromosomal band 18q11.2 of 13.4Mb. The propositus was born at 37 weeks via caesarian section after a pregnancy complicated by gestosis. Her birth weight was 2400g (<10th centile), birth length was 45cm (3-10th centile) and occipital frontal circumference (OFC) 34 cm (5thcentile). Family history revealed no abnormalities or congenital heart diseases. At the age of one month, cardiac echo revealed tight istmic coarctation of the aorta and the patient underwent surgery. Clinical evaluation showed a distinctive craniofacial apparence characterized by brachicephaly, plagiocephaly, telecanthus, strabismus, low nasal bridge, smooth philtrum, thin lips, high arched palate, low set ears with hypoplastic lobule, proximally placed thumbs. The development milestones were delayed and the propositus developed behavioral problems: poor concentration, hyperactivity and distractibility. CGH analysis showed an interstitial proximal microdeletion of chromosome 18 of 13,4Mb [arr 18q11.2(22,032,122-35,430,900)x1]. In this region some genes are present that are involved in development of the heart, DSC2 ,DSG2, TTR, DTNA, FHOD3. This is the first report of aortic coarctation mapping to a proximal microdeletion involving the chromosome 18q11.2q12.2 segment. The report also expands the spectrum of clinical phenotype associated with 18q11.2q12.2 deletion.

P11.033-S
A further case of pure 15q deletion: Clinical and molecular cytogenetic findings

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A deletion of the distal long arm of chromosome 15 is generally reported with the formation of ring chromosome 15, whereas an isolated 15q deleti-

on is rarely described. Here we report an 11 year-old girl, from non-consanguineous parents, was referred to the Pediatric Genetics Department with growth retardation and multiple congenital abnormalities. In her medical history, she was born at-term after an uncomplicated pregnancy. Her birth weight was 2200 g. She had a cleft palate, hip dislocation and crossed renal ectopia. On physical examination, her weight, height and head circumference were below the 3rd percentile. Dysmorphological evaluation revealed a triangular face, low-set ears, fissured cleft tongue, micrognathia, proximally placed hypoplasic thumbs, genu valgus, 2-3 toe skin syndactyly, clinodactyly and nail hypoplasia. Speech problems were also noticed. The complete blood count, basic biochemical parameters and hormones profile were within the normal range. The karyotype was normal. Subtelomeric fluorescene in-situ hybridisation (FISH) analysis showed a *de novo* terminal deletion of chromosome 15. BAC FISH analysis of the patient indicated that the deletion breakpoint was at 15q26.3 and the deletion comprised 700-870 kb. The deleted region includes the *CHSY1* gene that is responsible for Temtamy preaxial brachydactyly syndrome which shares clinical features with 15qter deletion syndrome. To the best of our knowledge, this deletion is the smallest among reported cases. It is considered that the case presented here significant contribution to phenotype-genotype correlation in 15q deletion patients.

P11.034-M
Association between HLA-G and non-syndromic Oral Cleft

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Non-syndromic oral clefts (nsOCs) affect 1 per 1,000 live births worldwide. There is some evidence suggesting that embryo-maternal interaction can play a relevant role in the etiology of nsOCs. HLA-G has a protective function at the maternal-embryo interface, protecting the embryo from destruction by mother's immune system.
In this study we investigated the association between a functional variant in HLAG gene and the risk of nsOCs. A 14 nt insertion in the 3'UTR of HLAG was genotyped in a group of 222 Italian nsOC trios, including affected children and their parents.
The analysis of transmission disequilibrium resulted in no evidence of association in nsCL/P (p=0.16) nor in nsCP trios (p=1.00). However, when considering the length of pregnancy, a significant overtransmission of the insertion was observed in the trios with pregnancy length >38 weeks (p=0.005). In trios with affected children born after 38 weeks or less, the transmission of minor allele was reverse (p=0.031), so that in the effect of the asymmetric segregation was canceled when the two groups were pooled. In children born after more than 38 weeks of pregnancy the ins/ins genotype resulted specifically associated with a three-fold increased risk of the more severe phenotype (cleft lip and palate, CLP) (p<0.0001).
This results suggests the existence of a link between HLA-G genotype, length of pregnancy and chance of developing CLP. We hypothesize that HLA-G could be involved in prenatal loss of abnormal embryos (teratothanasia) and drive selection against those which fail to fuse lip and palate.

P11.035-S
Association between chromosome 8q24.21 and susceptibility for nonsyndromic cleft lip with or without cleft palate in Iraqi population

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Cleft lip without or without cleft palate (CL/P) are the most common craniofacial congenital malformations. Most of CL/P cases are non-syndromic (nsCL/P), with a multifactorial etiology. Recent genome-wide association studies (GWAS) have identified susceptibility loci for nsCL/P, among which one of the most relevant is located in 8q24.21. In particular, the polymorphism rs987525 has been shown to be highly associated with nsCL/P in population of European origin, while yielding only marginal significance in Oriental populations.
We present the result of case-control study of a sample of Iraqi population from Nasiryiah: 418 affected children, and 273 unaffected controls. The rs987525 C>A variant was genotyped using TaqMan assay. The frequency of minor allele (A) resulted significantly higher among cases compared to controls. Odd Ratio (OR) for carriers of heterozygous (C/A) genotype was 1.69 (95% C.I. 1.24-2.31), while OR for homozygotes (A/A) was 1.95 (95% C.I. 1.16-3.29). In the studied Iraqi sample the rs987525 variant acts in a dose dependent fashion, in agreement to what resulted from association studies in population of European origin, suggesting that 8q24.21 locus confers risk for nsCL/P also in Middle-East population.

P11.036-M
Haploinsufficiency of MEIS2 is associated with orofacial clefting and learning disability

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MEIS2 is a homeodomain-containing transcription factor of the TALE superfamily that has been proven important for development. We confirm and extend a recent single case report stating that deletions in MEIS2 can cause cleft palate (Crowley et al, Am J Med Genet 2010, 152A:1326-7). Here we report five additional cases with 15q14 deletions of sizes 0.6, 0.6, 1.0, 1.9 and 4.8 Mb, respectively, all involving MEIS2. In addition, we present a family with four affected individuals and an intragenic 58 kb direct duplication disrupting MEIS2. In total, 7/9 cases had clefting, from mild (submucous cleft palate) to severe (cleft lip and palate), and 3/9 cases had ventricular septal defects. All cases had delayed motor development and most had learning disability, at worst in the mild intellectual disability range. Our results show that MEIS2 clearly is a gene needed for palate closure. In syndromic cases of cleft palate, MEIS2 should be considered among the candidate genes, e.g. in cases without 22q11.2 deletions.

P11.037-S
Familial Beckwith-Wiedemann Syndrome: Follow up

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Introduction: Beckwith- Wiedemann Syndrome (BWS)(OMIM 130650) is a genetic disorder, caused by mutation or deletion of imprinted genes within the chromosome 11p15.5 region; with a prevalence of 1 in 13,700 births and characterized by overgrowth, macroglossia, organomegaly, exomphalos and predisposition to embryonal tumor development. **Objective:** To present a BWS familial case follow up. **Case Report:** Family data: Mother with 4 pregnancies, she presents ear pits. Case 1: 9 years 7 months female, product of the 2nd pregnancy term, obtained by caesarian section from non-consanguineous parents, with 28 years (he) and 29 years (she) at birth time. Apgar 9-10, weight and height >pc97, macroglossia, depressed nasal bridge, posterior helical ear pits and exomphalos surgically corrected, presented neonatal hypoglycemia. At first year of age presented overgrowth, renal ultrasonography reported right pyelic duplication. At 3 years was performed transversal and anteroposterior reduction glossectomy. At 8 years hemihypertrophy was detected. At present she has no complications. Case 2: 3 years 9 months female, product of the 4th pregnancy, obtained by cesarian section at 30 weeks, weight at birth 2500g. Physical examination: weight and height > pct 97, posterior helical ear pits, macroglossia, umbilical hernia; has not required surgical procedure. Renal ultrasonography without abnormalities. Actually with weight and height > pct 97. **Conclusion:** Clinical findings meet criteria diagnosis for BWS. In case 1 treatment consisted of surgical reduction of exomphalos and anterior-transversal reduction glossectomy and case 2 not required surgical intervention. The opportune diagnosis allows a complete treatment, genetic counselling and appropriate follow up.

P11.038-M
Detection of PIK3CA somatic mutations in CLOVES syndrome

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CLOVES syndrome (congenital lipomatous overgrowth, vascular malformations, epidermal nevi, and skeletal abnormalities (MIM 612918) is a non-hereditary regional overgrowth disorder distinct from Proteus syndrome. Gain-of-function mutations in PIK3CA have been identified in affected tissues demonstrating somatic mosaicism of varying degrees. Activating PIK3CA somatic mutations have also been shown in further regional overgrowth conditions predominantly affecting the limbs like in isolated macrodactyly and fibrolipomatous gigantism. In the current study, hot spot mutations of PIK3CA were identified in six patients presenting with CLOVES syndrome. Analysed tissues included epidermal nevi, skin, fatty tissues, and connective tissue. Blood was available from five patients. Molecular analysis was performed for all six patients by Sanger sequencing. Mutant allele ratios of 30-

50% were observed in scrapings from epidermal nevi or affected fatty tissue samples. In none of the blood samples PIK3CA mutations were detected. Because detection levels and quantification of mutant alleles were limited to 10-15% by Sanger sequencing, fragment analysis and amplicon-deep sequencing on a GS Junior platform (Roche) were applied. This increased the mutant allele detection to 1-5%. In blood samples mutant allele ratios were 2% or less. Our data confirm that cells from affected tissue are essential for mutation analysis in CLOVES syndrome whereas blood is an inappropriate source. Improved detection methods may be required for other tissues with low level somatic mosaicism.

P11.039-S
Endocrinological study in a new case of Coffin Siris syndrome due to ARID1B gene deletion

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Coffin Siris syndrome (CSS) is a rare congenital anomaly syndrome (MIM135900), characterized by developmental delay with severe speech impairment, growth deficiency , coarse facial features, hypertrichosis, hypoplastic/absent fifth fingernails or toenails. Some CSS patients have mild microcephaly and hypoplasia/agenesis of the corpus callosum.
In 2012 Santen et al. showed that haploinsufficieny of ARID1B gene causes CSS and Tsurusaki et al. demonstrated that CSS can be due to de novo germline heterozygous mutations in one of five SWI/SNF subunit genes SMARCB1,SMARCA4, SMARCE1, ARID1A and ARID1B.
We describe a girl of four years of age, first daughter of healthy unrelated parents. She was born by caesarean section for IUGR, at 39 wks of pregnancy. Out first examination at the age of 18 months showed psycho-motor delay with absent language. Her weight, length and OFC were between 25th and 50th centile for age, but her facial and extremities features were suggestive of CSS. She had marked body hypertrichosis and moderate, non familial, joint laxity, usually not described in CSS phenotype.
Array-CGH analysis revealed a de novo 1,3 Mb interstitial deletion in 6q25.3, including ARID1B gene,
Because few hormonal data in CSS have been reported so far, we performed an extensive endocrinological study. Biochemical measurements showed normal values of electrolytes, venous blood gas, creatinine, glucose, fT4,TSH, aldosterone, renin activity,1-OH progesterone, DHEAS, cortisol, ACTH and normal slow response of cortisol and 17-OH progesterone to ACTH test. These results suggest that pituitary-adrenal axis anomalies are not responsible for hypertrichosis in CSS.

P11.040-M
Cohen syndrome is associated with major glycosylation defects

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Cohen syndrome (CS) is a rare autosomal recessive disorder with multisystemic clinical features due to mutations in the *VPS13B* gene, which has recently been described encoding a mandatory membrane protein involved in Golgi integrity. As the Golgi complex is the place where glycosylation of newly synthesized proteins occurs, we hypothesized that VPS13B deficiency, responsible of Golgi apparatus disturbance, could lead to glycosylation defects and/or mysfunction of this organelle, and thus be a cause of the main clinical manifestations of CS. The glycosylation status of CS serum proteins showed a very unusual pattern of glycosylation characterized by a significant accumulation of agalactosylated fucosylated structures as well as asialylated fucosylated structures demonstrating a major defect of glycan maturation in CS. However, CS transferrin and α1-AT profiles, two liver derived proteins, were normal. We also showed that ICAM-1 and LAMP-2, two highly glycosylated cellular proteins, presented an altered migration profile on SDS-polyacrylamide gels in peripheral blood mononuclear cells (PBMCs) from CS patients. RNA interference against *VPS13B* confirmed these glycosylation defects. Experiments with Brefeldin A demonstrated that intracellular retrograde cell trafficking was normal in CS fibroblasts. Furthermore, early endosomes were almost absent in these cells and lysosomes were abnormally enlarged, suggesting a crucial role of VPS13B in endosomal-lysosomal trafficking. Our work provides evidence that CS is associated to a tissue-specific major defect of glycosylation and endosomal-lysosomal trafficking defect, suggesting that this could be a new key element to decipher the mechanisms of CS physiopathology.

P11.041-S
A Novel Frameshift mutation in the PIEZO2 gene in a Turkish pediatric patient with Distal Arthrogryposis 5A

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The distal arthrogryposis (DA) are characterized by congenital contractures of two or more different body areas without a main neurological and/or muscle disease. Currently, DAs are subdivided into 10 types, depending on the number and nature of additional features. DA5 is unique among DAs because, in addition to contractures, affected individuals have ocular abnormalities. These abnormalities include ptosis, ophtalmoplegia, and/or strabismus. Recently, it has been shown that a subtype of DA5 that includes restrictive pulmonary disease is caused by gain-of-function mutations in the mechanically activated cation channel PIEZO2. We report 14 years old boy with DA5 who initially presented with multiple contractures at the age of 2 months. The main clinical findings included pitosis, ophtalmoplegia, micrognathia, clinodactyly, pectus excavatum, recurrant pulmonary infections, operated inguinal hernia/midpenil hypospadias, elbow extansion/bilaterale wrist supination restriction, knees lightly in flexion moode and pes valgus. Sequencing of the PIEZO2 gene in the proband revealed a de novo one base-pair deletion in Exon 52 of PIEZO2, which results in a frameshift mutation (c.8208delA). The mutation leads to a p.Y2737Ifs*7 change within the C terminal domain of PIEZO2. Recently electrophysiologic studies of E2727del variant showed that mechanically activated current inactivation were clearly slower and E2727del channels spend about two fold less time in an inactivated state following mechanical stimulation than in wild channels, thus can be reactivated quicker. Therefore because our pathologic variant is at the same domain as E2727del, it can be speculated that increased response to mechanical force may explain the phenotype of our patient also.

P11.042-M
The contribution of discrepant DNA variations in discordant monozygotic twins with Congenital Diaphragmatic Hernia (CDH) or Esophageal Atresia/ Tracheoesophageal Fistula (EA/TEF).

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Congenital Hernia of the Diaphragm (CDH) and Esophageal Atresia with/or without Trachea-esophageal Fistula (EA/TEF) are congenital anomalies that can either be present as isolated anomaly or in association with other birth defects. Both anomalies likely have a multifactor etiology, are associated with known (genetic) syndromes and can occur in combination with specific chromosomal aberrations, Copy Number Variations (CNV) or mutations. When evaluating the genetic component of a disease twin studies help to elucidate potential causal or predisposing genetic factors. Monozygotic (MZ) twins are believed to have the same genetic content and share the same environment during development. We hypothesize that *de novo* mutations arisen early in embryonic development could explain the phenotypical differences in discordant MZ twins. In total of six EA/TEF and four CDH discordant MZ twins are characterized with SNP-arrays and exome-NGS in order to detect, complete or mosaic, DNA discrepancies. We could not detect any CNV (mosaicism) differences in these twins. Both SNP-array genotyping and exome-NGS variant calling with the Genome Analysis ToolKit revealed numerous discrepant SNPs and InDels. Visual inspection of hundreds events with Illumina's Genomestudio and the Broad institute's IGV indicated that most discrepancies were actually false positive differences due to technical limitations, analysis settings (thresholding) or limitations of the variant calling. Therefore, we compared different exome capturing and alignment techniques in addition to various variant callers, statistics and analysis stra-

tegies to determine if we could distinguish false positive differences from actual ones. Currently, we are evaluating with whether these remaining discrepancies are true differences.

P11.043-S
Associated noncardiac congenital anomalies among infants with congenital heart defects

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BACKGROUND: Although the majority of congenital heart defects (CHD) occur in isolation, a significant number varying in previous reports from 6 to 66% occur with noncardiac anomalies. The purpose of this investigation was to assess the prevalence and the types of associated anomalies in infants with CHD in a defined population. METHODS: The associated anomalies in CHD were collected during 26 years in 346,831 consecutive births of known outcome. RESULTS: Of the 4005 infants with CHD (116 per 10,000), 1055(26.3 %) had associated anomalies. There were 354 (8.8%) patients with chromosomal abnormalities including 253 trisomies 21, and 99 (2.5%) nonchromosomal recognized dysmorphic conditions. There were no predominant recognised dysmorphic conditions, but VA(C)TER(L) association. However, other recognised dysmorphic conditions were registered including Di George and Noonan syndromes. 15.0 % of the patients had multiple congenital anomalies, non syndromic, non chromosomal (MCA). Anomalies in the musculoskeletal, the urinary tract, the digestive, and the central nervous systems were the most common other anomalies. CONCLUSIONS: The overall prevalence of associated anomalies, which was one in four infants, emphasizes the need for a thorough investigation of infants with CHD. The most commonly associated major noncardiac anomalies involved the musculoskeletal system, followed by the urinary, the digestive, and the central nervous systems. A routine screening for other anomalies may be considered in infants and in fetuses with CHD. One should be aware that the anomalies associated with CHD can be classified into a recognizable anomaly syndrome or pattern in one out of nine infants with CHD.

P11.044-M
Informational-analytical system of registration, systematisation and counting of congenital and hereditary diseases

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Creation of an informational system of congenital and hereditary diseases's registration is very acute in today's world, which provides the ability to schedule medical - diagnostically and preventive measures to reduce infant morbidity and mortality.

Primary source of information is the patient's electronic registration card, which includes personal data, life history and disease, genealogical information, a description of the phenotype, the results of research. Information system consists of four main sections: congenital malformations, prenatal diagnosis, chromosomal abnormality, monogenic pathology.

The database contains information about 2685 cases of patients and fetuses with congenital and hereditary disorders. The structure is dominated by pathology congenital malformations - 1323 (49.3%) cases. The most frequent defects of congenital malformations: the nervous system's - 236 (17.8%), multiple malformations - 191 (14.4%), the genitourinary system's - 183 (13.8%), the circulatory system's genitourinary system's - 169 (12.7%). Chromosomal abnormality was 744 (27.7%) cases. Monogenic pathology was defined in 618 (23.0%) cases. Most frequent are osteogenesis imperfecta - 43 (6.9%), chondrodystrophy - 36 (5.8%), congenital adrenal syndrome - 28 (4.5%), congenital hypothyroidism - 23 (3.7%), mucopolysaccharidosis - 22 (3.5%), spinal muscular amyotrophy - 17 (2.7%), Duchenne myopathy - 11 (1.7%).

Thus, the informational registration system of congenital malformations and hereditary pathology's spectrum helps to improve health system of medical genetic counseling and allows setting the frequency and structure of congenital and hereditary diseases.

P11.045-S
CNV analysis in a cohort of 174 patients with bladder-exstrophy-epispadias complex

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The clinical presentation of the bladder-exstrophy-epispadias complex (BEEC) ranges from epispadias (E) and classical bladder exstrophy (CBE), to the most severe form, cloacal exstrophy (CE), often referred to as the OEIS complex. The birth prevalence for the complete spectrum has been reported to be 1 in 10,000 live births, with a male-to-female ratio of 2.4:1. Although the etiology for the majority of cases remains elusive, there are several lines of evidence, that de novo copy number variations (CNVs) represent a major genetic contributor.

Here we array-based molecular karyotyping in a large cohort of 174 BEEC patients, aiming to identify disease related de novo CNVs. For array-based molecular karyotyping we used the Illumina HumanOmniExpress-12v1.1 bead-chip, comprising a total number of 719,665 markers. All genotype data were analyzed by QuantiSNP using an Objective-Bayes Hidden-Markov model. To narrow down the computed number of 13,828 putative CNVs, we used different filter criteria and implemented various procedures for data analysis.

In total, 17 putative disease related CNVs ranging from 2,52 kb to 6,08 Mb in size, including one duplication in the Cat eye syndrome relevant region (22pter-22q11.21) remained. Validation of the CNVs and testing for their de novo occurrence with parallel investigation of the parents using quantitative PCR and MLPA is currently performed. Array-based molecular karyotyping furthermore identified triple X syndrome in an isolated CBE patient.

P11.046-M
New point mutations in the HDAC8 gene: Cornelia de Lange syndrome and beyond

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Cornelia de Lange syndrome (CdLS) is a neurodevelopmental disorder caused by mutations in either regulators (NIPBL, HDAC8) or structural elements (SMC1A, SMC3, RAD21) of the cohesin complex. As regards X-linked CdLS, a higher prevalence of females is described for both SMC1A, partially escaping X-inactivation, and HDAC8, which is instead subject to X-inactivation. At present, 26 different mutations in 41 individuals with features partially overlapping with CdLS have been reported in the HDAC8 gene, confirming the key role of this lysine deacetylase in the proper functioning of the cohesin complex. We analyzed a group of NIPBL- and SMC1A-negative patients with CdLS by classical sequencing approaches and exome/gene panel next generation technology. Thus we identified eight de novo HDAC8-mutations. Interestingly, one of which is shared by two siblings. The R166* nonsense mutation, the frameshift deletion F207Nfs*2 as well as all six missense mutations C153R, N156K, P257L, T280I, C287Y, G320R all affect highly conserved residues and were predicted to be damaging by four bioinformatics algorithms. All patients show a mild to severe phenotype overlapping with CdLS; the craniofacial appearance is similar but with some distinctions like a broader nose, dental anomalies and delayed anterior fontanelle closure. Moreover, postnatal growth retardation is less severe, and limb malformations have not yet been observed. Given the high percentage of HDAC8 mutations identified in our cohort, we suggest the HDAC8 screening as an essential part of the routine molecular diagnostics for patients with CdLS-overlapping features.

P11.047-S
A new prognostic index of severity of intellectual disabilities in Cornelia de Lange syndrome

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Cornelia de Lange syndrome is a well-known multiple congenital anomalies/mental retardation syndrome with genetic heterogeneity and wide clinical variability, regarding the severity of both the intellectual disabilities and the physical features, not completely explained by the genotype-phenotype correlations known to date.
The aim of the study was the identification of prognostic features, ascertainable precociously, of a better intellectual outcome and the development of a new prognostic index of severity of intellectual disabilities in CdLS patients.
In 66 italian CdLS patients aged 8 years or more, we evaluated the association of the degree of mental retardation with various clinical parameters ascertainable before 6 months of life and with the molecular data by the application of cumulative regression logistic model. Based on these results and on the previously known genotype-phenotype correlations, we selected 7 parameters to be used in a multivariate cumulative regression logistic model to develop a prognostic index of severity of intellectual disability.
In the table the parameters selected and their relative scores.

Parameter	Score
Small for gestationa age	1,5
Length <50° centile on CdLS growth charts	2
Heart malformation	1
Limb reduction	1
Moderate-severe sensorineural hypoacusia	3,5
NIPBL truncating mutation	0
SMC1A mutation	1,5

The probability of a mild MR increases with the reducing final score less than 2, the probability of a severe MR increases with the increasing final score more than 3.
This prognostic index allows to define, precociously in the life of a baby, the probability of a better or worse intellectual outcome in CdLS patients.

P11.048-M
SNP arrays in the diagnostic strategy of corpus callosum agenesis associated with intellectual disability (an update)

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Corpus callosum agenesis is the most common cerebral malformation in patients with intellectual disability (CCA-ID) with a prevalence of 2-3% of cases. Known genetic causes are heterogeneous and in the majority of cases, no etiologies have been found. In order to achieve a genetic diagnosis, we performed chromosome analyses on microarrays (CMA) on 81 patients with CCA-ID and no known causes. We found 34 different CNVs (42%) which were not carried in control subjects of the Database of Genomic Variants (DGV). Among these CNVs, 14 (17%) were de novo and considered to be likely pathogenic, with sizes varying from 1,3Mb to 24Mb, including 13 deletions and one inverted duplication with terminal deletion. Moreover, 12 CNVs (4 deletions and 9 duplications) were also carried by healthy parents, and therefore, could not be considered as the main causes of the phenotype. We were not able to recover blood samples of the parents to verify the 8 remaining CNVs. Thus, CMA seems to be a powerful tool in the diagnostic strategy of patients with CCA-ID and no etiologies. However, most of the tested patients still remain with no identified genetic causes. In the near future, new techniques such as exome sequencing, or massively parallel sequencing on selected genes panels, could improve the detection rate of the genetic causes of CCA-ID.

P11.049-S
Molecular Diagnostic Algorithm of Syndromic Craniosynostosis

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Craniosynostosis(CS) is a birth defect, with a prevalence of 1/2100-1/2500, caused by the premature fusion of one or more cranial sutures leading to specific cranial base and vault abnormalities. It is a highly heterogeneous group of disorders occurring both in syndromic and non-syndromic forms, associated with approximately 180 different syndromes. The identification of the responsible gene largely depends on the fact if it is syndromic or non-syndromic. Although 85% of the cases are reported to be non-syndromic with unknown etiology, syndromic forms arise from chromosomal anormalies or single gene defects of Mendelian inheritance, both together comprising the etiopathogenesis only in 40% of the cases and single gene defects contributing to three/fourth. Noteworthy genes in this group are FGFR1, FGFR2, FGFR3, TWIST1, EFNB1, MSX2, RAB23 and FREM1. EFNB1 can be excluded from this group due to its association with Craniofrontonasal Syndrome. Thirty syndromic CS patients with normal karyotype were included in the study cohort. Stepwise screening algorithm was applied, initial step being the sequencing of FGFR2, FGFR3 and FGFR1, followed by full gene sequencing of FGFR2 and FGFR3. Samples with unidentified etiology were further screened for deletion/duplication by craniofrontonasal MLPA kit (P080). The last step consisted of sequencing of FGFR1, MSX2, TWIST1, RAB23 and FREM1 genes, when the cases showed distinct related clinical phenotype.
We highly suggest that our ongoing research will lead to better insight for the clinical diagnosis, molecular diagnostic flow charts in CS and will contribute to the genotype-phenotype correlation.

P11.050-M
Craniosynostosis and Heart defects: Possibly a new autosomal recessive syndrome due to ZDHHC13 mutations

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We report two sisters with congenital craniosynostosis, heart anomalies and minor digital defects. They were born to non-consanguineous Hispanic parents. The mother is macrocephalic, but without other dysmorphisms. The first child was born with complex craniosynostosis involving the metopic, left coronal, and left lambdoidal sutures requiring two craniotomies and leading to significant cranial deformity despite the surgeries. In addition she had truncus areriosus with interrupted aortic arch that was successfully repaired. The second girl presented with metopic craniosynostosis and large atrial septal defect, both requiring surgeries. Both girls have apparently normal development at 6 and 4 years of age, respectively, but no formal evaluation has been performed. In addition, the older child has minor digital anomalies and mild pectus excavatum and the younger child has mild enamel hypoplasia. Their older brother has severe pectus excavatum, but no other anomalies. Chromosomal microarray and sequencing analyses of the hot-spot regions in FGFR1, FGFR2, FGFR3, and the TWIST gene were normal for both girls. There were no detectable metabolic abnormalities. Whole exome sequencing documented rare compound heterozygous variants in the ZDHHC13 genes in both children - c.629A>T (Asn219Ile, maternal inheritance) and c.1135A>G (Ser379Gly, paternal inheritance). The ZDHHC13 (zinc finger, DHHC-type containing 13) gene is located on ch.11p.15.1 and has palmitoyltransferase activity with possible role in the FGFR/RAS/MAPK pathway. The functional significance of these variants is under investigation. We propose that this condition represents a novel autosomal recessive syndrome, possibly due to mutations of ZDHHC13.

P11.051-S
Deletions in 14q24.1q24.3 are associated with congenital heart disease, brachydactyly and mild intellectual disability

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Interstitial deletions of chromosome band 14q24.1q24.3 are rare. We report on three unrelated patients with overlapping de novo deletions of sizes 5.4 Mb, 2.8 Mb and 2.3 Mb in this region. While some clinical problems such as intestinal malrotation, cryptorchidism and ectopic kidney were only observed in single patients, all three patients had mild intellectual disability, congenital heart disease, brachydactyly, hypertelorism, broad nasal bridge, and thin upper lips. It appears likely that haploinsufficiency of one or several of the 19 genes in the common deleted interval (ACTN1, DCAF5, EXD2, GALNTL1, ERH, SLC39A9, PLEKHD1, CCDC177, KIAA0247, LOC100289511, SRSF5, SLC10A1, SMOC1, SLC8A3, ADAM21P1, COX16, SYNJ2BP, SYNJ2BP-COX16, ADAM21) was responsible for these problems, but apart from SMOC1, mutations in which cause autosomal recessive Waardenburg anophthalmia syndrome, no disease associations have so far been reported for the other genes. Functional studies and a systematic search for mutations or chromosome aberrations in this region will elucidate the role of individual

genes in the clinical manifestations and will provide insight into the underlying biological mechanisms.

P11.052-M
Non-mosaic 4p16.3 deletion concomitant with low-level mosaicism for deletion at 21q11.1q21.2

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Multiple chromosome abnormalities are occasionally detected in live-born children. Furthermore, concomitant non-mosaic and mosaic chromosome aberrations are even less frequent. In the resent report, we describe a case of autism, moderate intellectual disability, epilepsy, cerebral palsy, osteoporosis, strabismus, speech delay, cardiac defect, enlargement of the left brain ventricle and kidney abnormalities in a 9 year old girl. Cytogenetic analysis has demonstrated a mosaic deletion of chromosome 21: 46,XX,?mos del(21)(q21q21)[5]/ 46,XX[15]. Molecular cytogenetic analysis using oligonucleotide array CGH has confirmed the presence of mosaic deletion spanning 21q11.1q21.2 chromosome region (11.423 Mb). Additionally, a non-mosaic deletion at 4p16.3 (size: 3.712 Mb) affecting 83 genes, 40 of which are listed in OMIM was found. Mosaic 21q11.1q21.2 was also confirmed using multicolor chromosome banding (MCB), which has shown this deletion to affect 19% of cells. It is to note, that the index case has demonstrated a phenotype atypical for 4p16 deletions. Nevertheless, the main phenotypic outcome was likely to result from non-mosaic 4p16.3 according to bioinformatics analysis, whereas mosaic 21q11.1q21.2 was concluded to be an additional co-factor modulating the phenotype. Thus, one can conclude that phenotypic heterogeneity of recurrent chromosome aberrations can be produced by concomitant genomic rearrangements. In this instance, multiple molecular cytogenetic techniques are warranted for the appropriate molecular diagnosis. Supported by the Russian Federation President Grant (MD-4401 .2013.7).

P11.053-S
Juvenile polyposis associated to hereditary hemorrhagic telangiectasia in an adolescent with complex chromosomal rearrangement and intellectual disability

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Juvenile polyposis can be associated to hereditary hemorrhagic telangiectasia (HHT) due to *SMAD4* gene mutations. We describe the first case of juvenile polyposis and HHT in a patient with *SMAD4* gene loss due to a chromosomal deletion. The male patient presents moderated intellectual disability, limited verbal language repertoire, attention-deficit/hyperactivity disorder, corpus callosum agenesis and brain arteriovenous malformation. Colonoscopy revealed inflammatory intestinal polyposis, with typical juvenile polyp histology, fulfilling juvenile polyposis syndrome clinical criteria. Three small haemangiomas in scalp and bilateral telangiectasias in Kiesselbach area were found. The patient presents a 46,XY,t(6;18)(q13;q21)dn karyotype. Chromosomal microarray detected two non-continuous *de novo* microdeletions encompassing 2.7 Mb and 0,4 Mb as follows: arr 18q21.1q21.2(47,553,468-50,257,792)×1,18q21.2(50,644,595-51,052,896)×1. FISH with BAC probes confirmed both deletions and the presence of a segment between them. This is the first case of juvenile polyposis and HHT in a patient with a chromosomal rearrangement that resulted in interstitial deletions of 18q and loss of the *SMADA4* gene, among others. The loss of a copy of the entire *SMAD4* gene, added to the fact that the patient had intestinal polyps at a young age, prompted us to look for telangiectasia. Thus, we showed the importance of the detailed screening for these phenotypes in patients in whom cytomolecular studies indicate deletion of *SMAD4* gene. Additionally, we demonstrate the relevance of cytogenomic investigation in patients with juvenile polyposis, dysmorphisms and intellectual disability. Financial support: FAPESP, Brazil.

P11.054-M
Microdeletion 19p13.12 in a fetus with severe microcephaly and paraventricular cysts: case report and review of the literature

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Chromosome 19 is one of the densest chromosomes in genes. Consequently, rearrangements occurring in this chromosome, even small in size, can be lethal. This might explain why only a few cases of chromosome 19 rearrangements have been reported so far. 19p13.12 microdeletions of different sizes and partially overlapping, detected by Array Comparative Genomic Hybridization (Array CGH), have been described in nine patients. The associated phenotypes include a mild to moderate intellectual disability in seven patients. Among these patients, four of them have been reported with cerebral malformations (corpus callosum hypoplasia with vermis hypoplasia, pontocerebellar hypoplasia). Microcephaly, neurosensorial deafness, ear abnormalities, hypertrichosis or facial dysmorphism including synophrys have also been reported. All of them were diagnosed in postnatal, from the first months of life up to late childhood No prenatal case has been reported so far. We present the first case of a de novo 1.1 Mb 19p13.12 deletion including 29 genes in a fetus which has been interrupted at 38 weeks of gestation because of severe microcephaly associated with benign paraventricular cysts. Among the deleted genes, NOTCH3 seems to be a good candidate gene for cerebral abnormalities. NOTCH3 mutations are associated with CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoencephalopathy). It has been suggested that the mechanism involved in CADASIL was a gain-of-function of the mutated protein. The consequences of NOTCH3 haploinsuffisiency are poorly known. Considering its role in neurodegeneration, NOTCH3 haploinsuffisiency may contribute to cerebral malformations and to intellectual disabitility as observed in deleted patients.

P11.055-S
Molecular cytogenetic characterization of a 2q35-q37 duplication and a 4q35.1-q35.2 deletion in two cousins: a genotype-phenotype analysis

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Derivative chromosomes usually display variable phenotypes and clinical expression.
We report two patients: a 37-year-old man (proband-1) and a 17-year-old girl (proband-2), two second-degree cousins, with a derivative chromosome leading to a 4q3 deletion-2q3 duplication.
Conventional karyotype revealed in both the patients the same rearrangement derived from unbalanced segregation of parental reciprocal translocation involving the long arms of chromosome 2 and 4. Proband-1 father and proband-2 mother were detected to be carrier of a balanced translocation t(2;4)(q35;q35).
Array-CGH analysis, performed to characterize the rearrangement, documented in both the probands the presence of a 26Mb duplication of 2q35-q37.3 region of chromosome 2 and a 6.3Mb deletion of 4q35.1-q35.2 region of chromosome 4.
The 2q3 duplication and 4q3 deletion are two distinct conditions with variable phenotypes including developmental delay, intellectual disability, Pierre-Robin sequence and cardiovascular, craniofacial, digital and skeletal anomalies.
Both the patients showed developmental delay, minor facial and non-facial anomalies, hearing, ocular and genitourinary problems. In particular, proband-1 showed a severe bilateral hypoacusia and hypergonadotropic hypogonadism secondary to bilateral orchiectomy for testicular seminoma. Proband-2 displayed principally ocular (microphtalmia, coloboma and visual loss) and urinary problems (nephrotic syndrome). The clinical phenotypes were similar to that reported by Rashidi-Nezhad, who first described a patient with a combination of 2q duplication-4q deletion, and to those reported in other cases of 2q3 duplication or 4q3 deletion. Our study contributes to further delineate the genotype-phenotype correlation and the combined effect of partial 2q duplication and 4q deletion syndromes in adulthood.

P11.056-M

A novel micro-deletion 7p14.3 associated with complex neurocognitive phenotypes and distinct Cardiac malformations

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We report a 20 year old male with global developmental delay and a novel microarray finding. His past medical history included a diagnosis of supravalvular mitral ring moderate endocardial fibroelastosis at age two, following recurrent pneumonia and congestive heart failure. His neurocognitive history, in addition to global delay, also included a diagnosis of autism, attention deficit disorder and bipolar disorder with episodes of psychoses that required admission. Other medical issues included moderate bilateral hearing loss, eosinophilic esophagitis and sleep apnea. He had minor dysmorphic craniofacial features, a narrow palate and a bifid uvula, as well as hypoplastic nails. Psoriasis and striae were noted on skin exam. Chromosomal microarray analysis revealed a de novo deletion at 7p14.3p14.2 (chr7:33,453,828-36,924,450), confirmed by fluorescence in-situ hybridization. The genes in the deleted segment include BBS9, BMPER, NPSR1, DPY19L1, TBX20, HERPUD2, SEPT7, EEPD1, KIAA0895, ANLN, AOAH and ELMO1. BMPER mutations are thought to be associated with craniofacial dysmorphism. TBX20 is a transcription factor involved in the formation of cardiac chambers and valves. Other components within this 7p14.3 deletion with features of cardiac malformations and learning disabilities may also be implicated and will be discussed. By sharing these novel findings we hope to aid other healthcare providers in the care and management of similar patients.

P11.057-S

A futher case of de novo 10p14-pter deletion detected by multiplex ligation dependent probe amplification (MLPA) assay in a newborn

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Chromosome 10p terminal deletion accounts for a rare subset of patients presenting DiGeorge syndrome, and is designated as DiGeorge2 syndrome.
We report on a neonate female with DiGeorge like phenotype, facial dysmorphism and hypocalcemia in which a de novo deletion of distal 10p (p14-pter) was found by MLPA (Multiplex Ligation-dependent Probe Amplification) analysis.
Additional FISH study of the proband with locus specific probes confirmed the presence of only one 10pter signal. Further microsatellite marckers analysis in the patient and in both parents revealed a de novo deletion occurring on the paternal allele.
In conclusion, we suggest MLPA analysis as a rapid test to confirm a clinical suspect of DiGeorge syndrome associated to HDR syndrome (hypoparathyroidier, deafness, renal dysplasia) in newborns.

P11.058-M

Distal 10q26.3 monosomy; three new cases and review of the literature

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Pure distal monosomy of the long arm of chromosome 10 is a rare cytogenetic abnormality; the location and size of the deletions described in this region are variable. The associated phenotype associated with this deletion is also variable but reported features include developmental delay/learning disability/mild to moderate intellectual disability, speech and language delay, poor attention, strabismus and distinctive facial features. We report two siblings; a male aged 15 and a female aged 21 years, with intellectual disability, microcephaly, motor impairment and ataxia. MRIs showed mild dilatation of the lateral ventricles and a prominent cisterna magna. Karyotype was normal. We performed whole-exome sequencing (WES) and they were found to have a 5.5 Mb terminal chromosome 10q26.3 deletion using the program FishingCNV. The deletion was confirmed by FISH and the mother was found to carry a pericentric inversion. A third child, age 6 years, presented in early childhood with global developmental delay, poor coordination and ataxia. MRI showed no abnormality of the posterior fossa. A high-resolution (105 K) comparative genomic hybridization microarray identified a deletion of the terminal 4.6 Mb of the long arm of chromosome 10, within cytogenetic band 10q26.3. This finding was confirmed by FISH analysis. The parents were tested and this is a de novo change. Our findings add three new cases of 10q26.3 monosomy to the literature; we summarize the previous cases and highlight the features of this emerging cytogenetic syndrome.

P11.059-S

Oxidative stress a Phenotypic Hallmark of Fanconi anemia and Down syndrome: The effect of antioxidants

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Oxidative stress plays a major role in the pathogenesis of leukemia-prone diseases such as Fanconi anemia and Down syndrome. Objectives: To explore the oxidative stress state in children with Down syndrome and Fanconi anemia by estimating the levels of antioxidants (e.g., malondialdehyde, total antioxidant capacity and superoxide dismutase [SOD] activity) and DNA damage, and to evaluate of the effect of antioxidant treatment on these patients. Methods: The study included 32 children clinically diagnosed with Down syndrome (15 patients) and Fanconi anemia (17 patients) in addition to 17 controls matched for age and sex. Malondialdehyde, total antioxidant capacity, superoxide dismutase (SOD) activity and DNA damage were measured. Antioxidants including vitamin A, E and C were given to the patients according to the recommended daily allowance (RDA) for 6 months. Clinical follow-up and re-evaluation were conducted for all patients. Laboratory tests including complete blood count, karyotyping, DNA damage and oxidative stress were re-evaluated. Results: Children with Fanconi Anemia and Down syndrome had elevated levels of oxidative stress and more DNA damage than controls. Oxidative stress parameters and DNA damage improved in Fanconi anemia and Down syndrome patients after antioxidant administration. Conclusions: Early administration of antioxidants to Fanconi anemia and Down syndrome patients is recommended for slowing of the disease course with symptoms amelioration and improvement of general health.

P11.060-M

Report of the first case of robertsonian translocation in Down-Turner mosaicism (mos 45, X / 46,XX, + 21, rob (21;21)(q10;q10))

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Double aneuploidy involving both autosomal and sex chromosomes is very rare. Down's/Turner's mosaic, occurs in about 1 in 2 000 000. We report the first case of Down's/Turner's mosaic with robertsonian translocation. The patient was the first child of non consanguineous parents. It was a female whom born a term, by uneventful cesarean section, weighting 2,220g and with length of 46 cm, without neonatal complications. At birth, the pediatrician made the diagnosis of Down syndrome. At 3 months, in consultation with routine pediatric a heart murmur was heard, so the patient was referred to a cardiologist. There were performed two surgeries to repair the heart defect, one with 6 months of life and another with 2 years. She also had mild developmental delay. At 3 years old she was examined at our outpatient Genetic unit. The patient showed more clinical findings of Down syndrome than Turner syndrome: low weight and height for age, microcephaly, flat facial profile, upslanting palpebral fissures, epicanthal folds, short nose with depressed nasal bridge, hypotonia with tendency to keep month open and protrude the tongue, short neck, single palmar creases, and prominent ears. Cytogenetic analysis of peripheral blood preparations using G-banding revealed mosaicism with 2 cell lines (mos 45, X [21] / 46,XX, + 21, rob (21;21)(q10;q10)[9]). Additional genetic studies (karyotypes) were made to define the cause which probably originated this double aneuploidy with this translocation. So we present the first case related of Down-Turner mosaicism with robertsonian translocation and we review all the previous reports.

P11.061-S

Trying to define the phenotype of 16p12.2-p11.2 duplication syndrome

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The short arm of chromosome 16 is rich in segmental duplications rendering this region susceptible to rearrengement through non-allelic homologous recombination. Several syndromes resulting from microdeletions or microduplications in this region have been reported. The chromosome 16p12.2-p11.2 deletion syndrome, 7.1-to 8.7-Mb [OMIM#613604] is characterized phenotypically by dysmorphic facial features, feeding difficulties, recurrent ear infections, developmental delay and cognitive impairement. Reciprocal duplication of 16p12.2-p11.2 has been observed in few patients with dysmorphic features, short stature, developmental delay and intellectual disability but a specific phenotype hasn't been established and more cases are needed.
We report two new unrelated cases of chromosome 16p12.2-p11.2 dupli-

cation analysed by 180K oligonucleotide arrayCGH (Agilent Technologies). Both children showed a de novo 7.8 Mb duplication extending from 21.5 Mb to 29.3 Mb, comprising the same region involved in the deletion syndrome. We discuss the phenotype and molecular findings of our cases with respect to previous reported ones to further define the syndrome.

P11.062-M
Redefining the contiguous gene syndrome in the era of high-throughput sequencing

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We ascertained an Algerian consanguineous family in which two sibs present with psychomotor delay, progressive microcephaly, spasticity, thin corpus callosum, and severe and early onset obesity. Exome sequencing identified two homozygous substitutions cosegregating with the phenotype and locating 170 kb apart on 7q22.1: a c.1137+1G>T splice mutation in *AP4M1* previously described in a Moroccan family and a c.595A>T misense variation in *AZGP1* which encodes zinc-alpha2-glycoprotein (ZAG). Haplotyping analysis indicated that the *AP4M1* mutation was a founder mutation shared between both families, whereas the *AZGP1* mutation is secondarily and unique in our family.
Mutations in *AP4M1* cause AP4-deficiency syndrome, a condition characterized by severe intellectual disability, progressive microcephaly and spasticity. Notably, none of the 25 previously reported cases with AP4-deficiency syndrome exhibited obesity. On the other hand, ZAG is an adipokine stimulating lipolysis in adipocytes; ZAG likely regulates body weight since administration of human ZAG to ob/ob mice resulted in progressive weight loss. We propose that the phenotype of our patients resulted from the additional effects of the two mutations in *AP4M1* and *AZGP1* accounting for the neurological signs and the precocious morbid obesity, respectively.
The contiguous gene syndrome was proposed in 1986 to explain the association of multiple and unrelated clinical features due to the deletion of multiple adjacent genes: the phenotype results from the combination of the endophenotypes of each contiguous gene sensitive to haploinsufficiency. Today, high-throughput sequencing allows us to enlarge this concept to describe simultaneous transmission of independent mutations that are genetically linked.

P11.063-S
Insight into genetic heterogeneity of complex diseases by exome sequencing

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Exome sequencing has become a successful strategy for genetic diagnosis, particularly for largely heterogeneous diseases. We report 4 cases of example, presenting complex diseases, for which it was possible to reach rapidly a correct diagnosis only by exome sequencing.
Patient 1 was a one month-old female presenting dilated aortic root, extensive arterial tortuosity, mild dysmorphisms and several musculoskeletal features (joint laxity, arachnodactyly and pectus excavatum). Patient 2 (female, 3 years-old) presented absent pulmonary valve, ectasia of the pulmonary trunk and aorta, motor developmental delay, joint laxity, strabismus, hypermetropia and mental retardation. Patient 3 was a 3 years-old male referred for low-set ears, micrognathia, generalized hypotonia, clubfeet and tricuspid regurgitation. Finally, Patient 4 (female, 3 years-old) presented Marfanoid habitus, with aortic dilation. Because these features overlap with several neonatal rare disorders, we performed on patients' (and parents') genomic DNA, Illumina TruSight Exome sequencing, generating a mean target coverage of 98.6% at >20X and a mean read depth of 257X. In Patient 1 we identified a homozygous mutation in fibulin-4 gene, associated with autosomal recessive cutis laxa syndrome. Sequencing of Patient 2 surprisingly revealed a *de novo* missense mutation in ITPR1 gene, which causes congenital non-progressive spinocerebellar ataxia, resulting in altered development of cerebellum. Patient 3 and 4 carry *de novo* mutations in genes that cause Loeys-Dietz syndrome, TGFBR1 and TGFBR2 respectively. In conclusion, these examples are significative for stressing that exome sequencing represents a helpful approach for differential diagnosis and for more closely specify therapeutic approach for individual patients.

P11.064-M
Stable expression of mutant FANCA: is there any correlation with mild Fanconi anemia clinical?

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Fanconi anemia (FA) is an inherited disease characterized by congenital malformations, pancitopenia, cancer predisposition, and sensitivity to cross-linking agents. The molecular diagnosis of FA is relatively complex, due to several aspects including genetic heterogeneity with mutations in at least 16 different genes. In this study, we report the mutations identified in 100 unrelated FA probands enrolled into the National Network of the Italian Association of Pediatric Hematology and Oncology. We identified 108 distinct variants of FANCA, FANCG, FANCC, FANCD2, and FANCB genes in 85, 9, 3, 2, and 1 families, respectively. Particularly, in FANCA we found mainly private mutations of all different categories (large intragenic deletions, nonsense, frameshift, splicing and missense mutations). Expression level of FANCA protein was studied in 32 lymphoblastoid cell lines of complementation group FA-A and a correlation between the type of mutation and the expression level of FANCA was observed. In case of nonsense or frame-shift mutations FANCA is not detectable whereas it is expressed at the same levels as in controls when alleles are hit by missense or in frame mutations. Since it will be interesting to determine whether there is a correlation between stable expression of mutant FANCA and phenotype, we have been evaluated a series of different aspects at both clinical and cellular levels in order to provide insights into potential residual activities of altered but expressed proteins.

P11.065-S
A familial case of Fanconi anemia-related VACTERL-H association due to a mutation of *FANCF* gene, identified using a next generation sequencing approach

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VACTERL-H association is a rare and etiologically heterogeneous condition, characterized by a variable combination of many birth defects. It can represent a severe phenotype of Fanconi anemia (FA), a rare disease characterized by birth defects, bone marrow failure, cancer predispositions and increased chromosomal instability due to mutations in at least 16 genes. The genetic heterogeneity together with the wide spectrum of mutations make the molecular genetic testing in FA a complex and tiered task, which could benefit from application of next generation sequencing strategies (NGS). We describe a family with 3 aborted fetuses affected with hydrocephalia and radial ray defects, variously associated with cardiac, renal and other skeletal anomalies. Both healthy parents originate from the same alpine valley. A genetic counseling leading to a presumptive diagnosis of FA-related VACTERL-H was performed only after the second termination. Thus, a FA diagnostic diepoxybutane test could be performed only on cultured cells of the third fetus. The results confirmed the suspicion and complementation analysis excluded mutations in the two more frequent groups (FA-A and FA-G). We hence used the Ion PGM™ System and identified a homozygous c.484_485delCT mutation of *FANCF* gene. The same finding was found in DNA extracted from the first two fetuses. Only 1% of FA patients have mutations in *FANCF* and, to the best of our knowledge, the 3 fetuses represent the first cases of VACTERL-H association caused by mutations of *FANCF*. Application of NGS is suitable for a comprehensive molecular screening of FA and identification of rare disease-causing genes.

P11.066-M
Unique frontonasal dysplasia case with anencephaly with possible link to a novel gene

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Frontonasal dysplasia is a well-known developmental abnormality of the anterior neurocranium and viscerocranium, characterized by findings involving the craniofacial midline, such as anterior cranium bifidum, hypertelorism, and clefting of alae nasi. While the genetic etiology for a number of different syndromes described within the FND spectrum is identified, no gene is known to cause classical FND. Recently, patients with autosomal recessive FND were linked to homozygous loss-of-function mutations in the ALX homeobox gene family. Biallelic mutations in *ALX1* are associated with a

severe frontofacionasal dysplasia phenotype with extreme microphthalmia and oblique facial clefts. It is shown that mice homozygous for deficiency in Cart1, the mice homolog to *ALX1* are born with acrania and meroanencephaly. In the limited number of ALX1-related FND patients, neural tube closure defects have not been reported.
We report a unique FND case, a 20-week-old male fetus terminated due to anencephaly. Postmortem examination showed severe hypertelorism, clinical anophthalmia, bifid nose and midline upper lip cleft. The parents were first degree cousins, and their first pregnancy was also terminated due to anencephaly.
Array-comparative genomic hybridization using the NimbleGen CGX-3 1.4M DNA oligoarray set revealed normal results. Molecular analysis of *ALX1* did not reveal any mutations. Trio exome sequencing revealed a homozygous mutation in a candidate gene, linked to a human phenotype for the first time. While the mouse model for this gene is well described, further studies are needed to establish its role in human development.

P11.067-S
High throughput analysis in Goldenhar syndrome

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Goldenhar syndrome (GS) is a developmental disorder involving first and second pharyngeal arches during blastogenesis. It is characterized by craniofacial anomalies (oculoauriculo vertebral (OAV) dysplasia, hemifacial microsomia, facioauriculo vertebral sequence), epibulbar tumours, ear malformation and vertebral anomalies; cardiac, pulmonary, renal, skeletal, and central nervous system anomalies have also been described. The etiology of GS is not fully understood, it seems related to vascular disruption, predominantly of the stapedial and the external carotid artery, which alters the morphogenesis of structures derived from the first and second branchial arches. Although most affected individuals are isolated cases in otherwise normal families, some familial cases suggest that GS might have a genetic basis. Linkage and array-CGH analysis have detected several candidate loci for this genetically heterogeneous condition (including deletions in 1p22.2-p31.1, 2q11, 5p14, 12p13.33, 14q31.1q31.3, 15q24.1q24.2, 18p, 22q11.2, 22qter; duplications in 14q23.1, 22q11q13, unbalanced translocations, trisomies of chromosomes 7,8,9,10p, 22). No recurrent chromosomal abnormalities were identified. In our study genotyping by High Density SNP-array (HumanOmniExpress BeadChip, Illumina, CA) was performed on five GS unrelated cases. No specific CNVs were detected (the data were analyzed by GenomeStudio software Illumina, CA, and compared to the Database of Genomic Variants http://projects.tcag.ca/variation/). Whole exome sequencing is now in progress to identify possible single gene mutations potentially involved in the disease pathogenesis. Updated data will be presented and discussed.

P11.068-M
A case of Goldenhar syndrome and microrearrangements of chromosome 12

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We report a case of a 10 months boy. During pregnancy, the amniocentesis was performed after the detection of congenital heart disease, with a normal fetal karyotypel. The heart defect was characterized by situs ambiguous in destrocardia, left atrial isomerism, muscular apical ventricular septal defect, atrial septal defect in fossa ovalis. He was also affected by bilateral severe hearing loss, iris and chorioretinal coloboma in the right eye, dermoid cyst in the left eye, two preauricular tags on the left ear and one tag on the left cheek, facial asymmetry and growth retardation. SNP-array release hg19 showed a microdeletion of chromosome 12q13.3 [(57,160,163-57,317,932) x1 pat] and a microduplication of chromosome 12q22.33 [(90,562,016-91-,612,832)x3 mat]. In the duplicated region there are two genes, KERA and DCN. The protein encoded by KERA gene is a Keratan Sulfate Proteoglycan that is involved in corneal transparency. It may be important in developing and maintaining corneal transparency and for the structure of the stromal matrix. The protein encoded by DCN gene is Decorine, a component of the extracellular matrix, involved in the organization of collagens. Collagens play an important role in the cornea, which is the clear outer covering of the eye. Bundles of collagen called fibrils must be strictly organized for the cornea to be transparent. Decorin ensures that these collagen fibrils are uniformly sized and regularly spaced. The clinical features are indicative of Goldenhar Syndrome and the involvement of these microrearrangements as a contributory cause of the disease cannot be ruled out.

P11.069-S
Unusual presentation of Goltz syndrome with minimal ectodermal involvement in a 3-year-old Iranian girl

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Goltz syndrome (MIM 305600) is a rare genetic disorder characterized by distinctive skin abnormalities and a range of defects affecting the eyes, teeth, limbs, skeletal, urinary, gastrointestinal, cardiovascular and central nervous system. It is inherited in an X-linked dominant mode with lethality in males. One of the main features in this syndrome is the skin changes which are usually present at birth. Ectodermal features include symmetric linear reticulated thin skin, linear hyperpigmentation, ulcerations, telangiectasias, inflammation, hernialike outpouchings of fatty tissue, and papillomas. Here we report on a 3-year-old girl, with asymmetric involvement, greater severity of findings on the right side. She had, sparse hair, hyperkeratosis on 2/3 of the right side of the forehead, lacrimal duct stenosis, hypoplastic alae nasi, hyperkeratosis of the nose, simple ear, narrow auditory canal and hypoplastic tragus,, partial cleft of upper lip and pitting on lower lip and slight defect on tongue, hypoplastic nipple, ectrodactyly of hand and foot (on the right side). She has bilateral dysplastic nails on feet, scoliosis, syndactyly of third and 4th toes on the left side. We only had a chest X-ray from our patient which did not show striated bones. Initially Ectrodactyly-Ectodermal dysplasia was suspected and genetic testing for TP73L did not reveal any pathogenic mutation. We then sequenced PORCN gene and identified c.611T>C (p.Leu204Pro) mutation. In conclusion we present a patient with Goltz syndrome with unusual findings. Our patient did not have any of the common ectodermal, skeletal or ocular findings seen in such patients.

P11.070-M
Xq26.2q26.3 microduplication in a boy with developmental delay, distinct facial appearance and genitourinary abnormalities

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Microduplications involving the long arm of chromosome X are rare and the phenotypic consequences of functional disomy of X-chromosome genes in men remain mostly unknown. We here report on a 4-year-old boy with a Xq26.2q26.3 microduplication. The boy was born at 41 weeks after an uneventful pregnancy, with birth weight 2580g (<1st centile), length 48 cm (3rd centile), head circumference (HC) 36 cm (75th centile). He sat at 13 months and walked at 18 months. At the age of 4.5 years he spoke no words and showed some autistic-like behaviour. He had normal growth parameters (75th centile). Examination revealed hypertelorism, small, low-set ears, small mouth, clinodactyly of toes, micropenis, hypoplastic scrotum, right-sided duplicated collecting system and bilateral ventriculomegaly. Chromosomal microarray analysis (180K oligo) showed a 0.77 Mb duplication of Xq26.2q26.3, encompassing the whole coding sequence of the PHF6, HPRT1, PLAC1 genes and the first exon of the GPC3 gene. The result from the array was confirmed by MLPA analysis The duplication was inherited from the phenotypically normal mother and not detected in two healthy maternal brothers. The phenotype of the reported patient shares some features with recently reported microduplication syndrome of Xq25q26 (Møller et al., 2014), like prenatal growth retardation, genital abnormalities, digital malformations and intellectual disability, but not the short stature, microcephaly, and facial features. The smaller size of duplication with only partial duplication of the GPC3 gene is a possible cause of phenotypic discrepancies. These observations suggest that the Xq26.2q26.3 duplication represents a distinct clinical entity.

P11.071-S
Three new patients with Hamamy syndrome: expanding the phenotype

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Hamamy syndrome is a very rare, autosomal recessively inherited malformation syndrome characterized by craniofacial findings, bone fragility, conductive heart defects and sensorineural hearing impairment. Patients have common dysmorphic features such as severe telecanthus with hypertelorism, sparse lateral eyebrows, thin upper vermilion border, flat philtrum and dysplastic, protruding ears. High myopia with retinal changes, absent or dysfunctional nasolacrimal structures, teeth anomalies, mycrocytic hypochromic anemia and mild intellectual deficit may also be present.

The syndrome was clinically described as a new syndrome by Hamamy et al. in 2007, in two brothers born to double first cousin parents of Jordanian-Arabic origin. Identification of two further patients from a Turkish family led to the localization of the causative gene to 16q12.2-q21, using homozygosity mapping. By locus re-sequencing, two different homozygous missense mutations were identified in the *IRX5* gene. A member of the Iroquis family of transcription factors, *IRX5* plays an active role in face, heart, blood, brain, bone and gonad development.
We report here a clinical and molecular evaluation of three new patients carrying two novel homozygous mutations in *IRX5*, along with previously unreported clinical findings, including acute myeloid leukemia with maturation. The phenotypic and molecular features of the Hamamy syndrome patients will be reviewed to further delineate the clinical and mutational spectrum.

P11.072-M
Novel de novo heterozygous *FGFR1* mutation in two siblings with Hartsfield syndrome: suggesting gonadal mosaicism

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We describe two siblings with Hartsfield syndrome(association of holoprosencephaly, ectrodactyly, cleft lip and palate) and a novel de novo *FGFR1* mutation suggesting gonadal mosaicism. The proband presented at age 6 years for genetic evaluation.He was a product of a non-consanguineous union.Multiple congenital anomalies were detected at birth.His phenotype was consistent with Hartsfield syndrome (global developmental delay with spastic quadriplegia due to holoprosencephaly, ectrodactyly of bilateral hand and feet and bilateral cleft lip and palate).Previous genetic evaluation included normal karyotype, oligonucleotide array and single gene testing for non-syndromic holoprosencephaly (*SHH, SIX3, ZIC2, TGIF*).At the age of 6 years, exome sequencing was performed on the patient *at* BCM, *Houston,TX* and a *de novo* novel missense variant was identified in *FGFR1* (coding for fibroblast growth factor-1) on chromosome 8p12:c.1880G>C (p.R627T).This variant affects a highly evolutionarily conserved area of the gene, replacing arginine with theorine.Online prediction programs suggest this variant is a deleterious mutation.Subsequently a younger sibling was born with the same phenotype (holoprosencephaly, ectrodactyly of bilateral hand and feet and bilateral cleft lip and palate).Sequencing of *FGFR1* revealed the identical variant. We report a novel heterozygous *FGFR1* mutation in Hartsfield syndrome in two siblings, making this as the first case of familial recurrence of this rare syndrome. Both parents were negative for the sequence variant in *FGFR1*, thus suggesting gonadal mosaicism. This report also expands the phenotypic spectrum associated with loss of function mutations in *FGFR1* gene to include Hartsfield syndrome and confirms autosomal dominant inheritance of this condition.

P11.073-S
Clinical and molecular dissection of two novel cases of hemihyperplasia

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Hemihyperplasia (HH) term describes an asymmetric body overgrowth, generally due to an increased or unregulated cell proliferation, which can involve one or both sides of the body, a single limb or a half of the face. There is clinical overlap between HH and Beckwith-Wiedemann syndrome. Both conditions have been associated with molecular abnormalities of the imprinted cluster of genes at 11p15. We report on two pediatric patients with typical features of asymmetric body overgrowth. The first subject is an 8 month-old baby having only one body side manifesting HH and left asymmetric macroglossia. Polyhydramnios, umbilical hernia and omphalocele were also noted during pregnancy. Molecular testing conducted using MS-MLPA analysis identified a mosaic hypermetilation of the imprinting center IC1-H19 and hypometilation of the imprinting center IC2-KVDMR1. Uniparental disomy testing with microsatellite markers and SNP-array analysis confirmed mosaic uniparental isodisomy of 11p15 region. The second patient, a 10-year-old girl, presented a crossed asymmetry in the length of upper and lower limbs. At one year of age, she developed a left hypochondrial lipoblastoma. In this patient, MS-MLPA analysis of 11p15 region identified a mosaic KVDMR1 hypomethylation in both blood and saliva. To our knowledge, this is the first description of a lipoblastoma in a HH patient with a chromosome 11p15 region-imprinting defect.
The present results highlight the importance of performing genetic testing for 11p15 region imprinting defects in subjects with clinical diagnosis of HH. Considering the high tumor risk, a careful clinical surveillance, regardless the different methylation patterns is mandatory in these individuals.

P11.074-M
Hepatoblastoma and severe neurodevelopmental phenotype in neurofibromatosis type I: a case report and review of the literature

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A male patient presented with severe neonatal hypotonia including bulbar muscle involvement, necessitating tracheostomy. Severe feeding difficulties and gastro-oesophageal reflux led to gastrostomy placement. Hepatoblastoma was diagnosed at 5 months, and successfully treated with surgery and chemotherapy. Clinical genetic examination revealed features suggestive of a Ras-MAPK pathway disorder: relative macrocephaly, ptosis, downslanting palpebral fissures, curly hair, thickened ear helices, short stature and moderate to severe developmental delay. Cardio-facio-cutaneous (CFC) syndrome was suggested as the most likely diagnosis, due to the severity of developmental delay and one previous report of hepatoblastoma in CFC syndrome. Genetic testing of exons of *BRAF, KRAS, MAP2K1* and *MAP2K2* was normal, as was testing of *HRAS* and Noonan syndrome associated genes. Features of neurofibromatosis type I developed in later childhood: skinfold freckling and café au lait patches were present, and a plexiform neurofibroma was identified in his left arm at 10 years of age. The SureSelect 50Mb exome enrichment kit and Illumina HiSeq were used to reveal a *de novo* 4 basepair deletion in *NF1*, with no other known pathogenic variants being identified in this analysis.
The degree of neurodevelopmental delay in this patient was highly atypical for NF1. There has been only one previous report of hepatoblastoma in NF1, suggesting that this too is a rare association. These two highly atypical phenotypic aspects led to difficulties in making the diagnosis. This patient's presentation emphasises the related nature of Ras-MAPK pathway disorders and the potential of massively parallel sequencing for effective diagnosis of these conditions.

P11.075-S
13q14.2 duplication in a patient with alobar HPE phenotype associated with large ears

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Holoprosencephaly is a common developmental defect; affecting both the forebrain and the face and presenting a high mortality rate with only the minority of patients surviving after 1 year. The etiology of HPE is complex, with both environmental and genetic factors being implicated. Here we presented a girl with a classic alobar HPE phenotype associated with large ears. Investigation of copy number changes was performed by array-CGH using the whole genome Cytosure™, ISCA V2 array 4X180K (Oxford Gene Techology, OGT, UK) containing ~180.000 oligonucleotides. Result showed ~14Kb duplication at 13q14.2 (47.948.518-47.963.274pb) with partially duplication of RB1 gene (based on UCSC Genome Bioinformatis, Hg18, http://genome.ucsc.edu). Reports on partial 13q duplication are unusual and the phenotype resulting is very heterogeneous. Duplication of the 13q distal chromosomal region has been also reported in a fetus that showed dysmorphic features such as postaxial polydactyly of the right hand and left foot with short fingers, malformatation of the gut, and a micropenis with hypospadias. Cerebellar hypoplasia had been noticed at ultrasound examination in the 14-week of gestation. The main purpose of the present report is to stress the importance of array CGH in patients with midline defects, since it represents a paramount point concerning genetic counseling and management to families.

P11.076-M
Holt-Oram syndrome: new TBX5 exonic deletion leading to extreme variability among affected family members

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Introduction: Holt-Oram syndrome (HOS) is an autosomal dominant disorder characterized by upper-limb malformation in combination with congenital heart defect. More than 70% of patients who meet diagnostic criteria have an identifiable mutation in *TBX5*.
Clinical report: 32-year-old woman. Second gestation. Abortion at 20 weeks because of bilateral upper-limb amelia in a male fetus. Normal karyotype. No necropsy.
The patient had a healthy son. Her partner had required surgery to correct atrial septal defect in childhood. He displayed low-set thumbs with no major upper-limb anomalies.
In a further gestation, ultrasound detected again bilateral upper-limb amelia in a male fetus at 12 weeks. Karyotype and heterochromatic repulsion analysis were normal. The patient underwent abortion. Post-mortem examination didn´t reveal heart defects. No environmental hazards were identified.
A new pregnancy was followed closely by high-resolution ultrasound, without detecting anomalies in a female fetus. At birth, triphalangeal thumbs and atrial septal defect were present. TBX5 gene sequencing analysis revealed no mutations. MLPA showed a deletion in exon 9, not previously described. The same deletion was detected in the second affected fetus and the father, co-segregating with the disease.
Discussion: Upper-extremities abnormalities are variably expressed, even within families. Although phocomelia has been described in HOS, there are few reports of frank amelia. TBX5 analysis should be therefore considered in such finding, even in the absence of heart defect. We report on a new exonic deletion in TBX5. Upcoming expression and exome analysis will help elucidate the mechanisms and modifying factors underlying its remarkable variable expressivity.

P11.077-S
Three TBX5 gene mutations resulting in Holt-Oram syndrome

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Holt-Oram syndrome (HOS) is characterized by the congenital malformations of the heart, limbs, often accompanied with variable skeletal abnormalities. HOS-associated limb malformations usually involve absent or hypoplastic radial bone(s) and often abnormal thumb(s), which may be digitalised, absent, hypoplastic, triphalangeal or bifid. In a subset of patients, the syndrome results from TBX5 mutations. To date, about 60 different TBX5 gene lesions resulting in HOS have been identified. In this report, we describe three index cases of Polish ethnicity suspected of HOS, in whom we performed TBX5 Sanger sequencing. In all three probands we identified heterozygous TBX5 causative mutations: c.255-256delCA(p.P85fs94X), c.C524T(p.S175F), c.C668T(p.T223M). The first two mutations were novel, whereas the latter one represented previously identified variant. In the first two probands we observed severe bilateral hypoplasia of the radial bones and absent thumbs. Both probands presented with complex congenital heart defect composed of ventricular septal defect and persistent foramen ovale. The third patient was born with inborn heart defect in the form of atrial septum defect with patent ductus arteriosus and skin syndactyly of thumbs with second fingers. Upon MRI of the head, hypoplasia of corpus callosum was noted. Similar hand anomaly was observed in her father without heart involvement. Although based on a small sample, our study shows that TBX5 mutations may account for a significant proportion of HOS causative alterations and points to the importance of TBX5 mutational screening in the patients clinically suspected of this syndrome.

P11.078-M
Diamond-Blackfan anemia and intellectual disability: a new contiguous gene syndrome at 15q25.2

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15q25.2 microdeletion is an emergent CNV locus for intellectual disability, dysmorphic features and congenital anomalies. Two distinct microdeletions have been described at this locus: 1) a distal deletion (11 cases) responsible for neurodevelopmental and neuropsychiatric disorders and 2) a proximal deletion (8 cases) which is a susceptibility locus for cognitive deficit, diaphragmatic hernia and Diamond-Blackfan anemia (DBA). This proximal deletion is said to predispose to DBA because it contains the gene RPS17, encoding for a ribosomal protein, responsible for 2% of DBA. Until now, however, DBA has been diagnosed with certainty in only one case of 15q25 proximal deletion. The additional case reported here had a history of intrauterine growth retardation. Aged 18 months, the patient had a moderate developmental delay, dysmorphic features and musculoskeletal anomalies. He had a normochromic macrocytic aregenerative anemia with elevated erythrocyte adenosine deaminase activity and elevated HbF (3.2%) highly suggestive of DBA. A 15q25.2 microdeletion of 2.2Mb including RPS17 was identified using SNP array. The deletion of RPS17 was confirmed by FISH using a specific probe. The deletion was absent in the patient's father and was impossible to test in his mother. To date, only a few mutations in RSP17 have been reported in patients with DBA. Anemia was mentioned in 4 cases among the 8 previous reported cases of 15q25.2 proximal deletion but the definite diagnosis of DBA was made in only 1 case. The present report confirms that patients with 15q25.2 deletion involving RSP17 are at risk of DBA and possibly DBA-associated malignancies.

P11.079-S
Moderate intellectual disability, speech delay, strabism, pseudo Hirschprung disease and mild abnormalities of extremities in a girl with a 2q24.3q31.2 duplication.A new syndrome ?

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We report on a 15-year-old girl presenting with moderate intellectual disability, delays in speech and language acquisition, **strabism** and severe chronic constipation looking like **Hirschsprung** disease in spite of the presence of ganglion cells. Her parents are not consanguineous and in good health but her paternal half-brother presents speech difficulties and moderate intellectual disability. Conventional chromosome analysis was considered as normal, but array CGH showed a 10,2Mb interstitial duplication of the 2q24.3q31.2 region. In situ hybridization of paternal metaphases revealed a direct intrachromosomal insertion of the long segment of chromosome 2 at band q32.3 between bands 2q24.3 and **2q31.2**. The duplication observed in the patient results from an abnormal meiotic recombination of the father's insertion. Given its risk of recurrence for another child, an abnormality of the same chromosomal region has now to be searched in the girl's half brother. The function of several duplicated genes can explain the phenotype of the patient. As it is, to our knowledge, the first 2q24.3q31.2 duplication reported, additional patients are needed to improve the description of the phenotype.

P11.080-M
Comprehensive sequencing of all known Joubert genes in a large Joubert syndrome cohort in search of oligogenicity

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Joubert syndrome (JS) is a ciliopathy characterized by a distinctive hindbrain malformation, ataxia and cognitive dysfunction. It is typically inherited in an autosomal recessive manner caused by biallelic mutations in one of >20 genes, but despite the large number of causal genes, the underlying cause remains unknown in more than one third of affected individuals. Affected individuals carrying multiple heterozygous rare deleterious variants (RDVs) in known JS-associated genes raise the possibility of more complex genetics. To search for evidence of oligogenicity (defined as disease causation through combined effects of two or more heterozygous RDVs) and genetic modifiers, we used a novel molecular inversion probe-based (MIP) capture technology to sequence all known JS-associated genes in a large JS-cohort. Using a recessive model where biallelic RDVs in any of the known JS-associated genes were considered causal, we were able to determine the cause in less than two thirds of our subjects. In the remaining subjects, only a small number carried heterozygous RDVs in two or more JS-associated genes, and this proportion was not significantly different from that observed in a control population. Moreover, Sanger sequencing identified a second RDV that had been missed by MIP sequencing in several subjects, thereby establishing a recessive cause in these individuals. Thus, our data do not support the hypothesis that a significant proportion of JS cases are due to oligogenicity involving RDVs in the known JS genes. Current analyses focus on determining whether genetic burden correlates with disease severity.

P11.081-S

Multi-gene-panel diagnostics detects MKS1- gene mutations in a boy with Joubert-Syndrome

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Mutations in the MKS1 gene are known to be a major cause for Meckel-Gruber-Syndrome, a genetically heterogeneous condition and the most common form of syndromic neural tube defect . The MKS1 gene also accounts for a minor fraction of the total mutational load in Bardet-Biedl-Syndrome.
We report the phenotype of a five year old boy from Austria with episodes of apnoe during the first 7 months of life, severe hypotonia, psychomotor retardation, congenital nystagmus and a molar tooth sign detected in the MRI of the brain, which suggested the diagnosis of Joubert syndrome.
Next-Generation sequencing based multi-gene-panel diagnostics from a blood sample revealed two mutations located in the MKS1 gene: The first mutation, c.1407-7_1408_35del29, is known to be a major cause for Meckel-Gruber-Syndrome in homozygous state. The second mutation is a missense-mutation that has not yet been reported. Five bioinformatic tools predict an alteration of protein function caused by the mutation. We therefore assume that these two mutations in the MKS1 gene in compound heterozygous state are causative for the phenotype.
Mutations in the MKS1 gene are primarily reported in patients with Meckel-Gruber-Syndrome, a phenotype which denotes the most severe (usually lethal) end of the spectrum of ciliopathies with occipital encephalocele and other early embryonic malformations. Here we describe a patient with classical Joubert syndrome which underlines the genetic heterogeneity in Joubert syndrome and illustrates the pleiotropy of MKS1 mutations.

P11.082-M

Kabuki syndrome: clinical and molecular diagnosis in the first year of Life

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Objective: To review the clinical and molecular characteristics of 18 patients presenting a suspected diagnosis of Kabuki Syndrome (KS) in the first year of life, in order to outline the clinical handles leading to a prompt diagnosis of KS in newborns. Clinical diagnosis of KS can be challenging during the first year of life, as many diagnostic features become evident only in subsequent years.
Methods: All patients were clinically investigated by trained clinical geneticists. A literature review was performed using the Pubmed online database and Diagnostic criteria suggested by DYSCERNE_ Kabuki Syndrome Guidelines (2010) were used. Molecular analysis of the known causative-genes of KS, MLL2 and KDM6A, was performed through targeted resequencing, using MiSeq® sequencing platform. All mutations identified were validated by Sanger sequencing standard protocols.
Results: Facial dysmorphisms (94%), feeding difficulties (100%) and hypotonia (100%) suggested the clinical diagnosis of KS. Notably, long palpebral fissures and large anteverted ears were present in 94% and 100% of the cohort, respectively. Other abnormalities such as brachydactyly, joint laxity and nail dysplasia were present in 15 (83%) patients. Congenital heart diseases occurred in 14 (77%) cases, the most common defects being septal defects (11/18;61%) and left-sided obstructive lesions (4/18; 22%). Mutations in MLL2 gene were identified in 16/18 (89%) of the patients, while none of the patients had KDM6A mutations.
Conclusions: We present an overview of patients with KS diagnosed during the first year of life. Early diagnosis is serviceable in terms of clinical management and for targeted genetic counselling.

P11.083-S

Congenital heart defects in Kabuki syndrome, revisited after new molecular knowledges

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Kabuki syndrome (KS) is associated with CHD in 28%-80% of the cases, including left-sided obstructive lesions (LVOTOs), septal defects, and conotruncal anomalies. Our group has performed a clinical review of CHDs diagnosed in patients with KS in 2001, when molecular basis of the syndrome were unknown. At present, two causative genes for KS have been identified, the *MLL2* and the *KDM6A* genes. In this study we analyzed the prevalence of CHD, cardiac anatomic types and molecular characteristics of 41 patients with KS from a single institution. All patients underwent cardiological evaluation. Analysis of *MLL2* and *KDM6A* genes was performed through targeted resequencing, using MiSeq® sequencing platform. All mutations were validated through Sanger sequencing. *MLL2* mutations were found in 34/41 (83%), including nonsense, frameshift, in-frame duplication, and missense variants. Two/41 (5%) had *KDM6A* mutations. Additional chromosome rearrangements were detected in 3. CHDs in *MLL2* mutated patients (23/34=68%) included LVOTOs (bicuspid aortic valve, aortic coarctation, Shone complex) in 11/23 (48%), subaortic ventricular septal defect in 5/23 (22%), atrial septal defect in 4/23 (17%), abnormal pulmonary venous return (APVR) in 2/23 (9%), double outlet right ventricle (DORV) in one (4%). The patients with *KDM6A* mutation had normal heart. In conclusion, the high proportion of left-sided obstructions and the cardiac overlap with Turner syndrome (LVOTOs and APVR) have been confirmed. No specific hot spots for CHD have been identified, but mutations are preferentially located in proximal and terminal *MLL2*. Unusual CHD (DORV) is diagnosed in association with an additional chromosomal rearrangement.

P11.084-M

Hypermobility in Individuals with Kabuki Syndrome

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Kabuki Syndrome (KS; OMIM 147920) is a well-known congenital anomaly/intellectual disability syndrome caused by a mutation in the KMT2D gene (OMIM 602113). Hypermobility has been described as one of the major features of KS. However, no prevalence of hypermobility is known within the population of children with KS. On assessment of KS children in our clinic, we noticed that the degree and the pattern of hypermobility varies greatly amongst patients. Therefore, we aimed to assess the degree and pattern of hypermobility in the KS individuals.
Twenty individuals (age 3-30 years old) with KS and a known KMT2D mutation were assessed. The persons were evaluated using two systems: the Beighton and the Bulbena score.
The prevalence of hypermobility in this cohort was 25% using the Beighton score, and 45% using the Bulbena score. The difference between these two percentages is due to the items within each system and the pattern of hypermobility in the KS patients. These patients have a non-generalized pattern of hypermobility with the small joints of the hands and feet, hips and knees most affected. In contrast with the general population, boys with KS have a higher degree of hypermobility than girls with KS.
We present the data in a graphical way.
In conclusion, persons with KS have a specific non-generalized pattern of hypermobility with hips, patellae and the small joints of the hands most affected. Furthermore, boys are more severely affected than girls.

P11.085-S

Novel KDM6A (UTX) point mutations and a review of the X-linked Kabuki syndrome (KS2)

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Autosomal dominant or *de novo KMT2D* (MLL2) mutations account for nearly one-third cases of Kabuki syndrome (KS1, MIM 147920). Large deletions and nonsense or frameshift point mutations involving *KDM6A* (UTX) on Xp11.3 are another very rare cause of this condition (KS2, MIM 300867).
Here, we describe seven new patients with *de novo KDM6A* mutations, which doubles the number of reported cases with point mutations in KS2. We report, for the first time, germline missense and splice-site *KDM6A* mutations and confirm their pathogenicity via multiple lines of evidence. Combined analysis from two centres shows that less than 5% cases of Kabuki syndrome are due to *KDM6A* mutations.
Our findings enable a detailed review of the clinical features of KS2. We demonstrate that the developmental delay and learning disability in KS2 is generally moderate-severe in boys and mild-moderate in girls. Speech and cognition tend to be more severely affected than motor development. Some girls with *KDM6A* mutations may have a normal developmental profile. Similar to the commoner KS1, KS2 patients are characterized by hypotonia and feeding difficulties during infancy and poor postnatal growth and short

stature. Increased susceptibility to infections, join laxity, heart, dental and ophthalmological anomalies are common. Hypoglycaemia is more common in KS2 than in KS1. Importantly, diagnosis on facial gestalt alone may be difficult in many patients because the facial dysmorphism with *KDM6A* mutations is highly variable. Hypertrichosis, long halluces and large central incisors may be useful clues to an underlying *KDM6A* mutation in some patients.
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P11.086-M
Examining Kabuki syndrome causing mutations in Czech population

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Kabuki (make-up) syndrome (KS) is an autosomally dominant disorder caused by *de-novo* mutations and has an estimated prevalence of 1:32 000 newborns. KS1 is caused by mutations or deletions in *KMT2D* (lysine/K/specific MethylTransferase 2D) (formerly *MLL2*) gene, while KS2 is due to similar molecular defects in *KDM6A* (lysine/K/specific DeMethylase 6A) gene. Here we present molecular genetic analysis in a cohort of 14 Czech patients with clinical symptoms indicative of KS by DNA sequencing of coding regions of *KMT2D* and *KDM6A* genes, including MLPA-based analysis (kit P389-A1) of intragenic rearrangements within *KMT2D*. Mutations in *KMT2D* were detected in 6/14 (43%) of patients. All detected mutations were truncating, thereby predicting haploinsufficiency. Three mutations were previously published (c.16371_16374del, c.8743C>T, c.5627_5630del) and three are novel (c.2488G>T, c.4549_4549delG, c.6349_6350delinsA). No mutations were detected within the *KDM6A*, as well as intragenic rearrangements were not found in *KMT2D* gene (MLPA assay for *KDM6A* is not available, thus far). Our results substantiated the disease association with *KMT2D* gene. Since the clinical features of "*KMT2D* mutation-positive" cases did not differ from those, where aforementioned methods did not detect any DNA alterations, we plan to utilize next generation sequencing in an attempt to identify other loci that are potentially contributing to the genetic heterogeneity in KS.
Supported by CZ.2.16/3.1.00/24022OPPK and 00064203.

P11.087-S
A novel mutation in KAT6B in a patient with genitopatellar syndrome and some features of SBBYSS

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Genitopatellar syndrome (GPS) and Say-Barber-Biesecker-Young-Simpson syndrome (SBBYSS) are two clinically overlapping syndromes. Recently de novo heterozygous truncating mutations in KAT6B have been identified in SBBYSS, and independently also in GPS.
KAT6B encodes lysine acetyltransferase 6B, a part of histone H3 acetyltransferase complex. KAT6B is highly conserved and expressed in adult neural stem cells. Most mutations are in exon 18 encoding the acidic (A) and transcriptional activation (TA) domains of KAT6B. Genotype-phenotype correlation showed that patients with mutations in the 5´ region of exon 18 leading to loss of both domains suffer from GPS, while patients with mutations in the 3´ region leading to loss of the TA domain suffer from SBBYSS.
We present an 8-year-old girl with intellectual disability, autism and multiple developmental anomalies. She has corpus callosum agenesis, flexion contractures, hypoplastic patella, renal cysts and tracheomalacia (symptoms frequent in GPS but rare in SBBYSS), as well as blepharophimosis, lacrimal ducts stenosis, long toes, hypotonia and normal head circumference (frequent in SYBBYSS but rare in GPS) and atrial septal defect, feeding difficulties and bulbous nose (common to both syndromes).
Karyotyping and aCGH analysis of the patient yielded normal results. Whole exome and Sanger sequencing of the family trio showed de novo heterozygous truncating mutation KAT6B:NM_001256469:exon18:c.3295G>T:p.E1099X located in the 5´ region of exon 18, which should cause GPS. However, our patient manifests also many features of SBBYSS. Therefore this case demonstrates that the phenotypic overlap between GPS and SBBYSS could be broader than expected previously.
Supported by 00064203, CZ.2.16/3.100/24022 and NT/14200.

P11.088-M
Familial case of KBG syndrome caused by a novel *ANKRD11* gene mutation

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Background: KBG syndrome is an autosomal dominant disease characterized by intellectual disability, seizures, short stature, skeletal anomalies and distinct craniofacial features. It is caused by mutations in *ANKRD11* gene.
Methods: We report the cases of a 19-year-old woman and her 41-year-old mother presenting with intellectual disability, short stature and short 5th fingers. While the mother had already lost most of her teeth, we observed that her daughter had macrodontia of the upper central incisors. The diagnosis of KBG syndrome was suspected. Hence, we proceeded with mutation screen of the coding region of the *ANKRD11* gene.
Results: *ANKRD11* gene sequencing revealed a heterozygous pathogenic nonsense mutation, c.1318C>T [p.(Arg440*)], previously not described in the literature.
Conclusion: Our patients showed clinical features of KBG syndrome. The molecular analysis of *ANKRD11* gene confirmed our clinical hypothesis, which allows a more precise genetic counselling for our patients and their family.

P11.089-S
X chromosome-linked copy number variations in Klinefelter syndrome

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Klinefelter syndrome 47,XXY (KS) is the most common sex-chromosome aneuploidy in men, characterized by at least one supernumerary X chromosome. The wide spectrum of clinical manifestations in KS often varies in severity. The clinical features of KS commonly include hypergonadotropic hypogonadism, gynecomastia, small testes and azoospermia, with a varying degree of androgen deficiency, gynecomastia, cognitive dysfunction, increased central adiposity, increased height with eunuchoid proportions, and increased frequency of diabetes, metabolic syndrome, osteoporosis, autoimmune disorders and psychosocial/behavioural abnormalities. The mechanism by which the supernumerary X-chromosome determines the clinical phenotypes in KS is poorly understood, although genetic background in conjunction with the parental origin of the supernumerary X chromosome and consequently with gene-dosage effects may contribute to the variability of the phenotype and increase risk of certain diseases in KS.
In order to understand the role of X-linked Copy Number Variations (CNVs) in Klinefelter subjects, we recruited 93 patients having non-mosaic KS and 85 healthy controls. We performed SNP array analyses using the Human OmniExpress-12 Bead Chip (Illumina Inc.). By the analysis of the X chromosome, we observed CNVs patient-specific not reported in Database of Genomic Variants (DGV). Furthermore the total length of duplications and deletions, and the length of duplications and deletions per patient were significantly different from controls.
This is the first study that shows CNVs on X-chromosome in KS patients and represents an important step forward a better understanding of clinical features of this syndrome.

P11.090-M
KMT2D and *KDM6A* mutations in Kabuki syndrome

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Kabuki syndrome (KS; MIM 147920) is a congenital anomaly syndrome characterized by developmental delay, intellectual disability, specific facial features including long palpebral fissures and ectropion of the lateral third of the lower eyelids, prominent digit pads, and skeletal and visceral abnormalities. As mutations in *KMT2D* and *KDM6A* are known to cause KS, we screened 81 individuals with KS for mutations in these genes by conventional methods (n = 58) and/or targeted resequencing (n = 45) or whole exome sequencing (n = 5). We identified a mutation in *KMT2D* or *KDM6A* in 50 (61.7%) and five (6.2%) cases, respectively. Thirty-five *KMT2D* mutations and two *KDM6A* mutations were novel. Non-protein truncating-type *KMT2D* mutations were mainly located around functional domains, while truncating-type mutations were scattered through the entire coding region. The facial features of patients in the *KMT2D* truncating-type mutation group were typical based on those of the ten originally reported patients with Kabuki syndrome; those of the other groups were less typical. High arched

eyebrows, short fifth finger, and hypotonia in infancy were more frequent in the *KMT2D* mutation group than in the *KDM6A* mutation group. Short stature and postnatal growth retardation were observed in all individuals with *KDM6A* mutations, but in only half of the group with *KMT2D* mutations. The genetic basis of the patients who tested mutation-negative (20-45%) remains elusive. Further studies are necessary to understand the whole picture of the genetic aspects of KS and its genotype-phenotype relationships.

P11.091-S
Large cryptic genomic rearrangements with apparently normal karyotypes detected by array-CGH

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Chromosomal abnormalities that result in genomic imbalances, such as numerical or structural changes, are one of the most common causes of congenital and developmental anomalies. Conventional karyotyping with a resolution of at least 550 bands should be able to identify chromosomal aberrations down to 5-10 Mb. In contrast, a-CGH analysis is known to detect submicroscopic imbalances increasing the diagnostic yield to 15-20% in patients with an cognitive impairment and/or multiple congenital anomalies.
Here we report a subgroup of patients referred for multiple malformations, developmental delay / cognitive impairment, and an apparent normal standard karyotype. Whole genome array-CGH analysis (60 K, Agilent Technologies) identified six patients with large terminal complex rearrangements, all beyond the threshold of 5Mb (6 to 18 Mb). Results were suggestive for the presence of an unbalanced translocation derivative, confirmed by FISH analysis. Five were inherited from a parent with a balanced translocation, and one was apparently de novo.
Comparison of the karyotype and array-CGH showed that cytogenetic rearrangements were all almost indistinguishable from a normal karyotype, swapping similar band patterns.
Only one case was recurrent in an affected brother with the same rearrangement and an identical phenotype. Miscarriages were reported in two families.
In conclusions, large complex rearrangements involving chromosomal regions with similar size and band appearance may be missed by conventional karyotype and detected by array CGH to allow a precise chromosomal diagnosis and recurrence risk definition.

P11.092-M
Family cases of Leopard syndrome

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Background. LEOPARD syndrome is a complex disorder characterized by multiple dysmorphogenetic features. According to molecular studies, LEOPARD syndrome and Noonan syndrome are caused by different mutations in PTPN11 gene. Aims. Authors emphasize diagnosis peculiarities in two relatives with facial dysmorphism. Methods. Authors presents a 10 year-old boy admitted for airway infection symptoms. Family history: non-consanguineous parents; father and sister with face dysmorphism. Clinical exam: short stature, impaired nutritional status, axillary freckles, widespread café-au-lait spots, face dysmorphism (hipertelorism, mandibular prognathism, broad nasal root, high arched palate, large and posteriorly rotated ears, down slanted palpebral fissures), webbed neck, skeletal anomalies (thorax anomalies, bilateral wide hallux with exostosis), interdigital webs between hallux and 2nd toe, mental retardation. Results. Blood investigations didn't reveal anomalies. Cardiac ultrasound exam: no pulmonary stenosis. Differential diagnosis includes Noonan syndrome (see webbed neck, short stature), Greig syndrome (due to wide hallux, feet cutaneous sindactily), type 1 neurofibromatosis (because of café-au-lait spots, axillary freckles), Albright syndrome (see skin pigmentation). The patient was evaluated from genetic point of view: normal karyotype. Suspicion for Noonan syndrome has justified DNA sequencing that revealed mutation in PTPN11 gene (c.1403C>T, p.Met468Thr) suggestive for LEOPARD syndrome. Authors also found same mutation for probant's father. Conclusions. 1.Authors described two related cases with dysmorphic skull, skeletal anomalies, skin pigmentation, mental disabilities and short stature, justifying further genetic evaluation and revealing a very rare genetic disorder; 2.Genetic counseling and examination of other family members is important (probant's sister).

P11.093-S
Phenotype of two patients with mandibulofacial dysostosis with microcephaly (MFDM) associated with esophageal atresia and choanal atresia caused by EFTUD2 mutations

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Mandibulofacial dysostosis (MFD) causes malar and mandibular hypoplasia, cleft palate and hearing loss. Several distinct MFD syndromes are recognized, one of them is MFD with microcephaly (MFDM), sometimes associated with major defect: choanal atresia (CA), esophageal atresia (EA), kidney and heart defects. We present two unrelated patients with MFDM confirmed by EFTUD2 mutations and associated with EA and CA.
1. Female, 36hbd, birth weight-1720g. EA, cleft palate and hearing loss were diagnosed. Facial dysmorphism includes microcephaly, asymmetric face, hyperplastic supraorbital ridges, broad base of nose, retromicrognatia, microtia, preauricular tags. The patient had gastrostomy and tracheostomy. Psychomotor, somatic and speech development is delayed but social development is correct. CHARGE, facio-auriculo-vertebral spectrum and syndrome described by Megabrane et al. were considered after birth.
2. Female, 37hbd, birth weight-2400g. Respiratory distress due to CA and hearing loss were diagnosed. Facial dysmorphism includes microcephaly, microtia, asymmetry, preauricular tags, hypertelorism, narrow palate, micrognathia. Psychomotor and speech development is delayed but social contact with child is correct. CHARGE and Bohring-Opitz syndromes were considered after birth..
MFDM in both patients was suspected. EFTUD2 gene was sequenced. The mutation c.1435dup was identified in patient 1 and c1859A>T in patient 2.
The full clinical spectrum of MFDM is heterogeneous. Facial phenotype with ear abnormalities and microcephaly of presented patients was distinctive, but observed major defects in neonatal period were considered as leading symptoms and correct diagnosis was delayed. We suggest that MFDM should be taken in the differential diagnosis of child with craniofacial malformations accompanied by EA or CA.

P11.094-M
A new case of MDP syndrome caused by recurrent single-codon deletion in the POLD1 gene

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Progeroid features with concomitant cardiac, skeletal, and muscular anomalies, lipodystrophy and insuline resistance define rare genetic diseases named laminopathies, caused by mutations in genes encoding nuclear proteins.
Here, we report a case of a 13-yr-old girl, second-born of non consanguineous parents, who showed retarded growth, anemia and xerotic skin at age 1. She showed triangular facies, micrognathia, low-set and small ears and sensorineural deafness at age 8. Other clinical features included hypertrichosis, a notable subcutaneous fat loss from her extremities with accumulation at abdomen level and insuline-resistance. She also presented generalized hypotonia, severe muscular hypotrophy and joint contractures. Given that the clinical overlapping with progeroid disorders, genetic analysis of known mutated genes was performed. We identified an in frame single codon deletion (c.1812_1814delCTC, p.S605del) in POLD1 gene, encoding DNA polymerase δ, has been recently associated to a multisystem disorder named MDP syndrome, characterized by Mandibular hypoplasia, Deafness and Progeroid features, detected in other four MDP patients.
The case underlines the clinical and genetic heterogeneity of patients with adipose tissue, skeletal and muscular anomalies, associated to progeroid features.

P11.095-S
Loss-of-function mutations in MED13L cause a distinctive clinical phenotype mimicking the 1p36 deletion syndrome

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Exome sequencing (WES) has already been proven to be an effective method for the identification of the causative gene in small groups of subjects clinically selected by sharing homogenous phenotypes.
We selected a group of 8 patients with overlapping phenotype, resembling

the 1p36 deletion syndrome and with normal array-CGH (15kb resolution). By means of WES in two of them, we found two causative mutations: p.M303K in SYT1 (subject 1) and p.P1255Pfs*3 in MED13L (subject 2). The missense mutation in SYT1 involves an essential functional domain. By Sanger sequencing of both genes in the other subjects, we identified another MED13L mutation (p.S203Sfs*32) in one. All mutations were de novo. Missense mutations involving MED13L have already been identified as responsible for isolated congenital heart defects. One only literature report deals with complete or partial gene deletion, which were also associated to intellectual disability (ID). Mutations in SYT1 have never been described in humans.
Our three patients presented with moderate ID and shared facial features, including brachycephaly, horizontal eyebrows, high forehead, long eyelashes, depressed nasal bridge, mid-face hypoplasia. Additional clinical signs were epilepsy (subject 1), cleft palate, clubfoot and conductive hearing loss (subject 2) and atrial septal defect (subject 3). Patient 2 was diagnosed with acute lymphoblastic leukemia.
These results suggest that the haploinsufficiency of MED13L causes a typical phenotype that should be considered in the differential diagnosis of the 1p36 deletion syndrome. We tentatively suggest SYT1 as another candidate gene for the same clinical presentation, but it needs to be further confirmed.

P11.096-M
An interstitial microdeletion of 20q11.21 in a boy with cheilognathopalatoschisis, anorectal malformation, severe microcephaly, craniofacial features, feeding difficulty, mild growth impairment, and mild intellectual disability

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Interstitial microdeletions involving 20q11.2 are very rare with only four reported patients. We
have identified a de novo interstitial 20q11.2 microdeletion in a 7-year-old boy, clinically showing cheilognathopalatoschisis, anorectal malformation, severe microcephaly, craniofacial features (triangular face, hypertelorism, hypoplastic alae nasi, long philtrum, low set ears), feeding difficulties, mild growth impairment, mild intellectual disability (IQ67), and attention-deficit hyperactivity disorder. G-banded chromosomes were normal, cytogenomic microarray (135K Oligo, Roche) revealed a de novo 1.15 ~ Mb microdeletion at 20q11.2 [arr[hg18] 20q11.21(29,297,619-30,447,117)x1 dn]. The deleted segment encompassed 15 OMIM genes (*COX4I2, MYLK2, ASXL1,* etc). The sizes of deleted segments in four reported patients were 2.6Mb, 6.5Mb, 6.6Mb, and 6.8Mb[Iourov et al.2013; Hiraki et al.2011; Callier et al.2006;Iqbal et al.2007;]. Clinical features shared by those with > 6Mb deletion included feeding difficulty, facial features (triangular face, hypertelorism, hypoplastic alae nasi, long philtrum, and low set ear), developmental delay and/or intellectual disability, though the other with 2.6Mb had a milder manifestation. The present patient with the smallest deletion showed clinical features of previously reported patients with 20q11.2 microdeletion. Although long-term follow-up and collection of additional patients is needed to delineate the phenotypic spectrum of the condition, we propose the microdeletions at 20q11.2 to be a clinically recognizable syndrome characterized by craniofacial features, feeding difficulty, growth impairment, and intellectual disability.

P11.097-S
The clinical phenotype of 3q29 microdeletion and 3q29 microduplication syndrome in three female patients

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It has been demonstrated that whole genome scanning technologies (array-CGH) is especially suited to identify chromosome abnormalities in individuals with unclear or variable presentations. Application of this technology resulted in the delineation of several previously unrecognized microdeletion/microduplication syndromes such as recently described del3q29 (OMIM 609425; ORPHA65286) and dup3q29 (OMIM 611936; ORPHA251038) syndromes with highly variable clinical phenotypes.
The most common features of del3q29 phenotype include mild-to-moderate intellectual deficit and slightly dysmorphic facial features: microcephaly, long and narrow face/asymmetric face, short philtrum, large posteriorly rotated ears, high nasal bridge, crowded/dysplastic teeth, tapered fingers with occasionally observed autism and gait ataxia. Clinical features of dup3q29, included micro/macro-cephaly, round face, bulbous nose, short or downslanting palpebral fissures, excessive hand creases, obesity and pes planus.
Herein, we report on two unrelated female patients with different phenotypic consequences (high interindividual phenotypic variability) due to de novo deletion of an identical segment [spanning ~2.0Mb (start 195,438,699bp - 197,404,251bp)] and one female patient with de novo duplication of 3q29 which overlap this region [spanning ~1.58Mb (start 195,740,387bp - 197,317,074 bp)].

P11.098-M
Molecular analysis of ACTG2 in a cohort of patients with Megacystis Microcolon Intestinal Hypoperistalsis Syndrome suggests locus heterogeneity

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Megacystis Microcolon Intestinal Hypoperistalsis Syndrome (MMIHS) was recently classified as an actinopathy: a disease caused by mutations in ACTG2, one of the 6 human actins. ACTG2 encodes for the enteric smooth muscle actin γ-2; heterozygous de novo mutations were found in 2 MMIHS patients1. Our aim was to study a cohort of MMIHS patients to determine whether a locus other than ACTG2 could be involved. Sanger sequencing of ACTG2 was performed in a cohort of 7 patients (5 single cases and 2 families: one consanguineous family and one family originating from a genetic isolate). We identified heterozygous ACTG2 mutations in 5/5 single cases. In both families a mutation in ACTG2 was excluded. All identified mutations were non-synonymous, affecting an arginine residue. These mutations were located in different exons of ACTG2: exon 3 (R40C), 4 (R63Q) and 7 (R178C and R178H) and were considered to have a pathogenic effect by at least 2 out of 3 prediction programs. Although several sib-pairs have been published, the majority of the patients were sporadic, consistent with the presence of de novo mutations in a single gene. Interestingly, the mutation affecting residue R178 has also been reported in 2 other MMIHS patients1, suggesting that this position could play a major role in MMIHS pathogenesis. Moreover, the fact that ACTG2 mutations were excluded in the 2 families, points towards the involvement of a second locus. Whole-exome sequencing of these families should prove the involvement of another gene.
1. Thorson et al. Hum Genet 2013.

P11.099-S
Mosaic deletion of 18q in lymphocytes represents a diagnostic challenge for clinicians and underlines the importance of CMA testing in children with ID

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We report on a 2½ year-old boy with mental retardation, muscular hypotonia, deafness with atresia of the external auditory canal and a preauricular tag on the right side. The boy was referred for global intellectual disability with very limited speech capabilities and autistic traits. The pregnancy had been uneventful except for an emergency Cesarean section because of breech position. Birth measurements were normal, however, the baby was hypotonic and exhibited difficulties in feeding. At physical examination at the age of 2½ years measurements were still normal but we noted discrete dysmorphic features: long eye lashes, broad mouth with cupid bow lips, small fingers with broad short endphalanges, clinodactyly of toes V on both sides, tender translucent skin, fine hair and cryptorchidism on the right side. On the MRI at the age of 2 years delayed myelinisation was visible. As the atresia of the external auditory canal is typical for a deletion 18q we performed a karyotype on lymphocytes which was first reported with no pathological findings.
The array (2.7 Affymetrix SNP-oligo array platform) analysis revealed a deletion in mosaic form of 20.7 Mb of the long arm of chromosome 18, encompassing 69 genes in total. The mosaic was confirmed with FISH, which also revealed that the mosaic ratio in this case was 13% in cultivated blood and 81% in buccal swab. Reevaluating the karyotype the deletion was visible.
This case demonstrates the higher sensitivity of SNParrayCMA testing for mosaic aberrations.

P11.100-M
Molecular diagnostics of rare hereditary diseases using next generation sequencing

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Next generation sequencing (NGS) is a rapidly evolving method for the molecular diagnostics of hereditary genetic disorders, NGS is based on parallel sequencing, capable of reading the sequences of whole genes in a single run. Using this technology, we analysed two causative genes - NF1 (neurofibromatosis) containing 58 exons, and PKHD1 (polycystic kidney disease) with 67 exons. Neurofibromatosis is an autosomal dominant disorder of the nervous system, characterised by café au lait spots, cutaneous neurofibromas and Lisch nodules in the iris, affecting 1 in 3-5,000 people. Polycystic kidney disease is an autosomal recessive disorder, characterised by the development of cysts affecting the collecting ducts. The population prevalence is 1:85,000 individuals. Methods: DNA was isolated from peripheral blood and amplicons prepared by PCR were analysed on a GS Junior system (Roche). Data were analysed by AVA (Roche) and Sequence Pilot (JSI medical systems) software. Results: We analysed 11 patients for PKHD1, and found the following mutations: Thr36Met, Leu2128X, Ile2957Thr, Ile2331Lys, Thr36Met, Gly112Arg, Arg92Trp, Gly1712Arg, Gln1122Ser (not described) and Ser3505Arg (with unknown effect). Four patients were negative. Of 5 NF1 patients, three were negative and two patients (who were related) had a deletion of TAACTT in exon 48. Conclusion: NGS is a very useful method for the analysis of large genes with many exons, such as NF1 and PKHD1. These genes can be sequenced in a single run, instead of multiple single reactions. In the future we intend to analyse more genes using NGS, namely COL2A1 (Stickler syndrome) and USH2A (Usher syndrome).

P11.101-S
Unusual RASopathy due to a novel and severe mutation in the NF1 gene.

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We report a Caucasian man first seen at age of 25 months, referred with the diagnosis of Cerebral Palsy due to prematurity. Furthermore, he presented severe developmental delay, hypotonia, seizures, failure to thrive, dysplasia of the right optic nerve, and pulmonic stenosis. We also found dolichocephaly, open fontanel, hypertelorism, large ears with posterior pits, wide neck, mild pectus excavatum, short 4th metacarpals, and minor ridge dysplasia and radial loops in the indexes and right 3rd finger. Chromosomes and metabolic tests were normal. Because features of a RASopathy, sequence analysis of the PTPN11, RAF1, SOS1 and KRAS genes was performed, but no mutations were found. At age 21, his older sister was diagnosed with plexiform neurofibromas, requiring amputation of a leg. CT scan of the proband after accident unexpectedly showed massive plexiform neurofibromas infiltrating the spine, pelvis and extremities. Molecular testing showed an NF1 mutation, c.2326-6T>G, resulting in in-frame skipping of exon 20 (r.2326_2409del), therefore expected to result in a protein lacking 28 amino acids from within the Cystein-Serine Rich Domain (CSRD) (p.Trp777_Ala804del). This is a "private" mutation, not previously reported and observed in 1/6,500 NF1-positive unrelated patients (UAB cohort). This was also found in his sister and father who both developed spinal neurofibromas but no classic skin findings (cutaneous neurofibromas, CALMs, freckling). This family illustrates the importance of molecular confirmation in patients with minimal or no pigmentary manifestations and with features of other RASopathies. Our proband, who was exceptional despite of all his limitations, died at age 26 (January 2014).

P11.102-M
Unexpected karyotypes in patients with non-syndromic phenotype studied in the postnatal period

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We present 3 cases of patients with mild phenotypes, seen at the medical genetics clinical service of the Romagna AUSL, in which analysis of high-resolution karyotype showed rearrangements of about 10 Mb.
The first case concerns a boy who on examination showed obesity, overgrowth, flat feet, brachydactyly, reduced elbow extension, large testes and mild facial features with maxillary protrusion, but no cognitive defect. Cytogenetic analysis revealed a duplication in the long arm of chromosome 9 with karyotype: dup(9)(q32q33). The array-CGH analysis confirmed the duplication of the long arm of chromosome 9, locating it more precisely between bands q33.1 and q33.3 of 9.2 Mb not present in the mother (father not available).
The second case concerns a child who has no obvious dysmorphic features, cognitive levels borderline, anteverted nostrils, tendency to keep open his mouth, slight retrognathia, ears with large pinna and small lobe. Cytogenetic analysis revealed a karyotype: 46,XY,del(1)(q31.1q32.1) mat confirmed by the array-CGH analysis of 13Mb deletion. Mother with the identical deletion has normal cognitive profile and no relevant duymorphisms.
The last case concerns an adult come to our attention for couple infertility; karyotype analysis showed: 46,XY,del(18)(q21.3q22).ish del(18)(pter+,wcp18+,bcl2-,qter+) confirmed by FISH The array-CGH analysis confirms the deletion of about 9.4 Mb of maternal origin .
CONCLUSIONS
Generally chromosomal regions deletions of this magnitude are associated with malformations, dysmorphic features or with a pathology of the psychomotor development. However, chromosomal imbalances in specific regions can also be asymptomatic, due to the small number of dosage-sensitive genes present in these regions.

P11.103-S
RASopathy syndromes in Polish patients: a molecular study of Noonan, Cardiofaciocutaneous and Costello syndromes

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We present a group of 106 Polish RASopathy cases, including 81 unrelated patients of various age (from infancy to adulthood) with 52 different mutations in genes encoding components of the Ras/MAPK signaling pathway. About 65% of 61 unrelated patients with Noonan syndrome (NS), including 4 cases of NS with multiple lentigines (NS-ML), had mutations in *PTPN11*, 21% in *SOS1*, 12% in *RAF1* and 2% in *KRAS*. Nearly half of NS cases were family occurrences. In the group of 15 patients with cardiofaciocutaneous syndrome (CFCS) about 80% of all mutations occurred in *BRAF*, while 20% in *MAP2K1* or *MAP2K2*. The five cases of Costello syndrome had two most common mutations in *HRAS*. All identified changes were missense, 8 of them being novel substitutions in different Ras/MAPK genes, causative of NS, NS-ML or CFCS phenotype and one *RAF1* mutation so far reported only in a patient with t-AML. The aCGH studies of a group of 21 patients with Noonan-like phenotype (including cardiac defects, musculoskeletal and facial abnormalities) and no causative mutation detected in the known Ras/MAPK genes revealed in one patient a 8p23.3-p23.1 deletion and in the other a 12q12-q13.11 deletion. The molecular findings of our study mostly correspond to the data reported worldwide. We presume that our results will contribute to the existing databases of RASopathy patients and enable detailed phenotype-genotype correlations and differential diagnostics among the patients suspected of RASopathies. The research was supported by Projects: NCN UMO-2011/03/N/NZ2/00516, MNiSW PB 0056/B/P01/2008/35 and POIG.02.01.00-14-059/09.

P11.104-M
A neuronal phenotype characterized in a mouse model for Noonan Syndrome

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Constitutional dysregulation of the Ras-mitogen activated protein kinase (MAPK) signaling pathway can lead to Noonan Syndrome (NS) or similar disorders, the so-called "RASopathies", which are characterized by an overlapping pattern of physical abnormalities and cognitive impairment. Their molecular basis is an overactive Ras-MAPK signaling pathway caused by gain-of-function-mutations. In animal models, it has been shown that mutations in homologous genes can lead to impaired cognitive function and reduced synaptic plasticity. However, the molecular pathogenesis for the intellectual disability still remains unknown. This study was aimed at investigating the consequences of dysregulated Ras-MAPK signaling in neurons of a mouse model for NS expressing the oncogenic allele Ptpn11D61Y.
In the brain, reconfiguration of expression of neuronal genes represents an

important mechanism that underlies persistent activity-induced changes in the brain function in the process of neuronal plasticity. While the Ptpn11-D61Y mutation is usually found to evoke higher levels of phosphorylated ERK (pERK), we found no differences in the nuclear level of pERK comparing wild type and mutant cells under basal conditions. However, neuronal activity-driven induction of nuclear translocation of pERK was affected in the mutant neurons, suggesting a dysregulation of the activity-induced signaling. In line with this finding, we found differences in the size of total recycling synaptic vesicle pools, which is a subject of regulation during homeostatic adaptation in neurons.
The lacking response to stimulation found in the Ptpn11D61Y neurons suggest a deficit in cellular signaling underlying usage-dependent neuronal plasticity and might contribute to the intellectual disability found in patients with NS.

P11.105-S
Cryptorchidism and pulmonary stenosis as the most important features in Noonan patients with mutation in PTPN11 in Slovak population

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Introduction: Noonan syndrome (NS) is an autosomal dominant disorder which together with Cardio-facio-cutaneous syndrome (CFC), Costello syndrome (CS) and Noonan-Neurofibromatosis syndrome (NFNS) belongs to a group of genetic syndromes called 'RASopathies'. These disorders are characterised by occurance of similar phenotypic features. Among the most common are short stature, specific dysmorphic facial features and congenital heart anomalies. 'RASopathies' are caused by mutations in genes resulting in dysregulation of RAS-MAPK signaling pathway. Mutations in PTPN11 account for approximately 50% of all cases of Noonan syndrome.
Aims: The comparism of phenotype features in patients with PTPN11 mutation and without PTPN11 mutation.
Methods: Mutation analysis of PTPN11 gene was performed in 19 Slovak patients with phenotype of NS. It consisted of polymerase chain reaction and direct sequencing of 15 coding exons and exon/intron boundaries of the PTPN11 gene.
Results: We identified PTPN11 mutations in 47% of our patients (N=19) with clinical diagnosis of Noonan syndrome. Comparing the prevalence of phenotypic features in patients with PTPN11 mutation with prevalence of the same features in patients without PTPN11 mutation, we found the most significant differences in cryptorchidism (100% vs. 0%), pulmonary stenosis (78% vs. 10%), pterygium colli (0% vs. 70%) and birth length which was short only in 22% of patients with PTPN11 mutation in comparison to 80% in patients without PTPN11 mutation.
Conclusions: Our findings show that cryptorchidism, pulmonary stenosis, pterygium colli and short birth length are the most important features that distinguish patients with mutation in PTPN11 from patients without PTPN11 mutation.

P11.106-M
Noonan syndrome - the usefulness of whole exome sequencing in the identification of known and new causative genes and disease differential diagnosis.

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Noonan syndrome (NS) is a rare disease belonging to the group of RASopathies caused by the germline mutations in genes encoding proteins of RAS/MAPK signaling pathway. The majority (about 50%) of cases is caused by *PTPN11* mutations. Also mutations in *RAF1* and *SOS1* are quite common (up to 17 and 13%, respectively). The aim of the study was the identification of molecular defect responsible for the NS phenotype using whole exome sequencing (WES). Forty two patients with primary clinical diagnosis of Noonan syndrome and excluded mutation in *PTPN11*, *SOS1* and *RAF1* genes were included in the study. The WES was performed using Illumina sequencing platform. The WES analysis allowed for the identification of mutation in RASopathies-related genes in 7/42 (16.7%) NS patients. The p.Ser2Gly mutation in *SHOC2* was identified in 2 patients. The mutations in *NF1* (p.Met1035Arg, p.Tyr2556*), *BRAF* (p.Gln257Arg, p.Thr241Pro) and *KRAS* (p.Asp153Val) genes were found in 2, 2 and 1 patients, respectively. In two patients, mutations (p.Phe82Val and p.Met90Ile) in new NS-related gene *RIT1* were found and subsequent analysis of follow-up cohort identified p.Gly95Ala mutation in further two patients. In several cases, the primary NS diagnosis was refined to Kabuki syndrome (2 patients, *MLL2* mutation), Andersen-Tawil syndrome (1 patient, *KCNJ2* mutation) and Aarskog syndrome (1 patient, *FGD1* mutation). Our results support the usefulness of whole exome sequencing in the identification of known and new mutations related to specific disease as well as in the differential diagnosis. Supported from NCN research projects no. 2011/01/D/NZ5/01347 and 2013/09/B/NZ2/03164.

P11.107-S
Noonan syndrome-like disorder with loose anagen hair (mazzanti syndrome): a new case with neuroblastoma

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Mazzanti syndrome also known as Noonan-like syndrome with loose anagen hair (OMIM #607721) associates facial features resembling Noonan syndrome, cardiac defects, cognitive deficits, reduced growth generally associated with GH deficit, and hair anomalies. It is caused by an invariant mutation of the *SHOC2* gene. We report on a subject with molecular confirmed diagnosis of Mazzanti syndrome with neuroblastoma, first suspected at the age of 3 months by abdominal ultrasound.
At first evaluation he showed a round face, blond, thin and sparse hair, slightly down-slanting palpebral fissures, slightly posterior rotated ears, evident palmar creases, mild generalized hypotonia and dystrophy, especially in his face and legs. Because of severe feeding difficulty and failure to thrive he underwent an abdominal ultrasound and subsequently abdominal MRI, which confirmed the presence of a circumscribed retroperitoneal nodular lesion between inferior cava vein and aortic artery at the level of the liver (2.7 x 1.5 x 2.2 cm), hyperintense in T2 and isointense in T1, with slight enhancement with contrast. The mass was completely removed and the histological analysis documented a neuroblastoma, poorly differentiated with intermediate MKI. Tumoral cells were negative for the *MYCN* amplification and bone marrow aspirate was N-Myc negative. The patient did not require any subsequent chemotherapy or radiotherapy.
The present finding emphasizes the importance of monitoring these patients to obtain a precocious diagnosis of possible malignancies. The growing evidence of a variably increased cancer risk in apparently all RASopathies demands further studies to substantiate and specify risk figures and tumor spectrum.

P11.108-M
A de novo microduplication 3q29 in a patient with oculo auriculo vertebral spectrum

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Oculoauriculovertebral spectrum (OAVS; OMIM 164210) is a phenotypically and probably also a genetically eterogeneous disorder derived from an abnormal development of the first and second branchial arches. Main clinical characteristics include defects of the aural, oral, mandibular, and vertebral development. Anomalies of the cardiac, pulmonary, renal, skeletal, and central nervous systems have also been described. We report on a 25 year-old male with preauricular pits and tags, unilateral absence of the auditory meatus, dysgenesis of the inner ear and unilateral microphthalmia, clinical features fitting with the oculoauriculovertebral spectrum. Using SNP array analysis we identified a de novo microduplication of 723Kb on chromosome 3q29, which was absent in 52 OAVS patients and in 80 ethically matched non-OAVS individuals. This de novo microduplication was proximal to the 3q29 microdeletion syndrome region and reciprocal microduplication. The identified microduplication encompassed 9 genes including ATP13A3 and XXYLT1, which are involved in organogenesis and regulation of Notch pa-

thway, respectively. The present observation is in accordance with the hypothesis that OAVS is a genetically heterogeneous condition, underlying the importance of SNP array analysis in patients with OAVS features.

P11.109-S
Screening of CD96 and ASXL1 in twelve patients affected with Opitz C or Bohring-Opitz syndromes

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Opitz trigonocephaly (or C syndrome, OTCS) and Bohring-Opitz syndrome (or C-like syndrome, BOS) are two rare genetic disorders which show phenotypic overlap. The genetic bases of these diseases are not well understood. Two genes have previously been associated with OTCS or BOS with a dominant pattern of inheritance. Whereas the CD96 gene has been related to OTCS (one case out of 25) and to BOS (one case in 4 patients analyzed), ASXL1 (Additional Sex Combs-Like 1) has been related to BOS only (around half of the patients). In this study we analyzed CD96 and ASXL1 in a cohort of twelve individuals, including two sibs. Eight of them were diagnosed with OTCS, two displayed a BOS phenotype and another two could not be accurately diagnosed. Exome sequences were available for six patients with OTCS and three couples of parents, in which CD96 and ASXL1 were inspected using bioinformatic tools. For the remaining patients, Sanger sequencing of all exons in these genes was carried out. Detailed scrutiny of the sequences allowed identification of only one potentially pathogenic mutation in one of the patients. In this subject, affected with BOS, we identified a de novo mutation in ASXL1 (c.2100dupT). By nature and location within the gene, this insertion resembles those previously described in other BOS patients and we conclude that it may be responsible for the disease. Our results indicate that for eleven out of the twelve patients, the disease (OTCS or BOS) is not caused by mutations in CD96 or ASXL1.

P11.110-M
A case of Opitz GBBB syndrome: clinical presentation

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Opitz G/BBB Syndrome is a multiple congenital anomaly disorder affecting midline structures, characterized by variable expressivity of clinical signs. Primarly clinical features are facial anomalies (including ocular hypertelorism, broad nasal bridge and cleft lip and/or palate), laryngotracheo-esophageal abnormalities and hypospadias. Imperforate anus and congenital heart defects are also present; patients may also show developmental delay and brain abnormalities. We report the case of a patient born prematurely with cleft lip and palate. Some dysmorphic features were evidenced: hypertelorism, flat nasal bridge and bifid nasal tip, macrocephaly. Systolic murmur and mild hypospadias were noted on clinical examination. An echocardiogram defined congenital heart disease (patent ductus arteriosus together with persistent left superior vena cava and coronary sinus). During follow-up neurological examination and EEG were normal, but delay in speech and motor development emerged. MRI examination showed inferior vermis hypoplasia and small pituitary gland. Differential diagnosis among a number of clinical entities was considered, but the hypospadia and the marked hypertelorism oriented towards the diagnosis of Opitz GBBB syndrome. The analysis of the MID1 gene confirmed a partial deletion, spanning from exon 3 to the end of the gene. Opitz Syndrome is genetically heterogeneous presenting with X-linked and autosomal dominant form; the two forms cannot be distinguished on the basis of clinical manifestations. The autosomal dominant form is linked to a still unidentified gene located on a large region of chromosome 22q11.2. The X-linked one is associated with mutations in the MID1 gene located on the short arm of the X chromosome (Xp22.2).

P11.111-S
Two cases of Opitz GBBB syndrome

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Opitz GBBB syndrome is a rare congenital syndrome characterized by hypertelorism, hypospadias, cleft lip/palate, laryngotracheoesophageal abnormalities, imperforate anus, developmental delay, and cardiac defects. Opitz GBBB syndrome is genetically heterogeneous, with both X-linked and autosomal dominant forms. X-linked form of Opitz GBBB syndrome is caused by mutation in the *MID1* gene (OMIM 300552).
We here report two male cases with Opitz GBBB syndrome. One of them was referred with preliminary diagnosis of FND due to hypertelorism at the age of 14 months. On physical examination he showed hypertelorism, notched nares, bifid incisory tooth, pectus excavatus, accessory right nipple, Simian line, bilateral fifth finger clinodactyly, glandular hypospadias and hypoplastic scrotum. Clinical evaluation prompted the diagnosis of Opitz GBBB syndrome and *MID1* sequencing revealed homozygous c.1798dupC [p.His600ArgfsX12]. Since the mother had one of the major signs of the syndrome, widows peak, X-linked inheritance was suspected.
Second patient, at the age of three and a half, displayed hypertelorism and penoscrotal hypospadias on physical examination and displayed additional findings; such as sparse hair, telecantus, depressed and broad nasal bridge, micrognathia, long philtrum, v shaped upper lips, diastasis recti and umbilical hernia. He had a striking resemblance to the first one and his mother also had widows peak. He clinically diagnosed as Opitz GBBB syndrome and MID1 analysis is still pending.
We will discuss Opitz GBBB syndrome in view of the literature.

P11.112-M
Mutations in a new gene cause a novel overgrowth syndrome with macrocephaly, hypoglycemia, enlarged ventricles, mild/moderate intellectual disability and recurrent inflammatory diseases

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Overgrowth syndromes (OGS) are a group of disorders in which all parameters of growth and physical development are above the mean for age and sex. Partial, localized, or regional OGS are those disorders in which excessive growth is confined to one or a few regions of the body. We evaluated a series of 270 families from the Spanish Overgrowth Syndrome Registry with no known overgrowth syndrome. We identified a deletion and three missense mutations in a new gene in six patients from 4 families with overgrowth, macrocephaly, intellectual disability, mild hydrocephaly, hypoglycemia and inflammatory diseases resembling Sjögren syndrome. Our studies of this gene point to disruption of three important signaling pathways as the putative final effectors.

P11.113-S
An apparently balanced translocation in a boy with multiple congenital anomalies of the eye

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A 2 year old boy product of the 1st pregnancy was referred for karyotype testing because of multiple congenital anomalies, including bilateral cataracts diagnosed at birth, hypospadias and right cryptorchidia, hypotonia and failure to thrive Glaucoma was diagnosed at 3 months of age. MRI showed generalized brain atrophy. At one year of age developmental delay was noted and height, weight, and head circumference had fallen below the 3rd percentile. There was no family history of similar findings and the parents are first cousins. Karyotype showed an apparently balanced translocation 46,XY,t(3;6)(p25;q15). Karyotype of both parents was normal. Further testing for genes involved in anterior segment dysgenesis and/or congenital cataracts including PITX3, FOXE3, and CYP1B1 was done and all were negative for mutations. Sequencing the breakpoints of the translocation did not find genes at or near the direct breakpoint. Whole exome sequencing ruled out mutations in known cataract genes, but identified a homozygous mutation in the PAH gene (NM_000277:c.1139C>T, p.(Thr380Met). This mutation has been reported to cause Hyperphenylalaninemia (HPA) when combined with another deleterious mutation. Homozygosity for this mutation has not been reported previously, to our knowledge. We are confirming this result by Sanger sequencing and analysis of parental samples is ongoing. We are still investigating the link between HPA/PKU and congenital cataracts (and

reviewing variants in other genes in the WES data), but untreated PKU has been clearly linked to microcephaly and learning disabilities.

P11.114-M
New severe learning difficulty syndrome with skeletal features due to de novo missense mutation in the polycomb group ring finger protein 2 (*PCGF2*) gene

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Two UK patients, each known to a clinical genetics service (Northern, Peninsula), were independently recruited to the Deciphering Developing Disorders (DDD) study, Cambridge. Patient 1 presented with poor weight gain and hypotonia in infancy, and subsequently global developmental delay. He had relative macrocephaly, enlarged cerebral ventricles, and normal myelination on brain MRI at 17 months. At 2½ years a skeletal survey revealed delayed epiphyseal ossification, particularly carpal bones, pseudo-epiphyses of many metacarpals, hypoplasia of L1, and thoraco-lumbar kyphosis. He has severe intellectual disability, no clear speech, drools heavily, has short tapering fingers and valgus deformity of the feet, and multiple pigmented naevi. Patient 2 presented at 6 months of age with poor weight gain. Subsequently her development was delayed with a moderate learning disability. She has relative microcephaly; an MRI scan performed at 7 years of age was normal. She developed constipation which was sufficiently severe to require an ACE procedure aged 12 years. She has fine hair, a long face with high arched palate and prognathism, long thin hands and fingers and mild generalised joint hypermobility. She does not have spine or foot abnormalities. Both subjects were found through DDD to have a de novo p.P65L (c.194C>T) missense variant in exon 4 of the *PCGF2* gene (17q12). Previously, *PCGF2* had no associated human phenotype. This identical de novo finding in 2 subjects is strong evidence for a new syndrome associated with a novel morbid gene, despite some divergence of clinical features.

P11.116-M
A nonsense mutation in BMP2 causes a syndromic form of Pierre Robin sequence

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Pierre Robin sequence is a disorder of craniofacial development comprising mandibular hypoplasia, cleft palate and glossoptosis. It can be isolated or associated with other malformations in a syndromic form, with a high genetic heterogeneity.
Here we report on a 7 years old boy presenting with Pierre Robin sequence, developmental delay, microcephaly, growth retardation, brachydactyly, deep palmar flexion creases and supra-ventricular tachycardia. Exome sequencing revealed the heterozygous, nonsense mutation c.460C>T (p.R154X) in exon 2 of BMP2 (Bone morphogenetic protein 2). Microdeletions at 20p12 encompassing BMP2 have been described in 3 other patients with similar phenotype, and have been also implicated in Wolff-Parkinson-White syndrome. Duplications of the region located downstream the BMP2 gene and containing a cis-acting enhancer element were described in patients with brachydactyly type A2. BMP2 is also known to stimulate SOX9 expression, and contribute to the postnatal development of normal growth plate and articular cartilages.
We suggest that BMP2 causes a new syndromic form of Pierre Robin sequence, associating intellectual disability, microcephaly, growth retardation, brachydactyly and risk of heart rhythm disorder.

P11.117-S
Functional validation of a novel germline mutation in the *PIK3CA* gene in a child without the typical segmental overgrowth

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We report on a 6 year boy with body overgrowth at birth, megalencephaly and motor delay, who is a carrier of a de novo heterozygous mutation in PIK3CA (NM_006218.2):c.335T>A, p.Ile112Asn. The mutation appeared germline in peripheral blood, buccal swabs and skin fibroblasts (Sanger sequencing); it has not been observed in published PIK3CA-related segmental overgrowth patients. Phosphatidylinositol-3,4,5-triphosphate immunostaining of patient cells and Epidermal Growth Factor-mediated PI3K-AKT-mTOR pathway stimulation proved that p.Ile112Asn leads to increased PI3K activity. The p.Ile112Asn change lies adjacent to the p85 (PIK3R2) regulatory binding domain on the p110 (PIK3CA) catalytic subunit of PI3K. Altered stoichiometry within the p85-p110 complex could underlie the hyperactive PI3K-AKT-mTOR signaling in this instance. Further experiments to investigate this are currently ongoing.
Interestingly, the phenotype of this child appears unique comparing to published PIK3CA-related segmental overgrowth patients. The boy did not show somatic asymmetry, focal overgrowth, capillary malformations, epidermal nevi, or digital abnormalities. His brain MRI at 6 years showed a thick corpus callosum, large cerebellar vermis and possible restricted right posterior persiylvian polymicrogyria but his cerebral cortex otherwise looked largely normal.
Our observation adds an isolated megalencephaly with mild body overgrowth to the PIK3CA-associated clinical spectrum. The identified novel mutation is among the few known germline PIK3CA mutations. We demonstrate robust and rapid functional assays to enable interrogation of PI3K-activity in this context from patient-derived cells. The constitutional distribution of PIK3CA mutation might be of prognostic advantage regarding somatic overgrowth, cortical malformations and consecutively favorable development. However, this hypothesis needs further assessment.

P11.118-M
A clinical score system as useful tool in selecting subjects with clinical presentation in the spectrum of Pitt-Hopkins syndrome

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Pitt-Hopkins syndrome (PTHS) is characterized by ID, typical facial gestalt, and additional features, including breathing abnormalities.
The overlapping phenotype of severe ID, epilepsy, and constipation makes it difficult to differentiate the PTHS phenotype from that of Angelman (AS), Rett (RTTS), and Mowat-Wilson (MWS) syndrome. However comprehensive analysis of many patients led us to define a checklist of the most consistent phenotypic manifestations of this condition, and to suggest a clinical score ≥13 as tool for enrolling patients into *TCF4* analysis first.
We analyzed a total of 176 patients, of whom 22 had a proven mutation in *TCF4* (and on average a clinical score of 14-15), and 154 did not. Of them, 53 were selected by means of the same score system, as having a clinical presentation within PTHS. These 53 subjects were grouped in two clinical categories:
1) Group A (tot 11), clinical score > 13 and facial phenotype highly consistent with PTHS;
2) Group 2 (tot 42), clinical score of 10-11; 2A (tot 16): PTHS-like phenotype that appears homogenous to large extend to each other; 2B (tot 11): PTHS-like phenotype that appear different from 2A, but homogenous as well; 2C (tot 15): with certain clinical heterogeneity. We performed semiquantitative analysis of *TCF4* mRNA in 5 subjects in Group A, and found that it was significantly increased in two. Sequencing of the promoter region and UTRs regions is ongoing. Strategies and preliminary results of the diagnostic approach in the remaining patients, including saliva analysis and exome sequencing, are presented.

P11.119-S
Xq22.1-q22.3 deletion including PLP1 gene in a girl with global developmental delay, hypotonia, and brain retarded myelination

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We describe the case of a 3 years 6/12 old girl presenting marked developmental delay, hypotonia, and dysmorphisms. She was born at term from apparently healthy, non consanguineous parents. Pregnancy and delivery were uncomplicated except for polyhydramnios. Neonatal growth parameters were in the normal range. She showed right choanal stenosis and velopharyngeal incompetence which made feeding difficult. A brain magnetic resonance imaging at age 14 months showed a retarded myelination in parietal and periventricular areas.
At last evaluation general hypotonia was still present, with global developmental delay and absent speech. Weight and height were in the upper part of the normal range, while cranial circumference was slightly under the average. She presented facial and skeletal dysmorphisms, including depressed nasal bridge, alternating esophoria, protruding tongue, pectus excavatum, hyperextensible ligaments, thoracic kyphosis, bilaterally recessed IV toes, broad halluxes.

Array-CGH analysis disclosed a de novo 5.6 Mb microdeletion of Xq22.1-q22.3 chromosomal region. The pattern of X-inactivation was markedly skewed. The microdeleted region includes 52 genes 3 of which are OMIM morbid: PLP1, RAB40AL and SERPINA7.
There have been only a few reports of whole PLP1 gene deletions. PLP1 mutations have been associated with a continuum of neurologic phenotypes from severe forms of Pelizaeus-Merzbacher disease (PMD) to spastic paraparesis. Null alleles tend to associate with milder PMD features in hemizygous males but higher rate of neurological manifestations in heterozygous females. PLP1 haploinsufficiency could explain at least part of the girl's neurological phenotype since it encodes for a primary constituent of myelin in the brain.

P11.120-M
Dissecting the genetic bases of Poland Syndrome by exome sequencing

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Poland syndrome (PS) is a congenital disorder presenting with agenesis/hypoplasia of the pectoralis major muscle variably associated with thoracic and/or upper limb anomalies. Incidence was reported to be 1/30000 births, with higher prevalence in males. Most cases are sporadic. Familial recurrence has been observed but PS genetic etiology remains to be clarified. Since PS involved structures may originate from the same embryonic tissues, genes controlling cell proliferation, migration, and differentiation of these tissues might be involved. Alternatively, one common assumption is that PS may origin from an embryonic vascular insult indicating genes controlling vessels development as indirectly involved.
We recruited a large cohort of PS patients (more than 250, about 10% familial). Karyotype analysis in 128 patients did not show any relevant alterations. ArrayCGH revealed the presence of chromosome anomalies in 19 out of 119 analyzed patients (10 duplications and 9 deletions); bioinformatic data analysis indicates gene enrichment in different pathways including those involved in cell-cell adhesion and muscle structure development. We selected 3 sporadic and 6 apparently dominant familial cases for whole-exome sequencing. Patients' parents, affected and non-affected siblings to a total of 31 subjects were sequenced. Preliminary data from one trio confirm involvement of genes regulating cell-cell and cell-matrix interaction and also indicate a possible contribution by genes implicated in vascular development. Analysis of other sequenced families is now ongoing in order to check sharing and segregation of newly identified variants between different patients, and to clarify their role in pathogenesis of PS.

P11.121-S
PTPN11 mutations in Noonan and LEOPARD syndromes: molecular spectrum, structural and functional insights on pathogenic mechanisms, and genotype-phenotype correlations

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Noonan syndrome (NS) is a genetically heterogeneous developmental disorder characterized by reduced growth, dysmorphic facial features, congenital heart defects, skeletal and hematological anomalies, and variable cognitive deficits. NS is caused by aberrant signaling through the RAS-MAPK cascade, and we previously identified *PTPN11* as the major gene underlying this condition. *PTPN11* encodes SHP2, an SH2 domain-containing protein tyrosine phosphatase functioning as a signal transducer that positively modulates RAS function. Mutations in the same gene are implicated in LEOPARD syndrome (LS), a disease clinically related to NS, or contribute to leukemogenesis. Here, we explored further the molecular spectrum of germline *PTPN11* lesions and their associated phenotype. Mutation scanning of the entire *PTPN11* coding sequence was performed in large NS/LS cohorts collected in the frame of the NSEuroNet Consortium. Among the 452 mutation-positive subjects, 68 different variants deemed to be of pathological significance, including 12 novel missense changes and 2 in-frame indels, were identified. Besides the previously characterized mutations destabilizing SHP2's inactive state or increasing binding to phosphotyrosyl-containing partners, a novel mutation cluster was recognized. Specifically, eleven changes affecting Leu^{261}, Leu^{262} and Arg^{265} were identified in ten unrelated subjects with clinical features fitting NS. Biochemical and structural characterization of these mutants provided novel insights on disease pathogenesis. Finally, these data and published records were used to define more accurately the mutational spectrum of germline and somatic *PTPN11* mutations in human disease.

P11.122-M
A paternally inherited interstitial deletion of 15q11.2 causing clinical features of PWS: refinement of the PWS-IC

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Imprinting occurs on chromosome 15q11.2-q12 by differential methylation of genes on the paternal and maternal chromosomes. Prader-Willi syndrome (PWS) is caused by the absence of paternally imprinted genes at chromosome 15q11.2, whether by deletion, uniparental disomy, or imprinting center (IC) defect. The PWS-IC is defined by the 4.1 kb shortest region of overlap (SRO) of reported deletions, which includes the promoter and exon 1 of the SNRPN gene. Mutation of the PWC-IC blocks the switch from maternal-to-paternal imprint within the male germ line.
We present an infant with a classical clinical presentation of PWS. As a neonate, he was hospitalized for severe hypotonia and required tube feeding. He has bitemporal narrowing, and typical facial features and hand morphology. Methylation-sensitive (MS) PCR across the differentially methylated CpG island of SNRPN showed amplification of only the maternal-specific product. High-resolution chromosome microarray analysis detected a copy loss at 15q11.2 which overlaps the 4.1 kb PWS-SRO; this copy loss is at least 1.6 kb in size and may be as large as 5.8 kb. MS-MLPA confirmed the deletion and showed a methylation pattern identical to that seen in other individuals affected with PWS due to paternal deletion. Familial studies showed that the father carried the mutation but the paternal grandparents did not, consistent with a *de novo* deletion occurring on the grand-maternal allele. To the best of our knowledge, this is the smallest copy loss causing PWS reported to date, and further refines the PWS-IC to a 1.8 kb region.

P11.123-S
Clinical phenotypes in patients with genomic anomaly detected by aCGH

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High resolution molecular karyotyping has been implemented recent years in our group of 11 outpatients as a diagnostic test for nonsyndromic dysmorphia or developmental delay. Genomic imbalance in different chromosomal region was detected in seven cases. Principally there were detected deletion/duplication in the range of 110 kb to 2,8 Mb. Clinical features and aCGH data will be summarised. In two patients was found a genomic rearrangments in more than one region. In one child patient with skeletal dysplasia like phenotype was identified a duplication at three different chromosomes. Genotype/Phenotype correlation in our patients with different genomic anomaly as a deletion/duplication has a direct impact on a health care managment including reproductive outcome in the family. An assesment of significance of genomic imbalance along with the incidence of benign CNV in the light of acquiring new knowledge is still a considerable dynamic process A broadening spectrum of aCGH data, clinical phenotypes in probands and their parents will be provide in the registry. Many of them may have a important role in creating potentional new nomenclature for this extremely rare genomic diseases.

P11.124-M
An alternate unbalanced recombinants of chromosome 10 due to familial pericentric inversion

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13 years old female was referred for genetic counseling because of intellectual disability (ID), short stature, microcephaly, dysmorphic features (low frontal hairline, left convergent strabismus, wide nose root, abnormal shape of the pinnas, flat philtrum, large mandible, hypodontia, malocclusion of teeth), club-foot, partial syndactyly of II-III th toes. A sister of proband`s mother presented with ID, delay of speech, club-foot and additional features not common to proband: normal stature, obesity, short philtrum, high narrow palate, crowded teeth, IIth toes overlapping thumbs. Mother's second sister died in infancy because of multiple congenital anomalies. Array-CGH of proband`s DNA revealed trisomy of the distal bands of chromosome 10 short arm (10pter-->10p15.1) and monosomy of the distal bands of chromosome 10 long arm (10q26.12-->10qter). Subtelomeric FISH analysis confirmed a rearranged chromosome 10 [rec dup(10p] due to a large, maternal pericentric inversion. A reverse segmental imbalance [rec dup(10q)] was detected in proband`s aunt by subtelomeric FISH. The high frequency of recombinants in this family and data from literature review suggests a high recurrence risk in similar cases with large pericentric inversions comprising almost entire chromosomes. The research leading to these results was funded by the Lithuanian-Swiss cooperation programme to reduce economic and social disparities within the enlarged European Union under project agreement No. CH-3-ŠMM-01/04, UNIGENE project.

P11.125-S

Functional characterization of L440Xfs mutation in the thyroid hormone receptor beta (TRβ) in an individual with RTH syndrome

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Resistance to thyroid hormone is an autosomal dominant disorder that affects 1 in every 40,000 births. It is characterized by reduced soft tissue responsiveness to thyroid hormone, with increased levels of T4 and T3 and non-suppressed thyroid hormone (TSH), due to mutations present in the thyroid hormone receptor β gene (TRβ), particularly in its T3 binding domain. The clinical phenotype varies both between different families and between affected family members. Individuals with RTH can have variable resistance in different tissues, as a consequence of mixed features of hypo- and hyperthyroidism. Our goal was to assess whether there was a functional alteration due to the mutation L440Xfs in TRβ gene that may generate RTH in our patient. In this study, we performed molecular and functional characterization of the L440Xfs mutation found in a male patient, who was diagnosed with RTH at 15 months of age. The patient harbors a new mutation in exon 10 of the TRβ gene, which consists of a deletion of a cytosine at nucleotide 1609 in the position 440, leading to a stop codon. The mutation was found in neither his parents nor his two healthy sisters, indicating a *de novo* mutational event. Transfection studies showed that the mutant TRβ was unable to carry out the transcription of luciferase gene in the presence of T3. Therefore, it is likely that the impaired receptor generates the severe RTH phenotype in our propositus.

P11.126-M

Restrictive Dermophaty: report of two new cases

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Restrictive dermopathy (RD) is a very rare, lethal genetic condition with peculiar phenotypic, histologic and genetic characteristics. The first case we describe was clinically diagnosed after death and confirmed thanks to precise histological and molecular characterization. The fetus was stillborn at 31+1 weeks of gestation by cesarean section. He did not show any spontaneous respiratory activity at birth nor after cardiopulmonary resuscitation. Post mortem, clinical examination showed multiple joint contractures and peculiar facial features like thick hair, absent eyelashes and eyebrows, hypertelorsim, small nose, small mouth in the "O" position with thin lips, micrognathia, low-set ears. The skin was thin, erythematous and translucent with evident superficial vessels. Fissures and erosions were evident in particular in the axillary and inguinal folds, nails were long. It was hypothesize the diagnosis of RD, confirmed by the microscopic examination of the skin. Molecular analysis performed on fetal DNA, showed a homozygous duplication in ZMPSTE24 gene, already described as causative for RD. The second patient is a SGA boy, born at 32 weeks of gestation by urgent cesarean section because of premature membrane rupture and pathologic CTG. Apgar was 3-0 and cardiopulmonary resuscitation was effective only after orotracheal intubation because of choanal stenosis, midface hypoplasia, micrognathia, microstomia and tight trismus. At this moment, spontaneous breathing is maintained through respiratory support. The clinical diagnose of RD is supported by the typical facial dysmorphisms, multiple joint contractures, arthrogryposis, thin, tense and translucid skin with some erosions, long nails. Molecular characterization is still in progress.

P11.127-S

Rubinstein-Taybi syndrome lymphoblastoid cells show impaired DNA damage response to oxidative stress

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The Rubinstein-Taybi syndrome (RSTS) is a genetic disorder associated with growth defects, intellectual disability, and increased risk of tumors. About 60% of RSTS individuals carry heterozygous mutation/deletion of CREBBP gene, while ~3-8% of RSTS are caused by mutations in the EP300 gene. CREBBP and p300 proteins play a key role in many aspects of DNA metabolism, such as DNA repair. However, the efficiency of DNA damage response (DDR) in RSTS is not yet elucidated. Here, we have investigated DDR in lymphoblastoid cell lines from RSTS patients carrying monoallelic deletion, or mutations of CREBBP gene. RSTS cells showed signs of histone H2AX phosphorylation, indicating an endogenous DNA damage. In addition, all RSTS cell lines tested were more sensitive to treatment with the oxidative agent KBrO3. No significant differences were observed in protein expression levels of PCNA, and DNA repair proteins, such as XP proteins, PARP-1, XRCC1 and DNA polymerase β. The analysis of the recruitment of DNA repair proteins (PCNA, DNA polymerase β, XRCC1) to DNA damage sites, suggests that in RSTS the DNA repair process is slowed or impaired. Preliminary results on the efficiency of DNA repair, as assessed by the Comet test, has suggest a delayed kinetics in the repair of oxidative lesions in RSTS cells, in particular at the level of DNA incision. These results suggest that RSTS cells show a reduced efficiency in DNA repair, explaining the greater sensitivity to oxidative DNA damage, and accounting for the increased susceptibility to cancer of RSTS patients.

P11.128-M

Report of five novel Schinzel-Giedion patients with mutation in SETBP1. Further delineation of the neuroradiological phenotype

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Schinzel-Giedion syndrome was first described in 1979, and is characterized by typical facial gestalt, severe developmental delay, frequent epilepsy and various malformations (urinary tract, genitalia, heart and skeletal). The reported cerebral malformations are ventriculomegaly, thin or absent corpus callosum, cortical atrophy, gyration anomalies. Hypoplastic pons was found in 2 patients.
In 2010, SETBP1 gene was identified as responsible for Schinzel-Giedion syndrome, with identification, by exome, of de novo missense mutations, some recurrent, all localized in the SKI oncogene homologous domain.
We present 5 novel patients with Schinzel-Giedion syndrome (2 girls and 3 boys) with mutation in SETBP1 (proven de novo for 3). Two mutations were previously reported (c.2602G>A, c.2608G>A) and the 3 others were novel (c.2607C>A, c.2608G>T, c.2606G>C). All patients had typical facial gestalt, severe developmental delay, epilepsy, genitalia anomalies. Hydronephrosis was present in 4 patients, and the 5th patient had coralliform kidney stones with recurrent infections. Cerebral MRI showed constant corpus callosum anomalies, and frequent cortical atrophy, ventricular dilatation, septal rupture. Two patients had gyration anomalies. Four patients displayed similar posterior fossa anomalies with hypoplastic pons and enlarged medulla oblongata, 3 patients had dysplastic cerebellar hemispheres. Atrophic caudate nuclei was noted in 4 patients.

These novel cases further delineate the cerebral spectrum of malformations in Schinzel-Giedion syndrome, in particular with similar posterior fossa anomalies.
The 3 novel mutations, as all the previously described mutations, are localized in the known mutational hotspot.

P11.129-S
Towards a better understanding of *de novo* germline mutations in *SETBP1* in Schinzel-Giedion syndrome

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Schinzel-Giedion syndrome (SGS) is a rare and severe developmental disorder characterized by malformations in multiple organ systems. Exome sequencing recently identified the cause of SGS as *de novo* germline mutations clustering within a 12-bp exonic region in *SETBP1*. Mutations in this *SETBP1* hotspot are presumed to be gain-of-function mutations which disrupt a degron (a consensus motif for protein degradation), disturbing normal SETBP1 proteolysis. Interestingly, somatic mutations in this hotspot have also been found in leukemic cells and shown to increase cell proliferation in this context.
We have collected clinical information and samples from over 25 SGS patients, which to our knowledge constitutes the largest cohort of SGS patients yet. To better understand germline *de novo* mutations in *SETBP1*, we investigated the parental origin of the allele in which these mutations occur in SGS. Using a combination of long range PCR and single molecule real time sequencing (Pacific Biosciences), we determined the parent of origin for four out of five trios analyzed. In three out of four cases, the *de novo* mutation in *SETBP1* occurred in the paternal allele and only in one in the maternal allele. Additionally, we examined the molecular and cellular consequences of *SETBP1* mutations in the context of SGS. When compared to controls, fibroblast and lymphoblastoid cell lines of SGS patients have alterations in downstream targets of *SETBP1*, such as higher levels of SET protein and deregulated gene expression.
Our studies provide insight into the occurrence and molecular consequences of *de novo* germline *SETBP1* mutations causing SGS.

P11.130-M
5p15.33-31 deletion and unilateral open lip schizencephaly: causal or casual association?

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We report on a case of a 3 year-old girl, with left hemiplegia, psychomotor delay and high pitched neonatal crying, epilepsy with MRI evidence of right open-lip schizencephaly; CGH array analysis disclosing a paternal inherited 5p complex rearrangement consisting of a 7.6 Mb deletion (del5p15.33-31) and a 2,6 Mb duplication (dup5p15.31). Indeed, the 5p15.2 critical Cri du Chat (CdC) region is spared and accordingly our patient does not present the typical dysmorphic and severe developmental features of CdC syndrome.
The proband's father has a high pitched voice, a history of hyperactivity and poor school performances and normal EEG. Intrafamilial phenotype variability is not uncommon in contiguous gene syndromes and it can be explained by differences in modifying genes and in critical allele polymorphisms.
Schizencephaly is a full-thickness cleft within the cerebral hemispheres, that can result from disruption or malformation. It has never been reported before in patients with 5p- syndrome, who typically show a variable degree of hypoplasia, especially of subtentorial structures.
Based on the already published phenotypic data and CNV databases as well as on the increasing knowledge on the function of the genes located in the deleted fragment, we discuss the genotype-phenotype correlation in our patient and the consequence of haploinsufficiency of NSUN2 in determining speech delay and hyperactivity, and of ADAMTS16 in causing abnormal larynx cartilages development. We also speculate on a potential pathogenic mechanism linking loss of Iroquois homeobox gene cluster and/or TPPP leading to schizencephaly.

P11.132-M
Novel Deletion within the translated sequence of the SHOX gene leading to Leri-Weill Syndrome with emphasizing severity in females

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Leri-Weill dyschondrosteosis (LWD) is a dominantly inherited genetic disorder characterized by short stature, mesomelia and Madelung wrist deformity. The LWD is caused by haploinsufficiency of the Short Homeobox containing gene (SHOX), located in the 2.6 Mb pseudoautosomal (PAR1) region. At the heterozygous level most mutations found are of different size deletions, point mutations within the coding sequence or mutations on regulatory enhancers downstream of the SHOX gene. We present the clinical and molecular data of a three generation family with LWD. The index patient is a 43 year old female with disproportionate short stature, reduced arm span to height ratio (particularly mesomelic shortening), a muscular body habitus and no evidence of a classic Madelung deformity. We report for the first time a novel 15kb deletion detected by microarray-CGH using a chromosome X exon-specific array (OGT) in the index patient. The deletion resides in the coding region of the SHOX gene, includes exons 3-6 containing the homeodomain region. RT-PCR studies confirmed the deletion and revealed the same 15 kb deletion in her sister, father and son. The youngest male member of the family has a very mild form of LWD, a phenomenon previously observed, that SHOX deficiency is more pronounced in females than in males and it can also exhibit inter-familial and intra-familial variable expressivity. Extended family studies (paternal side) have identified another 5 members to carry the deletion. Detailed clinical evaluation of these family members is ongoing which will shed more light in the phenotypic effect of this deletion.

P11.133-S
SKI gene analysis clarifies the diagnosis in cases suspected of Shprintzen-Goldberg syndrome

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Shprintzen-Goldberg syndrome (SGS) is a genetic disorder characterised by craniosynostosis, dysmorphic facial features, a marfanoid habitus and cardiovascular, connective tissue, neurological, and skeletal anomalies. The phenotype overlaps with Marfan (MFS) and Loeys-Dietz syndrome (LDS). Recently, mutations in *SKI* have been described as causative for SGS. All mutations are located in the SMAD binding or the DHD domain of the SKI protein, a repressor of TGFß-activity. TGFß signaling is also affected in MFS and LDS explaining some of the shared features.
Here we report on the *SKI* analysis in four patients suspected of SGS (Table, n/a no information available). Two of them (T7178, T7424) have previously been described (Robinson et al. Am J Med Genet A. 2005;135:251-62).

Patient ID	T6857	T7178	T7386	T7424
Sex, Age	female, 2y	male, 28y	male, 46y	male, 29y
SKI sequence variant	c.95T>A (p.Leu32Gln)	c.100G>T (p.Gly34Cys)	none	c.185C>G (p.Ala62Gly)
Craniosynostosis	+	n/a	n/a	-
Dolichocephaly	+	+	+	+
Hypertelorism	+	+	+	-
Down slanting palpebral fissures	+	-	-	-
Exophthalmos	+	+	-	-
High arched or cleft palate	+	+	+	+
Micrognathia or retrognathia	+	+	+	+
Low set posteriorly rotated ears	+	+	+	+
Arachnodactyly	+	+	+	-
Camptodactyly	+	-	-	-
Scoliosis	-	+	-	-
Pectus deformity	-	+	+	-
Joint hypermobility	+	+	+	+
Mitral valve prolapse	-	-	-	n/a
Aortic dilatation	-	-	-	n/a
Hernias	-	+	-	-
Muscular hypotonia	+	+	-	+
Developmental delay	+	+	+	+

Sanger sequencing of the whole coding region of *SKI* (NM_003036.3) revealed heterozygous mutations in two patients. The mutation c.100G>T (p.Gly34Cys), but not the sequence alteration c.95T>A (p.Leu32Gln) has already been described as a pathogenic mutation for SGS. However, the description of two other pathogenic missense mutations in the same codon 32 within the SMAD binding domain as well as *in silico* predictions underline the role of the sequence alteration c.95T>A as a pathogenic mutation. Furthermore, we identified an unclassified heterozygous variant, c.185C>G (p.Ala62Gly) in the third patient (Table).

In conclusion, the molecular analysis of *SKI* should be considered to differentiate between marfanoid phenotypes such as MFS, LDS and SGS.

P11.134-M
Submicroscopic genomic alterations detected by array CGH analysis in a cohort of patients with Silver Russell syndrome found negative to classical genetic and epigenetic tests

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Silver Russell syndrome is characterized by pre- and postnatal growth retardation, variable facial dysmorphisms, clinodactyly of the fifth fingers, and sometimes asymmetry of face, trunk and extremities. Genetic and epigenetic aberrations on chromosomes 7 and 11 are commonly found in SRS, leaving out however, a fraction of up to 50% of cases with unknown genetic aetiology. A cohort of 32 clinically selected SRS patients, without detected genetic or epigenetic alterations was analyzed by high resolution array CGH analysis to identify possibly pathogenetic CNVs. Twenty-eight patients (87.5%) were found to carry one or more rare CNVs, according to the Database of Genomic Variants and, overall, 55 rare CNVs, 25 gains (45.5%) and 30 losses (54.5%) were identified. Inheritance, established for 36 of the identified rare CNVs, showed that 7 occurred de novo (19.5%). Interrogation of public databases allowed us to pinpoint genomic regions containing genes, either imprinted or not, that according to their function, appeared plausible candidates for SRS. Interestingly 4 CNVs span genomic regions already associated with growth defects, 3 CNVs contain known genes found altered in previously described SRS patients and 3 CNVs include genes implicated in growth control pathways not yet associated with SRS. These results confirm the genetic heterogeneity of SRS and the high percentage of potentially causative imbalances, attesting the wide clinical expressivity of patients with a phenotype strongly suggestive for this syndrome. Genome-wide scan is reconfirmed an appropriate and powerful tool to achieve a differential diagnosis between SRS and SRS-like patients.

P11.135-S
Mosaicism of the H19 hypomethylation in a patient with very low weight and severe insulin resistance

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A 6-year-old boy, born to healthy non-consanguineous parents, presented a history of severe staturo-ponderal deficiency, bilateral cryptorchidism and hypogenitalism. The propositus was delivered at 38 weeks by cesarean section for intrauterine growth retardation (IUGR). Birth weight was 1400 g (-3SD), birth lenght 38 cm (-4SD) and OFC 32 cm (-2SD). He had moderately delayed developmental milestones, a poor appetite and feeding difficulties since the first year. Physical examination showed dysmorphic features suggestive of Silver-Russell Syndrome (SRS): triangular facies, relative macrocephaly with frontal bossing, prominent forehead, mild low-set prominent ears, micrognathia, downturned mouth, thin lips, fifth finger brachidactyly/clinodactyly. Psychomotor development was normal.
At 6 years he was 90 cm (-5.12 SD) tall and weighed 8,900 kg (-9.22 SD). His basal levels of free thyroxine, TSH, cortisol and coeliac disease screening were normal. Plasma concentrations of IGF-I and IGFBP-3 were low for age and sex (-2.30 and -1.95 SD). Growth hormone stimulation tests revealed a classic growth hormone deficiency. He also had severe insulin deficiency and normal glucose response after OGTT, negative diabetes autoantibodies and normal glycated haemoglobin, features not reported in association with SRS.
Molecular studies revealed a hypomethylation of the paternal H19/IGF2 Imprinting Control Region. Insulin like growth factor 2 (IGF2) is an imprinted gene, which has an important role in foetal growth. IGF2 is downregulated through hypomethylation of a differentially methylated region in SRS, characterised by growth restriction. Insulin metabolism abnormalities have never been described in SRS: could it be a specific manifestation of reduced IGF2 expression?

P11.136-M
Regulatory element deletion cause a down-regulation of *ZDHHC15* gene in a proband with Smith Magenis syndrome phenotype

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Here, we describe a boy aged 3 years who showed mild facial minor anomalies such as brachycephaly, square face, thick eyebrows, broad palate, generalized hypotonia, developmental delay, behavioural problems (self injury), sleep disorders and congenital heart defect.
Based on suspected Smith Magenis syndrome (SMS), chromosomal analysis was performed and showed a 46,XY karyotype. FISH analysis of the SMS locus at 17p11.2, as well as MLPA, mutational analyses and quantitative expression of the *RAI1* gene, gave normal results. High-resolution array-CGH analysis disclosed two rare maternal deletions at 4q35.2 and Xq13.3, both yet unreported in healthy subjects according to the Database of Genomic Variants. Whereas it is not possible to assign a pathogenetic role to the 4q35.2 deletion, the Xq13.3 loss of 54 kb involves a predicted conserved insulator that maps 29 kb far from the 5' end of the *ZDHHC15* gene. *ZDHHC15* acts as a palmitoyl-transferase in brain, and its null expression has been reported in a syndromic patient. We thus investigated in the patient's blood the causative role of the identified CNV on *ZDHHC15* by RT-qPCR. The *ZDHHC15* expression level was significantly reduced in the male proband compared to controls, whereas the expression level in the mother was within the control range.Our results suggest the involvement of *ZDHHC15* perturbation in the onset of the proband's phenotype and point to this gene, sharing interactors with *RAI1*, as a novel candidate gene for SMS.

P11.137-S
A novel NSD1 mutation in Sotos syndrome with constriction of vena cava

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Background: Sotos syndrome, first described in 1964, is characterized by typical facial appearance, overgrowth (height and/or head circumference ≥2 SD above the mean), and sometimes other features such as learning disability behavioral problems, congenital cardiac anomalies, neonatal jaundice, renal anomalies, scoliosis, and seizures.
The occurrence of Sotos syndrome is 1 in 10,000 to 1 in 14,000 newborns. However, many cases are assumed to be undiagnosed.
Methods: Genetic analyses including karyotype, array Comparative Genomic Hybridization and exome High Throughput Sequencing (HTS) was performed in a two year old girl with an unknown syndrome.
Results: HTS analysis revealed a novel de novo nonsense mutation in the NSD1 gene.
Her facial appearance was consistent with Sotos syndrome whereas her associated balloon dilated vena cava inferior constriction has not previously been assigned to Sotos syndrome.
Conclusion: We describe a novel NSD1 nonsense mutation causing Sotos syndrome. To our knowledge, this is the first time a Sotos patient has presented with a vena cava constriction.

P11.139-S
Unbalanced translocation t(8;17)(q23;q24) in a patient with developmental delay and epilepsy

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We present a 2 year-old male patient with poor growth, developmental delay and epilepsy. He is the second-born child of healthy non-consanguineous parents, with an unremarkable family history. He was born at gestational week 40 after an uncomplicated pregnancy. Seizures appeared at 16 months and the EEG revealed focal abnormalities in the left frontal and parietal regions during sleep. The array-CGH analysis identified a de novo 2.32 Mb

deletion on chromosome 8q23.1q23.2 due to an unbalanced translocation t(8;17)(q23;q24). The analysis detected two additional anomalies which were both inherited from the healthy father: a 165.8 Kb duplication involving chromosome 7p15.2 and a 914.6 Kb duplication involving 22q13.2. In the deleted region, SYBU and KCNV1 appear to be candidate genes for the patient's neurologic and electroclinical phenotype. In fact, SYBU encodes a protein which is part of a kinesin motor-adaptor complex that is critical for the anterograde axonal transport and contributes to activity-dependent presynaptic assembly during neuronal development. KCNV1 encodes a neuronal modulatory subunit of a voltage-gated potassium channel and it is predominantly expressed in the brain.

P11.140-M
TBC1D7 mutations are associated with intellectual disability, megalencephaly, patellar dislocation and celiac disease

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Mutations in both TSC1 and TSC2 cause the tuberous sclerosis complex (TSC), a multisystemic disorder characterized by the development of hamartomas or benign tumors in various organs as well as epilepsy, intellectual disability (ID) and autism. Whereas the binding of TBC1D7, the third constitutive subunit of the TSC1-TSC2 complex, is required to maintain its integrity, sequencing of TSC patients with no TSC1-TSC2 mutations indicated that TBC1D7 is unlikely to represent a "TSC3" gene. Loss of function of TBC1D7 results in an increase in mTORC1 signaling, and consequently a delay in the induction of autophagy.

Mutations in TBC1D7 were recently reported in a family with ID and macrocrania. Using exome sequencing we identified two sisters homozygote for a novel TBC1D7 truncating mutation. In addition to the already described macrocephaly and mild ID, they share osteo-articular defects, patella dislocation, behavioral abnormalities, psychosis, learning difficulties, celiac disease, prognathism, myopia and astigmatism. Consistent with a loss-of-function of TBC1D7 the proband's cell lines show an increase in the phosphorylation of 4EBP1, a direct downstream target of mTORC1 and a delay in the initiation of the autophagy process.

This second family allows enlarging the phenotypic spectrum associated with TBC1D7 mutations and defining a TBC1D7 syndrome. Our work reinforces the involvement of TBC1D7 in the regulation of mTORC1 pathways and suggests an altered control of autophagy as possible cause of this disease.

P11.141-S
Temple syndrome - introducing a new name for a characteristic chromosome 14 imprinting disorder

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Chromosome 14 harbours an imprinted locus at 14q32. Maternal uniparental disomy of chromosome 14, paternal deletions and loss of methylation at the intergenic differentially methylated region, result in a human phenotype of low birth weight, hypotonia, early puberty and short stature. This rarely diagnosed imprinting disorder has considerable overlap with other better known imprinting conditions such as Russell Silver and Prader Willi syndromes.

We have reviewed the world literature of 51 cases to identify the key diagnostic features to increase awareness, enhance diagnosis and improve treatment.

Key findings

1) Small for gestational age: The median birth weight standard deviation score (SDS) was -1.88 and none had a birth weight SDS >0. The median birth length SDS was -1.64.

2) Hypotonia and motor delay (93% and 83%).

3) Mildly reduced intellectual ability; IQ, 75-95.

4) Small hands and feet (87% and 96%)

5) Early puberty (86%)

6) Short stature in adulthood; the median final height SDS was -2.04

7) Metabolic syndrome; the median final adult weight SDS was -1.07 demonstrating a relatively greater weight for height in adults; median BMI of 27. Of 15 patients over the age of 11 years, three developed non-insulin dependent diabetes mellitus at the ages of 12 years, 19 years and 20 years.

The facial appearance distinguishes this condition from other imprinting disorders.

The use of the name Temple syndrome is not universal, as yet, but rather than the somewhat cumbersome use of 'maternal uniparental disomy of chromosome 14 related conditions' we propose this name change.

P11.142-M
Testicular regression and cerebral abnormalities: a new syndrome

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We report three male siblings affected by a Multiple Congenital Abnormalities/Intellectual Disability (MCA/ID) syndrome which, to the best of our knowledge, has not been reported to date. Patient 1 presented with a clinical phenotype including micropenis, anorchia and a very low serum level of antimullerian hormone, consistent with progressive testicular regression; severe developmental delay; spasticity; epilepsy; dysmorphic features; deafness; retinopathy; cerebral atrophy, corpus callosum hypoplasia and periventricular cysts. The two subsequent pregnancies were terminated at 34 weeks gestation because of recurrences. The fetuses (patients 2 and 3) showed cerebral cysts (2/2), thin corpus callosum (1/2), very low levels of antimullerian hormone in the amniotic fluid (2/2) associated with ambiguous genitalia in patient 2. Pathological examination of patient 2 showed a testicular atrophy, micropenis, a thin corpus callosum, white matter abnormalities (gliosis, cysts), and cerebellar focal neuronal migration anomalies. A large genetic and metabolic assessment performed in patient 1 was normal: standard karyotype and array CGH (105k, Agilent®); molecular analysis of the genes *SRY, SF1, ARX, ATRX, KAT6B* (exon 18); analysis of cholesterol precursors, screening for peroxysomal disorders (blood and fibroblasts), CDG syndromes, creatine deficiencies, adenylosuccinate deficiency, blood lactate and pyruvate, maternal X inactivation pattern. Magnetic resonance spectroscopy was normal. An exome sequencing analysis has been performed and we are interested to study other possible patients showing a similar clinical picture. In conclusion, we report the preliminary results of the clinical and molecular study of a previously unreported MCA/ID syndrome, characterized by testicular regression and cerebral abnormalities.

P11.143-S
Autosomal recessive *POLR1D* mutation with decreasing of *TCOF1* mRNA is responsible for Treacher Collins syndrome

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Purpose: Treacher Collins syndrome (TCS) is a mandibulofacial dysostosis caused by mutations in genes involved in ribosome biogenesis and synthesis. *TCOF1* mutations are observed in around 80% of the patients and are inherited as an autosomal dominant manner. Recently, two other genes have been reported in less than 2% of patients, *POLR1D* in patients with autosomal dominant inheritance, and *POLR1C* in patients with autosomal recessive inheritance. **Methods:** We performed direct sequencing of *TCOF1*, *POLR1C* and *POLR1D* in two unrelated consanguineous families. **Results:** The four affected children shared the same homozygous mutation in *POLR1D* (c.163C>G, p.Leu55Val). This mutation is localized in a region encoding the dimerization domain of the RNA polymerase. It is supposed to impair RNA polymerase resulting in a lower amount of mature dimeric ribosomes. A functional analysis of the transcripts of *TCOF1* by RT-qPCR was performed in the first family demonstrating a 50% reduction in the index case compatible with this hypothesis. **Conclusion:** This is the first report of *POLR1D* mutation responsible for an autosomal recessive inherited Treacher Collins syndrome. These results reinforce the concept of genetic heterogeneity of Treacher Collins syndrome and underline the importance of combining clinical expertise and familial molecular analyses for appropriate genetic counseling.

P11.144-M
Molecular and cytogenetic characterization of an unusual case of partial trisomy 13q mosaicism

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Trisomy 13 (Patau syndrome) is a rare multiple malformation syndrome and includes anomalies of the central nervous system, cardio-vascular and urogenital system. The probability of survival for live-born to one year is 3%. Partial or mosaic forms of trisomy 13 can occur and they cause a variable phenotype.
We report a case of a live-born baby with multiple congenital anomalies including severe Fallot tetralogy, bilateral postaxial polydactyly of fingers and toes, monolateral hydronephrosis, microcephaly with trigonocephaly and brain anomalies (hypoplastic corpus callosum and dismorphic cerebellar vermis). The child also presents some facial anomalies (sloping forehead, hypotelorism, low set ears and microretrognathia) and scalp defect (aplasia cutis of vertex). Cytogenetics, fluorescence *in situ* hybridisation and array-CGH analysis showed the presence of two cell lines in which a normal chromosome 13 was replaced in one by a isochromosome (13q) and in the other by a isochromosome 13 derivative, with a deletion spanning from 13q11 to 13q14, resulting in partial trisomy 13q. The two cell lines were present either in peripheral blood lymphocytes or skin fibroblasts.
Chromosomal mosaics made up of cell lines with distinct intrachromosomal structural rearrangements are unusual. Two cases reported by Reardon *et al.* (1981) and by Fogu *et al.* (2008) showed similar karyotypes with rearrengements of the same region on isochromosome 13 derivative. We suggest a common mechanism producing the coexistence of distinct structural rearrangements at common breakpoints on chromosome 13.
Phenotype of the baby is discussed with respect to other cases showing partial trisomy mosaicism in Patau syndrome.

P11.145-S
Classical karyotyping vs molecular karyotyping (arrayCGH) in a case of trisomy 9 mosaicism

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Trisomy 9 mosaicism is considered to be a rare chromosomal abnormality with limited survival and a characteristic pattern of multiple anomalies. The features commonly associated with trisomy 9 include growth retardation, facial dysmorphisms, skeletal abnormalities, congenital heart disease and intellectual disability. More than 50 cases have been reported, most of which were diagnosed after birth. We report a case of a one month old baby girl with craniofacial abnormalities, hydronephrosis and multiple contractures. ArrayCGH analysis was performed and detected a trisomy 9 mosaicism in approximately 40% of the cells. Classical karyotyping of lymphocytes revealed trisomy in about 3% of the metaphases. Previous prenatal analysis of cell cultures from amniotic fluid had not shown the mosaic trisomy 9 constitution. Mosaicisms are expected to be exhibited at different levels in different tissues. In addition, cell culturing leads to a bias in terms of the ratio between trisomic and disomic clones and very likely underestimates the percentage of trisomic cells in conventional karyotyping. Our data show the importance of using uncultured samples (amniotic fluid and blood) and the value of microarray technology in the assessment of mosaicisms.

P11.146-M
Cytogenetic-molecular characterization of an idic (Xq) in a female with Turner syndrome and her daughter

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The following is the case of a patient born 2 weeks preterm weighing 2.800 kg. She had a multicystic kidney that was removed immediately after birth. At age 9, due to a significant growth delay, a cytogenetic analysis was made on peripheral blood revealing a homogeneous 45,X karyotype thus leading to Turner syndrome. Growth hormone treatment contributed to stature increase but with the development of secondary sex characters and the menstrual cycle, it became necessary to repeat blood cytogenetic analysis that confirmed the 45,X karyotype. At 30 years, following absence of menstrual cycle , she realized she was pregnant. She had to undergo a caesarian delivery and was simultaneously operated for ovariectomy. Karyotype obtained from gonadic tissue was mos 45,X/46,X,der(X). The insufficient amount of cells however did not help to investigate on marker nature. As the daughter presented the same stature growth delay, at 5 years her constitutional karyotype was analyzed and was the same one found in her mother's ovarian culture: 46,X,der(X), in this case present homogeneously. Cytogenetic-molecular characterization by FISH with various X specific probes allowed to define, beyond doubt, that the X derivative is a dicentric isochromosome with an inactive centromere and with symmetric point in Xp21: 46,X,idic(X)(qter→p21::p21→qter). This data allowed to demonstrate : 1) the presence of phenotype atypia. 2) ovarian functionality, 3) the short stature of both mother and daughter due to SHOX gene deletion.

P11.147-S
SNP array revealed maternal UPD16 in a boy with short stature, craniosynostosis and psychomotor retardation.

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We report on a 15 months old boy (gemini after IVF, healthy sister) with positive prenatal anamnesis (IUGR, oligohydramnion), craniosynostosis, short stature, hypotonia, hypospadia, cryptorchidia, pulmonary vein stenosis and delayed psychomotor development. Chromosomal examination revealed normal male karyotype 46, XY. The whole genome genotyping was performed using SNP array (300K, Illumina) and no pathogenic CNV was found. However, three extensive blocks with loss of heterozygosity spanning almost one third of the whole chromosome 16 were found. Parental DNAs were analyzed and the maternal UPD16 was confirmed. Chromosome 16 showed the mixture of isodisomy and heterodisomy with homozygous haplotype within the centromere. This haplotype arrangement implies the following origin of the UPD16: meiosis II nondisjunction, recombination and mitotic loss of the paternal chromosome 16 (trisomy rescue). Data for UPD16 are inconsistent, prominent phenotype of maternal UPD16 is IUGR with or without catch-up growth. Other features may include heart defects, inguinal hernia, hypospadia, pulmonary hypoplasia, although these features may be partly due to hidden mosaic trisomy 16. Mental development ranges from normal to severely delayed. We consider a potential impact of two imprinted genes and five predicted imprinted genes on chromosome 16 to the distinct phenotype of the UPD16.

P11.148-M
Whole exome sequencing identifies novel genetic variants in the PRDM5 gene in Brittle cornea syndrome and Axenfeld-Rieger syndrome

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Background: Brittle cornea syndrome (BCS) and Axenfeld-Rieger syndrome (ARS) are disorders affecting the anterior segment of the eye, often leading to secondary glaucoma. BCS is an autosomal recessive disorder associated with mutations in the *PRDM5* and *ZNF469* genes, while Axenfeld-Rieger syndrome is inherited in an autosomal dominant fashion that has been associated with genetic defects in *PITX2* and *FOXC1.* The purpose of current study is to identify the underlying genetic causes in a family with autosomal recessive BCS and in a family with autosomal dominant ARS by whole exome sequencing (WES).
Methods: WES was performed for a single affected individual of both families. Missense, nonsense and splice site variants identified by WES were prioritized based on pathogenicity prediction scores (PhyloP, Grantham). Segregation analysis of selected variants was performed in family members.
Results: A homozygous splice site variant (c.93+5G>A) was identified in the *PRDM5* gene, which was found to segregate with the disease in the BCS family. In the ARS family, a novel heterozygous *PRDM5* missense variant (c.877A>G; p.Lys293Glu) was prioritized from the WES data, and was found to segregate with the disease in an autosomal dominant fashion. Both variants were absent from population-matched controls, the Exome Variant Server and an in-house exome variant database.
Conclusions: In the current study we identified a homozygous splice site variant in a BCS family and a heterozygous missense variant in an ARS family in the *PRDM5* gene. This suggests that genetic variants in *PRDM5* can lead to different disorders affecting the anterior segment.

P11.149-S
WHSC/NSD2 is the major candidate gene for growth delay and facial dysmorphisms in Wolf-Hirschhorn syndrome: expression analysis and functional studies on primary fibroblasts and immortalized peripheral lymphoblasts from three patients.

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Wolf-Hirschhorn Syndrome (WHS, OMIM194190) is a contiguous gene syndrome caused by partial deletion of the short arm of one chromosome 4. The core WHS phenotype includes growth delay, intellectual disability, distinctive facial appearance and seizures. It maps within the terminal 1.9 Mb region on 4p16.3, where the critical region, WHSCR-2, was described. With respect to pathogenic genes falling within WHSCR-2, WHSC1 is the major candidate gene for both facial characteristics and growth delay. WHSC1 is expressed mainly in embryonic tissues and presents homology with Drosophila dismorphy genes. Its PWWP, HMG-box, PHD and SET structural domains suggest an histone methyltransferase activity and a role in the epigenetic regulation of morphogenetic transcriptional programmes. However, to which extent whsc1 expression and activity are reduced in patients'cells due to 4p16 deletion has not been thoroughly investigated. Additionally, while very little is known about whsc1 regulation, it is intriguing that this gene is a potential target of hsa-miR948, that also maps to 4p16.3, and whose concomitant deletion may, in a subset of patients, reduce the effect of WHSC1 monosomy by increasing the expression of the residual allele. Based on these consideration, we decided to evaluate at a mRNA and protein level the expression of WHSC1 and of hsa-miR948 in primary and immortalized cell lines from selected patients and healthy individuals as controls. Furthermore, the expression levels have been correlated with biochemical characteristics (H3K36 and H4K20 methylation) and cellular phenotypes (DNA damage response, inflammatory signaling) reportedly related to WHSC1 biological activity at least in tumor cells.

P11.150-M
Coeliac disease in Williams syndrome

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Williams syndrome is a multi-system disorder characterized by distinctive facial features, growth delay, mental retardation with typical neurobehavioral profile, cardiovascular anomalies, endocrine anomalies including autoimmune pathologies, and hypercalcemia. Coeliac disease is an autoimmune, gastrointestinal disorders characterized by intolerance to the dietary grain protein gluten. An increased prevalence of Coeliac disease has been reported in Down syndrome and Turner syndrome, but there has been only few previous report which respect to the association of Coeliac disease in Williams syndrome.
The aim of this study was to estimate the prevalence of Coeliac disease in our 24 molecularly confirmed Williams syndrome patients (12 girls, 12 boys, mean age 9.4 years). All patients were analysed by the dosage of tissue transglutaminases IgA and IgG HLA genotyping and intestinal biopsy was performed to the patients with positive serology. Celiac disease symptoms and gastrointestinal problems were recorded. Biochemical profile (calcium, urine calcium/creatinin ratio, thyroid functions), IgA tissue transglutaminases (IgA tTG) and IgG tissue transglutaminases (IgG tTG) were assessed in all cases. Serum total IgA levels assessed only in tTG positive patients. Patients were selected for the haplotypes in the HLA class II region (HLA DQ), endoscopy and intestinal biopsy on the basis of tTG positivity.
The results show that the prevalence of Coeliac disease in Williams syndrome was higher than in the general population. As our results, Coeliac disease questioning and serologic screening is recommended at certain intervals in all cases with Williams syndrome to explain the cause of growth retardation and gastrointestinal problems.

P11.151-S
A novel ZCD2 intragenic deletion in Wolfram Syndrome 2

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Wolfram Syndrome type 2 (WFS2) is considered a phenotypic and genotypic variant of WFS, whose minimal criteria for diagnosis are diabetes mellitus and optic atrophy.
The responsible gene for WFS2 is named CISD2, a highly conserved zinc-finger gene encoding for the Endoplasmic Reticulum Intermembrane Small (ERIS) protein, which plays a pivotal role in calcium homeostasis. It was identified for the first time in three consanguineous families of Jordanian descents who carried a point mutation in exon 2 that disrupts messenger RNA splicing by eliminating exon 2, causing a premature stop codon. After this first report of Jordanian patients, no further CISD2 mutations have been reported worldwide.
We describe the first case in Europe of WFS2 with a novel homozygous CISD2 exon 2 deletion in a 17 year-old girl, identified using both PCR and high density SNP array. She presented diabetes mellitus, optic neuropathy, intestinal ulcers, sensorineural hearing loss, and defective platelet aggregation. Her brother and parents carried the heterozygous mutation and were apparently healthy, although they showed subclinical defective platelet aggregation.
Since the mutation had never been reported, consanguinity was hypothesized. Long runs of homozygosity analysis from SNP-array data did not show any degree of parental relationship, while microsatellite analysis confirmed the hypothesis of a common ancestor.
This report provides novel clinical and molecular insights about WFS2.

P11.152-M
Early diagnosis of Kallmann syndrome in a 4 year old boy with a familial X;Y translocation

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X;Y translocation (tXY) is a rare chromosomal rearrangement typically inherited from a tXY carrier mother. Patients with tXY commonly display 46, X or Y, der(X),t(X;Y)(p22.3;q11). Women with tXY show a mild phenotype due to X inactivation and are fertile. Men with tXY show a range of phenotypes with variable degree of severity according to the size and position of the deletion of the X chromosome, comprising short stature, chondrodysplasia punctata, ichthyosis, ocular-albinism, mental retardation, and Kallmann syndrome (KS). Here we describe a boy with tXY inherited from a carrier mother. His phenotype was characterized by mesomelic short stature, midface retrusion, ichthyosis, micropenis, bilateral undescended testes, hypoplastic scrotum, agenesis of right kidney and central hypogonadism as assessed by gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG) stimulation tests. Magnetic resonance imaging (MRI) of the olfactory tracts showed hypoplastic olfactory bulbs bilaterally, aplasia of the left olfactory sulcus and hypoplasia of the right olfactory sulcus. The patient's mother and sister showed mesomelic short stature and his mother displayed Madelung deformity. Single nucleotide polymorphism (SNP) array analysis defined the breakpoint in intron 7 of the KAL1 gene. Conclusion: For early diagnosis in patients with KS, it would be useful to search for other symptoms including renal abnormalities (agenesis of right kidney) and to assess the presence of olfactory tracts by MRI (hypoplastic olfactory bulbs bilaterally).

P11.153-S
Chromosome Xq21 deletion syndrome - rare cause of deafness and mental retardation

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Our case is a family, where Chromozome Xq21 deletion syndrome was diagnosed.
Genetic counselling was recommended because of mental retardation, bilateral hearing loss and paleocerebellar syndrome at 5-year boy. He also had hypotonia, developmental delay and hyperactivity. We started genetic examination and we found normal karyotype 46,XY,9qh+. Molecular genetic examination excluded X-Fragile syndrome, Prader-Willi-Angelman syndrome and mutations in the GJB2 gene. Microarray comparative genomic hybridisation identified a 5-Mb deletion of Xq21, which include the POU3F4, ZNF711 and CHM genes. The boy did not have any symptoms of choroideremia. Multiplex ligation-dependent probe analysis (MLPA) showed that the unaffected mother also carried the deletion. The boy had no siblings.

Next time the family was examined in the following pregnancy of the mother, but the case was complicated because of twin pregnancy. Prenatal diagnosis was recommended and chorionic villus sampling was performed with the result one male and one female foetus. Molecular genetic examination of the Chromozome Xq21 deletion syndrome in the male foetus was performed and the same familiar deletion was identified. Mother underwent selective fetocide of the affected male foetus at 16 weeks' gestation. Further course of gravidity is normal.

P11.154-M
17q21.31 microdeletion syndrome: neurocognitive functioning in two italian young patients

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The 17q21.31 microdeletion syndrome causes a wellknown syndrome recently described in the scientific literature. Clinical features are: neonatal hypotonia, low birth weight, craniofacial dysmorphism, developmental delay, intellectual disability and amiable behaviour disposition. The present study analyzes neuropsychological assessment in two young italian patients with a genetically described 17q21.31 microdeletion. Case 1: 18-year-old male. Case 2: 13-year-old female.
Neuropsychological Assessment Wechsler Adult and Children Intelligence Scales Revised, Italian Neuropsychological Battery, Peabody, Vineland Adaptive Behavior Scales, Adaptive Behavior Inventory, Parent Stress Index, Brief Cope, Multidimensional Scale of Perceived Social Support, Child Behavior Checklist. Case 1: severe mental retardation (Intelligence Quotient, IQ = 32), immature representative capacity, impairment of receptive and expressive communication, anxiety and apprehension. The Adaptive functioning is impaired in all areas (communication, skills of daily living, socialization and motor skills). Mental Age 4y2m. Case 2: moderate to severe mental retardation (IQ<40), expressive and receptive language deficits, decline in all areas of adaptive functioning (M.A. 5y7m), socially indiscriminate attachment behavior, hyperactivity. Reading and writing disabilities, semantic memory deficits, verbal and visuospatial short-term memory deficits, ideational and motor dyspraxia, deficit in scheduling tasks and spatio-temporal organization were present in both patient. The emotional systems are characterized by trust in peers and adults, kindness, dependence and poor individual autonomy. The attachment is secure with important people.To our knowledge these observations can be added to preliminary evidences already present in literature: 17q21.31 microdeletion syndrome correlates to low intellectual capacities, learning disability, good interpersonal skills and an approaching behaviour.

P12.001-S
Impact of Aberrant Promoter Hypermethylation on down-regulation of MTS2 and MTS1 in Childhood ALL

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The tumor suppressor genes MTS1 and MTS2 are cycline dependent kinas inhibitors inactivated in some human neoplasms via several mechanisms such as hypermethylation. We have investigated the methylation status of MTS1 and MTS2 in its effect on transcriptional down-regulation in 125 bone marrow aspirate (7 cases T-cell and 118 cases B-cell phenotypes) from childhood ALL patient and 100 healthy control in north Indian population by using MSP-PCR ,bisulfite sequencing, SQRT-PCR and RT-PCR. There were significant differences in pattern of hypermethylation between patients and healthy controls of MTS2 (p=0.000) and MTS1 (p=0.001) and also when both genes methylated. Patients with hypermethylated of both genes showed an increasing risk for 2.33 fold (95%CI=2.33(1.97-2.77), p=0.03). Significant association of MTS1 hypermethylation was observed only among the male patients (p=0.004) in contrast hypermethylation of MTS2 was significantly associated with increasing risk of ALL among both genders. Down-regulation of mRNA expression was found in cases in which MTS1 and MTS2 were hypermethylated. In conclusion our data also indicate the impact of hypermethylation mediated inactivation of these genes which is associated with risk of childhood ALL. This abnormality particularly in promoter of MTS2 occurs in leukemogenesis and can be considered as an important factor in predicting the clinical outcome of ALL and it is able to suggest a diagnostic tool for some stage of ALL and even provide the better prospective for treatment strategy by applying some demathylating agents.

P12.002-M
The A785G CYP2B6 germline polymorphism may affect the risk of de novo Acute Myeloid Leukemia

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The etiology of acute myeloid leukemia (AML) is currently unknown although genetic background and environmental exposure postulated to be a possible cause of AML development. The CYP2B6 enzyme plays a vital role in the degradation of many genotoxic compounds, protecting cells from oxitative damage. CYP2B6 gene is subjected to the A785G germline polymorphism which reduces enzyme activity. Thus, individuals homozygous or heterozygous for mutant allele (G/G or A/G) present decreased enzymatic activity. The purpose of this study was to investigate the role of the A785G polymorphism in the AML susceptibility. Possible associations with specific AML-chromosomal abnormalities were also investigated. CYP2B6 genotyping was performed in 220 de novo AML patients and 243 healthy donors by RCR-RFLP and Real-Time PCR assays. Cytogenetic analysis was successful in 98% of patients. Among them, 72.7% presented an abnormal karyotype. A significantly higher incidence of the mutant genotypes (A/G and G/G) was observed in de novo AML patients compared to the controls (p<0.0001). The mutant allele frequency was similar between the different gender and age groups. Interestingly, a higher frequency of heterozygotes A/G was observed in normal karyotypes compared to abnormal (51.7% vs 30.0%, p=0.010). Furthermore, a significantly higher incidence of G/G genotype was observed in patients with t(8;21) and MLL rearrangements compared to patients with normal karyotypes (28.5% and 20.0% vs 6.6%, respectively). Our study comprises the first investigation of the A785G CYP2B6 polymorphism in AML susceptibility. Our results reveal a possible implication of this genetic variant in AML development and its specific chromosomal aberrations.

P12.003-S
Higher incidence of co-existing Anaplastic Lymphoma Kinase (ALK) rearrangements and Epidermal Growth Factor Receptor (EGFR) mutations in Non-Small Cell Lung Cancer (NSCLC) in a South-East Asian population

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Both EGFR mutations and ALK rearrangements in NSCLC are associated with sensitivity to EGFR and ALK tyrosine kinase inhibitors, respectively. The incidence of ALK rearrangements is about 5% worldwide and EGFR mutations and ALK translocations are generally known to be mutually exclusive. The aim of this study is to determine the incidence of ALK rearrangements and incidence of co-existing EGFR and ALK mutations in a South-East Asian population. FISH using an ALK break-apart probe was performed on formalin-fixed paraffin embedded (FFPE) tumor tissues from 1183 NSCLC cases from year 2011 till 2013. EGFR mutation test was performed using direct sequencing of EGFR exons 18-21. ALK FISH results were obtained from 1152 samples (97.4%). A total of 105 cases showed a rearranged ALK gene (9.1%) of which 76.2% showed the typical FISH pattern while 21.9% showed an atypical pattern with loss of the 5' ALK gene segment and 1.9% showed loss of the 3'ALK gene. Among the ALK rearranged patients, 95 cases had concurrent EGFR mutation assays performed out of which EGFR mutations were identified in 17 cases (17.9%). The incidence of concomitant ALK and EGFR alterations in the entire cohort was 1.48%. In our cohort study, the incidence of ALK gene rearrangement is relatively similar to the reported incidence. The co-existence of ALK rearrangements and EGFR mutations has been found to be rare in Western populations (0.33-6%). The higher incidence of concomitant mutations in our South-East Asian population suggests ethnic differences in terms of genetic alterations.

P12.004-M
FISH analysis of four unbalanced translocations involving chromosome 1q in hematologic neoplasms

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Unbalanced whole-arm translocations (WATs) of the long arm of chromosome 1 result in complete trisomy 1q. These are rare chromosomal abnorma-

lities detectable in both solid tumors and hematologic neoplasms including cases of myelodysplastic syndrome and acute myelogenous leukemia (AML), in most cases described as dicentric derivative chromosomes. We report on 1q unbalanced WATs detected at diagnosis in 4 patients as resulting from a t(1;15)(p10;p10) in a case with myelofibrosis (MF) and in one with AML, t(1;14)(p10;p10), and t(1;22)(p10;p10) in two other AML patients. Conventional cytogenetics was supplemented by FISH analysis using specific probes for the centromeric alpha-satellite region of chromosome 1 (D1Z7; 1p11.1-q11), chromosomes 14/22 (D14Z1/D22Z1; 14p11.1-q11.1/22p11.1-q11.1), and chromosome 15 (D15Z4; 15p11.1-q11.1). FISH analysis showed that the signal of chromosome 1 alphoid region D1Z7 was absent on derivative chromosomes in all patients suggesting that breakpoint on chromosome 1q is distal to the centromere, while D14Z1 on t(1;14), D15Z4 on t(1;15), and D22Z1 on t(1;22) were present. Therefore all derivative chromosomes showed unique centromere (monocentric) derived from the acrocentric chromosome rearranged with 1q, then resulting in reclassification of 1q WATs as der(15)t(1;15) (both cases), der(14)t(1;14) and der(22)t(1;22). Moreover, during follow up in the MF case cytogenetic analyses revealed progressive expansion of the clone with der(15) up to homogeneity, thus suggesting a proliferative advantage for the der(15)-related clone. Although studies on a large cohort of patients are needed, present results confirm 1q pericentromeric region instability in leukemia, and support trisomy 1q role to favor leukemogenesis and hematopoietic tissue alterations.

P12.005-S
Involvement of APC gene in Acinar Cell carcinomas of the Pancreas

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Pathogenetic mechanisms of pancreatic acinar cell carcinomas (ACCs) are poorly characterized. There is no data about gene hypermethylation and chromosomal aberrations in ACCs. In a subset of ACCs is reported the impairment of APC/β-catenin pathway including mutations of APC gene. However, it is not known whether the loss of APC function can occur even through alternative genetic and epigenetic mechanisms. In this study, we investigated the methylation profile of 34 tumor suppressor genes, Copy Number Alterations (CNA) of 52 chromosomal regions, and APC alterations (mutation, methylation, and loss) together with the measurement of APC mRNA level in 45 ACCs and related available peritumoral pancreatic tissues using different methodologies: MS-MLPA, FISH, mutation analysis, and reverse transcription-droplet digital PCR. ACCs did not show an extensive global gene hypermethylation profile. RASSF1 and APC were the only two genes frequently methylated (60% and 56% of cases, respectively). APC mutations were found in 7% of cases, while APC loss and methylation were more frequently observed (48% and 56% of ACCs, respectively). They were also found in pancreatic tissues adjacent to ACCs showing these alterations. APC mRNA low levels were found in 58% of cases and correlated with higher levels of CNAs. In conclusions, ACCs are frequently polysomic and mainly characterized by CNAs and gene methylation. APC alterations are crucial events in the pathogenesis of ACCs and the reduction of APC mRNA levels mainly depends on gene loss and promoter hypermethylation rather than gene mutation.

P12.007-S
The role of BIRC5 polymorphisms in oral and oropharyngeal squamous cell carcinoma

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Survivin, encoded by *BIRC5* gene, belongs to the family of inhibitors of apoptosis (IAP) proteins. In healthy organisms it is not expressed in differentiated tissues, while its expression is markedly increased in tumors. *BIRC5* polymorphisms have been previously associated with increased expression, stability and localization of survivin, all of which can affect tumor development.
In this study we investigated the role of *BIRC5* polymorphisms in oral and oropharyngeal squamous cell carcinoma. Genetic testing of 38 patients and 74 healthy controls was conducted using high resolution melting analysis and Sanger sequencing.
Results showed different significance of individual *BIRC5* polymorphisms. c.-235G>A showed significantly higher frequency in patients compared to control samples. For c.-644T>C and c.-625G>C, minor homozygous genotypes were significantly associated with higher Broders' grade, which was also observed for c.-235G>A. The minor homozygous genotypes of either c.9194G>A or c.9809T>C were associated with higher TNM stage. For c.-644T>C and c.-625G>C, the presence of minor allele was associated with higher survivin mRNA expression. Besides eight frequent, six rare polymorphisms were found, c.9349G>C found in 3' UTR previously unpublished.
This was the first study in Croatia which demonstrated correlation between *BIRC5* polymorphisms with the level of survivin expression and the risk of oral and oropharyngeal squamous cell carcinoma. Since it is known that increased expression of survivin is associated with increased resistance to chemotherapy and radiation, as well with poorer survival, *BIRC5* polymorphisms could be used as predictive and prognostic biomarkers in the etiology of head and neck squamous cell cancer.

P12.008-M
Bladder cancer risk in relation to DNA repair capacity, gene expression and epigenomic profiles.

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The ability to repair DNA damage is strongly associated with the risk of cancer and inter-individual variation in DNA repair capacity (DRC) might account for different susceptibility of developing cancer. DRC represents a complex marker comprising the sum of several factors such as gene variants, gene expression, stability of gene products, and effect of inhibitors/stimulators. Individuals with low DRC will tend to accumulate more damage than those who have a better ability to repair such damage. This variability is modulated by the genetic background to which SNPs/haplotype combinations in DNA repair genes, as well as epigenetic regulation are likely to contribute.
Phenotypic assays to evaluate DNA repair activity (in particular NER comet assay, H2AX phosphorylation and micronucleus assay) were performed on cryopreserved lymphocytes from 159 bladder cancer cases (collected before treatment) and 159 controls matched by age and smoking habits, enrolled in Turin Bladder Cancer Study (TBCS). We aimed at studying the relationship between DRC (evaluated by comet assay, micronuclei and H2AX phosphorylation assay) and bladder cancer integrating, gene expression and epigenetic profile data (methylation levels and microRNA expression). In particular, we performed an integrated analysis on the genotype/phenotype correlation in a population of bladder cancer cases and controls provided with detailed description of the follow-up for response to therapy/recurrence/survival. This integrated approach will help in elucidating the role of DRC (and its determinants) as predictive and prognostic marker and to identify DNA repair phenotypic assays to be performed in blood cells as non-invasive predictive method.

P12.009-S
Mutation detection in urines from bladder cancer patients as non-invasive prognostic tool

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In Europe, bladder cancer (BC) is the sixth most commonly diagnosed tumour and the second most common cause of death among patients with genitourinary tract malignancies. Patients with non-muscle-invasive bladder cancer (NMI-BC) have excellent survival; however two-thirds develop recurrences. Tumour specific mutations can be used to detect recurrences in urine assays, presenting non-invasive diagnostic procedure respect to cystoscopy. The present study involves male subjects recruited between 1994 and 2012 and diagnosed with BC. Urine samples have been collected for the first time at the time of diagnosis and exfoliated cells have been extracted. A subgroup of patients have been followed up for three years, collecting a urine sample every six months. The mutation spectrum of hTERT, FGFR3, HRAS, KRAS, NRAS and PIK3CA in 286 patients has been investigated at diagnosis. Thirty-eight patients have completed six samplings, twenty-five patients have five samplings and nineteen patients have four samplings, for a total of 454 follow-up samples. Mutations are detected using three different multiplexed SNaPshot assays. The first allows to detect in one reaction the nine most frequent FGFR3 mutations. The second multiplex screens simultaneously for nineteen RAS mutations. The last multiplex detects seven mutations, three in hTERT gene and four in PIK3CA gene. Preliminary analyses show that mutations in hTERT gene promoter and in FGFR3 gene are the most frequent somatic mutations in tumours of the urinary bladder. Mutational profiler will be correlated with recurrences and survival in order to verify the usefulness of urinary exfoliated cells to follow-up BC progression.

P12.010-M

SEPT9/SYHR1, novel fusion gene identified in bladder cancer by RNA-seq.

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Bladder cancer is one of the most common genitourinary malignancies in the world. The urothelial carcinoma (UC) has multiple genetic alterations, but only a few low-frequency fusion genes have so far been reported for this disease. In this study, we present a thorough search for novel fusion transcripts in UC sample using high-throughput RNA sequencing.
Sequencing was performed according to the paired-end RNA sequencing protocols from Illumina for Solexa sequencing on a Genome Analyzer II. We used the fusion discovery software tool Chimerascan. From 51 million paired-end sequence reads, we identified 563 candidate fused transcripts.
By stringent requirements, we nominated the one candidate fusion transcript for further experimental validation, which was positive by RT-PCR and Sanger sequencing. The transcript was intrachromosomal SEPT9/CYHR1 fusion gene. Septin 9 (17q25) is a member of the septin family of GTPases that have diverse cellular activity, including roles in cytokinesis, apoptosis, and vesicle trafficking. A chromosomal translocation involving this gene and MLL gene is described for acute myelomonocytic leukemia. CYHR1 (8q24) (cysteine and histidine-rich cytoplasmic protein) is involved in cellular trafficking transport of galectin 3. We founded two transcript variants: SEPT9 exon 2 was fused to CYHR1 exon 3 and SEPT9 exon 1 was juxtaposed to CYHR1 exon 3. We also revealed SEPT9/CYHR1 in 1/12 of UC FFPE samples.
Further investigation of functional and clinical relevance of novel fusion gene remains to be elucidated to reveal the role of SEPT9/CYHR1 in the carcinogenesis of bladder.

P12.012-M

Phenotypic and clinical characteristics of 3300 Israeli BRCA1 and BRCA2 mutation carriers

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Background: BRCA1/BRCA2 mutations are the most common cause of hereditary breast and ovarian cancer (HBOC). The HBOC-Consortium was created for unified data collection on Israeli BRCA carriers.
Methods: A uniform computerized-database was used by 12 cancer-centers
Results: Data were collected on 2145 BRCA1, 1131 BRCA2, and 22 double-mutation carriers.
BRCA1 vs. BRCA2 mutation carriers had more breast cancer (BC) (40.0% vs. 36.3%, p=0.06), ovarian cancer (OC) (17.1% vs. 10.6%, p<0.001), were younger at cancer diagnosis (BC 45yrs. vs. 49 yrs., p<0.001, OC 53yrs. vs. 62yrs., p<0.001), had more familial BC (64.5% vs. 59.0%, p<0.001), and familial OC (36.7% vs. 27.3% p<0.001).
BRCA1 carriers of 185delAG vs. 5382insC had less BC (38.6% vs. 46.4%, p=0.02), at older age (46yrs. vs. 40yrs., p=0.023), but more OC (17.6% vs. 12.9%, p=0.04).
Risk-reducing-bilateral-salpingoooporectomy (RR-BSO) rates were assessed in carriers 38-80yrs. BC patients had higher rates than unaffected carriers (92.6% vs. 78.1%, p<0.001), but underwent RR-BSO later (51yrs. vs. 48yrs., p<0.001). Carriers with familial OC had higher rates than carriers without (83.0% vs. 74.7%, p=0.05). Carriers with familial-cancer had RR-BSO earlier than those without (48 yrs. vs. 50yrs., p=0.037), especially BRCA2 carriers with familial OC (47 yrs. vs. 51yrs., p=0.018).
18/497(3.6%) male carriers had BC. 1/3 were BRCA1 carriers, and were younger at cancer diagnosis compared to BRCA2 mutation carriers (50yrs. vs. 57yrs., NS).
Conclusions: RR-BSO rates in Israeli carriers are very high, particularly in women affected with BC and family history of OC. Consortium data are consistent with lower cancer rates for BRCA2 mutations.

P12.013-S

Setting up the basis for translating "omic" data for BRCA1/2 into clinical practice

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Germline mutations in BRCA1 or BRCA2 genes account for about 60% of inherited breast cancer and, subsequently, for approximately 3% of all breast cancer (BC) cases. It is of chief importance that women who carry a hereditary mutation are identified and given options for prevention; for this reason BRCA testing is increasingly offered to women with suspected BRCA mutations, implying a rapid growth of testing requests to the molecular laboratory. In this regard, we are currently comparing the analysis of these genes with the Ion AmpliSeq next generation sequencing technology with the classic Sanger method. Preliminary data on 14 samples, already analysed using Sanger method, were obtained using the BRCA1/2 Ion Ampliseq kit on Ion Torrent PGM: data indicated a reliable and robust variant identification and sequence coverage ranging from 99.995 to 100%. Data comparison will be presented for 40 patients. In addition, recent data suggest that constitutive epimutations in BRCA1 might be relevant to breast carcinogenesis. We identified in a pilot study on Sequenom MassARRAY system that blood DNA from BC patients (negative for BRCA1/2 mutations) was consistently more methylated in a region corresponding to chr17:38531627-38532076, compared to controls (60-90% vs 30-40%). Therefore an epigenetic and quantitative expression analysis of BRCA1 is currently carried out in a larger group of patients with or without BRCA mutations, and controls, in order to establish a correlation between the degree of methylation and the expression levels in our cohort of patients. This study is supported by grant PRUa1GR-2012-001 "DIANE".

P12.014-M

Characterization of *BRCA1* and *BRCA2* variants of unknown clinical significance identified in a Norwegian cancer cohort

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The most commonly mutated high penetrance genes in hereditary breast and ovarian cancer (HBOC) are *BRCA1* and *BRCA2*. Germline loss-of-function mutations in these genes confer a high risk of breast cancer to the individual carrier. However, besides the clear loss of function mutations, there are still a large number of sequence variants of uncertain clinical significance (VUS) in both genes. These VUS generate a huge challenge both for genetic counseling and prophylactic surgery.
In the current study, the consequences of some *BRCA1* and *BRCA2* VUS identified in Norwegian cancer patients were evaluated. Initially, their possible influence on RNA splicing was investigated by targeted sequencing of cDNA. In addition, the complete *BRCA1* and *BRCA2* cDNA were sequenced in order to uncover eventual alternatively spliced transcripts. Some of the variants affecting the BRCT domains of BRCA1 were tested for their influence on BRCA1 trans-activation function. In total, 19 individuals with *BRCA1* VUS and 18 individuals with *BRCA2* VUS from families with HBOC were included in this study. Three of the variants, *BRCA1* c.213-5T>A, *BRCA1* c.5434C>G and *BRCA2* c.68-7T>A, were shown to influence RNA splicing. Investigation of the full-length cDNA of *BRCA1* and *BRCA2* proved to be challenging due to the presence of several alternatively spliced transcripts which were also present in the controls. A functional assay, developed to assess the trans-activation ability of BRCA1, indicated that some of the variants may have a deleterious effect.

P12.015-S

Two new cases of double heterozygosity for BRCA1 and BRCA2 gene mutations identified in a cohort of Italian breast and ovarian cancer families

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Double heterozygosity for BRCA1 and BRCA2 mutations is a very rare finding, particularly in non-Ashkenazi individuals, and only a few cases have been reported to date. Here we describe genetic and clinical data of two female double heterozygotes for both BRCA1 and BRCA2 mutations found in a cohort of 201 mutated Italian breast/ovarian cancer families out of 942 cases analyzed. The first one is a female patient affected by bilateral breast cancer at 47 and 49 years of age, carrying both a BRCA2 nonsense mutations (c.7408A>T - p.Arg2394X) and a BRCA1 proven splicing defect (IVS5-12A>G or c.331_332ins11 - p.Arg71SerfsX21). The second one is a female patient affected by ductal breast cancer at 42 years of age, carrying both a BRCA1 nonsense mutations (c.3726C>T - p.Arg1203X) and a BRCA2 frameshift mutation (c.3036_3039delACAA - p.Ala938ProfsX21). Although this event is rare (2/201: 1% in our clinical records, consistent with literature

data) and the phenotype is not worse than carriers of a single mutation, it has to be considered in the assessment of the biological effect of variants of uncertain biological effect. Furthermore the presence of a second mutation has important consequences for genetic counselling of relatives. We suggest that mutation analysis of index cases should always be extended in order to avoid missing a second BRCA mutation.

P12.016-M

BRCA mutation testing in all newly diagnosed patients with breast- or ovarian cancer: The DNA BONus study

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Introduction: *BRCA*-mutation carriership affects treatment, follow-up and further cancer prevention in patients newly diagnosed with breast- or ovarian cancer. Therefore indications for *BRCA* mutation testing grow rapidly. We investigate prospectively 1) the frequency of *BRCA* mutation carriers among unselected breast- and ovarian cancer patients, 2) current criteria for *BRCA* mutation testing by age and family history, and 3) psychosocial effects of *BRCA*-mutation testing in newly diagnosed patients.
Materials and methods: *BRCA*-mutation testing is offered consecutively since September 2012 (ongoing) to unselected newly diagnosed patients with breast- or ovarian cancer at four hospitals in Western Norway. Initial testing is performed by a selected panel of 40 most frequent *BRCA*-mutations, covering more than 90% of *BRCA*-mutations in the native Norwegian population. Additionally, patients with a positive family history are invited for genetic counseling and further genetic testing.
Results: By February 2014 N=208 breast cancer patients (40% response rate) and N=40 ovarian cancer patients (67% response rate) have been included in the study. A pathogenic *BRCA*-mutation has been found in 5 (2.4%) breast cancer patients and 6 (15%) ovarian cancer patients; 9 *BRCA1* and 2 *BRCA2* mutations. Only one of these mutation carriers would have been missed by current guidelines. 42% of the participants have been invited for genetic counseling.
Conclusion: Less than half of the patients with breast cancer and two thirds of patients with ovarian cancer choose genetic testing at diagnosis. The incidence of *BRCA*-mutations is particularly high among unselected patients with ovarian cancer. The updated results will be presented.

P12.017-S

Involvement of the *RNU2* macrosatellite in breast cancer susceptibility

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The *RNU2* macrosatellite is a tandem array of a 6.1 kb repeat unit containing the 190 bp-long gene coding for snRNA U2, *RNU2-1*, and 3.8 kb of interspersed repetitive DNA. Although located 124 kb telomeric to the breast and ovarian cancer susceptibility gene *BRCA1*, the *RNU2* locus is absent from the human genome assembly due to the inherent difficulty to assemble repetitive sequences. Thus, the influence that these large tandem repeat arrays might exert on neighbouring gene expression, stability or architecture and their contribution to the genetic basis of common human diseases remains largely unexplored. We have recently characterized in more details the *RNU2* locus and tested the association between *RNU2* copy number and breast cancer risk. Indeed, the proximity of this macrosatellite to *BRCA1* combined with its high degree of polymorphism raises the interesting possibility that it could be involved in breast cancer susceptibility, especially as no mutation is identified in *BRCA1/2* in 80% of the families tested in a diagnostic setting. We conducted a case-control study by genotyping the *RNU2* macrosatellite with a qPCR assay in 1452 familial breast cancer cases, 1457 affected and unaffected relatives and 1241 unrelated controls of the French study GENESIS. We found that mean *RNU2* global copy number (GCN) is higher in cases (52 copies) than in controls (50 copies). In addition, we found more very high *RNU2* GCN (>100) in affected women. We are presently investigating the functional impact of extremely large *RNU2* GCN on the *BRCA1* locus.

P12.018-M

Investigating the management of symptomatic and pre-symptomatic BRCA gene mutation carriers in an Irish Tertiary referral centre

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Aims: Germline mutations in BRCA1 and 2 confer a high risk of breast cancer. The aim of our study was to outline the disease phenotype and management of BRCA gene mutation carriers in the West of Ireland.
Methods: A longitudinal cohort study was undertaken. The study group included patients proven to carry a single gene mutation in BRCA1 or BRCA2 between 2000 and 2013. Clinicopathological information was obtained by chart review.
Results: Fourteen pathogenic mutations were identified in BRCA1 in 45 individuals from 24 families. The most common mutations were large genomic rearrangements, with deletion of exons 1-23 in 5 families, deletion exons 14-20 in 3 families and deletion exons 21-24 in 2 families. Fourteen pathogenic mutations were identified in BRCA2, in 32 individuals from 19 families. The most common was frameshift mutation 8525delC, in 5 families. Of forty-one patients affected with breast cancer, 17 carried mutations in BRCA1 and 24 mutations in BRCA2. Median age of onset of breast cancer in BRCA1 mutation carriers was 40years(25-67), and 45years(35-64) in BRCA2 mutation carriers (p=0.02, Mann-Whitney). Eight patients developed bilateral breast cancers including 6 BRCA1 mutation carriers. Five of six patients with ovarian cancer carried BRCA1 mutations. Ten of 51 pre-symptomatic carriers underwent surgical prophylaxis, including 9 prophylactic mastectomies and 5 oophorectomies.
Conclusions: BRCA gene mutations account for a small proportion of inherited predisposition to breast cancer. Carriers of these mutations require intensive surveillance or surgical prophylaxis. Counselling and testing of pre-symptomatic family members can facilitate intervention and modify disease phenotype.

P12.019-S

Characteristics of Greek patients with breast cancer rearrangements in BRCA1 gene

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In most countries large genomic rearrangements (LGRs) in *BRCA1* and *BRCA2* genes occur in a small percentage of patients tested for hereditary breast-ovarian cancer. Instead, in the Greek population, four specific LGRs have been identified in *BRCA1* [deletions of exon 20 (4kb), exon 20 (3kb), exon 24 (4,5kb), and exons 23-24 (11kb)], the latter three of which have been characterized as founder mutations. Several factors may be associated with LGRs, such as younger age at breast cancer (BC) diagnosis, bilaterallity and estrogen receptor–negative status.
The study's objective was to assess the possible establishment of criteria leading to targeted screening, as well as to increase our insight into the role and clinical significance of specific *BRCA1* pathogenic findings.
In a cohort of 2.100 hereditary BC or OC patients, 74 (3,5%) were found to carry one of the four LGRs. We have investigated the possible association between *BRCA1* LGRs and the aforementioned factors. The mean age at diagnosis was 40,6 years. Among the 74 patients, 43 (60%) developed BC, 15 (20%) both BC and OC, where 16 (20%) developed OC only. 14 out of 74 developed bilateral (3/14), contralateral (9/14) and ipsilateral (2/14) BC. Histopathology data was available for 40 out of 58 BC patients, demonstrating that 29/40 (72%) were triple-negative, 6/40 (15%) were ER+/PR+/Her2-, 3 (7,5%) were ER-/PR-/Her2+, 1 (2,5%) was ER+/PR-/Her2- and 1 (2,5%) was ER+/PR+/Her2+.
In conclusion, LGRs compared to other loss-of-function mutations of *BRCA1* gene do not seem to be associated with specific clinical or histopathological features.

P12.020-M

TAp73α regulates Otx1 expression during breast cancer stem cells differentiation and in response to cisplatin treatment

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Breast cancer is the most fatal disease for women in Western countries, despite mammography screening and adjuvant therapy with tamoxifen and polychemotherapy. Knowing the molecular mechanisms underlying this

disease may contribute to the identification of new targets for future therapies. Tp53, Tp63 and Tp73 tumor suppressor family members encode for transcription factors which control genome integrity. They take part in cell response and in tumor suppression. Wild-type p53 protein is a growth modulator and its inactivation is a critical event in malignant transformation of breast cancer stem cells (CSCs). Otx1 is an homeobox gene involved in central nervous system development, and when deregulated, plays a role in tumorigenesis. We showed that Otx1 is over-expressed in ductal and lobular invasive breast cancers and that is involved in adult mammary gland development. We demonstrated that p53 directly regulates Otx1 gene expression binding to the 3'p53 responsive element (RE) on its promoter, and that this pathway regulates the LA7 breast CSCs differentiation. Here we will show that the TAp73α isoform of p73 is able to bind the 5'p53RE on the Otx1 promoter, leading to the LA7 cell differentiation and the asymmetric division of breast CSCs. Furthermore we will demonstrate that Otx1 and TAp73 are over-expressed in ductal and lobular invasive breast carcinoma. Finally we will show the functions of the TAp73α/Otx1 pathway in differentiation of mammospheres obtained from wild-type and c-ErbB2 transgenic mice, and the response to cisplatin treatment in MCF7 and MBA-MB-231 breast cancer cells.

P12.021-S

One in three Greek patients with early onset or familial breast cancer carries a loss of function mutation in a known or candidate breast cancer gene

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The antiquity of the Greeks as a population defined by language and culture, and the complexity of Greek historical demography, present challenges to genetic testing for predisposition to cancer. The Greek population harbors ancient founder mutations in many genes, including five BRCA1 damaging alleles, as well as many disease alleles that are specific to one or a few families. The aim of this study was to identify loss-of-function mutations in 22 genes in 736 patients with breast cancer diagnosed at a young age (<35 years) or with a strong family history of breast, ovarian, and/or pancreatic cancer.
Targeted capture and multiplexed sequencing was carried out using BROCA, which captures the entire loci of 22 genes. Of 736 patients with young onset or familial breast cancer, 252 (34%) carried loss-of-function mutations in one of 11 genes. Frequencies were: 103 BRCA1 founders, 61 other BRCA1, 45 BRCA2, 29 CHEK2, 7 PALB2, 7 ATM, 2 PTEN and 1 in each in RAD51C, FAM175A, BRIP1, PIK3CA and TP53. PALB2 p.R753X was observed in four Greek families and may be a founder allele. We conclude that among Greek patients with familial or early onset breast cancer more than a third carry loss-of-function mutations in a breast cancer-related gene. Founder mutations account for about 50% of the BRCA1 and BRCA2 mutational burden and about 40% of the mutational burden in all known and candidate breast cancer genes. Given genetic heterogeneity, patients benefit from an approach that detects all classes of mutations in known breast cancer genes.

P12.022-M

Mutations in CHEK2 and NBN genes among Macedonian breast cancer patients

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Heterozygotes for mutations in CHEK2 and NBN genes were found to be associated with increased risk of developing cancers, including breast cancer (BC). The aim of our study was to determine the frequency of three common mutations in CHEK2 gene (1100delC, I157T and IVS2+1G>A) and two mutations in NBN gene (R215W and 657del5) among Macedonian BC patients and controls from the general population. For this purpose we have designed a multiplex PCR followed by SNaPshot analysis. A total of 299 BC patients, of whom 112 with a cancer family history and 283 controls were included in the study. Mutations were more frequent among BC patients (n=13, 4.3%) than among controls (n=5, 1.8%), although without statistical significance. Twelve patients were heterozygous for one of the analyzed mutations, while one patient had two mutations (NBN R215W and CHEK2 I157T). The most frequent mutation was CHEK2 I157T, found in 10 BC patients and 4 controls. The frequency of this mutation was statistically higher among BC patients with a cancer family history when compared to the controls (p=0.028). NBN R215W was found in one BC patient and one control, while CHEK2 1100delC and NBN 657del5 were found each in one BC patient and no control. CHEK2 IVS2+1G>A has not been found in our study. In conclusion, our study suggests that mutations in CHEK2 and NBN genes might play a role in the development of breast cancer among Macedonian patients. A study including larger number of patients and controls are needed to confirm these results.

P12.023-S

BRCA1 and BRCA2 mutation detection by a Next Generation Sequencing approach: an epidemiological study conducted in Southern Italy

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Hereditary breast and ovarian cancer (HBOC) accounts for about 10% of all breast cancers and BRCA1 and BRCA2 are the most prevalent genes causing this pathology. BRCA1/BRCA2 germline mutations escalate the risk of developing HBOCs by up to 20 fold. Testing for BRCA gene mutations is important to improve the clinical management of the high-risk patients and of their mutation carriers family members.
A NGS screening for BRCA1/2 germline mutations of 300 patients, with early-onset breast cancer ("*under forty*") and/or with positive family history, is reported in order to identify mutation carriers. BRCA1/BRCA2 coding regions were amplified using the BRCA MASTR v2.1 Assay (Multiplicom). Sequencing reactions were performed with the 454 GS FLX System (Roche) and the downstream data analysis was carried out through the SeqNext tool (JSI Medical Systems).
More than 15% of the analyzed patients, including a few men, carried a causative mutation. Several novel variants were identified: 1 splice variant, causing the loss of a canonic donor splice site at position +2 in the intron 21 of BRCA1 gene, and 1 missense variant falling in the BRCA2-DNA binding region; one double mutation including 1 missense mutation on BRCA1 gene causing a premature stop codon; 2 synonymous variants and 1missence variant predicted without clinical significance were found. Functional *in vitro* evaluations are ongoing to assess their pathogenicity.
Subsequent analysis of the mutation carriers families, allowed the identification of at higher risk subjects, including men healthy carriers, that have been involved in surveillance program of preventing health care.

P12.024-M

Rare key functional domain missense substitutions in MRE11A, RAD50, and NBN contribute to breast cancer susceptibility

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The MRE11A-RAD50-Nibrin (MRN) complex plays several critical roles related to repair of DNA double-strand breaks. Inherited biallelic mutations in any one of the three components predispose to genetic instability disorders. Inherited heterozygous mutations in the MRN genes have been implicated in breast cancer (BC) susceptibility, but the underlying data are not entirely convincing. In order to verify if rare MRN variants are intermediate-risk BC susceptibility alleles, we mutation screened the coding exons and proximal splice junctions of the MRN genes in 1,313 early onset BC cases and 1,123 population controls.
Considering the extremely low frequency of likely pathogenic variants in these genes in the general population, we have decided to evaluate the genes as if they constitute one relatively large gene (though still smaller than ATM, another gene in the same pathway).
Limiting our analyses to variants with MAF<0.1% and combining protein truncating variants, likely spliceogenic variants, and key functional domain rare missense substitutions, we found significant evidence that the MRN

genes are indeed intermediate-risk BC susceptibility genes (OR= 2.88, P= 0.0090). In addition we found that key domain missense substitutions were more frequent (24 vs 12 observations) than the truncating variants and conferred a slightly higher OR (3.07 vs 2.61) with a lower P-value (0.029 vs 0.14). Thus, the spectrum of pathogenic variants in MRN genes includes, as for ATM and CHEK2, a relatively high proportion of missense, and differs notably from the BRCA1/2 pattern where most susceptibility alleles are protein-truncating variants.

P12.025-S

Search for recessive cancer predisposition genes: the advantage of multiple primary cancer cases vs. family history-positive patients

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Virtually all known tumor predisposing genes have been identified via the analysis of familial cancer cases. Here we argue that this approach is likely to miss recessively acting cancer genes and suggest the analysis of family history-negative patients with multiple primary malignancies for identifying homozygous at-risk genotypes. We performed calculations showing that in cases of transmission of a recessive cancer predisposing allele (a), which has a frequency of 0.1 and a penetrance of 100%, only a minority of patients with the aa genotype (19%) will have one or both parents with the same genotype and hence report a family history of cancer. Therefore, while the focus on familial cancer clustering is a powerful tool for identifying dominant mutations, the cases of disease caused by the homozygous recessive at-risk alleles may be easily missed. We further revealed that the c.2515_2519delAAGTT homozygous mutation in a Holliday junction resolvase, GEN1, was over-represented in women with bilateral breast cancer (BC) as compared to healthy controls [11/360 (3.1 %) vs. 18/1305 (1.4 %); OR = 2.25 (1.02 - 4.75); p = 0.031], although this trend was not pronounced in unilateral BC patients. Noticeably, the presence of biallelic c.2515_2519delAAGTT mutation was associated with the absence of BC in mothers of both bilateral and unilateral BC patients [7/239 (3.0 %) vs. 0/41 (0 %) and 21/1,558 (1.3 %) vs. 0/215 (0 %), respectively; p = 0.041]. This study confirms that patients with multiple cancers may be particularly fruitful for the identification of recessive determinants of cancer predisposition.

P12.026-M

High-throughput genetic analysis in 100 hereditary breast cancer patients

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Objective: Here, we report our first results from our HBOC-NGS-panel that includes 56 genes associated with breast and ovarian cancer. So far, 100 samples were analyzed in a diagnostic setting.
Methods: A custom NGS-panel (HaloPlex, Agilent) was used to target 56 genes. Depending on the patient's family history, a set of "diagnostic" genes (mostly BRCA1/2, RAD51C/D) as well as "screening" genes were defined. DNA was isolated from all samples, enriched and sequenced on the Illumina MiSeq (2x 150 bp paired-end) following standard protocols. For diagnostic genes, regions with low depth (< 20x) were complemented by Sanger sequencing as well as MLPA. All mutations were confirmed by conventional Sanger sequencing.
Results: So far, we have sequenced 100 hereditary breast cancer patients. Overall 91-99 % of all targeted exons were represented with a "diagnostic" average depth of > 20x. Roughly 70 SNVs were identified per sample and stringent filtering resulted in less than seven variants for validation.
We identified 15 mutations that are known to cause HBOC as well as mutations likely to cause HBOC. Further experiments and segregation analysis is required to determine the pathogenicity in the latter. In addition heterozygous mutations were found in genes relevant for different autosomal recessive cancer syndromes.
Conclusion: Taken together, we demonstrate that NGS is a fast and cost efficient genetic screening tool to analyze for variants in genes associated with the development of hereditary breast cancer. By applying this approach we were able to uncover both known and novel sequence variants

P12.027-S

BRCA1 and BRCA2 germline mutational spectrum among Macedonian women with breast cancer detected by next generation sequencing

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The contribution of *BRCA1/2* mutations to hereditary breast cancer (BC) in women from R. Macedonia is largely unknown. Our previous small study revealed the presence of seven mutations (c.181T>G, c.1102G>T, c.5266dupC, c.81-?_547+?del and c.5407-?_5467+?del in *BRCA1* and c.8167G>C and c.1-?_6937+?del in *BRCA2* gene) in 10 unrelated BC families.
Here we describe the next generation sequencing analysis of *BRCA1* and *BRCA2* genes in 94 BC patients using TruSeq Custom Amplicon for library preparation and sequencing on Illumina MiSeq personal sequencer. In addition to the standard inclusion criteria, patients with triple negative tumors and *BRCA1*ness profile determined by MLPA analysis were included in this study.
Eight BC patients have been previously analyzed by Sanger sequencing; all sequence variations previously determined were correctly identified. Among the 94 analyzed BC patients we detected a total of 78 sequence variations (30 in *BRCA1* and 48 in *BRCA2*). Thirty five of these variants were found with a frequency between 2.7% and 42.9% among the analyzed patients, while 43 variants were found in only one or two patients. A total of 14 pathogenic mutations were detected of which 11 have been previously described (c.5266dupC, c.3700_3704delGTAAA, c.2596C>T in *BRCA1* and c.5351dupA, c.5848_5851delGTTA, c.7811_7814delTGTG, c.7879A>T, c.7916 C>T, c.8168A>G, c.9104A>C, c.9350_9351delAT in *BRCA2*), while three mutations were novel (c.4360delT in *BRCA1* and c.3186_3189delTCAG and c.8315_8328delAATCTCTTAT in *BRCA2*).
In conclusion, our work demonstrates that using TruSeq Amplicon technology and sequencing on MiSeq system is a fast, reliable and cost effective approach for mutational screening of *BRCA1* and *BRCA2* genes.

P12.028-M

Testing for genetic predisposition to breast cancer by NGS

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About 5-10% of breast cancer is due to inherited mutations. Genetic testing for predisposition in individuals with family history is recommended to determine their risk for developing this cancer type. Next generation sequencing enables us to perform analysis for large number of predisposing genes.
In this study, DNA samples were obtained from 27 individuals with positive family history – affected or still not affected. Informed consent was obtained from all the subjects. Sequence analysis for BRCA1/BRCA2 or 94 cancer-predisposing genes and 284 variants associated with cancer was performed on Illumina MiSeq system.
A wide range of variants were identified in the BRCA1 and BRCA2 genes. Four pathological variants were detected (14.8%): mutation in BRCA2 at the chromosomal (chr) position chr13:32890665, which affected the first position of the 5' splice region following exon 2; mutation in BRCA1 at chr17:41219635, causing an inframe triple nucleotide deletion of valine 1688 (8.3%); mutation in TP53 - c.775T>AT; 193H>LH; frameshift mutation in BRCA2 - c.2808_2811delACAA, p.Ala938ProfsTer21.
To the best of our knowledge, this study was the first to identify 3 common polymorphisms in BRCA2, characteristic solely of the Bulgarian population, including chr13:32973737, T/, a singlenucleotide polymorphism (SNP) within the 3'UTR of exon 27; chr13:32973280, A/, mononucleotide deletion within the 5'UTR of exon 27; and chr13:32973924, T/, mononucleotide deletion downstream of the gene sequence. Furthermore, this study was the first to apply nextgeneration sequencing of cancer-predisposing genes in Bulgarian population, prompting further investigation for local founder mutations and variants characteristic for this particular region.

P12.029-S

A PALB2 high-risk mutation recurrent in familial breast cancer cases from the province of Bergamo

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Germline deleterious mutations of PALB2 are associated with breast cancer risk and have been reported in several populations. In our initial survey, truncating PALB2 mutations were detected in 12/575 (2.1%) familial breast cancer cases negative for BRCA gene mutations (BRCAX), recruited at two large cancer centres in Milan (Istituto Nazionale Tumori and Istituto Europeo di Oncologia). One mutation (c.1027C>T; p.Gln343X) recurred in three cases, two of whom originally from the province of Bergamo. The genotyping of the c.1027C>T in 113 BRCAX cases ascertained at Azienda Ospedaliera HPG23 of Bergamo, detected a total of 6 carriers (5.3%), while only 2 carriers were observed among 477 female blood donors recruited in the same area (0.4%). The estimated age-adjusted odds ratio of these frequencies was 13.4 (95% confidence interval: 2.7–67.4). This value is similar to those previously observed for a few PALB2 mutations recurrent in other countries and suggests that the c.1027C>T is associated with a relatively high breast cancer risk. Of note, we also found that among breast/ovarian cancer families recruited in Bergamo the mutational spectra or both BRCA1 and BRCA2 were much less heterogeneous than in families ascertained in Milan. In particular, one BRCA1 founder mutation was observed in >8% of Bergamo families (Caleca et al., 2014). Further analyses are required to verify whether genotyping for specific recurrent mutations can be proposed as a cost-effective strategy for the rapid identification of individuals genetically predisposed to breast/ovarian cancer in the Bergamo area.

P12.030-M
RNA-Sequencing in MCF-7 cells: identification of a new transcript of SEMA3F and its expression in breast cancer

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Breast cancer is the most common tumor in women, and the second leading cause of death. Tumor cells invasiveness is mainly due to an alteration of cell-cell and cell-matrix connections. Thus, an altered expression of adhesion molecules and their receptors is a crucial event in this process. Among them, semaphorins, a large family of transmembrane or secreted molecules that regulate cell migration and adhesion, are of peculiar interest. A growing number of studies - and our recent work on *SEMA6B* in breast cancer among these - has demonstrated their involvement in cancer progression, often with divergent functions.
In order to simultaneously investigate gene expression and alternative splicing for all the genes encoding adhesion molecules, and particularly for semaphorins, their receptors and co-receptors, we performed RNA-Sequencing experiment on MCF-7 cells, a well-established and widely used cellular model of breast cancer. Interesting preliminary results were obtained, particularly for *SEMA3F* gene. Indeed, we identified a novel transcript generated by alternative splicing, predicted to encode for a truncated semaphorin. Moreover, through semiquantitative PCR and quantitative Real-Time assay we measured SEMA3F expression on a panel of breast cancer tissues compared to their healthy counterpart. The analysis revealed a strong and significant up-regulation of *SEMA3F* in breast cancer and, intriguingly, the sole presence - in the healthy tissues - of the newly identified transcript. These evidences suggest *SEMA3F* to be a potential biomarker for onset/progression of breast cancer.

P12.031-S
Investigating the importance of variants at 12p11, 12q24 and 21q21 in breast cancer in the west of Ireland

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Introduction: Recent genome-wide association studies have identified novel breast cancer susceptibility loci in women of European ancestry at 12q24 (rs1292011); 12p11 (rs10771399) and 21q21 (rs2823093). The aim of our study was to investigate the prevalence of variants at these three loci in a specific Irish subpopulation, and to examine the association between these variants and breast cancer in this cohort.
Methods: DNA was extracted from the whole blood of patients with breast cancer and from healthy female controls using a salting out method. Genotyping of each sample for each of the three targets was carried out using a Taqman®-based platform. Statistical analysis was performed using SPSS software after testing for Hardy-Weinberg equilibrium.
Results: A total of 1639 samples were included in the study group, comprising 1191 cases and 448 controls. The minor allele at locus 12p11 was found to confer a significant protective effect, with a per allele odds ratio of 0.7 (0.5-0.9, p=0.002, X2). The minor allele at 12q24 had a slight protective effect (per allele OR =0.9 (0.8-1.1, p =0.29, X2)). The minor allele 21q21 did not confer a protective effect, and was in disequilibrium as per test of Hardy-Weinberg.
Conclusion: All three genetic variants were detected in the population in the west of Ireland. The G allele at 12p11 was associated with reduced breast cancer risk. Population-specific genome wide association studies are required to identify susceptibility loci specific to the Irish subgroup

P12.032-M
DNA-diagnostics for inherited breast and/or ovarian cancer: standard approaches and novel technologies

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Modern molecular techniques allow revealing the most characteristic genetic changes responsible for hereditary breast and/or ovarian cancer (hBC/OC), calculating the risk of neoplasia development, defining treatment and prevention. In routine diagnostics of hBC/OC two methods: biochip-based approach and real-time PCR were used for the analysis of founder mutations in Russian Federation, namely, 185delAG, 300T>G, 4153delA, 4158A>G, 5382insC, 6174delT, 1100delC. However, only 15% of patients were found to carry the BRCA1/2 or CHEK2 mutation. Other genes, like TP53, PALB2, ATM, BRIP1, RAD50, BLM and so on, are widely tested in patients with hBC/OC susceptibility. The aim was to study an impact of TP53 germline mutations and polymorphisms into predisposition to bilateral BC and/or secondary-primary multiple neoplasia (SPMN). Genomic DNA was isolated and exons 4-11 of TP53 gene were amplified with commercial primer set SeqPlateTP53. Next-generation sequencing was performed using a platform GS Junior (454/Roche). The results were validated by Sanger sequencing. Exons 4-11 of TP53 gene in 22 patients with SPMN including BC or bilateral BC and without founder mutations in BRCA genes (wtBRCA) were analyzed. The findings included several rare variants with population frequency less than 0.001%. Previously found nonsense mutation 2637C/T, leading to stop-codon instead of arginine (codon 306) in patient with bilateral BC was confirmed by NGS. A heterozygous germline missense mutation in codon 241 (c.722C>A) was diagnosed in patient with SPMN (Li-Fraumeni syndrome). The mutation was confirmed by Sanger sequencing. The data demonstrated the clinical utility of TP53 testing in selected patients with SPMN and/or bilateral BC.

P12.033-S
The experiences and views of health care professionals and researchers regarding the feedback of results in the context of next generation sequencing in oncology

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Next generation sequencing (NGS) allows the production of large volumes of sequence data (and potentially genetic results) and the ethical and practical issues regarding feedback of results become particularly pertinent to address. Should (any) results be given to research participants? If so, which results and who should provide them? Within two EU funded projects in oncology (CAGEKID and EUROTARGET), in order to gather researchers' and health care professionals' views and experiences on providing results we distributed a questionnaire to attendees of genetics meetings in Europe in 2013.
Of the 95 respondents, 88% work as researchers and/or clinicians in a field related to oncology and half (52%) use NGS in some aspect of their work; 56% of respondents state that they provide specific information about NGS to participants or patients before enrolling them in a study or using their samples for sequencing. The majority, 83% had never received requests from physicians or patients for access to NGS data to inform treatment decisions. Regarding feedback of results in a research setting, 54% or respondents think that results stemming from NGS studies should be provided to individual participants and 72% think that actionable incidental findings should be disclosed to participants. Finally, 53% of respondents think that specific measures and/or limitations should be implemented for the sharing of NGS data/results with colleagues in the scientific community. Such empirical data from stakeholders is a valuable contribution to the ongoing discussion of how to responsibly handle and feedback results to patients and research subjects.

P12.034-M

Whole-genome profiling of cervical carcinomas patients with CGH+SNP microarrays: correlations with clinical outcome

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Alterations in the genome that lead to changes in DNA sequence copy number are characteristic features of solid tumors. In this study, we used GGH+SNP microarray technique for detailed screening of copy number alterations (CNAs) in a cohort of 26 patients with uterine cervical carcinoma (UCC), and the findings were correlated with the incidence of lymph node metastasis and lymphangioinvasion. The whole-genome screening discovered CNAs in 73.1% of samples. Frequent areas of gains were observed in 3q (50.0%), 1q (42.4%), 19q (23.1%), while losses were commonly observed in 11q (30.8%), 4q (23.1%), 13q and 2q (both 19.2%). Regions of loss of heterozygosity were observed in 15.4% in 11q23, 14q21, 18q12.2 and 8q21. The incidence of gain 3q was associated with gain 1q (P=.033), while loss of 4p was commonly observed with loss of 13q (P=.010). Higher occurrence of CNAs was associated with patients under 45 years (P=.016). Patients with adenocarcinoma have statistical trend to carry genomic profiles without CNAs (P=.051). Incidence of lymph node metastases was associated higher number of CNAs (12 vs 9; P=0.452) and patients without LVSI had trend to higher incidence of gains in chromosome 15q. Taking together array-CGH techniques allowed us to precisely detect specific cytogenetic lesions in UCC, which can be used for prognosis of the disease as well as novel genomic markers associated with the development of invasive cervical cancer.

Supported by OPVK CZ.1.07/2.3.00/20.0183.

P12.035-S

Human bile contains microRNA-laden extracellular vesicles that can be used for cholangiocarcinoma diagnosis

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Cholangiocarcinoma (CCA) presents significant diagnostic challenges, resulting in late patient diagnosis and poor survival rates. Primary Sclerosing Cholangitis (PSC) patients pose a particularly difficult clinical dilemma, since they harbor chronic biliary strictures that are difficult to distinguish from CCA. MicroRNAs (miRs) have recently emerged as a valuable class of diagnostic markers; however, thus far, neither extracellular vesicles (EVs) nor miRs within EVs have been investigated in human bile. We aimed to comprehensively characterize human biliary EVs, including their miR content. Conclusion: We have established the presence of extracellular vesicles in human bile. In addition, we have demonstrated that human biliary EVs contain abundant miR species, which are stable and therefore amenable to the development of disease marker panels. Furthermore, we have characterized the protein content, size, numbers and size distribution of human biliary EVs. Utilizing Multivariate Organization of Combinatorial Alterations (MOCA), we defined a novel biliary vesicle miR-based panel for CCA diagnosis which demonstrated a sensitivity of 67% and specificity of 96%. Importantly, our control group contained 13 PSC patients, 16 patients with biliary obstruction of varying etiologies (including benign biliary stricture, papillary stenosis, choledocholithiasis, extrinsic compression from pancreatic cysts, and cholangitis), and 3 patients with bile leak syndromes. Clinically, these types of patients present with a biliary obstructive clinical picture that could be confused with CCA. These findings establish the importance of using extracellular vesicles, rather than whole bile, for developing miR-based disease markers in bile.

P12.036-M

Functional studies on post-transcriptional regulation of FAS/FASL in chordoma

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Chordoma, originating from notochord remnants, is characterized by chemoresistance. Since the apoptotic Fas/Fasl pathway is involved in notochordal cell apoptosis, we investigated its possible role in chordoma. Accordingly we detected the lack of FASL mRNA and the presence of both FAS anti- and pro-apoptotic isoforms and the inactive Caspases 8 and 3 forms in most of the tumors analyzed, suggesting the Fas/Fasl pathway inactivation in chordoma. The enhancement of apoptosis in U-CH1 chordoma cells, expressing Fasl and Fas anti- and pro-apoptotic isoforms after Soluble Fasl treatment, indicates that this pathway can be re-activated. These findings lead us to hypothesize that Fas receptor binds Soluble Fasl that, undermining Fas antiapoptotic isoform, activates apoptosis. Soluble Fas isoform could have a key role as antiapoptotic factor as the alternative splicing underlying the expression regulation of the two Fas isoforms. Knowing that HuR is one of the splicing factors leading to Fas transmembrane isoform, we are performing functional studies aimed at modulating the reciprocal amount of Fas isoforms by interfering with the expression of HuR. U-CH1 apoptosis, cellular vitality and migration will be evaluated after HuR iperexpression and silencing to assess the role of Fas antiapoptotic isoform in chordoma apoptosis regulation. We are also silencing miR-21 targeting FASL mRNA to verify whether the increase of endogenous Fasl may activate apoptosis. This study, providing findings on the involvement of Fas/Fasl pathway in chordoma could help to elucidate the role of apoptosis in chordoma tumorigenesis, addressing the identification of new potential pharmacological targets.

P12.037-S

Transcriptome analysis of cancer cells in chronic myeloid leukemia

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Long term observation studies confirm high efficiency of targeted therapy of chronic myeloid leukemia (CML) by tyrosine kinases inhibitors (TKI). However part of CML patients demonstrate primary resistance to TKI. This resistance appears to be connected with activation of alternative BCR-ABL-independent signaling pathways. Transcriptome analysis of cancer cell in CML is a perspective approach to elucidate molecular mechanisms of TKI-resistance and to find new approaches to CML treatment. We aim to find and to study differences in expression levels of cancer cells in primary CML patients, who demonstrated sensitivity or resistance to TKI. Gene expression profiles were analyzed using Illumina HT-12 Expression Bead Chip. These chips quantitate expression levels of more than 47000 transcripts. According to European Leukemia Net (2013) criteria patients were divided into resistant to TKI therapy - molecular response >10% in 6 months of therapy and optimal responders with molecular response <1% in 6 months of therapy. Comparative transcriptome analysis revealed 2672 of differently expressed genes in responders and non-responders. Enrichment analysis of the differently expressed genes showed the following molecular networks involved ($p < 0,05$): HTLV-1 infection (FZD10, ADCY1, MYB); PPAR signaling pathway (SCD-1, OLR1); Transcriptional misregulation in cancer (MPO, CEBPE, ELANE); Melanogenesis (ADCY1, FZD10). Detailed analysis demonstrated that all of the selected genes are overexpressed in cancer cells of patients sensitive to TKI therapy. Differently expressed genes and expression particularities of the selected pathways may be useful molecular markers for prognosis of the TKI therapy efficacy and may be used to optimize treatment of CML.

P12.038-M

XRCC4 gene (Intron 3 VNTR) polymorphism predisposition to chronic phase chronic myeloid leukemia (CML) and XRCC1 gene (399) polymorphism in associated with event-free survival in CML treated with imatinib in Turkish population

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The aim of this study was to explore the association between polymorphisms of three DNA repair genes (XPD, XRCC1 and XRCC4) and clinical parameters in patients with Philadelphia-positive (Ph+) Chronic Myeloid Leukemia (CML) treated with imatinib. Sixty-two patients with CML and 70 healthy controls were examined for XPD (A-751G), XRCC1 (A399G), XRCC4 (intron 3 VNTR and G-1394T) polymorphisms. DNA was extracted from peripheral blood samples using salting out procedure. All polymorphisms were genotyped by PCR and/or PCR-RFLP and analyzed. All data were analyzed using de Finetti program and SPSS version 14.0 for Windows. No significant differences were detected between CML group and healthy controls with respect to the distributions and numbers of genotypes and alleles in XPD, XRCC1 and XRCC4 (-1394). However, intron 3 VNTR polymorphism in

XRCC4 gene was showed an association with CML patients. The distribution of DD, DI and II genotypes for the gene was 3.2%, 51.6% and 45.2% in CML compared with 14.3%, 60% and 25.7% in the controls. In the univariate analyses of clinical parameters, there was no significant prognostic factor found to influence overall survival. The four factors that were predicted for better event-free survival from univariate analysis were younger age (< 60) (p=0.020), absence of splenomegaly (p=0.011), lower Sokal risk score at diagnosis (p=0.0148) and XRCC1 GG genotype (p=0.033). Our results suggest that XRCC1 GG genotype may be a useful marker for CML prognosis and II genotype of intron 3 VNTR polymorphism in XRCC4 may be associated with susceptibility.

P12.039-S
Polymorphism in genes CYP2C9 and ODC1 involved in aspirin handling influence colorectal cancer risk

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Long term regular use of aspirin is associated with a reduced risk of colorectal cancer (CRC) of up to 60%. However, not all aspirin users receive the beneficial chemopreventive effect. This is potentially attributable to the presence of single nucleotide polymorphisms (SNPs) in genes involved in aspirin's pharmacokinetic and pharmacodynamic pathways. We investigated the impact of 32 SNPs within 9 genes and 2 SNPs in intergenic regions on CRC risk using the Leeds Colorectal Cancer Panel which contains 1910 cases and 1276 controls; these SNPs were chosen for their potentially functional effect. Sixteen out of 34 SNPs were removed from analysis as they had frequency of <3%. All remaining SNPs were in Hardy-Weinberg equilibrium. P values for the tests of association were adjusted for sex and age. The presence of rs11694911 T allele (OR=0.79, 95% CI=0.63-1.0, p=0.04) and rs2302615 T allele (OR=0.81, 95% CI=0.67-0.99, p=0.024) at *ODC1* locus were associated with a reduced CRC risk. When the data was stratified (colon versus rectum), *CYP2C9* rs1799853 T allele (OR=0.73, 95% CI=0.60-0.90, p=0.003) and *ODC1* rs2302615 T allele (OR=0.76, 95% CI=0.61-0.93, p=0.005) showed significant association with colon cancer suggesting influence may be dependent on the tumor location. Both variant alleles have previously been shown to modulate chemopreventive activity of aspirin in colorectal adenoma occurrence, but this is the first dataset to indicate their effect on carcinoma risk. This data suggests that the presence of these variants modulates CRC risk which in turn may provide insight into the variation in aspirin's chemopreventive efficacy.

P12.040-M
Identification of novel candidate genes for early-onset colorectal cancer susceptibility

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Approximately 25-30% of colorectal cancer (CRC) cases are expected to result from a genetic predisposition, but only in 5-10% of these cases high-penetrant germline mutations are found. Therefore, the majority of CRC heredity is still unexplained. This missing heritability is thought to result from the presence of rare variants with a moderate-penetrant risk. Whole-exome sequencing (WES) has made it possible to identify such risk factors. As hereditary CRC is marked by an early age of onset, we performed WES on a cohort of CRC patients (*n*=55) with an onset of disease before 45 years of age. To identify potentially pathogenic variants, we searched for rare protein-truncating variants or highly conserved missense variants. A total of 272 protein-truncating variants and 1537 missense or in-frame insertions and deletions were identified. Recurrence filtering and selection of genes potentially involved in CRC development revealed three novel candidate genes: *EMR3*, *PTPN12* and *LRP6*. A screen of the coding region of these genes in a cohort of early-onset or familial CRC patients (*n*=181) revealed additional rare variants in *PTPN12* and *LRP6*. *PTPN12* encodes a protein tyrosine phosphatase and is described to be a driver of cancer development. *LRP6* encodes a component of the Wnt-Fzd-LRP5-LRP6 complex that triggers beta-catenin signaling. This study contributes to the further understanding of the heterogeneity of the genetic susceptibility for CRC. Follow-up studies on the frequency of mutations in these genes in larger cohorts, and their functional assessment, will provide conclusive evidence for the role of these genes in CRC development.

P12.041-S
Whole-exome sequencing identifies rare coding variants in new predisposition genes for familial colorectal cancer

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Colorectal cancer (CRC) represents one of the most frequent neoplasms in Europe. Besides classical hereditary forms, around 30% of cases present familial aggregation mostly with an unknown inherited cause. Next generation sequencing permits to characterize genetic variation in one individual and to discover new disease predisposition genes.
Material and methods: Patients were selected from high-risk clinics from Spanish hospitals as well as from the EPICOLON consortium. Forty-two individuals from 29 families with strong CRC aggregation compatible with an autosomal dominant pattern of inheritance and without alterations in the known CRC hereditary genes were selected. Exome sequencing was performed in germline DNA with subsequent quality control, removal of sequencing artifacts, data annotation and variant filtering. An automatic CRC-specific pipeline was used for prioritization.
Results: Sequencing mean coverage was >95x. Only very rare variants, producing a clear loss of function and located in genes with a function related to CRC or cancer were selected as final candidates. Afterwards, they were validated by Sanger sequencing and segregation was studied in additional affected family members when available. Loss of heterozygosity in tumor DNA was analyzed in variants with correct disease segregation. Best candidate variants included those located in interesting genes such as CDKN1B, XRCC4, EPHX1, NFKBIZ, SMARCA4, BRCA2 and BARD1. Three variants are expected to abolish protein function and 4 missense changes had strongly deleterious in silico predictions.
Conclusions: We identified variants in DNA repair genes that represent good candidates to become new CRC predisposition genes, some of them previously involved in other neoplasms.

P12.042-M
Whole Exome Sequencing of Hereditary Colorectal Cancer Families from Canada

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Familial Colorectal Cancer Type X (FCCTX) is an inherited predisposition to colorectal cancer (CRC) in families fulfilling the Amsterdam-I criteria. A syndrome of unknown etiology, FCCTX is genetically heterogeneous. FCCTX families appear to have a moderate to high lifetime risk of developing CRC. A cohort of 91 FCCTX patients (66 families) from the Canadian provinces of Ontario and Newfoundland & Labrador (NL) were selected for whole exome sequencing of genomic DNA. A pooled segregation analysis of all missense variants recurrent in patients within two or more families identified 93 candidate missense variants. We then prioritized missense variants with low minor allele frequency (<1%), deleterious prediction of protein consequence and absence of identified variants in population controls. This refined our list of genetic culprits to variants within seven candidate genes. An age, sex and ethnically matched case-control cohort will be analyzed for statistical enrichment of candidate variants. We further queried our exome data for all deleterious rare variants in a set of CRC susceptibility genes identified by review of the current literature. Among these variants we identified POLE p. L424V; a missense substitution described in several patients of families with predisposition for CRC. In a final query involving the proband of a single, large NL family, we screened the exome for variants within two regions of linkage disequilibrium on chromosome 19 (LOD 3.49, 4.51), to refine the disease locus using segregation analysis. Studies of whole exomes continue to highlight the potential for identifying rare deleterious variants in CRC families worldwide.

P12.043-S
Towards personalized cellular adoptive immunotherapy targeting immunogenic neo-antigens in microsatellite unstable colorectal cancers

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Colorectal cancers (CRCs) with microsatellite instability (MSI) represent 15 % of all CRCs, and occur in patients with Lynch syndrome. MSI CRCs have a more dense cytotoxic T lymphocyte (CTL) infiltration and a better prognosis than microsatellite stable CRCs. Stronger immunogenicity of MSI CRCs is commonly explained by the presence of frameshift mutations in genes containing repeated coding sequences which could lead to the synthesis of neo-antigens recognized by specific CTLs. First, to explore the direct link between infiltrating CTL density and frameshift mutations, we quantified within 121 MSI tumors from two independent series CTL (CD8+) density, using tissue microarrays, and we searched for frameshift mutations in repeated coding sequences of 19 genes. We found that infiltrating CTL density significantly increased with the number of frameshift mutations, and was particularly correlated with frameshift mutations in *ASTE1*, *HNF1A* and *TCF7L2* genes. Second, we undertook to stimulate, *in vitro*, patients' peripheral blood CTLs specific of their own tumor neo-antigens, using artificial antigen presenting cells (AAPCs) developed in our laboratory and expressing the corresponding neo-peptides derived from their own tumor specific frameshift mutations. We could indeed easily and efficiently activate such CTLs, in two tested Lynch syndrome patients. These results suggest that, in MSI CRC patients, and especially in Lynch syndrome patients, it should be possible to propose new personalized adoptive cell immunotherapy strategies based on the characterization of frameshift mutations within their tumour and on the construction of AAPCs expressing the corresponding neo-peptides.

P12.044-M

Fourfold increased detection of Lynch syndrome by raising age limit for tumour genetic testing from 50 to 70 years is cost-effective

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Objective: Recognizing colorectal cancer (CRC) patients with Lynch syndrome (LS) can increase life expectancy of these patients and their close relatives. To improve identification of this under-diagnosed disease, experts suggested raising the age limit for CRC tumour genetic testing from 50 to 70 years. The present study evaluates the efficacy and cost-effectiveness of this strategy.
Design: Probabilistic efficacy and cost-effectiveness analyses were performed comparing tumour genetic testing of CRC diagnosed at age 70 or below (experimental strategy) versus CRC diagnosed at age 50 or below (current practice). The proportions of LS patients identified and cost-effectiveness including cascade screening of relatives, were calculated by decision analytic models based on real life data.
Results: Using the experimental strategy, 4 times more LS patients can be identified among CRC patients as compared to current practice. Both the costs to detect one LS patient (€ 9,437/carrier versus € 4,855/carrier) and the number needed to test for detecting one LS patient (42 versus 19) doubled. When including relatives the experimental strategy was found to be highly cost effective because CRC prevention in relatives neutralised the extra genetic testing costs, resulting in lower costs (-€ 2180 per extra tested patient) and an average of 9 life years gained.
Conclusion: Testing all CRC tumours diagnosed at or below age 70 for LS is cost-effective. Implementation is important as relatives from the large number of LS patients that are missed by current practice, can benefit from life-saving surveillance.

P12.045-S

Clinical relevance of 8q23.3, 15q13.3 and 18q21.1 SNP genotyping to evaluate colorectal cancer risk

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The aim of this study was to determine if the at risk SNP alleles for colorectal cancer (CRC), previously identified by GWAS studies, could either alone or in combination contribute to clinical presentations suggestive of an increased genetic risk for CRC. We performed a prospective national case-control study based on highly selected patients (CRC in two first degree relatives, one being diagnosed before 61 years; or CRC before 51 years; or multiple primary CRCs, the first before 61 years; exclusion of Lynch syndrome, adenomatous and hamartomatous polyposes), and appropriate controls corresponding to healthy volunteers, between 45 and 60 years of age, without personal or familial history of CRC. We included 1029 patients and 350 controls. We confirmed the association of CRC risk with 4 SNPs, with odds ratio (OR) higher than previously reported : *rs16892766* on 8q23.3 (OR: 1.89; p=0.0006); *rs4779584* on 15q13.3 (OR: 1.45; p=0.0033), and *rs4939827* and *rs58920878/Novel 1* on 18q21.1 (OR: 1.49; p=0.0061 and OR: 1.48; p=0,0039). We found a significant cumulative effect of the at risk alleles or genotypes with OR at 1.62, 2.11, 2.92 and 3.95 for 1, 2, 3 and at least 4 at risk alleles and OR at 1.71, 2.32 and 6.31 for 1, 2 and 3 at risk genotypes. This study shows that genotyping of a limited number of SNPs, such as 8q23.3 *rs16892766*, 15q13.3 *rs4779584* and 18q21.1 *rs58920878*, should allow to identify subjects at increased risk of CRC who might benefit from early CRC detection.

P12.046-M

Diagnostic criteria for constitutional mismatch repair deficiency (CMMR-D) syndrome: suggestions of the European consortium "Care for CMMR-D" (C4CMMRD)

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Constitutional mismatch repair deficiency (CMMR-D) syndrome is a distinct childhood cancer predisposition syndrome that results from biallelic germline mutations in one of the four MMR genes, *MLH1*, *MSH2*, *MSH6*, or *PMS2*. The tumour spectrum is very broad, including mainly haematological, brain and intestinal tract tumours. Patients show a variety of non-malignant features that are indicative of CMMR-D. However, currently no clinical criteria that should entail diagnostic evaluation of CMMR-D exist. The recently established European consortium care for CMMR-D (C4CMMRD) proposes a 3-points scoring system for the suspected diagnosis CMMR-D in a pediatric/young adult cancer patient. Tumours highly specific for CMMR-D syndrome are assigned 3 points, malignancies overrepresented in CMMR-D 2 points and all other malignancies 1 point. According to their specificity for CMMR-D and their frequency in the general population additional features are weighted with 1 - 2 points. They include multiple hyper- and hypopigmented skin areas, brain malformations, pilomatricomas, a second childhood malignancy, a LS-associated tumour in a relative and parental consanguinity. According to the scoring system CMMR-D should be suspected in any cancer patient who reaches a minimum of 3 points by adding the points of the malignancy and the additional features. We expect that application of the here suggested strategy for CMMR-D diagnosis will increase the number of patients being identified at the time when they develop their first tumour. This will allow adjustment of the treatment modalities, offering surveillance strategies for second malignancies and appropriate counselling of the entire family.

P12.047-S
Constitutional mismatch repair deficiency due to a homozygous MSH6 mutation in a 14-year-old boy with colonic polyps, colorectal cancer and retinal lesions

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Constitutional Mismatch Repair- Deficiency (CMMR-D) is a rare cancer predisposition syndrome in patients with biallelic mutations in one of *MLH1, MSH2, MSH6* or *PMS2*. Heterozygous mutations cause the adult onset, dominant cancer syndrome Lynch syndrome. However, CMMR-D causes childhood onset cancers, particularly hematological, brain and gastrointestinal malignancies. Children with CMMR-D also may have skin pigmentation findings suggestive of neurofibromatosis type 1.
We report on a 14-year-old boy with a previous kaposiform hemangioendothelioma who was diagnosed with at least 100 colonic polyps and 6 invasive adenocarcinomas of the colon and rectum. He had café au lait macules, axillary and inguinal freckling and bilateral, multifocal congenital hypertrophy of the retinal pigment epithelium (CHRPE). Therefore he fit diagnostic criteria for both NF1 and Familial Adenomatous Polyposis. He also had a previous clinical diagnosis and family history of Cleidocranial Dysplasia. There was no family history of colon cancer and he had 2 siblings.
The colon cancers were MSI-H and by immunohistochemistry the tumour cells and adjacent normal mucosa were negative for MSH6 and positive for MLH1, MSH2 and PMS2. A homozygous pathogenic MSH6 mutation was identified (c.3202C>T) in the patient and both parents were found to be heterozygous. Genetic testing of the *APC* and *MUTYH* genes was normal.
To our knowledge, this is the first reported case of CMMR-D with CHRPE and demonstrates difficulties in making this diagnosis in a patient who fits the clinical criteria for both NF1 and FAP. It also illustrates ethical dilemmas around genetic testing of at risk siblings.

P12.048-M
Recurrent copy number alterations in prostate cancer: an in silico meta-analysis of publicly available genomic data

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We present a meta-analysis of somatic copy number alterations (CNAs) from eleven publications that examined 662 prostate cancer patient samples, derived from 546 primary and 116 advanced tumors. Normalization, segmentation and identification of corresponding CNAs for meta-analysis was achieved using established commercial software (Nexus). Unsupervised analysis identified five genomic subgroups in which ~90% of the samples had an abnormal profile characterized by 8q gains. The most common loss was at 8p (NKX3.1). The CNA distribution in other genomic subgroups was characterized by losses at 2q, 3p, 5q, 6q, 13q, 16q, 17p, 18q and 10q (PTEN), and acquisition of 21q deletions associated with the TMPRSS2:ERG fusion rearrangement. Parallel analysis of advanced and primary tumors in the cohort indicated that genomic deletions of PTEN and the gene fusion were enriched in advanced disease. A supervised analysis of PTEN deletion and the fusion gene showed that when PTEN was deleted the overall percentage of the genome altered was significantly higher, suggesting that this important genomic subgroup was likely characterized by intrinsic chromosomal instability. Predicted alterations in expression levels of candidate genes in each of the recurrent CNA regions characteristic of each subgroup showed that signaling networks associated with cancer progression and genome stability were likely to be perturbed at the highest level in the PTEN deleted genomic subgroup.

P12.049-S
In-depth comparison of available targeted resequencing strategies for BRCA gene panels

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Next-Generation Sequencing is currently the technique of choice for genetic testing in genetic heterogeneous diseases. The broad spectrum of available target enrichment methods complicates the selection of the most suitable technique considering the size of the targeted region, the required depth of coverage to correctly identified variants, the number of samples to be sequenced, the turnaround time and the overall cost. Here we report a comprehensive comparison of four different commercial targeted enrichment kits (Haloplex, Nextera, Multiplicom and SureSelect) for the selection of the most convenient strategy to fully characterize the coding regions of BRCA genes in an Illumina benchtop sequencer. Most of samples were analysed with 1-3 different kits. Following clinical standards, we evaluated the analytical sensitivity, the analytical specificity, false positive and negative rates, the assay robustness and the precision for each of the considered capturing techniques. Our results demonstrate that technical and functional differences exist between capturing methods that could compromise clinical diagnosis if specific method-associated deviations are not considered.

P12.051-S
Mutually exclusive RAS-RTK and JAK2 mutations drive sub-clonal expansions in the majority of acute lymphoblastic leukaemia cases in Down syndrome

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Children with Down syndrome (DS) and acute lymphoblastic leukaemia (ALL) have poorer survival and more relapses, than euploid ALL children, highlighting the need for a molecular understanding of DS-ALL. Using full exome or cancer genes-targeted sequencing of 42 ALL samples from 39 DS patients, we discover driver mutations in RAS (KRAS and NRAS) recurring to a similar extent (13/42) as JAK2 (10/42) mutations or P2RY8-CRLF2 fusions (14/40). RAS/RTK mutations were mutually exclusive with JAK/STAT mutations (p=0.018), driving a combined total of two thirds of analysed cases. Clonal architecture analysis shows that both JAK/STAT and RAS/RTK mutations are mostly sub-clonal. The study of clonal evolution in the cases with primary leukaemias and relapses shows that JAK-STAT mutations are exclusively associated with primary leukaemias and RAS mutations - with relapses. This indicates that JAK2-mutated sub-clones respond well to conventional therapy, but then is replaced with RAS-mutated sub-clones. The dominant clones (including those persisting in primary and relapse leukaemias) were a combination of mutations in chromatin modifiers (62%), tumour suppressors, lymphocyte differentiation factors, and/or CRLF2 rearrangements. These events were cumulatively present in 93% of cases, with no association with JAK/STAT or RAS/RTK groups. Additionally, novel differences in number and profile of driver events were observed between cALL and preB immunophenotypic subtypes of BCP-ALL.
Overall our data provide a better molecular understanding of DS-ALL and suggests a personalized therapeutic approach to driver-mutations, with individual mutation profile-tailored inclusion of RAS (particularly KRAS) inhibitors (such as deltarasin) and/or chromatin modifier enzyme inhibitors.

P12.052-M

Neoantigen in esophageal squamous cell carcinoma for dendritic cell-based cancer vaccine development

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Esophageal squamous cell carcinoma (ESCC) is a highly malignant tumor which usually is diagnosed in advanced stages due to its asymptomatic course of tumorigenesis. Current therapeutic modalities are not effective enough and the 5-year survival rate of the disease is still very low which prompts the urgent need for finding novel efficient therapeutic methods. In this study we evaluated ex vivo immune response of ESCC patients against our newly designed chimeric construct consisting of highly immunogenic cancer/testis antigens (CTAs). After confirming effective expression of the in vitro transcribed chimeric mRNA in ex vivo electroporated dendritic cells of the ESCC patients, the patients' CTLs were primed by DCs and cytotoxicity assay was performed to evaluate how the primed CTLs can recognize and target the chimeric mRNA-loaded cells. The chimeric protein was strongly expressed relative to the housekeeping gene expression in electroporated cells. The cytotoxicity of the CTLs were significantly higher in DCs loaded with chimeric mRNAs compared to mock DCs ($p < 0.05$) in all of the tested ESCC patients. We are introducing a novel construct that our functional study showed can stimulate and induce an effective immune response in ESCC patients. The designed chimeric mRNA-loaded DCs are capable of priming CTLs effectively, and induce cytotoxicity against tumor. Therefore, loading dendritic cells with chimeric epitopes of highly immunogenic antigens, such as cancer testis antigens are potentially interesting and effective therapeutic modalities for immunotherapy of ESCC.

P12.053-S

Aberrant expression of BMP signaling pathway target genes are involved in esophageal squamous cell carcinoma progression and development

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Esophageal cancer is one of the lethal malignancies in northeastern of Iran and esophageal squamous cell carcinoma (ESCC), as a frequent kind of cancer, shows striking variations in geographic distribution reflecting exposure to specific environmental factors which are remained to be completely defined. According to the importance of ESCC in this high risk area and the great attention in studying the malignant progression of cells through a converging pathway of oncogenesis, the present study aimed to estimate the expression analysis of VentX and Evx1, two critical target genes of bone morphogenic protein (BMP) signaling pathway. In this study a total of 50 tumoral and margin normal tissues from treatment-naive ESCC patients were enrolled to expression analysis using comparative real-time PCR. VentX was significantly underexpressed in 76% of ESCC tissues ($P<0.05$). Underexpression of VentX was significantly correlated with increased tumor size. The overexpression of Evx1 was detected in 82% of ESCC samples significantly ($P<0.05$). This overexpression was inversely correlated with the progress of tumor stage and increased depth of tumor invasion. Our results show that aberrant expression of VentX and Evx1 genes in ESCC tissues may have significant effects in the progress of ESCC tumorigenesis and may play roles in development of the disease through malfunction of BMP signaling pathway. To the best of our knowledge, this is the first report of VentX and Evx1 gene expression analysis in ESCC patients globally.

P12.054-M

Retrospective analysis of genomic and transcriptional changes in a case of Ewing sarcoma tumor progression determined by whole transcriptome and exome semiconductor-based sequencing

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Ewing sarcoma is a cancer that often presents in the second decade of life and is usually associated with a chromosomal translocation that results in a EWS/FLI1 gene fusion. Four independent cell-lines have been established from a subject who succumbed to metastatic disease following relapse after myeloablative chemotherapy. Whole-transcriptome and exome sequencing of normal primary bone marrow-derived stromal fibroblasts, Epstein-Barr Virus (EBV) transformed normal lymphoblasts, a pre-therapy primary tumor-derived cell-line, and a post-chemotherapy metastatic tumor-derived cell-line, was conducted on an Ion Torrent Proton™ system to profile the differences in gene expression and in exonic DNA sequence to characterize the molecular changes associated with primary tumorigenesis and disease persistence after treatment. The presence of the EWS/FLI1 fusion gene in the tumor cells was confirmed and the breakpoint determined from both observation of chimeric reads in the RNA-seq data and exome sequence analysis. Exome datasets indicate apparent loss of heterozygosity genome-wide in CHLA10 consistent with cytogenetic analysis that shows tetraploidy in this cell-line. Results from RNA-seq also indicate numerous instances, genome-wide, of differing transcript isoform expression and exon usage between normal, primary tumor, and metastatic tumor cells suggesting an increasing genomic mutational burden in the evolution of the disease, and pointing in particular toward aberrant regulation of RNA-splicing components. Taken together, the combination of RNA-seq and exome-sequencing on normal cells and primary vs. post-chemotherapy tumor is providing a broad and deep view of molecular signatures in tumor progression and indicating that a significant role is played by changes in non-coding RNA expression.

P12.055-S

Beyond BRCA1 and BRCA2: results from screening 94 genes in 100 patients with familial breast and ovarian cancer using panel sequencing and custom array-CGH

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Breast and ovarian cancer predisposition has been associated with a number of high-, moderate-, and low-penetrance susceptibility genes. Here we report on the results of high-resolution custom array-CGH for deletion/duplication analysis and panel-based screening of 94 genes associated with hereditary cancer predisposition.
Selection criteria for the 100 patients were defined by the German Consortium for Breast and Ovarian Cancer. NGS was performed on an Illumina MiSeq and target enrichment was done with the Illumina TruSight cancer panel, whereas custom array CGH was performed using Agilent technology. In 28 % of the patients, BRCA1 or BRCA2 variations have been found. These were either clearly pathogenic protein truncating mutations (12 %) or very rare, unclassified missense variations with high probability of effect (16 %). In 39 % of the patients we found rare, unclassified missense variants in low penetrance susceptibility genes, especially NBN and ATM. In one case with early onset of breast cancer and no familial history, a putative splice relevant mutation in TP53 could be identified, which is currently being investigated on cDNA level. 33 % of the patients did not reveal any convincing sequence variation. In 10% of the patients we identified deletions in either BRCA1, CHEK2, or ATM.
The extension of mutation screening beyond BRCA1 and BRCA2 reveals disease-causing mutations in high-penetrance genes, like TP53, as well as mutations in low-penetrance susceptibility genes. However, the enormous number of unclassified sequence variants and the detection of carriers for other hereditary diseases pose a huge challenge for genetic counselling.

P12.057-S

First evidence for *FANCM* as a novel breast cancer predisposing gene

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Several members of the FANC (Fanconi anaemia complementation group) gene family such as *BRCA2* (*FANCD*), *RAD51C* (*FANCO*), *PALB2* (*FANCN*) and *BRIP1* (*FANCJ*) have been associated with breast cancer (BC) and ovarian cancer (OC) predisposition. Given the fact that most risk families do not carry mutations in established BC/OC genes, we hypothesized that yet unknown disease genes exist. In the course of an exome sequencing project aimed to identify novel BC predisposing genes, we identified a heterozygous nonsense mutation (p.Gln1701Ter) within the *FANCM* gene in a *BRCA1/2*-negative patient affected by early-onset BC (46y). The FANCM protein is a central com-

ponent of the Fanconi anaemia core complex and promotes the recruitment of the S-phase checkpoint machinery after binding to DNA damage sites. The nonsense mutation removes the ERCC4-like domain (aa1802-1922) of the 2022aa FANCM protein. A subsequent Sequenom-based case-control study including 1896 independent *BRCA1/2*-negative BC index cases and 1726 controls revealed a significant association with BC occurrence (9/1896 cases vs. 2/1726 controls; p<0.05). The carrier frequency of the p.Gln1701Ter mutation in controls (0.12%) is similar to that identified in the framework of the NHLBI Exome Sequencing Project (5/4300; european ancestry; 0.12%). Taken together, our data provide first evidence for *FANCM* as a novel BC predisposing gene (9/1896 cases vs. 7/6026 controls; p=0.00242) at a moderate penetrance level (OR: 4.101; CI95%:1.401-12.199; RR=2.357; CI95%:1.27-3.326). Due to the low frequency of the *FANCM* p.Gln1701Ter mutation, however, large collaborative studies are required to quantify the RR of *FANCM* alterations for BC and potentially other cancer entities.

P12.058-M
First clinical experience with screening formalin fixed and paraffin-embedded (FFPE) archival tissue for mutations in BRCA1 and BRCA2 - A new paradigm in genetic investigation and counseling

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Background: Women carrying a germline mutation in BRCA1 or BRCA2 (BRCA1/2) have a very high lifetime risk of breast- and ovarian cancer. Men carrying the same mutations are facing an increased risk of prostate- and breast cancer. Until now, screening for BRCA1/2 mutations required high quality DNA (from blood or other fresh specimens). This has ruled out families in which the relative(s) suffering from breast or ovarian cancer have already died. Several attempts to screen for mutations in archival formalin-fixed, paraffin-embedded (FFPE) tissue have so far been with limited success. **Aim:** We present the first clinical data from our newly developed NGS based analysis, screening archival FFPE samples of non-tumor tissue for germline mutations in BRCA1/2. **Results:** In 33 FFPE samples we found 3 pathogenic BRCA1 mutations, 3 pathogenic BRCA2 mutations and 2 variants of unknown significance in BRCA2. In 17 samples coverage was sufficient to disclose a negative result and in 8 samples the coverage was too low, and hence the result was designated inconclusive. The quality of the data varied between samples with a strong negative correlation between age of the tissue and sequence quality. **Conclusions:** Mutations in BRCA1/2 can now be sought after in deceased relatives, in families with suspected germline mutations. For clinical use, mutations found in FFPE samples should always be confirmed in other samples from either the same individual, or from samples from close relatives.

P12.059-S
Comprehensive genetic characterization of pediatric patients with B-lineage ALL reveals novel mutations and gene fusions

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High-throughput sequencing technologies provide new opportunities for personalized treatment approaches as well as for the identification of novel genetic biomarkers in cancer. We analyzed a total of 9 tumor and reference samples of patients with primary and relapsed B-lineage ALL by whole-exome-/transcriptome sequencing and SNP-microarrays. The results were combined to obtain a comprehensive overview on the genomic landscape of B-lineage ALL. Only few genes were altered recurrently and our data confirms the hypothesis that the interindividual heterogeneity requires highly individualized therapeutic approaches. Besides common and well known genetic variations like mutations (e.g. in NRAS, JAK, ILR7), CNVs (e.g. a deletion of PAX5) and fusion genes (e.g. BCR:ABL) a novel gene, PYGO2, was identified to be altered in three patients. Besides a fusion gene (MEF2D:PYGO2), a mutation and a duplication of PYGO2 were found in different patients. Our data suggest that PYGO2 plays a role in leukomegenesis.

P12.060-M
Unraveling the genetic predisposition of early-onset and familial gastric cancer

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Introduction: Gastric cancer is a multifactorial disease and results from a combination of environmental factors and genetic alterations. Approximately 8-10% of cases are familial. Germline *CDH1* mutations explain a small part of these families. In the majority of cases with early-onset or familial gastric cancer the underlying genetic cause remains unknown. Therefore, we aim to find new genetic predisposition genes for gastric cancer. **Patients**: 41 patients with early-onset (<35 years of age at diagnosis) or familial gastric cancer (two first-degree relatives with gastric cancer diagnosed below the age of 50) were included in our study. **Results**: Exome sequencing was performed on germline DNA of the 41 patients. High-quality variants were selected and common variation was excluded from the data set. Next, both truncating variants and missense variants predicted to be damaging to protein function were selected. In total, 225 variants were validated by Sanger sequencing (average 5.5 variants per patient, range 1-12 variants): 63 truncating variants and 162 missense variants in 191 genes. Thirty-eight genes were recurrently affected in maximally three patients. Homozygous and compound heterozygous missense variants were identified in 8 and 3 patients, respectively. Three truncating and 26 missense variants are in known cancer predisposing genes. Other variants are located in genes previously associated with or functionally linked to gastric cancer. **Conclusion**: Using exome sequencing we identified several candidate genes for gastric cancer predisposition. Their contribution to gastric cancer predisposition will be investigated by screening larger cohorts of patients with early onset and/or familial gastric cancer.

P12.061-S
Impaired Th17 mucosal host defense against *Helicobacter pylori* in an early-onset gastric cancer patient with a homozygous germline variant in *MYD88*

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Introduction: The main causes for gastric cancer are *Helicobacter pylori* (*H. pylori*) infection, diet and genetic factors. Germline *CDH1* mutations underlie a gastric cancer predisposition syndrome with autosomal dominant inheritance, characterized by early-onset diffuse gastric cancer. In the majority of cases with early-onset or familial gastric cancer the underlying genetic cause remains unknown. We aimed to find new genetic predisposition genes for gastric cancer. **Patient**: A 23-year old female patient with diffuse-type gastric cancer and without a germline *CDH1* mutation also suffered from recurrent fungal infections. Family history revealed a consanguineous relation of her parents. There were no other cases of gastric cancer in the family. **Results**: Whole exome sequencing of the patients' germline DNA identified a homozygous missense variant (c.712C>T, p.Arg238Cys) in the gene encoding MYD88, which plays a central role in the immune response against *H.pylori* infections. Immunological assays on peripheral blood mononuclear cells revealed normal immune responses to *Staphylococcus aureus,* but impaired immune responses upon stimulation with *H.pylori* and *Candida albicans*, characterized by a specific defect in production of Th17 cytokines IL-17 and IL-22. This was due to defective IL1-beta responses, a crucial cytokine for Th17 development. **Conclusion**: Impaired Th17 mucosal host defense against *H.pylori,* which is associated with gastric cancer development, was observed in a patient with early onset gastric cancer and a homozygous *MYD88* variant. These defective Th17 responses were also likely the cause of fungal infections. Our data suggest that genetic defects leading to defective anti-*Helicobacter* innate pattern recognition predispose to gastric cancer.

P12.062-M
Prohibitin 3' untranslated region 1630 C>T polymorphism and copy number variation contributes to its gene expression deregulation in gastric cancer

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Gastric cancer is the second leading cause of cancer-related deaths worldwide. *PHB* has been reported as an oncogene and tumor suppressor in several neoplasias, including in gastric cancer. Here, we evaluated whether the *PHB*

copy number and the rs6917 polymorphism affect its expression in gastric cancer. Forty-eight pairs of gastric cancers and corresponding non-neoplastic gastric samples were evaluated. *PHB* expression was analyzed by real-time quantitative PCR and by immunohistochemistry. Gene copy number was investigated by quantitative PCR. Allele-specific expression was determined by sequencing and by TaqMan assay. Down-regulation and up-regulation of *PHB* was observed in the tumors (45.5% and 20.5%, respectively). Reduced *PHB* expression was associated with dedifferentiation (P=0.029), lower invasion (P=0.002), absence of lymph node metastasis (P=0.040) and early gastric cancer (P=0.002). In all cases, the PHB immunoreactivity was detected in neoplastic and non-neoplastic cells. PHB was mainly expressed in the cytoplasm. *PHB* amplification was observed in 34.2% tumors. *PHB* gain was associated with higher gene expression (P=0.003) and late-onset gastric cancer (P=0.022). In our sample, 22% patients were heterozygous and 4.17% were homozygous for the minor allele in the rs6917 polymorphism. The presence of the T allele in this polymorphism was associated with reduced expression in GC (P<0.001) and in non-neoplastic samples (P<0.001). Only one patient presented a higher C/T ratio in both tumor and non-neoplastic samples. In most cases, lower C/T ratio was detected. Thus, *PHB* copy number variation and differential expression of the rs6917 polymorphism may have a role in *PHB* transcriptional regulation.

P12.063-S
Extrachromosomal driver mutations in glioblastoma and low grade glioma

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Alteration of the number of copies of Double Minutes (DMs) with oncogenic EGFR mutations in response to tyrosine kinase inhibitors (TKIs) is a novel adaptive mechanism of glioblastoma. In this study we provide evidence that such mutations in DMs, called here Amplification Linked Extrachromosomal Mutations (ALEMs), originate extrachromosomally and could therefore be completely eliminated from the cancer cells. By exome sequencing of 7 glioblastoma patients we revealed ALEMs in EGFR, PDGFRA and other genes. These mutations together with DMs were lost by cancer cells in derived gliomaspheres. We confirmed the extrachromosomal origin of such mutations by showing that wild type and mutated DMs may coexist in the same tumor. Analysis of 4198 tumors from TCGA collection suggested the presence of ALEMs across different tumor types with the highest prevalence in glioblastomas and Low Grade Gliomas. In these tumors, driver oncogenic mutations in DMs were 13 - 25 fold more frequent as compared to the distribution of passenger mutations confirming the effective expansion of extrachromosomal drivers. The extrachromosomal nature of ALEMs provides a powerful mechanism of rapid regulation of the copy-number of mutated oncogenes including their massive increase or complete loss in response to extracellular stimuli such as RTK inhibitors or growth factors.

P12.064-M
New loci of loss of heterozygosity (LOH) in glioblastoma

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Despite recent advances in the diagnosis and treatment of glioblastoma, the prognosis for patients with this highly malignant tumor remains poor. Research of glioblastoma at the molecular genetic level can help reveal changes underlying this tumor, identify potential markers of prognosis of the disease and response to therapy. An efficient approach of identifying candidate genes, the disturbance of structure and functioning of which may be associated with the development of cancer, is the analysis of LOH. We have assessed 7 genomic loci for which LOH in glioblastoma have not been previously reported: 2q31.2, 3p25.1, 5q14.3, 7q21.2, 12q21.33, 18q11.2 and 21q21.1. None of these loci is associated with glioblastoma susceptibility according to OMIM. The selected loci were examined by microsatellite analysis in 86 glioblastoma samples. Four of the seven loci, 2q31.2, 12q21.33, 18q11.2 and 21q21.1 revealed no LOH cases. However, LOH was identified at 3p25.1, 5q14.3 and 7q21.2 with a frequency of 25.8% (8/31), 20.0% (3/15) and 30.3% (10/33), respectively. Identified areas of LOH can contain potential candidate genes that may be of interest in terms of the molecular pathology of glioblastoma. Some of these genes are SLC6A6 (3p25.1), CCNH (5q14.3), AKAP9 (7q21.2). Thus, we have identified new loci of LOH in glioblastoma, in which candidate genes that play a role in tumor development and represent potential molecular genetic markers may be located. We have also confirmed LOH at 8q21.3 and 14q13.3 with a frequency of 17.9% (5/28) and 60.0% (9/15), respectively, which other authors have reported.

P12.065-S
Effects of NDRG2 downregulation on glioblastoma patient survival

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Background. Glioblastoma multiforme (GBM) is the most lethal malignant human brain tumor with the poor survival prognosis exceeding to 12-15 months after diagnosis. No reliable molecular marker has been verified for GBM diagnostics up to date. Therefore, extensive research in molecular changes of GMB is essential to better understand this pathology. *NDRG2* gene has been reported to be downregulated in GBM, whereas overexpression of this gene represses glioblastoma cell proliferation *in vitro*. To further address the role of *NDRG2* in gliomagenesis, we analyzed *NDRG2* expression at mRNA and protein level in gliomas of different malignancy grade. **Material and Methods.** The study included 78 different malignancy glioma tumors (9 astrocytomas grade-I, 29 grade-II, 14 grade-III and 26 glioblastomas). *NDRG2* mRNA expression study was performed using quantitative real-time Reverse-Transcription PCR analysis, while protein expression was estimated by using Western blot technique. **Results**. We found a strong decrease of *NDRG2* mRNA expression in glioblastoma to about 10-fold compared to I-III grade gliomas (Kruskal-Wallis, p<0.0001). In line with the mRNA data, NDRG2 protein level was markedly reduced in glioblastomas (to about 3-4-fold) as compared to grade I-III gliomas (p<0.0001). Spearman correlation analysis demonstrated significant correlation of *NDRG2* transcripts and protein expression levels (r=0.662, p<0.0001). Survival analysis showed negative correlation between *NDRG2* expression and tumor progression (Log-rank p=0.0001). Our results highlight the importance of *NDRG2* for glioma tumorigenesis as well as being as an indicatory factor for glioma malignancy and patient survival.

P12.066-M
Genomic profiling reveals three molecular relapse patterns in IDH1/2 wild-type glioblastoma

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Molecular changes associated with the relapse of glioblastoma after standard radiochemotherapy remain poorly understood. Here we compared genomic profiles of 27 pairs of primary and recurrent *IDH1/2* wild-type glioblastomas by genome-wide array-based comparative genomic hybridization. By bioinformatic analysis, primary and recurrent tumor profiles were normalized and segmented, chromosomal gains and losses called taking the tumor cell content into account, and difference profiles deduced. Seven of 27 (26%) pairs lacked DNA copy number differences between primary and recurrent tumors (Equal pairs). The recurrent tumors in 9/27 (33%) pairs contained all chromosomal imbalances of the primary tumors plus additional ones, suggesting that a major subclone sequentially accumulated aberrations (Sequential pairs). In 11/27 (41%) pairs, the profiles of primary and recurrent tumors were divergent, i.e. the recurrent tumors contained additional aberrations but had lost others, suggesting a polyclonal composition of the primary tumors and considerable clonal evolution (Discrepant pairs). Losses on 9p21.3 harboring the *CDKN2A/B* locus were significantly more common in primary tumors from non-Equal pairs. Non-Equal pairs showed ten regions of recurrent genomic differences between primary and recurrent tumors harboring 46 candidate genes associated with tumor recurrence. In particular, copy numbers of genes encoding apoptosis regulators were frequently changed upon recurrence. In summary, approximately 25% of *IDH1/2* wild-type glioblastoma pairs have stable genomic imbalances. In contrast, approximately 75% of *IDH1/2* wild-type glioblastomas undergo further genomic aberrations and alter their clonal composition upon recurrence impacting their genomic profile, a process possibly facilitated by loss on 9p21.3 in the primary tumor.

P12.067-S
Targeted resequencing for analysis of gene mutations in pediatric Glioblastoma Multiforme

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Glioblastoma multiforme (GBM; WHO-grade IV), the most frequent primary malignant brain tumor in adults, accounts for approximately 7-9% of all central nervous (CNS) tumors in childhood. Adult and paediatric GBMs (pGBMs) have distinct genetic and molecular pathways of tumorigenesis and different studies, based on array-CGH analysis, reported that there are significant differences in Copy Number Alterations (CNA). In our previous study we identified, using array-CGH, recurrent CNA in 8 pGBMs establishing minimum common regions (MCR) of duplication/amplification and deletion. Based on these results, we developed a next-generation sequencing (NGS) approach to screen the genetic profile of tumors. NGS has provided a new paradigm in biomedical research to delineate the genetic basis of human diseases. The panel was designed to cover 420 genes selected within of MCRs to try to identify new genes involved in tumorigenesis and/or progression of pGBMs. In 8 patients we found 18 heterozygous mutations in different genes. The same mutations were also found in DNA extracted from blood and in two cases we demonstrated the parental origin of 12 mutations. Given the rarity of the disease and the scarcity of data in the literature, our findings may better elucidate if there is a genomic background in development and progression of pGBM. The recognition of candidate genes underlying this disease could then improve treatment strategies for this devastating tumor.

P12.068-M
Distinctive expression profiles of lncRNAs in glioma subtypes

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Background: Gliomas are the most malignant and common primary brain tumors, classified upon malignancy grade and histological characteristics. Correct diagnosis that relies solely on histopathological characteristics might be difficult and inadequate, particularly in cases that lack typical features. Glioma subtypes have distinct molecular features and gene expression analyses could uncover molecular biomarkers for deciphering glioma subtypes. Our purpose was to identify lncRNAs that might play role in gliomagenesis and investigate potential association of differential expression profiles with different glioma subtypes. **Patients and methods:** Sixty-four patients were included in the study. We used quantitative real-time PCR (qPCR) in order to determine differentially expressed lncRNAs using LncRNA array profiler on a smaller cohort of pathohistologically evaluated glioma samples normalized to human brain reference RNA. A subset of differential lncRNAs was further validated by qPCR approach on a biger cohort of glioma samples and statistically evaluated using Mann-Whitney and Kruskal-Wallis tests. **Results:** LncRNA profiling revealed a total of 74 among 90 analysed lncRNAs to be widely expressed and statistical tests identified a subset of 10 significantly differentially expressed lncRNAs. Further analyses of 7 dysregulated lncRNAs (*7SL*, *EGOA*, *HOTAIR*, *JPX*, *MEG3*, *RNCR3* and *ZNFX1-AS1*) have showed statistically significant differences in relative gene expression ratios implicating distinctive expression profiles in glioma subtypes. **Conclusions:** Our findings support the concept of lncRNAs as molecular biomarkers by determining distinctive subtype-related lncRNA expression patterns between glioma subtypes, which suggest an important role of lncRNAs in glioma biogenesis and implicate the potential use of lncRNAs in glioma histological classification.

P12.069-S
Genomic hallmarks in glioma

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Recent genome-wide studies have been developed to support hystologic classification of gliomas and different molecular abnormalities have been linked to tumor grading and disease progression.
In our study we investigated MGMT promoter methylation, 10q loss of heterozygosity (LOH) and IDH1 R132H mutation in tumor specimens from 103 patients with glioblastoma and II and III grade gliomas, to further understand: 1) correlation with grading, 2) prognostic value.
Pyrosequencing analysis of MGMT promoter showed that methylation levels decrease from low to high grade tumors (p <0,001), confirming that hypermethylation (methylation levels >20%) correlates with a better progression free survival (PFS). Interestingly, a mildly methylated subgroup (methylation 20%-40%) revealed a better PFS. Microsatellite and a-CGH analyses showed that 10q LOH is common in high grade tumors and associates with MGMT hypomethylation, while absence of LOH and hypermethylation correlate with low grade tumors. IDH1 mutation was investigated by pyrosequencing and detected in all of low grade gliomas (10 cases), in 7/11 II grade gliomas. We did not find mutations in IV grade gliomas, indicating a strong association between tumor grading and IDH1 mutation status (p<0,001). In addition, we found a correlation between IDH1 mutation and MGMT methylation level (p<0,001), both decreasing with increasing tumor grade. In conclusion, our data reinforced the role of MGMT methylation and IDH1 mutation as hallmarks of better prognosis, and conversely 10q LOH as a poorer one. Moreover, we evidenced the existence of a mildly methylated glioma subtype correlated with a better PFS.

P12.070-M
miRNA expression profile in tumor cell lines treated with the histone deacetylase inhibitor LBH589

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Introduction: The presence of HDAC proteins is necessary for the correct regulation of gene expression. Multiple studies have demonstrated that inhibition of these proteins can lead to deregulation of gene expression and abnormal cell proliferation. In our study we have analyzed the miRNA profile of three epithelial cell lines (HCT116, HT29 and HCC1937) and two mesenchimal cell lines (H929 and MM1S) after treatment with a histone deacetylase inhibitor, currently in phase III clinical trial.
Materials and methods: We have analyzed miRNA expression comparing the profiles before and after treatment. RNA was isolated from cell lines and miRNA labeling was performed using a conventional assays. An Exiqon chip miRCURY LNA Array microRNA ™ was used for analysis. Comparisons between the different study groups were performed using SAM to identify those miRNAs whose expression showed statistically significant differences.
Results: Comparing the expression profiles of the cell lines between 24h and 72h after treatment with the histone deacetylase inhibitor, we found that epithelial cells show a group of 18 up-regulated and 16 down-regulated miRNAs. Whereas mesenchymal cells show 20 up-regulated and 20 down-regulated miRNAs. Both types of cells share 3 up-regulared miRNAs at 24h, 8 at 72 hours and 1 down-regulated miRNA.
Conclusions: These data show that treatment with histone deacetylase modifies miRNA expression patterns. Specifically depending on the cellular origin of tumor cells, which could explain different outcome of patients treated with this approach.

P12.072-M
Whole-genome sequencing of matched primary and metastatic hepatocellular carcinomas

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To gain biological insights on tumor metastases, we used whole-genome sequencing at 33X-43X coverage to profile somatic mutations in primary HCC (HBV+) and metachronous lung metastases (> 2 years interval). In total, 5,027-13,961 and 5,275-12,624 somatic single-nucleotide variants (SNVs) were detected in primary HCC and lung metastases, respectively. Generally, 38.88-78.49% of SNVs detected inmetastases were present in primary tumors. We identified 65-221 structural variations (SVs) in primary tumors and 60-232 SVs in metastases. Comparison of these SVs shows very similar and largely overlapped mutated segmentsbetween primary and metastatic tumors. Copy number alterations between primary and metastatic pairs were also found to be closely related. Together, these preservations in genomic profiles from liver primary tumors to metachronous lung metastases indicate that the genomic features during tumorigenesis may be retained during metastasis.Additionally, a few mutations were found specifically in lung metastases, which may explain the clinical observationthat

both primary and metastatic tumors are usually sensitive or resistant to the same systemic treatments.

P12.073-S
Identification of a novel CDH1 gene mutation in a family with hereditary diffuse gastric cancer

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Introduction: Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant genetic predisposition syndrome caused by CDH1 germline mutations, with incomplete penetrance. HDGC is relatively uncommon, representing about 2% of gastric cancer. The majority of inactivating mutations in HDGC families are of the truncating type (75%), whereas the remaining are missense type. The estimated lifetime risk for DGC is more than 80% in mutation carriers and prophylactic total gastrectomy is recommended. **Subjects and methods:** A 47 year-old female patient diagnosed with DGC was screened for CDH1 gene mutations. DNA and RNA extraction was carried out from blood and tissue (gastric biopsy) and the entire coding sequence and flanking intronic portions of the CDH1 were sequenced. Other family relatives *(*three sisters, an uncle and a nephew*)* were also screened. **Results:** A deletion in exon 9 of CDH1 gene (c.1220delC, p.407*), not previously described, has been found. This mutation generates a stop codon that leads to a pathogenic variant. The presence of the mutation was corroborated both, at DNA and RNA level in blood and tissue. The proband died during the study. One of the sisters presented also the deletion and had a previous history of malignant colorectal polyp at the age of 49. Her son (nephew of the proband) presented also the mutation. Neither of the other relatives harboured the mutation. **Conclusion:** HDCG has a poorly prognosis, mainly because of its difficult early detection. The identification of CDH1 mutations offers the opportunity of carry out prophylactic strategies for unaffected at-risk individuals.

P12.074-M
Novel mutations in Juvenile polyposis syndrome

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Juvenile polyposis syndrome (JPS) is an autosomal dominant disorder characterized by multiple juvenile polyps (JP) mainly in the colon with increased risk of colorectal cancer. Subsets of patients develop severe gastric polyposis with increased risk of gastric cancer.
Of fifty patients evaluated for polyposis in 2013, two JPS patients with novel mutations were diagnosed.
1st: 27yrs after right colectomy for multiple hyperplastic, and JPs resulting in recurrent rectal bleeding and anemia, normal gastroscopy. Sequencing and MLPA for SMAD4 were negative. BMPR1A sequencing yielded c.367G>T (p.123E>*), a novel nonsense mutation.
2nd: At 32 yrs total colectomy for multiple adenomatous polyps suspicious of Familial Adenomatous Polyposis. Gastroscopy detected multiple hyperplastic polyps. APC sequencing and two common MUTYH mutations were normal. Due to severe bulky gastric polyposis a revision of the pathology was performed and JPS was clinically diagnosed. A total gastrectomy was performed for persistent anemia and cancer risk. Epistaxis and telangiectases on his back and chest raised the possibility of hereditary hemorrhagic telangiectasia (HHT). Sequencing of SMAD4 found c.406_407delGT (p.V136CfsX6), a de novo novel frameshift mutation.
JPS composes 10% of polyposis syndromes. About 20% of SMAD4 patients have JPS-HHT combined syndrome. Phenotypically, patients with SMAD4 mutations may have gastric polyposis with a significant risk of gastric cancer. These JPS patients illustrate the need for careful history collection and thorough pathological analysis, that should guide the genetic evaluation. Genetic workup of JPS patients is important for family counseling and to establish the need for HHT evaluation in patients with SMAD4 mutations.

P12.075-S
Targeting the functional relevance of the novel tumor suppressor gene FOCAD (KIAA1797) in glioma pathogenesis

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Malignant gliomas have a highly invasive phenotype, which is a main determinate for the poor prognosis of patients suffering from these tumors despite multimodal therapy. In previous studies, we used 24-color-FISH to characterize the chromosomal translocation events in glioblastoma cell lines, and detected a t(7;9) disrupting the FOCAD (KIAA1797) gene encoding a then uncharacterized protein. FOCAD was deleted in around 50% of primary glioblastomas, and its gene product, focadhesin, had an impact on glioblastoma growth in vivo and cell motility in vitro. The aim of this project was to further characterize focadhesin by targeting its unknown binding partners. Therefore, we performed two independent yeast two-hybrid screens using a human brain cDNA library. In total, we isolated 206 clones, from which we eliminated 155 clones, because they could grow without auxotrophic marker expression. The DNA sequences of the remaining 51 prey inserts were identified. In 26 prey plasmids, the cDNAs were protein-coding and in frame. Finally, 8 distinct potential binding partners were identified, which can be categorized into the functional groups of calcium signaling, mitosis, protein folding, RNA processing and cell metabolism. To confirm these interactions, we performed retransformation experiments using fresh yeast. Three candidates were also analyzed by pull-down assays and co-localization studies using human cells. Two interactions have been confirmed so far. In the next step, the functional relevance of the verified interactions will be investigated in glioblastoma cells with stable focadhesin knockdown and cells with homozygous deletion of the FOCAD gene with or without FOCAD-rescue by viral transduction.

P12.076-M
Targeted deep sequencing of fusion transcripts - developing clinical assays for leukemia samples

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One of the main focuses of the National Genomics Infrastructure-Sweden (NGI) is to bring high-throughput sequencing to clinical use. Here we describe recent work on PCR- and hybridization-based deep sequencing of specific targeted fusion transcripts using Pacific Biosciences RSII (PacBio) and Ion Torrent PGM/Proton instruments, methods that can help to guide the treatment of leukemia patients.
By long-read PacBio sequencing we are developing clinical workflow for detection of BCR-ABL1 tyrosine kinase inhibitor (TKI) resistance mutations in chronic myeloid leukemia (CML). Our assay enables rapid sequencing of a 1578bp *BCR-ABL1* cDNA amplicon without the need of a nested PCR. Mutations down to a level of 1% are clearly detected using this approach. Moreover, the long PacBio reads makes it possible to resolve the mutational composition of all different clones present in CML patient samples. Since compound mutations might confer cross-resistance to multiple TKIs, the information provided by our assay can directly influence the choice of therapy.
In another project we study MLL-rearranged leukemias, characterized by chromosomal translocations involving the *MLL* gene at 11q23. *MLL* can form fusions transcripts together with several different genes, some of which are still unknown. In some cases conventional assays like G-banding and FISH fail to detect these fusion partners. We are therefore evaluating a hybridization-based assay for targeted capture of *MLL* cDNA, in combination with Ion Torrent sequencing. Our preliminary results indicate that our method increases the sensitivity for detecting *MLL* fusion genes by several orders of magnitude compared to a regular RNA-sequencing approach.

P12.077-S
Results of 20 years of Li-Fraumeni syndrome diagnosis in France

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The Li-Fraumeni syndrome (LFS), due to germline *TP53* mutations, represents a remarkable cancer predisposition characterized by the extent of tumour spectrum. For 20 years, analysis of over 1700 families allowed us to identify 214 French families with *TP53* mutation, thanks to the Chompret's criteria gradually elaborated by the French LFS working group. Updated data from 322 LFS patients with 552 tumours revealed that the median age of first tumour onset was 27 years, that the most frequent tumours were breast cancers (167), soft-tissue sarcomas (103), osteo/chondro-sarcomas (58), brain tumours (43) and adrenocortical carcinomas (42), and that 43% of patients developed multiple primary tumours. Germline *TP53* mutations were detected in 50% of children with adrenocortical carcinoma, choroid plexus carcinoma, or rhabdomyosarcoma. Sixty-four percent of *TP53* alterations were missense mutations and 4% genomic rearrangements. We found that *TP53* mutation carriers harbouring the *MDM2* 285-309GG haplotype developed tumours 5 years earlier than others. The most striking observation was that patients with dominant-negative missense mutation developed first-tumour earlier (22 y) than patients with null-mutation (42 y). Twenty

years after the identification of LFS molecular basis, this report reveals that 2 forms of LFS probably exist: an aggressive, characterized by early-onset tumours, due to highly penetrant dominant-negative missense mutations, and a moderate, characterized by later age of onset, due to mutations with lower penetrance. The remarkable high incidence of multiple primary tumours is probably explained by genotoxic effects of chemo- and radio-therapy and the key role of p53 response to DNA damage.

P12.078-M
Identification of a characteristic copy number alteration profile by high-resolution SNP arrays associated with metastatic sporadic colorectal cancer

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Background: Metastatic dissemination is the most frequent cause of death in sporadic colorectal cancer (sCRC). The metastatic process is considered, at least in part, to be related to a specific background of genetic alterations accumulated in cells from primary tumors, the identification of such genetic alterations being critical for the identification of sCRC patients at risk of developing metastases.
Methods: In this study we used high-resolution 500K SNP-arrays for the identification of copy number alteration profiles present at diagnosis in primary tumors from metastatic (n=23) versus non-metastatic (n=26) sCRC.
Results: Our results showed a characteristic pattern of copy number alterations among metastatic sCRC which involved losses of 23 regions at chromosomes 1p, 17p and 18q, together with gains of 35 regions at chromosomes 7 and 13q.
Conclusion: As could be expected, such copy number profile involved multiple genes previously associated with sCRC (i.e. SMAD2) and/or the metastatic process (i.e. PODXL) and it was further associated with a poorer outcome.

P12.079-S
Oligogenic germline mutations predispose to early lung adenocarcinoma in non-smokers

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Despite the great bulk of research to identify genetic susceptibility genes in lung cancer by genome-wide association studies, only three loci have been identified and replicated consistently in subsequent studies. In addition to confer a very low risk, they have been associated with lung cancer in smokers, but not in non-smokers. The polygenic nature of common cancers has frequently been suggested, but its biological basis still remains elusive. We tested the hypothesis that genetic susceptibility may rely on germ-line mutations of a restricted number of genes. A combination between an advanced technical tool, i.e. the exome sequencing, and a new patient selection strategy was used. Among 964 lung adenocarcinoma patients we selected two patients with very early onset disease (mean age 43) in absence of cigarette smoking, and having a first degree healthy sibling available for genome comparison older than at least 7 years. Germ-line truncating mutations were detected in 8 and 5 different cancer predisposing genes in each affected subject, respectively, but not in the healthy sib (p=0.0026). Some of them are well known cancer players in lung tumors and others are genes previously identified in other cancer tissues. This study demonstrated for the first time that never-smoker patients with lung adenocarcinoma carry a specific and private oligogenic combination of germ-line mutations in cancer predisposing genes. Exome sequencing of another pair of sibs with slightly later age onset is ongoing. These findings, if replicated with further studies, support the hypothesis of an oligogenic nature of early onset common cancers.

P12.080-M
Lynch Syndrome in the Israeli population

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It is becoming evident that Lynch Syndrome (LS) is one of the most common cancer syndromes. Diagnosis is not always trivial and may be costly. Knowledge of incidence, spectrum of mutations and genes involved in specific populations, facilitates the diagnostic process and contributes to clinical work-up. AIM: To report a cohort of LS families in the Israeli population, gene distribution, mutations detected and co-occurrence of related syndromes. METHODS: Patients were studied at high risk clinics. Diagnostics followed a multi-step process. It included testing for founder mutations, tumor testing, gene sequencing and MLPA. LS was defined by positive tumor analysis and/or positive mutation testing. RESULTS: We describe a cohort of 318 carriers from 169 families with LS. We have identified 61 different mutations in 102 families; 3 founder mutations occurred in over 70% of Ashkenazi LS families, where mutations in *MSH6* were more common than in *MLH1*. We identified founder mutations also among Jewish families from Georgia, Iran and Afghanistan. *MSH2* was mutated in 62% of the cases, one third of these mutations were large deletions. Constitutional mismatch repair deficiency (C-MMRD) was identified in 6 families. CONCLUSIONS: Mutation spectrum and gene distribution in the Israeli population is unique. Mutations in *MSH2* and *MSH6* cause the majority of cases. Six founder mutations contribute to LS in 4 different ethnic sub-groups. C-MMRD occurs either due to founder mutations or consanguinity. These features affect the phenotype, the diagnostic process, risk estimation, and genetic counseling.

P12.081-S
Genotype and Parent of Origin Effect on Colorectal and Endometrial Phenotypes in Patients with a PMS2 mutation

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Introduction Mutations in the PMS2 gene are responsible for Lynch syndrome, a genetically inherited disorder with an increased risk of foremost colorectal cancer (CRC) and endometrial cancer (EC). Possibly, the reported variability in cancer prevalence and age of diagnosis between patients and families can partly be explained by genotype and/or parent of origin effects (POE). **Methods** The genotypes and clinical data of 383 European PMS2 mutation carriers were available for analysis. Mutations with loss of RNA expression (group 1) and retained RNA expression (group 2) were compared. A one-way Anova test and Cox regression were done to compare mean age of cancer diagnosis and calculate hazard ratios (HR). **Results** Mean age of CRC diagnosis was 50,99 years (CI 48,09-53,89) for group 1 and 60,00 years (CI 52,50-67,50) for group 2 (p=0,032). For EC no significant differences in mean age of diagnosis were found. Cox regression showed slightly higher non-significant HRs for both CRC (HR: 1,274, p=0,440) and EC (HR:1,216, p=0,722) when comparing group1 to group 2. No significant HR for CRC or EC depending on whether the mutation was inherited from father or mother was found. **Discussion** A higher mean age at CRC diagnosis and a non-significant higher CRC risk in the group with loss of RNA expression was identified. No significant evidence of a POE was found. Larger studies are needed to confirm findings. Possible information on genotype and other modifying risk factors might lead to individual risk stratification and surveillance programs in the future.

P12.082-M
Bi-allelic MSH6 mutations in a case of early onset multiple primary tumors resembling Lynch and Turcot syndrome

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Multiple primary tumors of the gastrointestinal tract, uterus, ovaries, central nervous system and other organs are hallmarks of Lynch syndrome, caused by heterozygous germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2. Bi-allelic mutations of the same genes have been associated with a rare childhood cancer syndrome characterized by hematological malignancies, sarcomas, brain and gastrointestinal tumors together with cafè-au-lait spots resembling Neurofibromatosis 1. Here we report on a female patient who developed multiple primary tumors between 21 and 27 years of age including two metachronous colorectal cancers, a fillodes tumour of the breast, a glioblastoma and a clear cell carcinoma of the ovaries. Genetic testing identified two germline heterozygote MSH6 mutations: a frameshift mutation (c.1610_1613delAGTA) inherited from the father and a suspected deleterious missense mutation (p.Arg1076His) inherited from the mother. No TP53, MLH1 or MSH2 mutations were found. Microsatellite analysis revealed instability of BAT26 and BAT40 in both the colon and the ovarian carcinomas. The MLH1, MSH2 and PMS2 proteins showed nuclear staining in all specimens while MSH6 was completely absent in tumor and normal cells, including the fillodes tumor of the breast and the normal mucosa of the colon. Germline mutations in the MMR genes, either mono-allelic or bi-allelic, can therefore underlie a wide spectrum of cancer syndromes characterized by variable age at onset and severity of the cancer risk. Development of multiple primary tumors in young adults appears to be suggestive for the presence of bi-allelic mutations of the MMR genes.

P12.083-S

Constitutional epimutation of *MLH1* gene coexisting with a genomic deletion in Lynch Syndrome

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A few Lynch Syndrome patients display constitutional epimutations of the *mismatch repair* genes. Two models of *MLH1* epimutations have been hypothesized: the "primary" type arises spontaneously and is reversible between generations; the "secondary" type is caused by an unknown cis-acting genetic-based alteration, with a classical Mendelian autosomal dominant inheritance pattern.

We report here a case with a heritable large genomic deletion of *MLH1* associated with constitutional promoter methylation of the same gene. Both aberrations were found by MLPA analyses in a patient with colorectal and endometrial cancers (30 and 40 years), displaying MSI-H and loss of the MLH1 protein. They were also detected in three other affected relatives and in tumors.

We have explored the link between *MLH1* deletion and methylation by different molecular approaches. The deletion of 997bp included the ATG codon, exon 1 and part of intron 1. Bisulfite sequencing demonstrated that CpG-methylation was present on the deleted allele and involved both flanking sequences. As expected, a lymphoblastoid cell line obtained from the proband expressed about half of the *MLH1* transcript levels, suggesting that the mutated/methylated allele was completely silenced. Treatments with the demethylating agent 5-AzaC was not able to revert the methylation status of the CpG analyzed and had only a little impact on *MLH1* expression.

In conclusion, in this family with a classical autosomal dominant pattern, we have obtained evidences that *MLH1* hypermethylation is not an independent event, but it is induced by a so far unknown mechanism related to the presence of the concurrent deletion.

P12.084-M

Appearances deceive twice. Lynch-like syndrome ends up being MUTYH-associated polyposis. Apparent homozygous MUTYH mutation ends up being compound heterozygous with a large deletion

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Significant phenotypic similarities among Lynch syndrome (LS) and MUTYH-associated polyposis (MAP) has been reported.

We describe a family suspicious of having LS. A 69 years old female diagnosed of an endometrial cancer (T1N0M0) and right CRC (T3N0M0) with multiple polyps at ages 60 and 65, respectively. Endometrial tumor had loss of MLH1 and PMS2 expression, MSI, no BRAF_V600E mutation, absence of MLH1 methylation, and KRAS_G12C mutation. No pathogenic variant was found in the MLH1 mutation screening and MAP suspicion was considered. MUTYH testing was approached by Sanger sequencing screening of recurrent variants at exons 7 and 13. Patient did show homozygous pattern for c.1187G>A (p.G396D) variant and was diagnosed as MAP syndrome. Predictive test for relatives unveiled an unexpected finding, we found one of her sons with an apparent wild type sequence. Further analysis evidenced a large deletion comprising exon 4-16 in both mother and son. Breakpoint characterization of this deletion allowed us to precisely define this alteration as c.348+33_*64+146del4285insTA with a precise size deletion of 4,285 kb. The same deletion has been published in other two unrelated families from French and Portuguese origin suggesting a founder effect. Large rearrangements at MUTYH locus are rare although probably under-diagnosed. Based on these findings we recommend first, to include MUTYH gene in the testing strategy for LS and second, to test for MUTYH large rearrangements in heterozygous cases after whole gene sequencing, as well as in apparent homozygous cases.

P12.085-S

Male breast cancer in the Netherlands: uptake and outcome of BRCA testing. Results of study the Dutch Breast Cancer Research Group (BOOG 2009-04) in collaboration with the EORTC 10085 study.

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Background: In male breast cancer (MBC) the prevalence of BRCA1/2 mutations varies considerably between countries. In the Netherlands data were collected of all MBC patients diagnosed in the last 20 years. The nationally agreed criteria for DNA testing are rather broad, implying that many MBC cases with or without a family history for breast cancer have been tested. Aim of this study is to get more insight in the percentage of BRCA1/2 mutations among an unselected cohort of MBC patients. **Methods:** All diagnosed MBC patients between 1989-2009 (n=1487) were linked to databases of all clinical genetic centers. Data of BRCA testing, family history and tumor characteristics were collected. **Results:** 334 (22%) of MBC patients were tested for BRCA1/2. Ten (3%) BRCA1, 51 (15%) BRCA2 mutations were identified and also 7 (2%) variants of uncertain significance (VUS). At least one first or second degree relative with breast cancer <50 yr was seen in 80% of BRCA1, 20% of BRCA2 and 6% of non-BRCA1/2 MBC patients. Preliminary studies did show that the majority (86%) of BRCA associated MBC were ductal carcinomas of no special type. **Conclusion:** Roughly one fifth of unselected MBC patients had undergone DNA testing, in 18% of these a BRCA mutation was found. Most striking family histories were seen in BRCA1 families, whereas the majority of BRCA2 MBC patients had no strong family history. Based on our results and previous studies genetic testing for BRCA1/2 should be recommended for any MBC case, regardless of family history for breast cancer.

P12.086-M

Role of MAP/Microtubule Affinity Regulating Kinase 4 in the regulation of cell cycle progression and cytoskeleton dynamics

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MAP/microtubule affinity regulating kinase 4 (MARK4) is a serine-threonine kinase that phosphorylates and regulates MAP proteins. MARK4 differs from the other members of the MARK protein family, for encoding two isoforms (MARK4L and MARK4S) differentially expressed in the nervous system, and for the peculiar sub-cellular localisation at the centrosome and the midbody. In order to better define the role of MARK4 in cell cycle and cytoskeleton dynamics, we performed cytofluorimetric analysis of fibroblasts and glioma cells, showing that MARK4 is expressed throughout the cell cycle and is preferentially activated during mitosis and cytokinesis. Using the same cell system we demonstrated the role of MARK4 in cell cycle progression and cytoskeleton regulation by knockdown and overexpression experiments. Upon MARK4S silencing, fibroblasts and glioma cells display altered morphology, matched with a reduction in proliferation rate and mitotic fraction. In addition, silenced cells show duplicated centrosomes positioned apically to the nucleus, a feature typical of the G1/S phase transition. Overexpression of MARK4L or MARK4S reduced the density of the microtubule network, confirming microtubules as the main target of MARK4. Furthermore in fibroblasts MARK4L was found to colocalise with vimentin and to reorganise

intermediate filaments. Overexpression of kinase-dead mutants indicated that the effects on both cytoskeleton compartments are due to MARK4 kinase activity. The overall data highlight MARK4 as a key component in the regulation of MT dynamics, demonstrate its role in cell cycle progression, particularly at the G1/S transition, and point to vimentin as a new plausible MARK4 interactor.

P12.087-S
Multiple primary melanoma (MPM) as a valid criterion for genetic assessment : an Italian IMI multi-center study

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The prevalence of mutations in the CDKN2A melanoma candidate gene correlates with number of affected family members and number of MPM/neoplastic events. International referral guidelines for genetic counselling and testing (GenoMEL) indicate that in low melanoma incidence populations individuals developing two melanomas may be candidate for consideration for genetic testing, even in the absence of family history (FH). Aims of this multicenter case-control study performed within the Italian Melanoma Intergroup were to verify the likelihood to identify mutations carriers in MPM vs single primary melanoma (SPM) patients recruited, to update the current Italian shared protocol for hereditary melanoma (SIGU-ONC recommendations, Bianchi et al, 2004) and to include the presence of MPM, in the absence of family history, as a criterion. Despite regional differences (i.e. founder mutations), 118/587 (20,1%) of the recruited MPM patients (including those with melanoma FH) and 52/443 (11,7%) of sporadic MPM harbored CDKN2A mutations, suggesting that the development of MPM (2 or more events) even in the absence of FH can be considered a criterion for genetic testing on national basis. The presence of MPM cases in a family was confirmed as a strong mutation predictive parameter, while CDKN2A mutations in sporadic SPM was under 5%. The search for the MITF E318K mutation, recently identified as a novel intermediate risk allele, showed that the mutation was associated with the risk of sporadic MPM (3,7% of sporadic MPM compared to 0.8% of SPM), underscoring the importance of the MITF mutation to the burden of MPM susceptibility.

P12.088-M
Genetic variants in the interleukin locus at 1q32.1 as markers of melanoma survival

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Interleukins play a critical role in immune regulation of tumor development. Because melanoma is a highly immunogenic cancer, and its progression often correlates with immune-related factors, in this study we have tested whether inherited genetic variants in interleukin pathways affect the clinical outcomes of melanoma patients. We performed a two-stage association analysis of 94 SNPs tagging 32 interleukin genes in 1,200 melanoma patients, ascertained at the New York University Medical Center between 2001-2013. The two stages (discovery and validation) were matched by tumor characteristics, age, and gender. Multivariate Cox regression models tested the associations with recurrence-free and overall survival (RFS and OS, respectively), including age, gender, stage, thickness, ulceration status, anatomic site, and histological subtypes as covariates. A region within 1q32.1 containing IL10, IL19, IL20, IL24 was significantly associated with melanoma OS. Specifically, two SNPs in IL10 (rs3024493 and rs2222202) showed the strongest associations with OS (HR=5.82, 95% CI=2.08-16.3, p=0.0009; HR=0.47, 95% CI=0.28-0.80, p=0.006 respectively). The association between rs3024493 and OS replicated among both stages (stage 1 p=0.028, stage 2 p=0.017). This study has identified novel associations of germline genetic risk variants in an interleukin locus at 1q32.1 with melanoma outcomes (rs3024493), and validated the associations from previous smaller studies that showed germline variants in IL10 (rs2222202) associate with worse OS. Pending multi-institutional meta-analysis and further genetic and functional investigations, this study strongly suggests that germline variants in the interleukin locus at 1q32.1 should be considered as novel prognostic markers with potential clinical utility.

P12.089-S
Germline variants and immunotherapy response in advanced melanoma

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BACKGROUND: Ipilimumab-based immunotherapy has substantially increased survival for patients with advanced melanoma, however, the benefit is observed only in a small portion of treated patients. It is highly plausible, yet completely unexplored, that germline genetic factors modulate immunotherapy outcome. In this study we performed whole-exome sequencing (WES) to discover novel germline determinants of response to ipilimumab. METHODS: Blood samples were collected from >60 metastatic melanoma patients treated with ipilimumab at the New York University Cancer Center. WES was performed on objective responders (OR) and non-responders (NR), defined by immune-related response criteria, using the Nextera platform (Illumina) at average 30x coverage. A novel method for testing the association between OR and NR by variant, gene and enrichment of molecular networks was implemented. Gene-Set Enrichment Analysis and Pathway Studio were used to test the pathway associations. RESULTS: The preliminary data comparing an initial subset of 30 ORs and 30 NRs identified significant associations with ipilimumab response for several loci including RPS6KB1 (p=0.001) and LNX2 (0.001). In addition, the pathway analysis showed significant associations for SMAD 3 (p=0.04) and interleukin 1 (p=0.04) related pathways. CONCLUSION: Preliminary findings provide promising evidence supporting the presence of germline genetic factors associated with response to ipilimumab therapy. The data suggest in part a role for immune-related pathways. As we continue to accrue patients, we anticipate an increase in the analytical power of the discovery phase and also an expanded validation of the current findings, suggesting for the first time that germline genetic factors modulate immunotherapy response.

P12.090-M
Functional consequences of cancer associated variants in six human microRNAs

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MicroRNAs are crucial post-transcriptional gene regulators whose strong sequence conservation leads to predict that nucleotide changes in these molecules may be related to disease. We analyzed human microRNA genetic variation and its possible involvement in cancer through a functional approach. From 284 common SNPs (MAF>0.05) located in 254 out of 1872 microRNAs (miRBase Release20.0), 191 were in the precursor microRNA (2.0 SNP/kb), 58 in the mature microRNA (1.5 SNP/kb) and 35 in the microRNA seed region (1.8 SNP/kb). Five of these SNPs, which were previously associated with cancer, were selected for further studies. Three of them (rs12416605, rs35770269 and rs2910164) were in the seed region of miR-938, miR-449c and miR-146a, respectively; one (rs11614913) in the mature miR-196a-2 and the last one (rs3746444) in the mature miR-499b and seed region of miR-499a. Target gene predictions using TargetScan for these microRNA variants revealed that major alleles had a larger number of predicted target genes than minor ones and very little overlap was observed between both alleles in all cases. Furthermore, morphological differences in HeLa cells were observed between the rs3746444 A and G alleles (miR-499a seed) after transfection experiments. Also different expression levels between the C and T rs11614913 alleles (mature miR-196a-2) were detected by RT-qPCR. We are currently investigating the effect of these variants in the spectrum of genes regulated by the studied microRNAs through transcriptome and functional experiments. Different alleles of common microRNA SNPs associated with cancer could lead to changes in microRNA expression and affect their gene regulatory networks.

P12.091-S
The expression of mature microRNAs let-7a, miR-155, miR-205 in non-small cell lung cancer and tumor-adjacent normal tissue

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Non-small cell lung cancer (NSCLC) is the major group of lung cancers. MicroRNAs (miRNAs) are a class of small non-coding RNAs that play a crucial role in the regulation of mRNA translation and degradation. miRNA expression is affected in many cancers. The expression levels of miRNAs let-7a, miR-155, miR-205 in tumor and adjacent normal tissue at 2 and 5 cm from

tumor were measured by real-time PCR with subsequent quantification using a 2-ddCT method. Obtained results were then analyzed for association with clinical-morphological parameters: age, cancer stage, and tumor cells differentiation. The expression of let-7a was significantly decreased in tumors comparing to adjacent tissue at both 2 and 5 cm. Let-7a and miR-155 levels in tumor were substantially lower than in adjacent tissue in patients under 63 years. The expression of let-7a and miR-155 in tumor was also suppressed in patients with III-IV stages of NSCLC. Besides that patients with poorly differentiated NSCLC had significantly lower let-7a level in tumor comparing to adjacent tissue. The levels of let-7a, miR-155, miR-205 were different even in adjacent tissue, in patients with differentiated tumors it was higher than in the group with poorly differentiated tumors. The study showed that let-7a expression is suppressed in tumors comparing to adjacent normal tissue at 2 and 5 cm from tumor. The decrease of let-7a and miR-155 is distinctive for younger patients, III-IV stages of NSCLC, and poor tumor cells differentiation. Altogether these findings consider miRNAs let-7a and miR-155 as markers of unfavorable prognosis for NSCLC patients.

P12.093-S

A germline mismatch repair mutation possibly leading to a de novo NF1 germline mutation

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We report on a female patient diagnosed with breast, colon, and endometrial cancer at the age of 38, 40, and 41 respectively, presenting with café au lait macules, multiple neurofibromas and axillary freckling.
With respect to the family history, her paternal grandmother died at the age of 63 for a colon cancer diagnosed when she was 35, whereas her father died at the age of 42 because of an accident.
As the proband met the criteria for Neurofibromatosis 1 (NF1), analysis of the NF1 gene was performed. A frameshift mutation (c.7096_7101delAACTTT) was identified confirming the clinical diagnosis.
The parents showed no clinical signs of NF1 nor did the two proband's siblings, which tested negative.
Because of the co-occurrence of endometrial and colon cancer in our patient, and the presence of an early-onset colon cancer in the paternal grandmother, expression of mismatch repair protein and Microsatellite Instability (MSI) were analyzed on endometrial and colon cancers.
Both tissue showed loss of staining for the MSH2-MSH6 heterodimer and high MSI, therefore analysis of MSH2 was performed, leading to the identification of a nonsense mutation of exon 2 (c.289C>T, p.Gln97*), consistent with the presence of the Lynch syndrome.
As women with NF1 have a fivefold risk of developing premenopausal breast cancer, no further analysis was performed.
Although it was not possible to analyze any member of the paternal family, an intriguing possibility is that the inherited germinal mutation affecting the mismatch repair system led to a de novo NF1 germinal mutation in our patient.

P12.094-M

Whole genome sequencing of mitochondrial DNA in low and high-risk neuroblastoma patients

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Mitochondrial DNA (mtDNA) mutations may contribute to tumor initiation and progression. mtDNA mutations have never been considered as causative or secondary events for neuroblastoma progression. We sequenced 36 full-length mitochondrial genomes belonging to 16 low-risk (LR) and 20 high-risk (HR) Italian neuroblastomas by Sanger method.
Mutations were selected considering variability <0.01 (SiteVar algorithm) with respect to 14,144 mitochondrial genomes from healthy individuals. To determine whether the selected mutations were somatically acquired, we sequenced the matched germ-line DNAs.
We found 3 somatic missense mutations in CO1, CO2, CYTB genes in HR patients and 1 in (mt)-tRNA gene in LR patients. Moreover, we identified 47 germ-line mutations: 5 were novel (2 missense and 2 tRNA mutations in HR and 1 insertion in LR patients); 10 (83.3%) rare missense mutations occurred in HR and 2 (16.7%) in LR patients. Genes with higher germ-line mutation frequency in HR patients than in LR ones included CYTB (20.0% vs 6.2%), ND1 (10.0% vs 0.0%), and (mt)-tRNA (25.0% vs 12.5%). All CYTB and ND1 mutations in HR patients were missense. The highest rate of rare mutations was found in mt-tRNA gene (19.4% of cases). In HR patients, two mutations (one novel) in tRNA-thr altered (mt)-tRNA secondary structure and one novel mutation occurred in the anticodon loop of tRNA-gly.
In agreement with recent high-throughput screenings of nuclear DNAs, the rate of somatic mitochondrial mutations in neuroblastoma is low. Rare and novel germ-line variants in CYTB, ND1 and (mt)-tRNA loci might be important contributors to HR neuroblastoma development.

P12.095-S

A heterozygous deletion in *BUB1B* predisposing to pediatric cancer, outside the context of classic Mosaic Variegated Aneuploidy syndrome

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Biallelic mutations in *BUB1B* cause Mosaic Variegated Aneuploidy (MVA) syndrome, characterized by microcephaly, growth retardation, intellectual disability and cancer predisposition. *BUB1B* encodes a kinase involved in spindle assembly checkpoint (SAC) function. We describe a boy with PDD-NOS who developed acute lymphoblastic leukemia at age 9 years and primary diffuse leptomeningeal gliomatosis at age 17 years. His father, aged 54 years, had a head circumference at -2 SD and developed a brain tumor at the age of 19 years. They both were found to carry a monoallelic deletion of exons 9-23 of *BUB1B*. Karyotyping of EBV-transformed lymphoblastoid cell lines (LCL) from both father and son, revealed an enrichment of aneuploid cells (10% in both) and a significant increase in premature sister chromatid separation compared to controls. Since these two patients presented with features of the MVA syndrome, but did not present the full-blown phenotype, we hypothesized that this could be explained by BUBR1 protein expression levels below 50%. To study this hypothesis, *BUB1B* mRNA and BUBR1 protein expression levels were measured in EBV-transformed LCLs of the two cases. Expression levels were ~50% and, therefore, in concordance with a deletion of one *BUB1B* allele. Our findings suggest a role for heterozygous germline mutations in *BUB1B* in cancer predisposition, outside the context of classic MVA syndrome. An explanation might be found in digenic inheritance. To study this hypothesis, we will perform exome sequencing on germline DNA of the son and initially we will search for aberrations in SAC genes in particular.

P12.096-M

Somatic mosaicism and transmission of an MSH2 pathogenic variant in a parent of a Lynch Syndrome proband

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We describe the finding of somatic mosaicism for a pathogenic variant of the MSH2 gene in the parent of a proband with Lynch Syndrome. The proband was diagnosed with a colorectal tumor at age 50 that was immunodeficient for MSH2 and MSH6. Sequencing and MLPA analysis of MSH2, MSH6 and MLH1 in a blood sample revealed the presence of a heterozygous pathogenic variant in the MSH2 gene, c.920_923delTCAG (p.Val307Glufs*23). The father was found to have a low level of mosaicism, <10%, for this variant in DNA from blood. This finding was confirmed on a buccal sample from him which showed a greater proportion of the pathogenic variant, ~20%. The father had been diagnosed with prostate cancer at age 70 and his family history is not suggestive of Lynch Syndrome. The mother's sample was negative and parentage was confirmed using microsatellite loci. BRCA1/2 analysis was negative in the proband's sister who was diagnosed with uterine and ovarian cancer at age 37. She was found to be a carrier of this MSH2 pathogenic variant. The results in this family suggest the pathogenic variant arose as an early postzygotic mutation in the father of the proband. Documented incidents of de novo mutation of the mismatch repair genes (MMR) in Lynch syndrome are rare. Our findings lend further support to the suggestion that HNPCC-like histopathological characteristics of tumours in addition to revised Bethesda criteria be used to improve ascertainment of individuals and families at risk of Lynch Syndrome.

P12.097-S

A study to investigate the genetic basis of multiple primary tumours

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Multiple primary malignant tumours (MPMT) are frequently taken as an indicator of potential inherited cancer susceptibility and occur at appreciable frequency both among unselected cancer patients and referrals to cancer genetics services. Analysis of a referral based series of 212 MPMT cases sho-

wed that only around 40% of those who underwent genetic testing and 20% of referrals overall were identified as having a pathogenic germline mutation conferring predisposition to malignancy. Comparison of individuals who tested positive and negative revealed considerable overlap between the two groups with respect to clinical characteristics indicative of an inherited cancer syndrome, suggesting that many of the latter group also have a genetic basis. Analysis of PTEN and TP53, however, did not reveal any significant variants. Failure to detect a germline mutation may result from mosaicism for a mutation in a known inherited cancer gene, an unusual phenotype that leads to the relevant gene being overlooked or mutation in a novel inherited cancer gene. To address these possible explanations, further cases are being identified and next generation sequencing techniques applied to blood samples from the series. Initial analysis is being performed using the Ilumina TruSight cancer panel (of known inherited cancer genes) with subsequent whole exome or genome sequencing if no mutation is identified. Tumour samples will also be analysed for loss of heterozygosity at the relevant locus where putative mutations are found. Recruitment is currently open.

P12.098-M

Colorectal cancer susceptibility alleles as a possible explanation for phenotype variance in *MUTYH* associated polyposis (MAP) patients

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Introduction. Biallelic *MUTYH* mutation carriers have a predisposition to colorectal carcinoma (CRC) and polyp development, but the severity of the phenotype is highly variable. We investigated whether this can be explained by the presence of single nucleotide polymorphisms (SNPs) which have previously been implicated in the susceptibility to CRC and polyps in genome-wide association studies (GWAS).

Method. 154 MAP patients from three countries (UK,NL and DE) were genotyped for 17 SNPs previously identified by GWAS. Data were analysed with ANOVA and cox regression analysis. Homozygotes for the non-risk allele were used as reference category.

Results. A CRC odds ratio (OR) was identified for rs10936599 of 5.101 (95% CI:0.761-34.206) for heterozygotes and 1.403 (CI:0.234-8.415) for homozygotes (p=0.022). The CRC OR for rs10795668 in this cohort is 5.470 (CI: 1.413-21.171) for heterozygotes and 4.684 (CI:1.200-18.289) for homozygotes (p=0.042). For rs961253 heterozygotes an OR for having more than 100 polyps of 2.887 (CI:1.274-6.541) was identified, this is 1.041 (CI:0.299-3.622) for homozygotes (p=0.021). For two SNPs (rs3802842 and rs16892766) previously associated with CRC risk in Lynch patients, a significant pair wise effect on difference in age of diagnosis (of 12 years) was identified between patients with zero and more than 1 risk alleles (p=0.015).

Discussion. There is evidence for an effect of GWAS SNPs in MAP patients. However, a larger cohort size is needed to truly determine this effect. Unravelling risk factors - like SNPs - in MAP patients might allow for individual risk stratification and personalised surveillance programs in the future.

P12.099-S

Identification by exome sequencing of a novel homozygous mutation in *NDRG4* in a family with infantile myofibromatosis

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Infantile myofibromatosis (IM) is a rare disorder characterized by the development of benign tumors in the skin, muscle, bone, and viscera. The incidence is 1/150,000 live births and the disease is the most common cause of fibrous tumors in infancy. Although the identification of mutations in genes that may cause IM is the first step towards the possibility of targeted treatments, the molecular pathogenesis of IM is still poorly understood. Recently, mutations in *PDGFRB* and *NOTCH3* have been implicated in families with the autosomal dominant forms of IM. We have performed whole-exome sequencing of a family with a probably autosomal recessive visceral multicentric infantile myofibromatosis. We studied two brothers and their healthy consanguineous parents. In the two brothers we identified a c.511G>C (p.Val171Leu) novel homozygous mutation in *NDRG4* (N-myc downregulated gene family member 4). The healthy parents were heterozygous for the mutation. Consistent with the phenotype of IM, *NDRG4* is a tumor-related gene. Its expression has been shown to be decreased in numerous types of neoplasia and there have been proposed that it might be a tumor suppressor gene. Additionally, studies have demonstrated that *NDRG4* may have a role in cell survival, tumor invasion and angiogenesis in tumors. We believe that this homozygous mutation in *NDRG4* may be a good candidate for the causative variant of the autosomal recessive form of IM in the family studied and we propose that it should be investigated in other cases of autosomal recessive infantile myofibromatosis.

Financial Support: CNPq and Capes.

P12.100-M

Next-generation panel based characterisation of breast/ovarian cancer genetic predisposition

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BACKGROUND. Genetic predisposition to breast and/or ovarian cancer is largerly confined to mutations in BRCA1/2 genes, although rarer mutations in other known genes (e.g. TP53, PTEN, STK11, CDH1, PALB2, BRIP1, CHEK2 etc.) are also important. Massivelly parralel (or next-generation, NGS) resequencing technology is attractive for identifing cancer predsposing mutations in known genes (panels) and discover new associations.

METHODS. We aimed to better characterize cancer predisposing landscape in clinicaly selected 96 breast and 96 ovarian cancer cases (with strong family history or early age at diagnosis and negative for previously tested BRCA1/2 genes mutations) by perfoming NGS based analysis of 94 genes previously associated with both common (e.g., breast, colorectal) and rare cancers (TruSight Cancer Nextera Custom hybridization-based target enrichment) on MiSeq (Illumina). VariantStudio software was used for anotation and filtering of genetic variants.

RESULTS. Of 192 tested subjects, 8% carried germline loss-of-function mutations (confirmed by Sanger sequencing) in 10 cancer predisposing genes, 3 of them were not previously implicated in hereditary breast/ovarian cancer predisposition.

CONCLUSION. NGS panel based resequencing is effective way for better characterising of cancer predisposition landscape.

P12.102-M

Role of inflammatory gene variants in oral cancer in an Indian population

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Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide. Alcohol, tobacco and smoking are well known risk factors for OSCC. During oral cancer development; inflammation, angiogenesis and thrombosis are involved which correlate with immune cells involved in the production of cytokines, growth factors and adhesion molecules. The study was to evaluate association of cytokine gene variants *viz. IL-1RN* Variable Number of Tandem Repeats (VNTR in intron 2), *IL-1β*-511C/T [rs16944], *IL-6*-597G/A [rs1800797] and *TNF-α*-308 G/A [rs1800629] with oral cancer in a north Indian population. Clinical and addiction details of healthy age/sex matched controls (n=140) and OSCC patients (n=77) were recorded after ethical clearance and consent. DNA was extracted and SNPs genotyped by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). Minor allele frequencies; genotype and allele frequencies were calculated by chi-square ($\chi 2$) analysis (SPSS v.15.0). Gene-gene interaction, pairwise linkage disequilibrium (LD) based on D' statistics and correlation coefficient (r2) of frequencies were analyzed using SHEsis (online version). Genotypic frequencies of *IL-1RN, IL-1β* and *IL-6* while allelic frequency of *IL-6* showed significant association with OSCC (P<0.001). *TNF-α* increases risk of OSCC upto 1.68 times in alcoholic subjects. GATI* and GGTII* haplotypes increased the risk up to 2.863 and 38.285 times respectively. This is the first report from India showing the effect of cytokine gene polymorphisms in OSCC to predict individuals at risk of oral cancer. The knowledge of risk alleles will enable individuals to take precautionary measures before hand and prevent or delay the onset of disease.

P12.103-S

Referral of ovarian cancer patients to genetic counselling by oncologists: room for improvement

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Aims and methods: *BRCA1/2* mutations occur in 10-15% of ovarian cancer (OC) patients, regardless of family history. In November 2012 a pilot protocol was developed by the Unit of Hereditary Cancer (UHC) and the Oncology Service (OS) caring most OC patients in our Institute, to assess the feasibility of offering genetic counselling (GC) to all OC women. Oncologists agreed to propose GC during their clinics and to refer all interested OC patients by directly arrange a contact with UHC. After the first year we evaluated oncologists' adherence to protocol, patients' compliance with GC and testing, and prevalence of *BRCA1/2* mutations.
Results: 104 OC women underwent an oncology visit from November 2012 to December 2013. Ten patients were excluded because they had GC in the past. Only 29 patients (29/94; 31%) were referred to GC; 22/29 attended GC (76%) and 21/22 had genetic testing (95%). Three pathogenic *BRCA* mutations were detected (3/21; 14%); among healthy female close relatives, seven were tested and three were mutation-positive. Referral was much higher for patients attending the first visit (14/26; 54%) than for follow up patients (15/68; 22%). The main differences in these two settings are the time schedule (one vs half an hour) and the checklist in the clinical record (including or not family history).
Conclusions: Patients' compliance to GC/testing was high, and *BRCA* mutation prevalence was as expected. However, oncologists' compliance was low, mainly because of practical barriers. Efforts are needed to integrate GC-focused tools and procedures in oncology practice.

P12.104-M
Targeted resequencing approach to investigate the mutational landscape associated to platinum resistance in EOC

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Introduction Despite initial response to first line platinum-based chemotherapy, more than 80% of high grade serous ovarian cancer patients relapse and develop resistance. The molecular and genetic features involved in drug resistance are still unknown. By gene expression profile in a cohort of patients from which matched biopsies were taken at primary surgery (PS-O) when tumor was sensitive to chemotherapy and at time of relapse (SCR) when the tumor was resistant, we identified EMT pathway as a key player in tumor relapse (Marchini et al., 2013). Here we investigate the genomic alterations driving drug resistance by performing targeted DNA resequencing on our cohort of SCR and PS-O samples.
Methods DNA libraries enriched in a selected panel of 30 genes encompassing key players of signal transduction, cell cycle and DNA repair were generated using TruSeq Custom Amplicon kit and sequenced on MiSeq (Illumina). Data were analyzed using a high performance cluster computing platform (Cloud4CARE project).
Results Analysis identified a total of 166 mutations (152 SNPs and 14 InDels), of which 51 affecting PS-O and 115 SCR. With a 500X coverage, we observed BRCA1, BRCA2 and TP53 mutated in the majority of cases. In addition, the PI3K pathway was found mutated in SCR samples only.
Conclusions Our preliminary results suggest two main conclusions:
1- genomic alterations (SNPs or InDels) were more frequent in SCR compared to PS-O;
2- most of the mutations affected genes belonging to DNA and PI3K pathway.

P12.105-S
Transcriptome and pathway analysis identifies IRF1 as a predictor of progression free and overall survival in ovarian carcinoma

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Epithelial Ovarian Cancer (EOC) is the most lethal female reproductive tract malignancy. Most patients present with advanced stage disease and the cornerstone of treatment is surgical debulking followed by platinum-based chemotherapy. The major contributor to the high fatality-to-case ratio is chemoresistant disease. We sought to identify candidate biomarkers/pathways which could distinguish between platinum sensitivity and platinum resistance and test their prognostic ability. Ovarian tumor samples from patients with primary high-grade serous ovarian cancer were divided into two groups based on response to platinum status. Transcriptome analysis was performed using RNA-Seq and Ingenuity Pathway Analysis (IPA) was used to explore differences between these two sets of samples. Findings were validated using qRT-PCR. Survival analysis was performed in two independent sample sets: gene expression data (GEO); relapse-free/overall survival information, (EGA, TCGA).
IPA highlighted that Interferon regulatory factor 1 (IRF1) was differentially expressed between the two clinical groups and was upregulated in the platinum-sensitive group. Validation studies performed on 31 patient tumor samples demonstrated a significant difference in PFS between the low and high IRF1 groups (P = 0.027) as well as a distinct difference in the probability of recurrence. In conclusion, we have shown that high levels of IRF1 strongly correlated with increased overall survival in late-stage disease regardless of debulking status and grade in those patients who received platinum therapy.

P12.106-M
Role of p53 gene in the pathogenesis of Acinar Cell Carcinomas of the pancreas

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Introduction: The role of p53 (17p13.1) in the pathogenesis of pancreatic acinar cell carcinomas (ACCs) has not been fully clarified yet. Few studies, mainly using immunohistochemistry (IHC), suggested that p53 is not involved in ACCs but molecular data are controversial. Recently, p53 mutation and a significant (>50% of cells) p53 nuclear immunoreactivity (IR) have been reported in 20% and 27% of ACCs, respectively. Aim: To clarify the role of p53 in the pathogenesis of ACCs we investigate p53 alterations (mutation, methylation, loss and nuclear protein expression) in 44 ACCs using different approaches: direct sequencing of exons 5-8, MS-MLPA, FISH and IHC. Results: p53 mutations were found in 8/44 (18%) cases, correlated with higher tumor stage (5 cases were at stage IV). In one case, p53 mutation was observed only in the metastasis of a primary p53 wild-type ACC. Methylation of p53 was observed in only one case. Loss of p53 gene, including 17p13.1 deletion and monosomy of chromosome 17, was found in 50% of ACCs and in 4 cases a concomitant mutation was identified. The simultaneous presence of both p53 mutation and loss correlated with worse prognosis (p=0.001). Conclusions: p53 alterations including mutations and cytogenetic loss are frequent in ACCs. p53 mutation and loss are correlated with higher tumor stage and with worse prognosis. Our results suggest that p53 is not an early event in ACC tumorigenesis, but it is involved in late phases of cancer progression.

P12.107-S
Do pancreatic cancer patients diagnosed with BRCA1/2 Ashkenazi mutations have less clinical risk factors?

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Aim: This study investigated the clinical background of Ashkenazi pancreatic cancer patients, either carriers or non-carriers of any of the three founder Ashkenazi mutation in BRCA1/2 genes namely, 185delAG, 5382insC and 6174delT.
Methods: Clinical characteristics including age at onset, smoking behaviour, physical activity, BMI, and chronic diseases e.g., hypertension and hyperlipidemia was available for 90 pancreatic cancer patients, consecutively referred to our oncogenetic clinic.
Results: Fifteen (16.7%) carried a founder Ashkenazi mutation in either BRCA1or BRCA2: of these, nine (60%) carried the 6174delT mutation in BRCA2; five (33.3%) and one (6.7%) harbored the 185delAG and the 5382insC mutations in BRCA1, respectively. Carriers compared to non-carriers were diagnosed at 61±7.5 and 63.0±11.3 years of age, respectively (p=0.520); and have higher BMI levels (28.9±7.3 compared to 25.1±4.9; p=0.112). Although, not significant, *carriers* smoked less than *non-carriers* (3, 20% compared to 28, 37.3%; p=0.197), were less physically active (3, 20% compared to 34, 45%; p=0.092), suffered less from the chronic diseases, hypertension (3, 20% compared to 31, 41%; p=0.072); and hyperlipidemia (0 compared to 11, 14.7%; p=0.080).
Conclusion: Our observation revealed that although not significant, non-carriers tend to have more known risk factors for the development of pancreatic cancer compared to BRCA1/2 mutation carriers.

P12.108-M
Germline FH mutations presenting with Phaeochromocytoma

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At least a third of patients with phaeochromocytoma (PCC) or paraganglioma (PGL) harbour an underlying germline mutation in a known PCC/PGL gene. Mutations in SDHx genes (SDHB, SDHD, SDHD and SDHA) encoding a component of the TCA cycle, succinate dehydrogenase (SDH), are a major cause of inherited PCC/PGL. SDHB mutations are also associated with inherited renal cell carcinoma (RCC). Inactivation of SDH in tumour cells results in abnormalities of cellular metabolism associated with activation of hypoxic gene response pathways and epigenetic alterations (e.g. DNA methylation). Similar findings have recently been reported in cases with mutations the FH gene, which encodes the TCA cycle component directly downstream of SDH, fumarate hydratase. However, the clinical phenotype of germline mutations in SDHx genes and FH is usually distinct with FH mutations classically associated with hereditary cutaneous and uterine leiomyomatosis and renal cell carcinoma. In order to identify potential novel PCC/PGL predisposition genes we undertook an exome resequencing study in a case of childhood PCC. After identifying a candidate FH missense mutation (p.Cys434Tyr) we sequenced FH in a further 71 patients with PCC, PGL or head and neck paraganglioma (HNPGL) and identified a further candidate missense mutation (p.Glu53Lys). We then performed In vitro analyses and demonstrated that both missense mutations were associated with elevated intracellular fumarate levels compared to a wild-type rescue construct. These findings (a) confirm that germline FH mutations may present, albeit rarely, with PCC or PGL and (b) extend the clinical phenotype associated with FH mutations to paediatric PCC.

P12.109-S

Molecular analysis of somatic mutations in Phaeochromocytoma and Paraganglioma

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At least a third of patients with phaeochromocytoma (PCC) or paraganglioma (PGL) harbour an underlying germline mutation in a known PCC/PGL gene. Identification of germline mutations and key somatic mutations is important for the application of personalised medicine. To investigate the molecular pathogenesis in PCC, PGL and head and neck paraganglioma (HNPGL) we analysed PCC/PGL/HNPGL by next generation (NGS) and Sanger sequencing strategies. For the NGS studies, 55 tumours were analysed with the Ion Torrent AmpliSeq Cancer Hotspot Panel v2 which targets 54kb of mutational hotspots in 50 oncogenes and tumour suppressor genes and exome resequencing was performed in 12 tumours. Confirmed somatic missense mutations occurring in a single tumour were detected in CDH1, APC and JAK3. Activating mutations in HRAS (p.Gln61Arg and p.Gly13Arg) and HIF2A (p.Pro531Thr) were detected in 9.3% and 2% of tumours analysed. HRAS and HIF2A oncogenic mutations were detected in 6/30 PCC, 0/21 HNPGL and 0/4 PGL (frequency in PCC vs HNPGL P=0.69). Ten tumours harboured mutations in inherited PCC/PGL/HNPGL genes and no HRAS or HIF2A mutations occurred in this group. Combining our data with a previous report of HRAS mutations in PCC/PGL (JCEM 2013;98:E1266-71) we find that the mean frequency of HRAS mutations (7/9 at codon 61) in sporadic PCC/PGL is 10.8% (9/83; 95% CI = 6.3 to 17.9%) and in PCC/PGL with an inherited gene mutation 0% (0/29; 95% CI 0 to 13.9%) suggesting that HRAS and inherited PCC/PGL gene mutations might be mutually exclusive.

P12.110-M

Germline mutations in SDHB, SDHD, VHL, RET and TMEM genes in patients with nonsyndromic pheochromocytoma/paraganglioma in Serbian population

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Background: Several susceptibility genes have been found to be associated with development of pheochromocytoma PHEO/ paraganglioma PGL : RET, VHL, SDHB, SDHD and TMEM. We investigated the frequency of germline mutations in these genes in patients with apparently sporadic PHEO/PGL.
Material and methods: Two hundred patients (F/M 112/88) with apparently sporadic PHEO /PGL were screened.Germline mutations were investigated by using direct sequencing for point mutations in RET, VHL, SDHB, SDHD and TMEM, and multiplex ligation - dependent probe amplification for gross deletions in VHL gene.
Results: In 30/200 (15%) probands, germ-line variants were identified: 12 heterozygous germline mutations (7 novel nonsense: W218X; frameshift: c.661delG, p.Asp221ThrfsX27; splicing:c.424-12delTCTT; missense: R116M; frameshift: c. 636_637 ins A, p. Met 213Asn fsX8 and missense: H132R; splicing: c. 424-1insG -7 delA) of the SDHB gene, 10 in VHL1 novel, V84M, in 3 families , 6 variants in SDHD, 1 in RET and 1 in TMEM gene. Familiy members were also tested. Most of the patients with mutation in SDHB gene were found to have malignant PHEO/PGL. Patients with mutations in VHL, RET and TMEM genes developed PHEO.
Conclusion:The most commonly mutated gene was SDHB, which carries the highest risk of malignancy. Our patients with extra-adrenal disease needs careful follow-up, since they are in higher risk for the development of metastases or novel adrenal/extra-adrenal PHEO. The patients with VHL mutation (V84M) is apparently classified as 2C. These patients may develop some other tumors than PHEO.

P12.111-S

Post-transcriptional regulation of *PHOX2B* gene expression

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Neuroblastoma (NB) is one of the most frequent and severe solid tumors in childhood.
Mutations in the genes coding for the transcription factor *PHOX2B* and for its transcriptional target *ALK* have been detected in sporadic and familial cases of NB and over-expression of the two genes have been identified in NB samples and cell lines.
In this work, in order to investigate the mechanisms underlying *PHOX2B* overexpression in NB, we report *in silico* and *in vitro* characterization of the *PHOX2B* 3' untranslated region (3'UTR).
First, the *in silico* search for elements known to regulate the mRNA stability allowed us to identify three AU-RICH elements (AREs) in the more distal region of the 3'UTR, in addition to several putative miRNAs binding sites. Regions of the distal *PHOX2B* portion likely responsible for mRNA regulation were eventually defined by combining the above predictions with results from the phylogenic conservation of the PHOX2B 3'UTR.
In vitro experiments in IMR32 NB cells have shown that *PHOX2B* mRNA is not stable, thus suggesting it as target of post-transcriptional regulation mechanisms. The successive cloning of the 3'UTR *PHOX2B* downstream the *Luciferase* gene in the pmiRGlo vector allowed us to confirm such a hypothesis and, following the generation of constructs containing progressively shorter deletions of the 3'UTR, to map position and extent of the regions responsible for the *PHOX2B* mRNA stability.
As a consequence of all the above described observations, we suggest that regulation and modulation of *PHOX2B* post-transcriptional stability may be considered a pharmaceutical target in NB.

P12.112-M

In vitro drug screening approaches targeting PHOX2B over-expression in neuroblastoma

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PHOX2B is a transcription factor involved in the regulation of neurogenesis and in the correct differentiation of autonomic nervous system. Several evidence report a pathogenetic role of PHOX2B in neuroblastoma (NB): (i) somatic and germline gain of function mutations in familial, sporadic and syndromic cases of NB; (ii) the finding of *ALK*, a transcriptional target of PHOX2B, as major familial NB predisposition gene; and (iii) the observation of *ALK* and *PHOX2*B over-expression in tumor samples and NB cell lines. Starting from these observations, we have performed an *in vitro* drug screening targeting PHOX2B over-expression as a potential pharmacological means in NB. First, we have evaluated the effects of a (i) small subset of molecules and an (ii) epigenetic library in a IMR-32 cell line stably expressing *Luciferase gene* under the control of *PHOX2B promoter* to identify molecules able in down-regulating PHOX2B expression. Curcumin, SAHA and trichostatin A showed a down-regulation of *PHOX2B* promoter activity and a decrease of both protein and mRNA expression. To deepen into curcumin mechanisms of action, we have investigated the role of transcription factors (TF) predicted by *in silico* analysis to bind the PHOX2B promoter.

Among these TF, we have demonstrated that treatments with 8 or 20 μM curcumin led to a decrease of *PBX1/MEIS1* expression and modulated the activity of NFκB and AP-1. Moreover, combined drug treatments showed successful effects of down-regulation of the expression of both PHOX2B and its target *ALK*, thus supporting the notion of the effectiveness of molecule combination in tumor therapy.

P12.113-S

PIK3CA mutations in non-small cell lung cancer

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Background: Most of the proteins that are encoded by oncogenes which play a role in molecular pathogenesis of cancer, function as protein kinases. Acquired constitutional activity of these proteins leads to the activation of signaling pathways which are involved in cell proliferation, apoptosis, protein synthesis, cell migration and many other cellular processes. One of the most important signaling pathways that plays role in the pathogenesis of cancer is phosphatidyl inositol 3 kinase (PI3K) signaling pathway. Especially class IA PI3Ks are known to play a role in the pathogenesis of cancer. Basically, p110α isoform of catalytic domain accounts for the enzymatic function of class IA PI3Ks. It is encoded by *PIK3CA* gene. *PIK3CA* mutations has been previously reported in many cancers.

Methods: All exons of the *PIK3CA gene* were sequenced in 40 NSCLC tumor samples. All individuals provided informed consent, and the study was performed in accordance with ethical guidelines.

Results: The 1634A>C mutation which has already been identified in many cancers and NSCLC was determined in 7,5% of the tumor tissue samples. This mutation rate is higher than reported in the literature. Interestingly a second mutation (1658_1659delGTinsC) was identified in these patients. The concurrence of these two mutations has been reported as Cowden syndrome in the literature which is known to be a cancer predisposition syndrome. **Conclusion:** This finding is quite important since it can be an indication of underlying cancer predisposition syndrome in NSCLC patients. Besides previously reported PIK3CA mutations some novel mutations have been defined.

P12.114-M

Development of Acquired Resistance to Anti-EGFR Therapy in Colorectal Cancer Identified by Whole-Genome Plasma DNA Sequencing

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Introduction: EGFR-targeting monoclonal antibodies, cetuximab and panitumumab, are important therapeutic options in *KRAS* wild-type metastatic colorectal cancer (mCRC) patients. However, secondary resistance inevitably ensues in all patients within 3-12 months from the start of therapy. The mechanism and timing of emergence of resistance which limit the efficacy of these drugs, is highly relevant for designing therapeutic strategies. **Methods:** We examined the plasma DNA of 10 *KRAS* wild-type mCRC patients who received anti-EGFR therapy, using a high-throughput whole genome sequencing (Plasma-Seq) and ultra-deep sequencing of genes associated with resistance to anti-EGFR therapy such as *KRAS*, *BRAF*, *PIK3CA* and *EGFR* with Illumina's MiSeq. **Results:** Genome-wide characterisation of the plasma DNA and corresponding primary tumor revealed several tumor specific aberrations such as over-representation of chromosomes 8q, 13 and 20q, and losses of 8p, 4 and 18. The development of resistance to anti-EGFR therapy was observed to be associated with novel focal amplification of *KRAS* (n=3), *MET* (n=2) and *ERBB2* (n=1) or high level polysomy of 12p which includes *KRAS* (n=1). Overrepresentation of *EGFR* gene was associated with initial good response to therapy. However, ultra-deep sequencing of *KRAS, BRAF, PIK3CA* and *EGFR* did not reveal any novel mutations. **Conclusion:** Overall, predictive biomarkers associated with the anti-EGFR therapy efficacy, correlating well with treatment response was identified in 70% of our patients. The tumor genome is prone to continuous changes and plasma-Seq, a fast and affordable tool enables identification of novel clones and guide real-time modification of treatment regimen to delay or prevent disease progression.

P12.115-S

PMS2 mutation detection and PMS2 mutation spectrum in the Netherlands

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Heterozygous mutations in the mismatch repair (MMR) gene *PMS2* cause Lynch syndrome (LS), an autosomal dominant predisposition for colorectal, endometrial and other cancers. Biallelic mutations lead to constitutional MMR-deficiency syndrome (CMMR-D) in which various types of malignancy occur early in life. Isolated loss of PMS2, detected by immunohistochemistry in tumours directs mutation scanning to *PMS2* to diagnose LS and CMMR-D. Mutation scanning of this gene has been notoriously difficult due to the presence of highly homologous pseudogene sequences and frequent gene conversion events. Here we present results of recently improved DNA-based and RNA-based mutation detection strategies for *PMS2* as obtained in the five Dutch accredited diagnostic laboratories that offer *PMS2* genetic testing. Altogether, 53 different deleterious *PMS2* mutations were discovered in 126 LS and 8 CMMRD index patients. Recurrent *PMS2* mutations, probably of founder origin, appear to be common in the Dutch cohort: 15 mutations found in at least 3 probands represent 68% (96/142) of mutated alleles. Notably, one such recurrent mutation, detected in 3 probands in our cohort, is the recently described retrotranspositional insertion of an SVA repeat, that is missed by current DNA-based protocols. Pathogenic *PMS2* mutations were found in 78% (57/73) of patients with isolated PMS2 loss, and another 10% (n=7) could be explained by an *MLH1* mutation (data from 2 labs with complete data set). Based on an estimated number of 700 Dutch proven LS families, 18% (126/700) is explained by a *PMS2* mutation. Our data substantially expand knowledge on the spectrum of disease-causing *PMS2* mutations.

P12.116-M

A new POLD1 germline mutation as cause of Familial Colorectal Cancer Type X

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The genetic basis for Familial Colorectal Cancer Type-X (fCRC-X) is unknown. Mutations at the proof-reading domains of DNA polymerase ε (POLE) and δ (POLD1) have been recently identified in families with multiple colorectal adenomas and CRC.

We aimed to assess the prevalence of POLE and POLD1 mutations in fCRC-X.

A total of 63 index cases fulfilling Amsterdam II criteria with normal expression of mismatch repair proteins and microsatellite stable tumors were included. Sanger sequencing of POLE-exon13 and POLD1-exon11 was performed.

None of the cases carried mutations in POLE. We found one case with a new POLD1 variant in heterozygosis: c.1421T>C (p.Leu474Pro). The patient was a woman who underwent a synchronous CRC and large bowel gastrointestinal stromal tumor at age 36. Her maternal aunt had metachronous CRC (dx33y) and endometrial cancer (dx56y) and was a carrier of this variant. Therefore, the index case's mother, who underwent endometrial cancer (dx52y), was an obligate variant carrier. It has been described that the homologous residue of POLD1 p.Leu479Pro mutation in S. cerevisiae causes a mutator phenotype. Furthermore, this is the paralogous residue of the hot spot at POLE (p.Leu424). In silico prediction analysis strongly suggests a pathogenic nature for this variant. Integration of all these evidences drove us to classify this new variant as probably damaging.

POLD1 mutations might explain about 1.6% of fCRC-X. POLD1 testing should be included in the diagnostic strategy for unexplained familial CRC.

P12.117-S

Polymerase Proofreading - Associated Polyposis (PPAP): an unusual family with the highly penetrant POLE p.Leu424Val mutation with colorectal cancer, polyposis, duodenal cancer and diabetes

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Mutations in the exonuclease domain of the polymerase E (POLE) gene have recently been identified as causing a rare but highly penetrant predisposition to colorectal cancer and colorectal polyps. Palles et al employed whole-genome sequencing and detected the p.Leu424Val (L424V) mutation in 12 families, with no evidence of a shared common ancestor. The phenotypes of these families were consistent with an autosomal dominant predisposition to colorectal adenomas and carcinomas, with some individuals having multiple tumours. Tumours from these subjects were all microsatellite stable (MSI-S) and did not show any preponderance of site within the colon or particular morphology. There were no extra-colonic tumours described in any affected individuals in these families. Following the identification POLE L424V in our family through the CORGI study, subsequent testing identified the familial mutation in an individual with an MSI unstable (MSI-H) tumour and synchronous duodenal adenocarcinoma, rectal carcinoma and adenomas. It was also noted that all the individuals affected with colorectal cancer in this family also have diabetes, consistent across 3 generations. The co-segregation of diabetes and colorectal cancer requires formal confirmation in some relatives. This association warrants further study. An increased risk of CRC in patients with diabetes mellitus has been recognised for some years. Emerging evidence that polymerase D (POLD1) gene has an important function in adipose tissue homeostasis lends weight to the association. This family provides evidence of a broader phenotype of malignancy in POLE, and opportunity for new investigations into the possible mechanism linking diabetes and colorectal cancer.

P12.118-M

Association of variant -765G>C in the PTGS2 gene promoter with melanoma in Italian patients and its relation to gene expression in dermal fibroblasts

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Aim: The production of prostaglandins, especially prostaglandin E synthetase (PGE2) is hypothesized to influence carcinogenesis by promoting cell proliferation, inhibiting apoptosis, stimulating angiogenesis, and mediating immune suppression. Cyclooxygenase-2 (Cox-2), the inducible isoform of cyclooxygenase coded by the PTGS2 gene, is the key enzyme in the production of prostaglandins involved in inflammatory processes including cancer. In melanoma skin cancer, Cox-2 is overexpressed in primary malignant melanoma and in their corresponding metastases. Aim of this study was to investigate if polymorphisms -765G>C (rs20417), and -1195A>G (rs689466) in the PTGS2 gene impact on its expression in dermal fibroblasts and are associated with individual susceptibility to malignant cutaneous melanoma.
Methods: Two hundred forty patients presenting melanoma and 342 control individuals were genotyped for polymorphisms -765G>C (rs20417) and -1195A>G (rs689466) by restriction fragment length polymorphism (PCR-RFLP) analysis. PTGS2 gene expression was performed by Real Time PCR using Sybr Green.
Results: The allele -765C was associated with an increased prevalence of melanoma. No association of -1195A>G polymorphism was observed. Haplotype analysis of both variations showed that the haplotypes carrying the minor alleles were associated to a higher risk of melanoma (p=0.02). Expression analysis indicated that allele -765C is associated to a higher gene expression and thus could represent a risk allele by affecting the functionality of the promoter.
Conclusion: In conclusion, variant -765G>C may be associated to malignant cutaneous melanoma with a low penetrance effect and this effect could be a consequence of altered gene expression.

P12.119-S

DNA methylation profiles of PDGFB and FGF2 are potential biomarkers of disease progression in primary myelofibrosis

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The primary myelofibrosis (PMF) is characterized by clonal proliferation of the hematopoietic precursors, fibrosis, osteosclerosis and angiogenesis. Bone marrow fibrosis is a reactive process, where the fibroblast proliferation is in response to cytokines, such as platelet derived growth factor (PDGF) and the basic fibroblastic growth factor (FGF2), produced by malignant megakaryocytes or monocytes. No specific prognostic markers are today available to refine the clinical classification and the risk to develop fibrosis. The purpose of this study is to investigate DNA methylation of PDGFB and FGF2, to verify possible associations among the epigenetic profile fibrosis progression and prognosis of PMF. The methylation is evaluated by pyrosequencing in a cohort of 58 PMF cases and 20 controls. The methylation percentages of PDGFB and FGF2 in PMF ranged from a complete demethylation to hypermethylation (PDGFB range: 3-95, mean value: 39; FGF2 range: 1-96%, mean value: 40), in controls the methylation values of both genes are clustered in more restricted intervals (PDGFB: 28-46% mean value: 35; FGF2: 16-43% mean value: 28). The methylation values of PDGFB and FGF2 are significantly increased in the prefibrotic cases compared to controls (PDGFB: 71 vs. 33, $p < 0.0005$ and FGF2: 56 vs. 27.5, $p < 0.0005$). Interestingly hypomethylated PDGFB was an indicator of better prognosis for fibrosis, International Prognostic Scoring System (IPPS) and Dynamic International Prognostic Scoring System (DIPPS) progression (p=0.03, p=0.02 and p=0.01 respectively).

P12.120-M

Synergistic effect and VEGF/HSP70-hom haplotype analysis: relationship to prostate cancer risk and clinical outcome

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Prostate cancer is a complex disorder resulting from the combined effects of multiple environmental and genetic factors. Our previous single locus analysis showed that *VEGF* and *HSP70-hom* polymorphisms were significantly associated with prostate cancer susceptibility and prognosis. Both genes encoding these proteins were located on chromosome 6p21, and combining the neighboring SNPs into haplotypes may increase the association with the disease. Three tagging polymorphisms, the *HSP70-hom* 2437 T/C, the *VEGF*-1154 G/A and the *VEGF*-634 G/C SNPs were genotyped in 101 cases and 80 controls. For the combined analysis of *VEGF* and *HSP70-hom*, we found a positive gradient in the ORs related to the number of high risk genotypes with a 3.53-fold increase of prostate carcinoma risk (OR= 3.53; *P*= 0.015). Furthermore, The TAG and CAG haplotypes at positions *HSP70-hom*, *VEGF* -1154 and *VEGF* -634 exhibited a twofold (OR= 0.46; P= 0.014) and a sevenfold (OR= 0.14; P= 0.00005) reduction in prostate cancer risk, respectively. Regarding prostate cancer prognosis, the TAG haplotype had a negative association with the aggressive phenotype as defined by the histopathological grade (OR= 0.28; P= 0.006). Our findings confirm the role of at-risk haplotype across the *HSP70-hom/VEGF* gene cluster in determining susceptibility to prostate cancer.

P12.121-S

Frequency and clinical implication of rare *BRAF* variants other than *BRAF* V600E mutation in a large cohort of consecutive thyroid fine needle aspiration cytology samples

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Background: *BRAF* mutation analysis is a useful adjunctive tool in diagnosing thyroid nodules. The *BRAF* V600E (c.1799T>A) mutation comprises over 95% of all *BRAF* gene mutations in papillary thyroid carcinoma (PTC). The clinicopathological association of rare *BRAF* variants other than V600E

mutation is still obscure.
Methods: We evaluated a total of 1067 consecutive patients with malignant or indeterminate thyroid nodules by ultrasonography. All fine needle aspiration cytology (FNAC) samples were tested for *BRAF* mutation using mutant enrichment with 3'-modified oligonucleotide (MEMO) sequencing with real-time PCR concurrently. Rare *BRAF* variants were evaluated with regard to cytology and/or histology results.
Results: *BRAF* mutations were detected in 37.9% (404/1067) of all samples. The V600E mutation was detected in 98.3% (397/404), and six rare variants were detected by MEMO sequencing. Three types of variants were identified: c.1799_1801del (p.Val600_Lys601delinsGlu), c.1794_1795insGTT (p.Ala598_Thr599insVal), and c.1801A>T (p.Lys601*). The former two are known mutations to be associated with PTC and three patients with these mutations were diagnosed as PTC histologically. The third variant was novel. The case with c.1801A>T was diagnosed as a benign follicular nodule.
Conclusions: Out of 1067 thyroid nodule cytology samples, six (0.56%) had rare *BRAF* variants. A novel variant was identified in a cytologically benign thyroid nodule. Targeted testing for detecting only the V600E mutation may be good enough to increase the diagnostic value of FNAC, however, identification of other variants in *BRAF* gene may provide additional valuable information on the nature of thyroid nodules in the future.

P12.122-M
Association between the cytogenetic profile of tumor cells and response to preoperative radiochemotherapy in locally advanced rectal cancer

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Neoadjuvant radiochemotherapy to locally advanced rectal carcinoma patients has proven efficient in a high percentage of cases. Despite this, some patients show non-response or even disease progression. Recent studies suggest that different genetic alterations may be associated with sensitivity vs. resistance of rectal cancer tumor cells to neoadjuvant therapy. We investigated the relationship between intratumoral pathways of clonal evolution as assessed by iFISH (51 different probes) and response to neoadjuvant radiochemotherapy, evaluated by Dworak criteria in 45 rectal cancer tumors before (n=45) and after (n=31) treatment. Losses of chromosomes 1p (44%), 8p (53%), 17p (47%), 18q (38%) and gains of 1q (49%), 13q (75%) as well as amplification of 8q (38%) and 20q (47%) chromosomal regions were those specific alterations found at higher frequencies. Significant association ($p<0.05$) was found between alteration of 1p, 1q, 11p, 12p and 17p chromosomal regions and degree of response to neoadjuvant therapy. A clear association was observed between cytogenetic profile of the ancestral tumor cell clone and response to radiochemotherapy; cases presenting with del(17p) showed a poor response to neoadjuvant treatment (p=0.03), while presence of del(1p) was more frequently observed in responder patients (p=0.0002). Moreover, a significantly higher number of copies of chromosomes 8q (p=0.004), 13q (p=0.003) and 20q (p=0.002) were found after therapy vs. paired pre-treatment rectal cancer samples. Our results point out the existence of an association between tumor cytogenetics and response to neoadjuvant therapy in locally advanced rectal cancer. Further studies in larger series of patients are necessary to confirm our results.

P12.123-S
Epigenetic Profile of Early Relapsed Childhood ALL

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Relapsed acute lymphoblastic leukemia (ALL) is one of the leading causes of death among children with cancer. The prognosis for early relapse children remains poor. To discover the underlying epigenetic pathways that may play a role in drug resistance and relapse, we performed Infinium HumanMethylation450K BeadChip arrays and to characterize the molecular evolution of relapsed childhood ALL, we used Illumina HumanCytoSNP-12 arrays to identify somatic copy number alterations (CNAs) in 16 diagnosis/relapse pairs. Flow-sorted normal B-cell progenitor subpopulations and CD4+CD8+ T-cell purified from thymus were used as controls.
Analysis variant CpG sites in an unsupervised manner, we identified three distinct DNA methylation profiles in samples according to their structural variations. ALL cases did not show significant differences between paired diagnose/ relapse samples though they had hyper DNA methylation than control samples. Aberrant DNA methylation had been detected in negative regulator of cell cycle genes and DNA damage repair genes in ALL. Copy number analysis of patients revealed varying numbers of genetic lesions ranging from 0 to 45 CNAs per sample. The vast majority of CNAs observed were shared between diagnosis and relapse in the same patients. One of the most frequent CNAs involved deletions of CDKN2A/B , occurring in 8 patients, 2 of 8 patients lost the CDKN2A/B at relapse time. Integration of methylation and genotyping data revealed high concordance.
Early relapse samples were more likely to be similar to their respective diagnostic sample that suggests early-relapse results from the emergence of a related clone.

P12.124-M
Genome-wide methylation analysis of tubulocystic and papillary renal cell carcinomas

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This study was undertaken to characterize and compare molecular signatures associated with tubulocystic renal cell carcinoma (TRCC) and papillary renal cell carcinoma (PRCC).
We performed methylated DNA immunoprecipitation (MeDIP) coupled with genome wide microarray analysis (Roche NimbleGen) in 6 PRCC and 2 TRCC together with analysis of control tissues of normal histological appearance adjacent to each examined tumor sample.
All examined tumors and their control tissues of histological normal appearance share alteration in regulation of specific pathways. In TRCC we found higher number (48) of methylated tumor suppressor genes (TSG) than in PRCC (35). Only TSG DPH1 and VHL were found to be distinctively methylated in all PRCC. The methylation of gene sequences SPIB, PLAT, PCDHB1 and FAF2 was found exclusively in TRCC tumor samples, the methylation in genes KIFC3, MDH2, PAG1, BICD1, PLG and CTRL was limited to all PRCC tumor samples only.
Using bioinformatics tools (DAVID and GSEA), we found the pathways and genes which are altered by differential methylation and which may be responsible for developmental divergence of both tumors from precancerous renal tissue. The genes involved in transmembrane transport of small molecules, lipoprotein metabolism and transmission across chemical synapsis are significantly overrepresented among genes methylated preferentially in TRCC, PRCC is characterized by overrepresentation of genes playing roles in RNA metabolism and processing.
Supported by grants no. PRVOUK P25/LF1/2 of the Ministry of Education, Youth and Sport of the Czech Republic and no.RVO-VFN 64165 of the Ministry of Health of the Czech Republic.

P12.125-S
Identification of mosaicism in patients with sporadic retinoblastoma using NGS

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Objectives: Retinoblastoma is the paradigm of hereditary cancer. Approximately, 40% of patients carry germline mutations in the retinoblastoma gene RB1 that predispose to develop retinoblastoma. These mutations can be detectable in blood DNA using standard techniques. However, these techniques are not sensitive enough to detect mosaicism in sporadic cases of the disease (without family history). This study was thus designed to determine the frequency of mosaicism in patients with sporadic retinoblastoma by using next generation sequencing (NGS) technologies.
Methods: We selected for this study 22 patients with sporadic retinoblastoma in which we had previously identified two inactivating mutations in the RB1 gene in tumor DNA, but in which none of those mutations were detected in blood DNA using Sanger sequencing. Blood DNA of these patients was analyzed by NGS (Roche/454) in order to identify in low proportion

(mosaicism) one of the mutations identified in the tumor.
Results: Roche/454 sequencing was able to detect mutations present at <1% in blood DNA. In our series, NGS was able to detect, in 5 out of 22 patients (22.7%), one of the mutation identified in the tumor in the blood DNA. The frequency of the mutations in blood DNA ranged between 0.8 and 16.8%.
Conclusion: Roche/454 sequencing is very sensitive allowing the identification of mosaicism in patients with sporadic retinoblastoma. The existence of mosaicism can modify the risk of retinoblastoma and the probability of transmitting the mutation to offspring. Therefore, the identification of mosaicism in these patients improves genetic diagnosis.

P12.126-M
BRCA1 and TP53 germline mutations are associated with gynecologic sarcomas

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Introduction: gynecologic sarcomas comprise less than 1% of all gynecologic malignancies and represent very heterogeneous group. Very little is known about etiology of these malignancies, the only documented etiologic factor in less then 25% of these tumors is previous pelvic irradiation and some have been linked to tamoxifen treatment. We report that BRCA1 & TP53 germline mutations are associated with gynecologic sarcomas.
Methods:
During the last ten years 16 patients diagnosed with gynecologic sarcoma were seen at Genetic Counseling Unit at Cancer Center & Institute of Oncology in Warsaw. All the patients were offered genetic testing, after genetic counseling and after obtaining the written informed consent. The consent protocol was accepted by the local ethical committee. BRCA1 analysis was performed on DNA from the peripheral blood leukocytes. The mutations in BRCA1 were detected using DHPLC and sequencing of exons 2, 5, 11 and 20, where the Polish founder mutations are found most frequently. TP53 gene was sequenced from exon 2 to 10.
Results:
Among 16 patients with gynecologic sarcomas, we found 6 (37%) BRCA1 germline mutation carriers.
We have found only one TP53 mutation carrier, who had been diagnosed with vulvar angiosarcoma at exceptionally young age.
Conclusions:
In women diagnosed with gynecologic sarcoma screening for the germline mutations in the BRCA1 gene is important strategy. In pediatric patients gynecologic sarcoma should prompt Li-Fraumeni syndrome diagnosis.

Table 1

patient ID number	diagnosis	age at diagnosis (years)	mutation in BRCA1	mutation in TP53
DN 121	oviductal carcinosarcoma and serous G3 ovarian adenocarcinoma	67	yes	no
DN 310	carcinosarcoma sarcoma stromale uteri	54	yes	no
DN 1430	carcinosarcoma uteri, adenocarcinoma uteri	52	yes	no
DN 1659	vulvar sarcoma fusocellulare	75	yes	no
AN 245	vaginal carcinosarcoma and uterine adenocarcinoma G3 and uterine stromal sarcoma	60	yes	no
AN 1263	uterine leiomyosarcoma	45	yes	no
DN 5698	vulvar angiosarcoma	9	no	yes

P12.127-S
Glutathione S-transferase P1 gene in secondary Acute Myeloid Leukemia

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Interactions between genetic, epigenetic and genotoxic factors play a pivotal role in secondary acute myeloid leukemia (s-AML) development. The GSTP1 is a well-known housekeeping gene engaged in the detoxification of a variety of carcinogens. We hypothesized that genetic and epigenetic mechanisms resulting in reduction or inactivation of GSTP1 expression may be implicated in s-AML pathogenesis. Thus, we investigated the possible implication of the A313G germline polymorphism in s-AML development and/or its specific chromosomal abnormalities. Moreover, we studied the possible contribution of GSTP1 promoter hypermethylation in s-AML development and the methylation status in respect to patients' genotype. Concerning GSTP1 genotyping, a case-control study in 75 s-AML patients and 185 controls was performed by Real-Time PCR. The GSTP1 hypermethylation was studied by methylation-specific PCR in 40 of the above cases and 15 controls. The genotypic distribution between cases and controls revealed a statistically higher frequency of the variant genotypes (A/G, G/G) in s-AML compared to the controls (p=0.001). Allele frequency distribution analysis showed that s-AML patients exhibited an almost 2-fold increased risk of carrying at least one variant G allele compared to the controls. Stratification of patients according to the karyotype revealed a significantly increased frequency of A/G heterozygotes in patients carrying -7/del(7q) (89.5%). The GSTP1 promoter was hypermethylated in a large proportion of s-AML patients (32.5%), but in none of the controls. No statistically significant associations were found between the methylation status and GSTP1 genotype. Our findings provide evidence for an important role of the GSTP1 gene in AML pathogenesis.

P12.128-M
TP53 gene mutation analysis in breast cancer: Our experience of six years

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Hereditary breast cancers account for 5-10 % cases and are predominantly due to BRCA1/2 genes and less commonly due to other high penetrant (TP53, STK11, PTEN) and less penetrant (CHEK2,ATM,PALB2, BRIP1) genes. Breast cancer is an important component of Li-Fraumeni Syndrome (LFS) cancer spectrum related to TP53 gene mutation. Genetic testing for BRC1/2 is generally not offered in young breast cancer women (<30 years) alone unless they meet the testing criterion (Manchester score etc). However TP53 testing is suggested for young breast cancer alone cases without family history of LFs tumours
We analysed our data of past 6 years (2008-2013) of TP53 gene testing in women with breast cancer. Information was obtained regarding family history of cancers, age of onset, receptor pathology and BRCA1/2 testing. No TP53 mutation was found in our breast cancer alone cohort. The solitary TP53 mutation was detected in a woman with family history suggestive of LFS. The TP53 gene mutation detection rate in our cohort was only 4.5 %. (1/22).
Although our sample size is small TP53 gene mutation rate is likely to be better in breast cancer cases with LFS family history and may be influenced by receptor pathology as published previously. Genetic centres with limited resources need to consider these factors before requesting expensive send away genetic tests. Availability of affordable NGS panel testing for breast cancer genes may address this issue in future and would also help in understanding the contribution of the above mentioned genes to hereditary breast cancer.

P12.129-S
Expression analysis of TNF related apoptosis inducing ligand and its receptors in gastrointestinal tumors

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TRAIL (TNF related apoptosis inducing ligand) is a member of the tumor necrosis factor superfamily. Due to its ability to selectively induce apoptotic death in transformed cells, TRAIL pathway has been considered as a promising drug target for cancer therapy. Our purpose was to examine TRAIL pathway components expression in gastrointestinal tumors.
mRNA relative expression levels in 45 colon, 11 esophageal, and 11 gastric cancers (37 RCL2-fixed, 30 fresh frozen,43 male and 24 female, median age 67 years) along with matching normal tissues were analysed using RT-PCR for TRAIL pathway genes, namely TRAIL, DR4, DR5, DcR1, DcR2, OPG. Colon cancer samples displayed elevated mRNA levels in 20% (9 of 45), 42% (19 of 45), 47% (21of 45), 64 % (29 of 45), 44% (20 of 45), 31% (14 of 45) of the cases for TRAIL, DR4, DR5, DcR1, DcR2, OPG genes respectively. Furthermore, TRAIL receptors were found simultaneously overexpressed in a subset of colon tumors. TRAIL overexpression was correlated with low stage tumors (p=0.03) in our cohort. Esophageal cancers overexpressed TRAIL and its receptors DR4, DR5, DcR1, DcR2 at 18%, 54.5%, 27%, 45.5% and 45.5% of the cases, respectively. Finally, in gastric cancer samples overexpression was found in 45% of the cases for Dcr2 and 36% for DR4/5, DcR1 and TRAIL.
TRAIL receptor mRNA levels were found elevated in a significant percentage of gastrointestinal tumors. In addition, TRAIL expression emerged as an early event, which may delay disease progression.

P12.130-M
Discordant molecular breakpoints in an apparent recurrent translocation (3;12)(q13;p13)

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Background: The identification of recurrent translocations in neoplasia has been used to identify the genes involved in the neoplastic process in order to develop new therapies.The objective of our study was to determine if the breakpoints determined by conventional cytogenetics in two apparently recurrent translocations (3;12)(q13;p13) were the same at the molecular level. Material and Methods. Two patients with myelodysplastic syndrome had a complex karyotype including a translocation (3;12)(q13;p13). In addition to the translocation, both karyotypes shared a deletion of chromosome 5 and a trisomy of the chromosome 8. A set of 35 Bacterial Artificial Chromosomes (BACs) were used to cover the 3p11.1-q13.32 region and a set of 12 BACs were used to cover the 12 p11.21-p13.31 region.Results. The breakpoints were precisely established only for the translocation t(3;12)(q13;p13) in patient 1, within the BAC RP11-491D12 at the 3p11.2 region and within the BAC RP11-705C15 at the 12p13.31 region. A region of 22.458 kbp was deleted including the 12p13.2 - 12p11.21 region. In patient 2 the breakpoints on chromosomes 3 and 12 were not found, but based on the BACs study they should be more centromeric than the breakpoints determined in patient 1. A deleted region of 23.163,9 kbp including the 12p13.2 - 12p11.21 region was found in patient 2. Conclusion: BACs studies revealed that the breakpoints in two apparently recurrent translocations were different at the molecular level. Both translocations showed a deletion of 22.458 kbp - 23.163,9 kbp near the breakpoint, including the 12p13.2 - 12p11.21 region.

P12.131-M
Prevalence of BRCA1 and BRCA2 germline mutations in triple-negative breast cancer

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Triple-negative breast cancer (TNBC) is a subtype of breast cancer that lacks ER, PR and HER2 expression. TNBCs have a gene expression profile similar to breast tumours BRCA mutations. However, despite this correlation, there have not been many studies showing the prevalence of BRCA mutations in TNBC patients. It has been proposed that women with TNBCs may be candidates for genetic screening due to their shared phenotype with breast tumours of BRCA mutation carriers.
The aim of this study was to investigate the prevalence of germline BRCA mutations in TNBC cases unselected for family history of the disease. This knowledge could aid in the selection of women who could be candidates for genetic testing.
The study cohort comprised of 347 TNBC patients. BRCA genes were amplified by Fluidigm Access Array. The amplicons were sequenced on Illumina MiSeq. The sequencing data were analysed with NextGENe 2nd Generation Sequencing Software. MLPA was also performed and the peaks heights were analysed on GeneMarker® software.
39 (11.2%) pathogenic mutations were detected in our cohort of 347 TNBC patients. 22 of these patients (56.4%) did not have a recorded family histories of breast and/or ovarian cancers.
Our study shows that in a large cohort of TNBC patients unselected for family history of breast cancer, approximately 11.2% of the patients harbour a BRCA mutation. This suggests that there may be an underestimation of BRCA mutations in a breast cancer population using current screening criteria, and TNBCs should be considered as an inclusion criterion for genetic testing.

P12.132-M
Align-GVGD, SIFT, PolyPhen, MAPP-MMR, Grantham Analysis and Condel are weak predictors of the clinical significance for missense variants

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Non-synonymous missense changes that result in amino acid substitutions represent the majority of variants of uncertain clinical significance (VUSs) identified by genetic testing. We sought to analyze whether commonly used in silico tools, which assess the phylogenetic conservation of specific amino acids throughout evolution, can accurately characterize the possible disease association of missense mutations. We compared the accuracy of six commonly used algorithms (Align-GVGD, SIFT, PolyPhen-2, MAPP-MMR, SIFT, Grantham Analysis and Condel) using a dataset of 1,118 BRCA1, BRCA2, MLH1 and MSH2 variants previously classified as clinically deleterious or benign by our laboratory's variant classification program. For all algorithms (except Align-GVGD), the false-positive (FP) rate compared to our laboratory's variant classification program was higher than traditionally accepted thresholds for clinical confidence, with a range from 30.6% - 58.5% for BRCA1, 27.1% - 40.1% for BRCA2, 17.9% - 67.9% for MLH1 and 17.1% - 56.1% for MSH2. Although the FP rates using Align-GVGD for all four genes were lower, including values of 2.2% for BRCA1 and 7.9% for BRCA2, the sample size was too small to provide a robust analysis. The high FP rates for Condel, which classifies variants based on a weighted average of scores from five in silico tools, suggests that the use of multiple models is not significantly more accurate than any of the individual models in isolation. The results of our study suggest that none of the commonly used in silico tools achieve the traditionally accepted minimum threshold of specificity for the clinical use of predictive tools.

P12.133-S
Comparison between splicing reporter minigene assays and patient blood RNA analyses used for the assessment of splicing defects caused by variants in the DNA mismatch repair genes

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A fraction of sequence variants found in disease-causing genes induce aberrant splicing. At the moment, reliable splice-prediction tools are only available for variants in the consensus splice site regions, and even these cannot predict the exact molecular nature of the aberrant transcription. Wet-lab splicing assays, either RT-PCR analyses of patient RNA or functional splicing reporter minigene tests, have to be performed to confirm aberrant splicing. Here, we present results of splicing reporter minigene assays performed for 37 disease-gene variants of unknown significance (VUS), mainly found in the four DNA mismatch-repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2* that are associated with Lynch syndrome. Twenty variants are located in the consensus splice site regions, 13 are exonic and 4 are deep-intronic variants. We used a previously described minigene vector, and transfected HEK293 and/or Hela cells with wildtype and variant constructs. For 32 variants also results from patient RNA analyses were available, either performed by our laboratory or presented in literature. For comparison with minigene assay splicing data, we especially included variants that showed multiple aberrant transcripts in patient RNA analysis, or another splice effect than the prevalent exon skip. We found 100% concordance between patient RNA analyses and minigene assays in terms of showing an effect on splicing or not. However, for 6 variants discrepancies in the molecular nature of aberrant transcription were observed. Possible explanations for these discrepancies, and implications for the assessment of pathogenicity of the variant are discussed.

P12.134-M
Genetic risk factors in a vulvar cancer cluster among young Indigenous women in Arnhem Land, Australia

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Vulvar cancer is usually rare, and occurs most often in postmenopausal women. Among young (<50 years) Indigenous women living in remote Aboriginal communities in Arnhem Land, however, the incidence of this malignancy is more than 70 times the national Australian rate for the same age group. Previously, we found that neither excess human papillomavirus (HPV) incidence nor a particularly virulent strain of HPV could explain the very high incidence of vulvar cancer in this population. Reports from the Gynaecology Outreach Service that cases appeared to cluster in family groups suggested that a genetic susceptibility, either to the effects of HPV or another cause of vulvar cancer, may be involved in this cluster. To investigate the role of genetic risk factors, 30 cases and 61 controls, matched on age and community of residence, were recruited to the study. DNA was extracted from saliva samples, and genotyped to provide information on approximately 2.5 million variants. These data were analysed using both genome-wide association and identity-by-descent techniques. We found clear evidence for the involvement of a genetic risk factor predisposing this population to vulvar cancer, and identified three genomic regions of interest. Bioinformatic analysis prioritised biologically plausible candidate genes within these regions and functional studies to further elucidate the role of genetic variants in the aetiology of vulvar cancer are currently underway. This is the first genetic

study of this population, and these findings continue to inform health care delivery in Arnhem Land, especially vaccination policy and screening strategies.

P12.135-S
Whole exome sequencing identifies novel mutations from DNA repair pathways in familial esophageal squamous cell carcinoma

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Introduction: Esophageal cancer (EC) is the fifth leading cause of cancer death in Iran and esophageal squamous cell carcinoma (ESCC) is an aggressive subtype comprises 95% of all Iranian EC cases. High existence of familial aggregation among ESCC cases necessitates the identification of new germline mutations for the purpose of surveillance and personalized medicine. **Methods:** We analyzed the mutation spectra from 100X whole exome sequencing (WES) of peripheral blood mononuclear cell's DNA extracted from 10 affected probands of familial ESCC using SureSelect target enrichment system capture process (Agilent, U.S). All family members directly sequenced for confirmation of targeted mutations in patients.
Result: We identified 9 mutated genes from DNA repair pathways including ATM, BRCA1, SLX4, FANCA, FANCB, FANCE, RAD51AP1, RAD51AP2, RECQL5 with the allele frequencies below 0.01 according to 1000 Genome project (Oct 2011). Novel non-synonymous substitution found in FANCE causing glutamate to aspartate amino acid change. All mutations identified as damaging variants using SIFT and PolyPhen softwares. **Conclusion:** The use of WES identified that familial cases of ESCC harbor mutations from DNA repair, especially homologous recombination and Fanconi anemia pathways. These findings have potential implications on the surveillance and treatment of ESCC patients. Particularly, these patients may benefit from treatment with DNA cross-linking chemotherapeutic drugs, such as cis-platin and mitomycin C, or from a PARP [poly (ADP-ribose) polymerase] inhibitor. Additionally, genetic consoling and mutational analysis of the proband's relatives will considerably benefit these individuals for their surveillance and early diagnosis leading to an improved disease management.

P12.136-M
Whole transcriptome analysis of testicular germ cell tumors

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Next generation sequencing of the whole transcriptome enables high resolution measurement of gene expression activity in different tissue and cell types. This methodology provides an in depth study of known transcripts and depending on the data analysis, allows identification of additional transcript types such as transcript variants, fusion transcripts, and small and long ncRNAs. In this study we performed RNA-Seq using the Ion Torrent Proton platform to compare the expression profile of testicular germ cell tumors (seminoma type, n=3) and normal testis (n=3). Using Partek Flow and Star or TopHat aligners, we aligned the reads to the human genome and mapped sequences to the RefSeq database. We identified a large number of genes that were up and down regulated with high degree of significance p<0.01, >2X FC). These included genes related to testicular tissue type, stem cell pleuripotency (NANOG; POU5F1) and proliferation (KRAS, CCND2). In addition, a number of differentially expressed noncoding RNAs were identified (SNORD12B, XIST). The method was validated on a small set of genes (>20) using qPCR (TaqMan Assays). We used the Open Array platform to quantitatively screen a larger number of differentially expressed genes (224) across a number of different testicular germ cell tumor types (non-seminoma).

P12.137-S
Upregulation of key wnt signaling molecules in human astrocytic brain tumors

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The knowledge on molecular profiles of astrocytic brain tumors still needs elucidation. In the present study key players of wnt signaling, beta-catenin (CTNNB1), TCF1 and LEF1, adenomatous polyposis coli (APC) and axin (AXIN1) were investigated in the set of human astrocytic brain tumors.
The investigation of beta-catenin demonstrated 10% of samples with potential activating mutations. The results on protein levels demonstrated that 50% of glioblastomas (WHO grade IV) and 56% of astrocytomas (WHO grades II and III) showed upregulation of beta-catenin and nuclear localization was found in 52.1% of glioblastomas. Transcription factors of the wnt pathway were also upregulated. Strong TCF1 and LEF1 expression was observed in 51.6% and 71% of glioblastomas. Analysis of variances performed on the total sample indicated significant differences in the values of TCF1 weak expression (F=2.804; p=0.045), LEF1 weak (F=4.255; p=0.008) and LEF1 strong expression (F=5.498; p=0.002) with regard to malignancy grade. The F-ratios for two variables (LEF1 strong and LEF1 weak) indicated that differences between astrocytomas (II, III) and glioblastomas were statistically significant (p<0.02).
Allelic losses of APC gene were frequent with glioblastomas showing 60% and diffuse astocytomas (grade II) 20%. Allelic losses of AXIN1 were found in 10% of glioblastomas. In 31% of glioblastomas and 22% of astrocytomas downregulation of axin proteins was detected. In 31% of glioblastomas axin was localized in the nucleus.
Our findings contribute to understanding of human astrocytic brain tumor genetic profile and suggest that molecular changes of wnt signaling play important roles in astrocytic tumor etiology.

P12.138-M
Exome sequencing and deep sequencing reveals „mutational storm" in xeroderma pigmentosum (XP) patients

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Xeroderma pigmentosum (XP) is a rare genetic disorder with molecular defects in the DNA repair machinery. Clinically, patients suffer from increased UV-sensitivity and a skin cancer risk that is increased more than 1000-fold. It has been postulated that due to the repair defect patients show a mutator phenotype with indicative UV-signature mutations. We have sequenced exomes from sun-exposed and sun-protected tissues from three patients with XPC. Moreover, two long-range amplicons covering the whole mitochondrial genome and a comparable nuclear DNA fragment have been sequenced at >1.000 x coverage. These three XP-C patients have been compared to young and old healthy controls. The bioinformatics analysis was tailored towards detection of exposition-specific variants with high sensitivity. A representative subset of identified exposure-specific variants has been validated by a subsequent deep-sequencing approach. The number of exposure-specific variants was very high in the XP-C patients (610, 2.088, and 6528, respectively compared to 95%), the third XPC patient had relatively low numbers of exposure-specific variants and no predominance for UV signatures (~75% in 610 variants). Interestingly, all three samples displayed increase variation in the nuclear DNA but not the mitochondrial DNA as expressed by the count of non-consensus bases in amplicon deep sequencing. As expected, XPC hypermutation arises in the nuclear DNA predominantly with typical signature mutations. Interestingly, not all XPC patients seem to share the signature phenotype although the hypermutation is a common feature. In contrast, mitochondrial DNA does not show any differences as compared with young and old controls.

P12.139-S
YAP1 acts as oncogenic target of 11q22 amplification in multiple cancer subtypes.

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The transcriptional coactivator YAP1 is a critical effector of the human Salvador-Warts-Hippo-pathway. Literature data report apparently discrepant results on the carcinogenic role of YAP1, which acts as oncogene or tumor suppressor in different in-vitro and in-vivo models. Furthermore, amplification events of 11q22 locus-encompassing YAP1 gene-have been detected in multiple tumor types but there is limited direct evidence about the oncogenic role of endogenous YAP1 within in the amplicon.
We screened a panel of human tumor samples and cancer cell lines and identified that the YAP1 amplification event is actually present in up to 23% of the cases. We exploited EKVX, CaSki and RO82 cell lines harboring both genomic YAP1 amplification and YAP1 protein overexpression, in order to study the effects of downregulation of endogenous YAP1 by RNA-interference strategies. Gene expression profiling data identified 707 statistically significantly modulated genes (p-value =0.002) that were functionally annotated for cell proliferation and cellular movement ontologies. Mechanistic studies of the identified perturbed pathways revealed that YAP1 silencing

significantly decreased cell proliferation and cell cycle perturbation associated with upregulation of p21 and p27 cell-cycle inhibitors, reduced cell migration (p<0.048) and anchorage-independent growth (p<0.02). In CaSki cell line, YAP1 silencing induced significantly increased sensitivity and cell-death response to cisplatin treatment (p=0.011) as well as reduction of in-vivo tumorigenic potential (p=0.027).
Overall, these results establish that YAP1 is a direct oncogenic target of the 11q22 amplicon in previously unreported cancer types and support the relevance of such genetic aberration in carcinogenesis in a fraction of multiple tumor types.

P12.140-M
No change in the rate of bilateral mammographies after BRCA1/2 testing among true non-carriers

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The majority of women who are true non-carriers of the BRCA1/2 familial mutation may be reassured that they are no longer considered at high risk for breast and ovarian cancer. For this reason, most should be encouraged to adopt the same cancer screening practices as those recommended to women of the same age in the general population. The aim of this study is to compare the rate of bilateral mammographies after BRCA1/2 testing to that prior among true non-carriers of BRCA1/2 mutation. Information from the Quebec Health Insurance Board was used to identify all registered bilateral mammographies done between May 1, 1998 and March 31, 2012 among a cohort of 143 French Canadian unaffected true non-carriers. The Cox proportional hazards model for repeated events, with women's age as the time scale, was used to obtain hazard ratios of bilateral mammographies. The rate of mammographies did not change after BRCA1/2 testing, neither globally (HR=0.93, p=0.22), nor by age (<50 years HR=0.81, p=0.13; ≥50 years HR=1.01, p=0.84). Although women <50 years had a lower rate of mammographies than women ≥50 years (HR=0.55; 95% CI = 0.43-0.70) after genetic testing, 74% still continued to be screened, which is not generally recommended to women of the same age group in the general population. In conclusion, genetic testing information did not have a significant effect on mammography screening in our cohort of true non-carriers of BRCA1/2 mutation. Clear-cut recommendations for the follow-up of true non-carriers of BRCA1/2 mutation are needed.

P12.141-S
EGFR and ALK genes mutation screening in Non-small-cell lung carcinoma (NSCLC) specimens

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BACKGROUND: Testing for genetic abnormalities in epithelial growth factor receptor (EGFR) and anaplastic lymphoma receptor tyrosine kinase (ALK) is a critical tool in the care of advanced NSCLC. We investigated the incidence of epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangements in Lithuanian patients with non-small cell lung cancer (NSCLC). MATERIALS AND METHODS: 326 NSCLC paraffin-embedded, formalin-fixed (FFPE) specimens were collected, DNA extracted, and using real time mutations analysis in EGFR gene and translocation in AKL gene by IHC following confirmation positive test by FISH analysis. RESULTS: We screened 326 consecutive patients with NSCLC for the presence of concomitant EGFR mutations and ALK rearrangements Mutations in EGFR gene appeared mutated in 17%; ALK translocation was found in 4% of NSCLC cases.

P12.142-M
miR-106b-5p may act as tumor suppressor by down-regulation C1orf24 expression in human thyroid carcinoma.

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We have previously shown that the C1orf24 gene is highly expressed in follicular thyroid carcinomas while is not expressed in benign thyroid lesions. However, little is known about the molecular mechanism involved in C1orf24 expression. It is widely demonstrated that microRNAs (miRs) are potent regulators of gene expression. The miRs expression varies in according of tissue, development stage, and tumor types. We therefore, investigated whether miRs could modulate C1orf24 expression in thyroid cancer. In this study, we show that the miR-106b expression is lower in thyroid carcinomas, than in benign thyroid lesions (p<0,01). Functional analysis was performed in follicular thyroid carcinoma cell line (WRO), which highly express C1orf24 gene. The ectopic expression of miR106b into WRO resulted in down-regulation of C1orf24 at both mRNA and protein levels, when compared to negative control. Mutations made at miR-106-5p binding sites in the C1orf24 mRNA 3' UTR showed that miR-106b directly interacts with C1orf24. Additionally, miR-106b overexpression significantly decreased the migration capabilities of cells whilst increased apoptosis rate. Our findings indicate that miR-106-5p may play a role in thyroid carcinogenesis by negatively regulating C1orf24 expression and, therefore, by acting as tumor suppressor. However, further analyses are needed to fully demonstrate the role of miR-106b-5p in thyroid carcinogenesis.
Financial Support: FAPESP (2012/02902-9) and CNPq (470441/2013-5)

P12.143-S
SOX2 gene expression and copy number variation in squamous cell lung cancer

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Transcription factor SOX2 might have an important oncogenic role in development and progression of lung cancer. The aim of our study was to estimate copy number variation (CNV) of SOX2 in primary tumours and to analyse SOX2 expression in primary tumour tissue and in blood samples in relation to CNV status in squamous cell lung cancer patients. 28 patients with metastatic squamous cell lung cancer were prospectively included between years 2010 and 2013. Total RNA was isolated from whole blood collected in PAXgene Blood RNA Tube before any systemic treatment. For 10 patients primary tumour tissue was available from the same time points; for those patients RNA and DNA were extracted from FFPE tumours. SOX2 expression levels and CNV status were determined by quantitative RT-PCR. Copy number analysis revealed high copy number of SOX2 in 3/10 (30%) tumours and gain of function in another 3/10 (30%) tumours. Median SOX2 expression in FFPE tumour tissue was 3.9 (0.1-29.1) and in blood samples 5.4 (0.4-17.9). Elevated copy number of SOX2 correlated with higher SOX2 expression in tumours (ρ=0.92). Moreover, correlation between SOX2 expression in blood samples and FFPE tumour tissue was observed (ρ =0.60); patients with high SOX2 expression in blood tended to have high SOX2 expression in tumour tissue. According to our observation genetic alterations in SOX2 seem to be common event in squamous cell lung cancer. Furthermore, good correlation between SOX2 expression in blood and tumour samples supports the idea of liquid biopsy in lung cancer.

P13.01-S
A French series of 731 patients with 22q11.2 microdeletion

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The phenotype of the 22q11.2 deletion syndrome (22q11DS) is higly variable with a wide spectrum of abnormalities. About 90% of patients have a 3Mb deletion spanning LCR22-A to LCR22-D in the 22q11 region containing four low copy repeats (LCR22).
Methods : On behalf of the ACLF, 27 French Cytogenetics laboratories collaborated to collect data of 731 patients with 22q11DS diagnosed between 1996 to 2013.
Results :
Clinical data: On average over the years 2010-2012, at least 70 patients were annually diagnosed postnatally. The symptomatology that led to genetic analysis changes with patient's age, eg :
Heart defect, facial dysmorphism, hypocalcemia and thymus hypoplasia or agenesis in newborns
Developmental and speech delay with facial dysmorphism and velopharyngeal insufficiency in children

Variable phenotype in adults : some had intellectual disability with typical gestalt, some had psychiatric disorders, and a minority was almost asymptomatic parents when the diagnosis was first made in their children.
Molecular data: Thirteen cases were diagnosed with array-CGH. The majority of these patients were referred for intellectual disability (n=10/13) and only 3 had heart defect. The size of the deletion was variable: 745 - 2904 kb and surprisingly only 46,2% had deletion between LCR22-A and LCR22-D. The 22q11 deletion was inherited in 22.2% of cases and mainly maternally (86.7%).
Conclusion : We report the largest series of 22q11DS postnatal diagnosis. We plan to study more patients using array-CGH to investigate whether the size of the deletion or the presence of other CNV may explain the phenotype variability.

P13.02-M
Transgenerational inheritance of 22q11.2 atypical deletion: instability of LCRs in a family with discordant clinical phenotype

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Genomic rearrangements in 22q11.2 region are caused mainly by LCRs elements or repetitive sequences, leading to different clinical phenotypes including velocardiofacial or DiGeorge syndrome. Most of the deletions encompass the ~3 (LCR A and D) or ~1.5 Mb (LCR A and B), however atypical deletions have been described in a few cases. Patients with genomic alterations in 22q11.2 region present a spectrum of phenotypic manifestations and this condition can be more severe compared with the relatives. We report on a family, including the maternal grandfather, the mother and daughter, with discordant clinical phenotype and 22q11.2 atypical deletion using SNP-array (Illumina 850K) in order to better delineate the size of deletion. In three generations we found a difference in the deletion size in the 22q11.2 region, range of approximately 0.1 Kb to 0.5 Kb, encompassing the A and B LCRs for mother and daughter, and also the A and C LCRs in the grandfather.
Although there are rare, atypical variant deletion endpoints could provides important insights related to the role of genomic architecture in chromosomal rearrangements, chromosome evolution, and in human disease.

P13.03-S
A catalog of hemizygous variation in 127 22q11 deletion patients

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The 22q11.2 deletion syndrome (22q11.2DS) is the most common chromosomal deletion syndrome in humans with an incidence of 1 in 2-4000 live births. The clinical presentation of 22q11.2DS is extremely variable, but the underlying reason for this variation remains unknown. Individuals with 22q11.2DS most often have a classically associated 3 MB or 1.5 MB deletion. However, nested deletions as well as atypical deletions also occur that can contribute to the broad spectrum of phenotypic abnormalities. It is also hypothesized that variations in the remaining allele could underly the phenotypic variability.
To investigate variation within the non-deleted allele we performed targeted resequencing of the 22q11.2 region for 127 patients, identifying multiple deletion sizes, including two atypical deletions. We cataloged over 18 thousand hemizygous variant positions, of which sixty percent were previously annotated. As expected, in this gene dense region more variants were intronic (52%) than intergenic (36%). Within the coding regions we identified 213 non-synonymous variants, 6 stop gains, and 5 frameshift insertions. In addition, the observed number of variants per gene was higher or lower than expected for some genes in both our data and 1000 genomes data, indicating some genes may tolerate variation more or less than others. This extensive catalog of hemizygous varaints will serve as a blueprint for future experiments to correlate 22q11DS variation with phenotype and serve as an analysis model as we extend to whole genome sequencing of 22q11DS patients.

P13.04-M
New splicing mutation in SERPINA1 gene causing severe alpha-1 antitrypsin deficiency

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Alpha1-antitrypsin (AAT) deficiency is a common hereditary disorder associated with reduced AAT serum level, predisposing to pulmonary emphysema or liver disease. It is caused by inheritance of mutations in the AAT gene (SERPINA1). Most of the deficiency alleles occur in the coding sequence of the gene due to aminoacid substitution or deletion resulting in reduced protein level or altered functionality. Rarely mutations affecting RNA splicing in the AAT gene have been described so far.
We have identified a new null allele, QOmadrid in two siblings with significantly reduced serum levels of AAT. QOmadrid allele results from a duplication of a timine in the position +2 of the donor splice site of exon 1C (+2dupT). In these patients QOmadrid occurred in combination with another previously described rare null variant, QOporto. These two variants correspond to splicing mutations in a regulatory region of the gene, both causing disruption of the normal splicing of intron 1C. Analysis of transcripts in patients' samples and in vitro assays using minigenes revealed abnormal splicing leading to absence of transcription from exon 1C, where the hepatocytes transcription start site is located. Thus, in these patients no normally spliced RNA products are expected to be produced in the liver, causing the AAT deficiency. This mutation constitutes a new null allele, QOmadrid, contributing to explain the disease.

P13.05-S
Upstream open reading frames regulate cannabinoid receptor 1 expression under baseline conditions and during cellular stress

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The endocannabinoid system (ECS) plays a crucial role in the regulation of a variety of physiological functions, such as learning and memory processing, vegetative control, energy homeostasis, immunity and stress response. It acts through different endogenous endocannabinoids which are able to bind to the cannabinoid receptor subtypes 1 and 2 (CNR1 and CNR2). The CNR1 is not only associated with phenotypes such as cognitive performance, addiction and anxiety, but is also known to be crucially involved in cellular responses to acute and chronic stress conditions. The molecular mechanisms leading to altered CNR1 expression under acute or chronic stress exposure are not completely understood so far. It is known that the 5'- and 3'untranslated regions (UTRs) of genes can harbor regulatory elements, such as upstream open reading frames (uORFs) that are capable of influencing the expression pattern of the main protein coding region.
In our study, we investigated the influence of putatively functional uORFs present in the five known mRNA variants of the human CNR1 gene on transcription and translation under baseline conditions and various stress conditions in vitro. The functional analysis performed with reporter gene assay and quantitative realtime PCR revealed that two of these variants contain upstream open reading frames that modulate gene expression both under baseline condition and conditions of cellular stress. Thus our findings suggest that the functionally relevant uORFs found in the 5'UTR variants of CNR1 are part of the cellular stress response mechanisms.

P13.06-M
Clinical, cytogenetic and molecular analyses in seven patients with a constitutive autosomal ring chromosome.

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Autosomal ring chromosomes have a frequency of 1/30,000 to 1/60,000

births. We report 7 patients identified from the cytogenetic registers of the HIMFG, who attended during the last 8 years. They corresponded to r(4)(p16q35), r(5)(p15.3q35.3), r(9)(q24.3q34.3), r(14)(p11?q32.33), r(19)(p13.3q13.4), r(21)(p11q22.3) and idic r(21)(p11.2q22.3) (cases1-7, respectively). Additional chromosomal analyses with GTG banding were performed in all cases, FISH with subtelomeric probes were applied to 4 cases and molecular karyotyping with SNP array were carried out in 2. As cases 2 and 5 are mosaic with a normal cell line, they should have been originated postzygotically. A dynamic mosaicism was present in all the cases. All the rings were originated by breaks on both arms with loss of subtelomeric material and subsequent reunion of free ends with the exception of cases 2 and 5. The r(14) was probably generated by an inv-dup-del mechanism as there are a deletion and a duplication of 14q. The large dicentric r(21) may have been formed by breakage and reunion of long arms of an isochromosome. The phenotypes described included short height, microcephaly, variable facial dysmorphism and psychomotor developmental delay, corresponding to autosomal ring syndrome. Some patients showed hirsutism, renal abnormalities and hypoacusia, data corresponding to monosomy of the specific chromosomal region implicated. Cases 1 and 7 presented characteristics of Wolf-Hirschhorn and Down syndromes, respectively. Patients 2 and 4 had difficult control seizures. We consider that the variable clinical data reported in these patients are due to the specific chromosomal region involved and to the ring chromosome instability.

P13.07-S

A new translocation involving 4 and 11 chromosomes in a adult man

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Chromosomal translocations occurring between the chromosome 11 (region q12) and other chromosomes are the most recurrent chromosomal aberrations observed in some subtypes of leukemia, lymphoma and sarcomas. In many cases, identification of these chromosome abnormalities is crucial to select appropriate treatment protocols.
To our knowledge we are the first group to describe a new translocation, involving 11q12 and 4q21 regions, in a 35 years old man.
The proband came to our observation to perform cytogenetic analysis because of a suspected infertility and we found 46,XY t (4;11) (q21;q12) karyotype. The man had a cortical dysplasia and seizures from birth.
We extended the cytogenetic analysis to the parents and sister of the proband and the same translocation was found only in the father.
It is known that some types of dysplasia are linked to the presence of genetic alteration but so far it has not been described any association between dysplasia and chromosomal aberration.
The region 11q12 is involved in many chromosomal translocations such as t(2;11) (q31; q12), t(11; 17) (q12; p11.2), and t(6; 11) (p21; q12) which are all related to tumor development . The common breakpoint region (11q12) has been delineated by FISH and contains the FOSL1 gene which has been associated with fibroblast growth or cancer development, such as colorectal, breast, prostate and lung cancer, head and neck squamous cell carcinoma. Since the region 11q12 seems to be linked to tumor development, would be appropriate to perform a follow-up to monitor the subject at risk.

P13.09-S

Stable segregation of apparently benign chromothripsis in three generations

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Chromothripsis (CTH) is a newly described phenomenon of chromosome shattering where multiple localized breakpoints result in catastrophic genomic rearrangements. CTH is found both as a somatic (cancer) and germline rearrangement (G-CTH). The limited G-CTH cases (n=16) described so far have all been associated with abnormal phenotype. In this study we present a familial G-CTH with 7 breakpoints and a deletion, stably segregating in three generations (10 individuals) without an apparent association with a disorder. Initial cytogenetic analysis suggested an apparently balanced translocation t(3;5)(q22.3;q23.1). Using chromosome array and next generation mate-pair sequencing followed by Sanger sequencing we discovered a G-CTH involving six breakpoints and a ~108kb deletion within a ~6,4Mb region on 3q22.3-q23. Although six protein-coding genes were affected by the breakpoints the 10 carriers of the G-CTH do apparently not have common associated disorders. However, there are several spontaneous miscarriages in the family records presumably resulting from unbalanced rearrangements due to G-CTH. Our study is the first report suggesting that G-CTH may not always be associated with an abnormal phenotype but may also have a neutral effect. It is therefore possible that G-CTH may be found not only among individuals with apparently balanced rearrangements but also karyotypically normal asymptomatic individuals. We describe CTH on the cytogenetic level based on recommendations of ISCN-2013 (International System for Human Cytogenetic Nomenclature) as: 46,XX,t(3;5)(q22.3;q23.1).arr ngs[hg19] 3q23.3(137735949-137845370)x1.ngs 3q22.3q23(135827611-142218722)cth fam. We suggest combining this with a molecular description following HGVS (Human Genome Variation Society) nomenclature (see ESHG abstract by Taschner P. et al.). *Shared first-authors

P13.10-M

A family-based genome-wide scan shows 10q25.2, LRFN2, TGFB2 and CRISPLD2 loci associated with cleft lip with or without cleft palate in a high-prevalence cluster in South America

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Background: In South America four cleft lip with or without cleft palate (CL±P) high prevalence regions was detected, one of them being the Patagonia (Argentina). We aim the identification of independent autosomal segments containing polymorphic markers that may contribute to CL±P with a family-based design for genome-wide association scan.
Methods: The study sample included 26 families with isolated CL±P (27 affected and 99 total individuals).They were genotyped on the Affymetrix Genome-Wide 6.0 array. Only „independent" SNPs were included in the association analysis. We calculated linkage disequilibrium (LD) between each pair of SNPs into a window of 50 SNPs, shifting the window 5 SNPs forward and repeating the procedure to scan all autosomes. Then we pruned the data removing one SNP of each pair that was in strong LD (r >0.8). We perform the transmission disequilibrium test (TDT). We identified segments of a maximum length of 250Kb with more than one SNP significantly associated with CL±P.
Results: A total of 88 genomic segments with two or more independent SNPs significantly associated with CL±P were identified. An intergenic region of 33Kb on 10q25.2 showed the most significant association with CL±P ($p< 0.00007$). Furthermore, we found other significant association with 6p21.2 including the marker rs4153154 ($p<0.00009$). Besides, others genomic segments including the genes TGFB2 (1.4Kb on 1q41) and CRISPDL2 (61.3Kb on 16q24.1) deserve our attention because they have previously been associated with oral cleft in others populations.
Conclusion: Our results suggest 10q25.2 and some genes spread across autosomes as candidate loci to CL±P.

P13.11-S

The FRA14B common fragile site maps to a region of frequent somatic and germ-line rearrangements within the GPHN gene

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Common fragile sites (cFS) are heritable chromosomal loci that exhibit non-random breaks on metaphase chromosomes in response to replication stress. Chromosome breakage at cFSs contributes to cancer genome evolution and de novo pathogenic germ-line alterations. Approximately 90 cFSs have been identified at cytogenetic band resolution, but just few of them have been molecularly characterized. Precise mapping of cFSs can reveal new rearrangement-prone candidate genes critical for the development of cancer and hereditary conditions.
We performed FRA14B mapping in cultured lymphocytes treated with a replication inhibitor, aphidicoline, using six-color FISH with contiguous BAC probes on metaphase chromosomes. FRA14B was restricted to a 765 kb region within 14q23.3, overlapping with a major part of the GPHN gene.

GPHN encodes a protein involved in molybdenum cofactor biosynthesis and clustering of postsynaptic neurotransmitter receptors. Computational analysis of the FRA14B sequence revealed a large hairpin-prone motif. Targeted oligonucleotide array CGH in 160 cancer cell lines and primary tumors detected 13 copy number alterations at FRA14B. Sequence analysis of cancer breakpoint junctions indicated an involvement of microhomology-mediated repair. In 2 cell lines, exonic deletions resulted in the expression of abberant GPHN transcripts. A survey of publicly available copy number profiles revealed that FRA14B is a hotspot of focal losses in cancer cell lines, loss-in-size rare CNVs, and rare and de-novo deletions in patients with neurodevelopmental diseases.
In summary, intrinsic chromosome instability at FRA14B may account for pathogenic germ-line and somatic GPHN alterations, providing new insight into the role of cFSs in cancer and neurological disorders.

P13.12-M
Signature of germline chromothripsis in a 14-break complex rearrangement associated with deletions at 7q33-q35/11p13 and CNTNAP2 gene disruption

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We report an apparently balanced complex chromosome rearrangement (CCR) t(7;10)(q35;p12.1)ins(7;11)(q35;p14.1p12) associated with intellectual disability, developmental delay, absent speech and aniridia. The breakpoints were mapped by FISH. The *CNTNAP2* gene was truncated by the breakpoint at 7q35 (chr7:146,808,760-146,922,794; hg 19). The breakpoint interval at 10p12.1 (chr10:27,897,058-28,081,590) contains the *MKX* gene. The distal insertion breakpoint interval at 11p14.1 includes the *BDNF* gene (chr11:27,718,086-27,929,856), whilst the proximal breakpoint was mapped to a segment devoid of genes at 11p12 (chr11:37,068,735-38,957,367). Array-CGH (180K; Agilent) detected four deletions of 2.3Mb, 1.6Mb, 724Kb and 610kb at 7q33-q35 (chr7:135,994,754-138,315,026; chr7:138,522,186-140,134,404; chr7:140,258,006-140,982,607; chr7:144,738,971-145-,349,495), encompassing 28 genes. The inserted fragment of chromosome 11 contains a 2.9Mb deletion (chr11:28,964,028-31,926,058), spanning *PAX6* and other nine genes. To characterize this CCR at the nucleotide level mate-paired sequencing is in progress. The der(7) nine breakpoints and der(11) four breakpoints are, respectively, within 10.9Mb (7q33-q35) and 11.2Mb (11p14.1-p12) segments. Considering the breakpoint clustering and chromosome reorganization, we speculate that chromothripsis underlies this CCR formation. Twelve germline chromothripsis were previously reported, most of them balanced (Kloosterman et al. Curr Opin Cell Biol 2013;25:341). Our case illustrates that localized shattering of chromosomes and assembly of resulting fragments, signature of chromothripsis, may result in several fragment losses. Therefore the balanced state of most germline cases might not be a feature of constitutional chromothripsis, but reflect embryonic viability. Among the deleted/disrupted genes likely contributing to the patient's clinical features are *PAX6* explaining aniridia, and *CNTNAP2*, implicated in neurodevelopment, and particularly language abilities.
Financial support: FAPESP (2011/14293-4;2013/01146-9;2013/08028-1).

P13.13-S
Saturation of the human genome with chromosomal breakpoints

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Even in the era of exome and full genome sequencing, it will take decades and tremendous resources to saturate the human genome with mutations linked to abnormal and normal phenotypes.
As a supplement to exome and full genome sequencing strategies, we will use already identified balanced chromosomal rearrangements (BCR) to establish a first, detailed map of mutations covering a significant fraction of the human genome. In the first reexamination of *unselected de novo* BCRs (BCRdn) detected by 40 years of prenatal diagnosis in Denmark, we have shown that BCRs truncate protein coding genes, ncRNA genes, unannotated transcripts detected by deep sequencing, as well as developmental regulatory genomic landscapes, mimicing random mutagenesis. In addition, our study revealed a ~20% long-term disease-risk of unselected BCRdn, 2-3 fold higher than previously assumed, suggesting that a genotype-phenotype relationship can be obtained for a significant number of the human genes by mapping of BCRs.
We have initiated clinical reexamination/mapping of all known BCRs in Denmark. Based on a population of just 5.5 mill, this will provide data on >1.200 breakpoints. By international expansion, we will extend this at least 10-fold to reach a proposed first goal of ~10.000 breakpoints. Unlike other large scale genomic efforts, all countries including undeveloped and developing countries can participate. The breakpoint-map will identify and confirm numerous genotype-phenotype associations, saturate the regulatory landscapes around evo-devo genes that specify the vertebrate body plan, reveal novel genetic mechanisms, and define genomic regions which can be mutated without any direct phenotypic consequence.

P13.14-M
Insight into the mutational mechanism of the recurrent CREBBP c.3832G>A p.(Glu1278Lys) mutation in patients with Rubinstein-Taybi syndrome

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Rubinstein-Taybi syndrome (RTS) is a rare disorder affecting approximately 1/100,000 newborns. The syndrome is characterized by mental and growth retardation and a particular dysmorphology mainly concerning the face, hands and feet. The most frequent cause of RSTS are de novo mutations in the CREBBP gene, leading to haplo-insufficiency and found in 30-50% of patients. Furthermore, micro-deletions involving the CREBBP gene have been found in ~10% of patients.
Mutations are distributed throughout the gene and most mutations are unique. The c.3832G>A p.(Glu1278Lys) mutation in exon 21 is one of the most frequent pathogenic sequence variants in the CREBBP gene. We have identified this mutation in six independent patients out of a group of 101 RTS patients with a CREBBP mutation. Five of these patients were from the Netherlands, and another was from Spain. The molecular mechanism underlying most Guanine to Adenine substitutions is deamination of Cytosine to Uracil and the subsequent replacement of the complementary Guanine for an Adenine during DNA replication. However, recurring disease mutations in totally independent individuals at the same position within the gene suggests a shared mutation mechanism. By the systematic analysis of known DNA mutation mechanisms such as gene conversion, homologous recombination and DNA replication faults involving loop formation one may get insight in more general disease causing mutation mechanisms and find an explanation for recurrent mutations. This may have implications for counseling when hotspots are predictable based on knowledge about mechanisms leading to disease causing mutations.

P13.15-S
In vivo and *in silico* analyses of impact of the p.Val322Ala mutation on *CFTR* protein in a Brittany family

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Background: Cystic Fibrosis (CF) is an autosomal recessive severe genetic disease. Mutations on CFTR (Cystic Fibrosis Transmembrane conductance Regulator) gene could induce CF or CFTR-RD (related disorder). A missense mutation detected in a Brittany family (c.965T>C or p.Val322Ala) was studied since this mutation is present in a family owning a risk ¼ to have a child carrying this mutation and another severe mutation in trans (p.Phe508del). To provide a wise genetic counseling to this family, *in silico* and biological studies of the impact of the p.Val322Ala on CFTR protein were realized. **Aim:** This mutation was studied to elucidate its impact on CFTR process *in cellulo* (maturation and localization), and in silico (protein structure) as the prediction of the severity of this mutation is evaluated as "high" by Polyphen-2. **Methods:** pTCF plasmids containing CFTR-WT, or CFTR-p.Phe508del, or CFTR-p.Val322Ala are transfected in eukaryotic cells for western blot and confocal microscopy. Polyphen 2 and swiss prot were used to *in silico* studies. **Results:** The p.Val322Ala leads to a normal processing and correct membrane localization. Both mutated protein and the WT-CFTR structures are similar, suggesting that this mutation is a mild mutation. **Conclusions:** These results emphasise the importance of molecular studies in elucidating the impact of mutations on clinical phenotypes and therapeutic strategies.

P13.16-M
High frequency of chromosomal anomalies and a novel chromosomal insertion associated with infertility and recurrent miscarriages(Reproductive Failure) in west Turkey

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Numerical and/or structural chromosomal abnormalities may be a reason of high infertility rates and recurrent pregnancy losses (RPLs) in humans. Karyotype and karyogram profiles of patients with RPLs are presented in current results. A total of 722 patients; 161(44.5%) infertile and 200(55.5%) RPL couples were included in the study.
Karyotype and structural chromosome analyses of both patient groups in Canakkale population were made between May 2011-December 2013, using peripheral lymphocyte cell culture and GTG banding technique.
High frequency of chromosomal abnormalities(%7.45) were detected in 24 patients of the infertility group(n:322). 10 patients(42%) of this group(n:24) had numerical and 14 patients(58%) had balanced structural choromosomal abnormalities. A novel choromosomal insertion was found in an infertile male, one of the 22th choromosome was totally inserted in 9th choromosome [ins(9;22)(9pter-q12::22q11.1-q13.33::9q12-9qter)]. This is the first report of germline total inserion of a chromosome. Interestingly, this insertion was inherited from father. Balanced structural chromosomal abnormalities was also detected in 17 patients (4.25%) of RPL group without any numerical abnormalities.
Current results constitute the first report on the high incidence of structural chromosomal aberrations in RPLs and infertile couples in Canakkale district.

P13.17-S
Combination of molecular techniques in evaluation of de novo chromosome rearrangements involving terminal regions

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Structural aberrations make a significant contribution to genetic disease. Balanced or unbalanced structural abnormalities may be inherited from a carrier parent or may occur as de novo rearrangements. When the abnormality occurs as a de novo event, the risk for genetic disease or phenotypic effects is increased, even when the rearrangement appears balanced. This may result from either submicroscopic deletions or duplications at the breakpoints.
We report three cases with structural chromosomal abnormalities that were detected using a combination of different techniques. Conventional karyotype revealed the presence of de novo chromosomal rearrangements in all three cases. The investigation continued with targeted array CGH (Comparative Genomic Hybridization) technique, using BAC (Bacterial Artificial Chromosome) clones for the identification of chromosome imbalances. Since the results obtained using cytogenetic analysis and targeted array CGH revealed the implication of terminal regions of abnormal chromosomes, we also applied MLPA (Multiplex ligation-dependent probe amplification) for subtelomeric regions, as an additional detection technique. In two cases additional information regarding the chromosome constitution was provided by MLPA.
Knowing the advantages and limits of each technique, complementary investigations were necessary to detect the architecture of chromosomal imbalances.
Further investigations for a specific case, although it means additional costs, are required by a low genotype-phenotype correlation. Any discrepancy between the clinical picture and the results of genetic testing imposes supplementary tests for an accurate diagnosis and a proper genetic counseling.

P13.18-M
Somatic mosaicism for deletion at 8p21.3p23.1: some new twists on generation of terminal 8p rearrangements

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Somatic mosaicism for terminal 8p deletions is rare. Here, we report on a case of a deletion at 8p21.3p23.1 affecting about 50% of cells addressed by SNP/oligonucleotide CGH. An 8 year old girl presented with intellectual disability, autistic features, microcephaly, large upper incisors, small lower jaw, hypertrichosis, and pectus excavatum. Cytogenetic analysis has indicated the presence of a deletion at the short arm of chromosome 8 in about 60% of cells. SNP/oligonucleotide CGH has confirmed the presence of mosaic terminal deletion at 8p21.3p23.1 spanning about 11.152 Mb affecting 178 genes. Molecular cytogenetic analysis showed that 48% of cells are affected by this deletion. Clinically, the index case resembles non-mosaic deletions of 8p23. Nevertheless, milder manifestations of intellectual disability, microcephaly and facial dismorphisms were noticed, whereas autistic features were found to be more prominent. Interestingly, genomic loci flanking the breakpoint of mosaic deletion were regularly deleted. The regular deletion spanned three olfactory receptor (OR) genes (OR7E158P, OR7E161P, OR7E160P) and three beta-defensin (DEFB) genes (DEFB137, DEFB136, DEFB134). Since OR gene clusters are known to be involved in generation of 8p chromosome rearrangements, we have hypothesized that at least in the present case DEFB gene cluster is also involved in chromosome abnormality formation. Finally, to our knowledge this is the first case of a mosaic 8p deletion addressed by SNP/oligonucleotide CGH and mediated by OR gene recombination. Supported by the Russian Federation President Grant (MD-4401.2013.7).

P13.19-S
Differential allelic expression of the SOS1 c.755C activating variant in a Noonan syndrome family

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Noonan Syndrome (NS) is a genetic condition characterized by congenital heart defects, short stature and characteristic facial features. We analyzed a girl with moderate learning disabilities, delayed language development, craniofacial features and skin anomalies reminiscent of NS. After a mutation screening of the known NS genes PTPN11, SOS1, RAF1, KRAS, GRB2, BRAF and SHOC2 we found the heterozygous c.755T/C variation in SOS1 causing the I252T substitution, which was considered possibly pathogenetic by bioinformatic predictions. The same mutation was present in the proband's mother and maternal grandfather, both displaying some NS features, but also by a healthy subject on 1000 genomes analyzed. The functional analysis revealed that the SOS1 c.755T/C activated the Ras effector Erk1, confirming the predicted pathogenetic substitution. To explain the incomplete penetrance of the reported mutation we hypothesized that SOS1 may be subjected to a differential allelic expression (DAE). Interestingly, after sequencing the cDNA from peripheral blood compared to genomic DNA, we showed a DAE of some known SOS1 SNSs in healthy individuals and observed the mutated allele C 50% more expressed than the normal allele T in all our NS familial carriers. The similar level of SOS1 mRNA, between mutated and control individuals, suggests that the mutation here described does not affect SOS1 expression. We are now evaluating the SOS1 promoter polymorphisms. This study, providing the first evidence of allelic imbalance of SOS1, pinpoint DAE as a possible mechanism underlying a different penetrance of some SOS1 mutated alleles in unrelated carriers.

P13.20-M
Association of genetic factors involved in folate metabolism and the occurrence of congenital heart defects in individuals with Down syndrome

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Down syndrome (DS) individuals with polymorphisms in genes involved in folate metabolism can have increased risk for developing congenital heart defects, which are the leading cause of death in the first years of life. Moreover, these polymorphisms in mothers may also be associated with the risk for congenital heart disease in DS offspring. This study investigated whether the presence of the *MTHFR* C677T, *MTHFR* A1298C, *MTHFR* T1317C, *MTR* A2756G, *RFC1* A80G, *MTRR* A66G, *TC2* C776G, *TC2* A67G, *CBS* 844ins68, *CBS* T833C, *BHMT* G742A, *MTHFD1* G1958A, *DHFR* del 19 bp and *SHMT* C1420T polymorphisms in DS individuals and their mothers is associated with congenital heart defects. We evaluated 86 individuals with free trisomy 21 and their mothers, attended by Genetics Service at Faculdade de Medicina de São José do Rio Preto at the period 2005-2008. The investigation of polymorphisms was performed by Polymerase Chain Reaction (PCR), Real time PCR and PCR followed by digestion. The *RFC1* 80G polymorphic allele increased the risk for interatrial communication (IAC) (OR=7.92, CI=1.21-51.84, P=0.03) when in DS individuals. The *BHMT* 742A maternal polymorphic allele increased the risk of atrioventricular septal defect (AVSD) (OR=10.50; C=1.36-81.06; P=0.02) and interventricular communication

(IVC) (OR=18.00; CI=2.0-159.09; P=0.009) in DS offspring. We conclude that the *RFC1* A80G polymorphism in DS individuals is associated with the risk for IAC and *BHMT* G742A maternal polymorphism is associated with risk for AVSD and IVC in DS offspring.

P13.21-S
Protective action of NADPH oxidase inhibitors and the role of NADPH oxidase in the pathogenesis of colon inflammation in mice

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AIM: To investigate the role of NADPH oxidase in colon epithelial cells in pathogenesis of acute and chronic colon inflammation using mice dextran sulphate sodium (DSS) colitis model.
METHODS: BALB/c mice were divided into three groups: 8 mice with acute DSS colitis, 8 mice with chronic DSS colitis and 12 mice without DSS supplementation as control group. The primary colonic epithelial cells were isolated using chelation method. The cells were cultivated in the presence of mediators (lipopolysaccharide (LPS), apocynin or diphenyleneiodonium). Viability of cells was assessed by fluorescent microscopy. Production of reactive oxygen species (ROS) by the cells was measured fluorimetrically using Amplex Red. Production of tumour necrosis factor- alpha (TNF- α) by the colonic epithelial cells was analysed by ELISA. Nox1 gene expression was assessed by real-time (RT) PCR.
RESULTS: Our study showed that TNF-α level was increased in unstimulated primary colonic cells both in the acute and chronic DSS colitis groups, whereas decreased viability, increased ROS production, and expression of Nox1 was characteristic only for chronic DSS colitis mice when compared to the controls. The stimulation by LPS increased ROS generation via NADPH oxidase and decreased cell viability in mice with acute DSS colitis. Treatment with NADPH oxidase inhibitors increased cell viability decreased the levels of ROS and TNF- α in the LPS-treated cells isolated from mice of both acute and chronic DSS colitis groups.
CONCLUSION: Our study revealed the importance of NADPH oxidase in pathogenesis of both acute and chronic inflammation of the colon.

P13.22-M
Centromere of chromosome 9 presents unusual behavior in rearrangements leading to complete 9p duplication

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Trisomy 9p is one of the most common partial trisomies found in newborn, possibly because this region is relatively poor in genes resulting in a high survival rate. We report four different chromosome rearrangements resulting in complete 9p duplication, three of them involving 9p centromere alterations. The rearrangements in the patients were characterized by G-banding, SNP-array and FISH (Fluorescent *in situ* Hybridization) with different probes. One presents 9p duplication concomitant to 18p deletion due to an inherited der(18)t(9;18)(p11.2;p11.31)mat. Two patients present de novo dicentric chromosomes: der(9;15)t(9;15)(p11.2;p13) and der(9;21)t(9;21)(p13.1;p13.1), respectively. Another patient presents two rearranged chromosomes: a der(12)t(9;12)(q21.13;p13.33) and concomitant i(9)(p10) which showed a FISH centromeric signal smaller than its homologous. Besides the duplication 9p24.3p13.1, array revealed deletion in 9q13q21.13 (7.3 Mb). This rearrangement may have been originated by a misdivision centric fission resulting in a smaller centromere in i(9p) and part of the 9q long arm being translocated to the distal 12p. The deletion 9q may have been caused during the rearrangement with the chromosome 12. The chromosome 9 is rich in segmental duplications, especially in pericentromeric region, with high degree of sequence identity to sequences in 15p, 18p and 21p, chromosomes involved in our rearrangements. In two patients the dicentric chromosomes formed may have been converted into stable functional monocentric chromosomes by epigenetic centromere inactivation followed by heterochromatinization. Thus, we suggest that chromosome 9 is prone to illegal recombination, either intra or interchromosomal, that predispose it to rearrangements, frequently involving pericentromeric regions (Financial support FAPESP, Brazil).

P13.24-M
Multiprobe FISH method for enhanced detection of chromosome 9 heterochromatin rearrangements

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Heterochromatin rearrangements are believed to be clinically insignificant variants of the human karyotype. However, several authors have studied the possible association of heterochromatin variants with certain clinical diagnoses, especially with reproduction failure. Variants of heterochromatin area of chromosome 9 are the most common. They involve enlargement (qh+) or shortening (qh-) of the heterochromatin block as well as the pericentric inversion - inv(9)(p12q13). More complex variants of this area may include duplication and/or combination of above mentioned rearrangements.
Distinguishing between benign and pathological rearrangement in this area can be challenging. The classical G and/or C-banding are not very specific and array methods like SNP-array/array-CGH are usually not able to analyse precisely this pericentric region, which is composed mainly of satellite DNA.
For enhanced analysis of heterochromatin area of chromosome 9 we implemented a special molecular cytogenetic method using three different FISH probes - centromeric alpha-satellite, centromeric III-DNA satellite and a specific BAC probe (hybridizing on 9p12 and 9q13 homologous sequences). The outcomes of this examination in 20 patients with different clinical indications are demonstrated.
Although believed to be benign, the heterochomatin variants of chromosome 9 have been repeatedly mentioned as potentially associated with reproduction failure. Since the majority of these variants are undoubtedly truly benign, presented molecular cytogenetic examination is able to analyse this region more precisely than standard banding methods and distinguish among different (sub)variants of chromosome 9 much better than karyotyping and point out these potentially harmful.
This study was supported by the GAUK 523456 grant project.

P13.25-S
De novo case of a mosaic small supernumerary marker chromosome leading to proximal partial trisomy 5p

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Small supernumerary marker chromosomes (sSMC) are structurally abnormal parts of the karyotype with unknown origin that may arise de novo or be inherited from parents. ~70% of people with sSMC grow and develop normally, while 30% show different clinical signs and symptoms. Here we present the clinical and cytogenetic findings in a 1-year-old female referred for genetic evaluation because of dysmorphic features, including hypotonia, umbilical hernia, hypertelorism, broad nasal bridge, microretrognathia, low set ears, and wide spaced nipples. Cytogenetic examination of GTG banded metaphases showed a female karyotype with mosaicism of an sSMC. Additional molecular cytogenetics analysis (cenM-FISH and subcenM-FISH) characterized the sSMC to be derived from chromosome 5 including heterochromatic and euchromatic material. The shape of sSMC was not clearly to define; either it is a ring or centric minute. The karyotype can be reported as : mos 47,XX,1qh+pat,+der(5)?r(5)(::p1?4→q11.1::)[3]/der(5)?min(5)(:p1?4→q11.1:)[1]dn[38]/46,XX,1qh+pat[62]. Proband's twin sister and parents have normal karyotypes with respect to the sSMC. According to the literature, there are several cytogenetically similar cases described but with different clinical features. The most cases of proximal partial trisomy 5p arose in connection with familial translocations, while just five cases are due to a pure sSMC(5). The critical region for trisomy 5p syndrome seems to be located in the distal part of the short arm as the symptoms are similar to those seen in cases with pure trisomy 5pter to 5p13. The most common features are mental retardation, facial dysmorphism and hypotonia according to webpage "Small supernumerary marker chromosomes" (http://ssmc-tl.com/sSMC.html).

P13.26-M
Defining the role of CGGBP1 protein in FMR1 gene expression

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Fragile X syndrome (FXS) is the most common heritable form of cognitive impairment and is caused by the expansion over 200 repeats and subsequent methylation of the CGG triplets at the 5' UTR of the FMR1 gene, leading to gene silencing. The epigenetic and molecular mechanisms responsible for FMR1 gene silencing are still unclear. To outline structure-specific proteins that could recruit components of the silencing machinery we investigated the role of CGGBP1 in FMR1 gene transcription. CGGBP1 is a highly conser-

ved protein which binds specifically unmethylated CGG tracts. The role of CGGBP1 on FMR1 transcription is yet to be defined. Sequencing analysis and expression studies through quantitative PCR of CGGBP1 were performed in cell lines with different allele expansions (wild-type WT, premutation, methylated full mutations FXS and unmethylated full mutation UFM), demonstrating no differences between them. ChIP assays showed that CGGBP1 binds unmethylated CGG triplets of the FMR1 gene proportionally to the length of the repeats. We also observed that CGGBP1 binding to the FMR1 locus was restored after pharmacological demethylation with 5-azadC of FXS alleles, suggesting a possible role for CGGBP1 in FMR1 expression. CGGBP1 silencing with siRNA (reaching ~ 85% of CGGBP1-mRNA depletion) did not affect FMR1 transcription in WT and UFM fibroblasts. Although the strong binding to the CGG tract could suggest a relevant role of CGGBP1 on FMR1 gene expression, our results demonstrate that CGGBP1 is not a direct regulator of FMR1 transcription.

Supported by Telethon Onlus, FRAXA Foundation and Italian Association for fragile X syndrome.

P13.27-S

G6PD-Meyer: a new mutation causing compensated chronic haemolysis

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An updated database reports 186 different G6PD mutations. Many of these are polymorphic in various populations; many are sporadic, having been discovered because they cause chronic non-spherocytic haemolytic anemia (CNSHA) associated with severe enzyme deficiency (WHO class I).
A little boy with a history of neonatal jaundice treated with phototherapy, and of anemia (Hb 80-90 G/L) during his first semester, was seen at age 3.4 years. He is clinically well, with no significant physical findings; Hb 110 G/L, MCV 95, MCH 31, MCHC 33, reticulocytes 271x109/L, bilirubin 22 µmol/L: indicating a well compensated haemolytic condition. G6PD activity was 0.89 IU/GHb (ref values 7-10 IU/GHb). Sequencing the G6PD gene revealed an A->G change in exon 7 at nucleotide 655, predicting an Arg->Gly change at codon 219. The mother was shown to be heterozygous for the same mutation: her G6PD assay was normal. This mutation is not present in the database and therefore it is a new sporadic class I mutation. We interpret the CNSHA phenotype as related to the fact that Arg->Gly is a rather drastic amino acid change, since it entails a charge change as well as a steric change: such changes are likely to cause decreased stability of the G6PD protein and enzyme deficiency. It is interesting that Arg219 is not a conserved residue: we have previously reported that in terms of clinical phenotype the consequences in such cases would not be so drastic, and this may explain why this child does have haemolysis but only mild anemia.

P13.28-M

Large duplication implicating only exon 1 of F8 gene in mild hemophilia A phenotype

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In 98% of mild hemophilia A (HA) patients, a missense mutation spread throughout the Factor 8 (F8) gene's 26 exons can be identified using complete gene sequencing. In this study, 12 French-speaking Belgian with mild HA to whom any mutation could be identified by complete sequencing, multiplex ligation-dependent probe amplification (MLPA) analysis was performed as a second step. This gene dosage technique allowed for the detection of exon 1 duplication of the F8 gene in three apparently unrelated subjects. Using array-comparative genomic hybridization, breakpoint analysis delimited the duplication extent to 210 kb in the F8 gene intron 1 and in the VBP1 gene intragenic position. We postulated that the rearrangement responsible for this duplication could have arisen due to symmetrical tandem inversion duplication resulting in resulting in a large rearrangement of F8 gene intron 1 of 233 kb. This intron revised should allow the production of small number of normal mRNA transcripts in relation with mild HA phenotype. Indeed, the F8 gene mRNA from Patient 1 unexpectedly exhibited normal amplification of the segment containing exons 1 to 9 and reduced cross-reacting material (CRM), compatible with the patient's mild disease severity. All patients displayed an identical F8 haplotype, despite not being related, which suggests a possible founder effect of a 210 kb-large duplication involving the F8 gene exon 1.
The present study will need to be confirmed on a larger cohort of same genetic origin/background to evaluate for prevalence of the duplication as a cause genotype in mild HA patients.

P13.29-S

Subtelomeric chromosomal breakages characterization in patients with intellectual Disabilities/Congenital Anomalies and mechanisms for formation

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Abnormal CNVs are frequently found in subtelomeres of patients with intellectual disabilities (ID) and congenital anomalies (CA). The subtelomeric rearrangements do not usually present recurrent breakpoints (BPs) and involve several different chromosomes ends. Although these regions encompass approximately 30% of pathogenic CNVs, the causes of subtelomeric breakages and repair have not yet been investigated comprehensively. We investigated 105 unrelated patients with ID/CA using MLPA, FISH and arrays (BeadChip-Illumina/CGH-array-Agilent) in order to characterize the subtelomeric BP. Within the set of subtelomeric rearrangement studied, the deleted regions ranged from 137 kb to 29 Mb, and the duplicated regions from 155 kb to 32 Mb. We identified 38 BPs, from 19 different regions. Our analysis showed repetitive elements in 31 BPs encompassing SINEs (Alu; MIR), LINEs (L1; L2), and LTRs. We also found six BPs presenting simple tandem repeats and two BPs with interstitial telomeric sequences (ITs). Rearrangements with exclusively deletions are suggested to be caused by non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ) mechanisms. Complex rearrangements are likely caused by fork stalling and template switching (FoSTeS) or microhomology-mediated break-induced replication (MMBIR). Misalignments during replication due the repetitive sequences in these loci can lead to MMBIR and generate the complex dup/del rearrangements observed in 7 of our patients. Furthermore, genomic architectural features, like sequence motifs, non-B DNA conformations, and repetitive elements may increase the susceptibility for DNA breakage or promote FoSTeS in these regions. Mapping subtelomeric BPs should help to clarify the mutational mechanisms involved in these genomic rearrangements.

P13.30-M

Two cryptic duplications detected in an apparently balanced inversion posing a diagnostic challenge

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Balanced rearrangements in patients with abnormal phenotype are often associated with cryptic copy number changes (CNCs). These CNCs might not always be the cause of the phenotype and one must be careful prior to interpretation especially in aberrations involving the X chromosome. A 22-year old female patient was referred for cytogenetic analysis because of certain non-specific unusual facial features, a history of hypothyroidism and bilateral hearing loss. Chromosomal analysis revealed a small paracentric inversion on chromosome X with breakpoints at Xq21.1 and Xq23 delineated by FISH using several BACs. Surprisingly, array CGH analysis with a high resolution exon specific X-chromosome array (OGT) revealed two cryptic duplications, 116kb and 184kb in size, on each breakpoint. Cryptic duplications associated with apparently balanced inversions are very rare events. The duplications include coding genes such as *POF1B, ZNF711, SATL1, APOOL* and *LHFPL1.* Therefore the duplication was suspected to be causative for the phenotype as some of these genes included in the duplicated regions might partially explain the phenotype of the patient, e.g *LHFPL1* which has been previously found to be associated with hearing loss. Family studies revealed the same duplication in the father and grandmother of the patient. The father and grandmother have no remarkable clinical features with the exception of hypothyroidism in the father, rendering the duplication as coincidental rather than pathogenic. This case highlights the need for extended family studies and careful detailed clinical evaluation of other family members carrying the same aberration prior to interpretation and genetic counselling.

P13.31-S

Oncogenic splicing switch to the L isoform of the MAP/Microtubule Affinity-Regulating Kinase 4 (MARK4) gene in glioma through PTB (Polypyrimidine Tract-Binding protein)-driven pre-mRNA alternative splicing

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MARK4, a centrosomal serine-threonine kinase that phosphorylates microtubule associated proteins, takes part in cell cycle regulation and proliferation. In glioma we pointed out an imbalance between MARK4 isoforms, proportional to cellular de-differentiation and tumor grade. This imbalance

is triggered by decreased expression of MARK4S, the canonical isoform, associated with overexpression of MARK4L, the alternative isoform with skipping of exon 16.
Having ruled out mutations, CNVs and transcription defects as cause of deregulated MARK4 expression, we searched alterations in alternative splicing at the origin of the observed isoforms imbalance.
Bioinformatic analysis of MARK4 genomic sequence revealed three binding sites for the splicing factor PTB in introns flanking MARK4 alternative exon 16. A functional role for these sites is suggested by the conservation in mouse and the surrounding polypyrimidine rich context. Since in glioma PTB overexpression drives an oncogenic splicing switch favoring exon skipped-isoforms, we performed Western blot on glioma and glioblastoma-derived cancer stem cell samples and found a significant overexpression of PTB, correlating with MARK4L expression. Splicing assay of the designed and differently deleted minigenes revealed that IVS15 contains a functional intronic splicing silencer (ISS). However, mutagenesis of the PTB binding site in this region does not affect minigene splicing, suggesting PTB binds to a non-canonical ISS. Electrophoretic mobility shift coupled to mass spectrometry confirmed the PTB binding to MARK4 IVS15 and its involvement in MARK4 alternative splicing.
Alternative splicing thus emerges as an oncogenic mechanism that through the PTB-mediated regulation of MARK4 isoforms expression fosters proliferation and de-differentiation in glioma.

P13.32-M
Normal and oncogenic proliferation under control of microRNAs: a functional high content screening

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Cancer cells share several characteristics with normal stem cells especially those concerning cell cycle regulation. Therefore it is believed that cancer cells might arise from stem cells possessing self-renewal capabilities. MicroRNAs (miRs) are small RNA molecules that act regulating gene expression post-transcriptionally by targeting hundreds of mRNAs simultaneously. With this in mind, we hypothesized that significant changes in the cell cycle could be a good indicator for miRs capable of regulating normal and oncogenic proliferation. To test this hypothesis, 2.000 BJ foreskin fibroblasts were transfected with 50nM of 28 microRNAs mimics (pre-miR) and inhibitors (anti-miR) in 96-well microplates. After 5 days, proliferation was measured by XTT assay. Cell cycle classification (EdU assay) and viability (Sytox Green staining) following miR transfection were performed in a High-Content Screening platform. Nineteen treatments significantly altered the cell proliferation. Interestingly, while transfection of pre-miR of miR-20b, miR-101 and miR-181d decreased the proliferation, the corresponding anti-miRs had the opposite effect. Pre-miR-181d, pre-miR-20b and pre-miR-101 had the most cytotoxic effect. Pre-miR-181d and pre-miR-101 induced a significant reduction in the percentage of cells in S phase. Cyclin D1 expression was elevated by anti-miR-101 and by pre-miR-24, indicating that this cell cycle regulator might be the mediator of these miR's effects. These results indicate that these miRs can be good candidates for inducing alterations of interest in the cell cycle, such as speeding it up or slowing it down as desired. Also miR-101 acts as tumor suppressor in colorectal cancer and this role is under evaluation in our lab.

P13.33-S
The evaluation of apoptotic gene expressions, telomerase activity and the effects of insulin like growth factor-I and erythropoietin on apoptosis in an experimental necrotizing enterocolitis mice model

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Necrotizing enterocolitis (NEC) is a major cause of mortality among premature infants. Apoptosis, which occurs after hypoxia/reoxygenation (H/R), has an important role in the pathogenesis of NEC. Telomerase activity may also be important in the recovery process. The aim of this study is to evaluate the apoptotic gene expressions and telomerase activity in the H/R-induced intestinal mucosa and to investigate whether pre-treatment with insulin-like growth factor-I (IGF-I) and erythropoietin (EPO) could protect the intestinal cells from apoptosis or intestinal injury.
The study set was divided into 4 groups, each containing 10 young Balb/c mice. Group 1 mice were exposed to H/R. Group 2 and group 3 mice were pre-treated with IGF-1 and EPO for 7 days respectively before H/R. Group 4 served as a control group. Intestinal injury was evaluated by histological scoring. TUNEL test and caspase-3 activity was perfromed to asses apoptosis. Pro-apoptotic (p53, Casp3, Tnf, Bax, Fas, Bad) and anti-apoptotic (Bcl2, Bcl-W, Bcl-XL, NF-kB) gene expressions and telomerase activity were studied by Real-Time RT PCR.
IGF-1 and EPO treated animals showed decreased histological damage and decreased apoptosis which was confirmed by TUNEL test and caspase-3 activity. Telomerase activity increased in these groups in addition to increased expression of anti-apoptotic genes ($p< 0.01$). However pro-apoptotic gene expressions were not statistically different ($p>0.05$). In conclusion, the protective effects of IGF-1 and EPO in H/R damage may be due to increased expressions of anti-apoptotic genes.

P13.34-M
Phenotype variability in Slovak NF1 patients related to some mutation characteristics

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One of the most frequent autosomal dominant disorders is neurofibromatosis type 1 (NF1). In NF1 patients are usually found at least two of seven consensus clinical features: "café au lait" macules, freckling, Lish nodules, bone dysplasia, glioma of optical pathway, different types of neurofibromas and the first degree relative with confirmed NF1. Our cohort included 108 unrelated Slovak NF1 patients and we identified in 39% (42/108) patients frameshift, in 13% (14/108) missense, in 18.5% (20/108) splicing, in 17% (18/108) nonsense mutations, moreover in 4,5% (5/108) deletion of entire gene type I, in 6% (7/108) large deletions and in 2% (2/108) small in frame deletions. 23% (25/108) of mutations were located in Ras-GRD (Ras GAP related) domain and 21% (23/108) in CSRD (cystein serine rich) domain. "Café au lait" macules were present in all patients, freckling in 87%, Lish nodules in 28%, glioma of optical pathway in 31%, neurofibromas in 56%, and bone dysplasia in 6% of patients. Patients with nonsense mutations show the lowest occurrence of Lish nodules (11%) and the highest incidence of neurofibromas (72%). There are no significant differences between clinical features of patient with mutations in Ras-GRD and CSRD domains. Complete mutation analysis in the first-degree relatives was performed in 51 families. Interestingly, we showed that 63% (32) of the patients from these families carry de novo NF1 mutation. Age of the patients was from 2 to 69 years, 41% patients were younger than 18 years.

P13.35-S
In vitro analysis of alternative splicing of OLR1, a gene involved in atherogenesis and tumorigenesis

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Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), encoded by OLR1 gene, is the major endothelial ox-LDL receptor and plays a role in the pathogenesis of atherosclerosis and tumorigenesis.
OLR1 is subjected to a physiological alternative splicing. Two different isoforms are known: Loxin (NM_001172633.1) lacks exon-5 and encodes for a putative truncated receptor (LOXIN) with impaired binding activity; OLR1D4 (NM_001172632.1) lacks exon-4 and the putative protein has a different C-terminus domain. Functional role of OLR1D4 is unknown, while LOXIN is a natural inhibitor of LOX-1-mediated signalling and its up-regulation may have a potential therapeutic effect.
We transfected HeLa cells with minigenes (HIGH/LOW-risk) carrying two different haplotypes of the six SNPs regulating OLR1/Loxin splicing. A dose-curve PMA treatment shows that 10nM PMA at 3h up-regulates Loxin expression (FC10nM=+1.6, $p<0.05$) in HeLa transfected with H-risk haplotype (Hela-H). Loxin increase was not followed by OLR1 up-regulation and the OLR1/Loxin ratio after 10 nM of PMA was lowered to 28.1% than HeLa-H. These results suggest a role of PMA in modulating OLR1 splicing in vitro, leading to an increase of Loxin isoform. Moreover, we performed a computational analysis from different databases (TargetScan, miRanda, Pita) that predicted two microRNAs that bind the 3'UTR of OLR1 gene in nucleotide region in which are located SNPs. So these miRNAs may act as regulators of OLR1 expression and alternative splicing.
These are the first evidences of a regulation of OLR1 splicing, and provide new data addressing a future more selective and personalized therapy for diseases caused by OLR1 over-expression.

P13.36-M
Exploring the role of a ceRNA-based regulatory network, involving GBA and GBAP1, in the pathogenesis of familial Parkinson disease

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Parkinson disease (PD) is a complex neurodegenerative disorder characterized by the loss of dopaminergic neurons of the substantia nigra, which causes motor impairment and resting tremor. To date, mutations in the glucocerebrosidase (GBA) gene represent the most frequent cause of genetic PD. A widespread deficiency of GBA activity was recently demonstrated in the brains of PD patients carrying GBA mutations. Most interestingly, also PD patients without GBA mutations had lower levels of GBA activity in the brain, suggesting that dysregulated GBA expression could contribute to PD susceptibility. Possible mechanisms modulating GBA expression include post-transcriptional networks involving microRNA (miRNAs) and competing-endogenous RNAs (ceRNAs). ceRNAs are a class of regulators that titrate away miRNAs from their targets, thus influencing mRNA expression. The highly-homologous and expressed GBA pseudogene (GBAP1), located 16 kb downstream of GBA, is particularly suited to act as a GBA ceRNA. To verify this hypothesis, we first bioinformatically selected five miRNAs potentially targeting both transcripts, and demonstrated that one of them is significantly overexpressed in whole blood from PD cases vs. controls (2-fold increase, P=0.0028). Then, the miRNA precursor was overexpressed in HeLa/Hek293/HepG2 cells; in all cases, both GBA and GBAP1 endogenous mRNA levels were significant decreased (up to 70%). The specific interaction between the miRNA and its targets was demonstrated by luciferase-based reporter assays. Finally, preliminary overexpression experiments showed that the GBAP1 3'-untranslated region is able to act as a molecular "sponge" of the identified miRNA, thus suggesting the actual existence of a ceRNA-based regulatory network modulating GBA expression.

P13.37-S
Non-coding PAH gene alterations act as new transcription regulators

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Phenylketonuria (PKU) is rare metabolic disease caused by mutations in phenylalanine hydroxylase gene (*PAH*). Mutations that abolish structure and function of PAH are main determinant of PKU phenotype. However, phenotype couldn't be always predicted precisely. Previously, we found a transcription enhancer in *PAH* intron 8 that could affect genotype-phenotype correlation. In this study, we functionally analyzed additional non-coding *PAH* gene alterations to propose new transcription regulatory elements.
In silico prediction for transcription factor binding sites pointed to a population-specific promoter alteration (*PAH*: c.-170delC) and VNTR alterations in 3' region, that have never been analyzed before. We transiently transfected HepG2 cell line with various CAT reporter constructs to determine the effect of a *PAH* gene non-coding sequences on transcription. We found that a construct with additional binding site in promoter and constructs with VNTR3, VNTR7 and VNTR8 alterations had a 50-60% reduction of CAT activity in comparison to pBLCAT5. EMSA supershift showed binding of KLF1 transcription factor to the analyzed promoter sequence, while the full structure of VNTR3 was needed to obtain binding of C/EBPalfa.
Our study pointed to two new elements in promoter and 3' region of *PAH* gene that could act as transcription silencers and thus influence genotype-based prediction of PKU severity. Given that these non-coding alterations are population specific, further validation of their relevance should be studied in different populations. New transcription regulators in non-coding regions, like these ones, will contribute to better understanding of PKU phenotype complexity and may become important for optimization of PKU treatment.

P13.38-M
Apparent chromothripsis in a child with a complex chromosome rearrangement resulting in an 11p11.2 microdeletion within the Potocki-Shaffer syndrome region

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Chromothripsis is a phenomenon of genomic rearrangement arising during a single genome-shattering event resulting in multiple chromosome breaks involving one or more chromosomes, followed by imprecise reassembly. We report a case of a 21 month old female presenting with global developmental delay, hypotonia, minor dysmorphic features, nystagmus and multiple café-au-lait spots. Array-CGH analysis identified a 1.15 Mb interstitial deletion within chromosome 11p11.2 that partially overlaps the proximal interval of the Potocki-Schaffer syndrome (PSS) critical region. This deletion does not overlap the EXT2 and ALX4 genes which are associated with the multiple exostoses and parietal foramina phenotypes, respectively, frequently observed in individuals with this syndrome. This deletion does, however, overlap a 137 kb region previously identified as the critical region sufficient to cause hypotonia in PSS. Furthermore, haploinsufficiency of PHF21A, which is deleted in this patient, has been implicated in the intellectual disability and craniofacial anomalies associated with PSS. Chromosome and FISH analyses revealed an unanticipated de novo complex rearrangement involving chromosomes 9, 10 and 11 with at least 8 chromosome breaks: 46,XX,der(9)(10qter->10q24.3::11p11.2->11p13::9p23->9qter),der(10)(10pter->10q24.3::11p13->11p14::9p23->9p24::11p14->11pter),der(11)(9pter->9p24::11q13.5->11p11.22::11q13.5->11qter)dn. Although the deletion within chromosome 11p11.22 was the only clinically significant copy number imbalance, it is unclear if the other chromosome breaks and possible gene disruptions contribute to the patient's phenotype. Similar to other reported instances of apparent germline chromothripsis, this case showed minimal DNA loss presumably reflecting a selection against massive copy number imbalances. This case highlights the importance of performing both array-CGH and conventional cytogenetic methods as either investigation alone would have provided partial information.

P13.39-S
'Unexpected' finding with QF-PCR.

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At 11^{+5} weeks of pregnancy, chorionic villus sampling was performed with referral reason 'DNA analysis for sickle cell anaemia'. The HbS mutation was not identified. As is standard in our laboratory a rapid test for aneuploidy detection, quantitative fluorescence (QF)-PCR. was performed. The QF-PCR pattern of multiple X/Y specific markers was consistent with the presence of two X chromosomes, as well as Y-specific sequence (SRY). This suggested that the fetus could have a XX male genotype. FISH showed that SRY was located on the derivative X chromosome. Additional karyotyping (46, XX.ish der(X) t(X;Y)(p22.3;p11.2)(SRY+)) confirmed the presence of a 46,XX, SRY+ disorder of sexual development. After karyotyping and FISH analysis of both parents, the abberation turned out to be *de novo*. Thus, additional aneuploidy testing resulted in an unexpected finding which complicated the prenatal counselling.
Approximately 1 in 20,000 individuals with a male appearance have a 46,XX testicular disorder. The SRY gene, normally located on the Y chromosome, provides instructions for making the sex-determining region Y protein, which causes a fetus to develop as a male. Ultrasound at 18 weeks of gestation and postnatal inspection and ultrasound showed normal male genitalia. There were no signs of ambiguous genitalia. Parents felt confused by knowing prenatally that their unborn male child will be infertile, because of absence of the Y-chromosomal AZF regions. It is likely that at puberty hormone treatment is necessary to induce development of male secondary sex characteristics with testosterone and to prevent development of gynaecomasty.

P13.40-M
Post-transcriptional control of RET gene expression: implications in Hirschsprung Disease and Thyroid Cancer

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Control of mRNA half-life plays a central role in normal development and disease. mRNA stability depends not only on *cis*-acting sequences, but also on *trans*-acting elements able to promote mRNA degradation.
Though the RET gene is the major known gene involved in Hirschsprung disease (HSCR), a congenital defect of gut innervation, and in Medullary Thyroid Carcinoma (MTC), its role in these disorders has not fully been explored yet. To this end, we sought to characterize molecular events leading to post-transcriptional regulation and to identify genetic variants of RET 3'UTR predisposing to HSCR and MTC development. Starting from a large set of DNA samples from patients affected with either HSCR or sporadic MTC, we focused on RET variants lying in candidate regulatory regions or miRNAs target sequences of the distal gene portion. To this end, a next generation sequencing approach for the whole RET gene has been undertaken. Alleles of variants located in proximity of Poly-Adenylation cleavage sites and putative regulatory elements have turned out differently distributed among HSCR, MTC and control samples. Moreover, linkage disequilibrium is apparently stronger in cases than in controls, thus further suggesting that functional

3'UTR variants may be responsible for variable susceptibility to RET-related pathologies. Furthermore, to identify AU-rich regions, microRNAs and binding factors involved in the post-transcriptional control, the RET 3'UTR was fragmented in three parts and cloned into a luciferase vector. Preliminary results show different effects on mRNA stability for these fragments both in RET-positive and RET-negative cell lines.

P13.41-S
Mosaic ring chomosome 17 : a new case

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Ring 17 syndrome is a rare disorder and only 19 cases have been reported so far. Severity of the associated phenotype is influenced by the presence or deletion of the Miller-Dieker critical region (MDCR) with presence of the MDCR being associated with milder phenotypes, including growth delay , intellectual disability, epilepsy, café au lait skin spots and minor facial dysmorphism. We report a patient born at 41 gestation weeks after an uneventful pregnancy, with normal birth parameters - weight : 3kg350, length : 49 cm-. The Initial psychomotor development was normal. When he was 3 years old the patient developed fronto-parietal epilepsy. Cytogenetic investigations from peripheral blood cells showed a mosaic ring chromosome 17 karyotype :mos 46,XY,r(17)(p13;q25)[42]/45,XY,-17[5]/46,XY[3]. At 6 years of age, he had generalised epilepsy, developmental delay, school difficulties, and attention deficit disorder. Clinical examination showed no facial dysmorphism and no growth delay. Skin changes showed sparse café au lait spots but no axillary or inguinal freckling, and 2 small achromic spots. Cerebral MRI, cardiac ultrasound and ophtalmological fundus were normal. Molecular cytogenetic investigations confirmed that no terminal deletion had occurred, and an array-CGH (Agilent 44K) did not detect any submicroscopic deletion or duplication. Chromosome analysis were also obtained on café au lait skin spot and normal skin, and are currently being studied. Data from the patient will be compared to the literature, including the recent reports which raised the hypothesis of telomere shortening and telomere position effect in mild ring 17 syndrome.

P13.42-M
Somatic structural genome variations (chromosome rearrangements) are common in children with intellectual disability, congenital malformations and autism

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Classically, common pathogenic somatic genome variations are attributed to mosaic aneuploidy or polyploidy. However, recent reports on somatic mosaicism have demonstrated that mosaic structural genomic rearrangements (chromosome abnormalities) are likely to underlie intellectual disability, congenital malformations and autism as low-level mosaic aneuploidy does. To get further insight into possible role of somatic structural genome variations in disease, we have analyzed 202 patients with intellectual disability, congenital malformations and autism using SNP/oligonucleotide array CGH (two array CGH platforms with a resolution of 1 and 15 kb). Low-level mosaic aneuploidy (additional chromosome Y in about 5% of cells) was found in 2 cases (1%). Mosaic structural genomic rearrangements associated with a phenotypic outcome (according to an original bioinformatic technology) were detected in 14 cases (6.9%). These were mosaic duplications at 1q21.1q21.2 (1.9Mb), 11p15.5 (1.3Mb), 11p14.3 (8.4Mb) and mosaic deletions at 1p36.33p36.23 (7Mb), 2q22.1q23.3 (10.2Mb), 2q23.3q24.2 (10.7Mb), 5q14.3q15 (6.3Mb), 7q35q36.3 (14.6Mb), 8p23.3p23.1 (11.1Mb), 11q23.3 (0.1Mb), 21q11.1q21.2 (11.4Mb) and Xq28 (MECP2) (0.09Mb). Moreover, two cases have demonstrated low-level mosaicsim for isochromosome 12p and small supernumerary chromosome, which represented min(17)(:p11.2->q11.1:) (8Mb). These rearrangements were further confirmed by FISH and/or multicolor banding. Additionally, two benign mosaic subchromosomal aberrations (deletion at 14q11.2 in 7 cases; duplication at Xq28 in 2 cases) were observed. To our knowledge, such somatic genome variations (recurrent and benign) were not reported. Our data evidence that somatic genome variations manifesting as structural rearrangements are relatively common among children with intellectual disability, congenital malformations and autism. Supported by the Russian Federation President Grant (MD-4401.2013.7).

P13.43-S
Assessing the impact of exonic variants on splicing regulation by functional analysis and bioinformatics predictions: BRCA2 exon 18 as a model system

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Sequence changes in exons can alter pre-mRNA splicing either by changing splice sites or by modifying exonic splicing regulatory elements (ESR). The effects on ESR are especially difficult to predict by using currently available bioinformatics tools. Here, we used as a model system the exon 18 of BRCA2 gene involved in breast-ovarian cancer predisposition, and tested the impact on splicing of 36 variants identified in patients or reported in public databases. By using a splicing minigene assay, we found that 13 (7 missense, 4 synonymous, 1 nonsense and 1 in frame deletion variants) out of these 36 variations induce an increase in exon 18 skipping, by potentially modifying ESR. When patient blood RNA was available (n=4), the effects of the variants were confirmed. Recently, we demonstrated the predictive value of ESR hexamers' scores established by Ke et al. (2011) in identifying splicing regulatory mutations within BRCA2 exon 7 (Di Giacomo et al, 2013). Here, we show that this approach is also able to predict the effect on splicing regulation of BRCA2 exon 18 variants. Together with segregation data, these results should contribute to the classification of variants of unknown significance in BRCA2 exon 18. In addition, this study extends the validation of a new in silico tool for predicting the impact of exonic variants on ESR. This tool may have important applications in the filtering strategy allowing the discovery of pathogenic mutations among the large fraction of variants detected by high throughput sequencing.

P13.44-M
„Stress Entropic Load" as a Transgenerational Epigenetic Response Trigger

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Epigenetic changes are generally based on the switching of alternative functional or structural states and result in the adaptation of cellular expression patterns during proliferation, differentiation or plastic changes in the adult organism, whereas some epigenetic information can be passed on other generations while other is not. Hence, the principal question is: why is some information reset or resolved during the meiosis process and other is passed from one generation to anohter, or, in other words: what "adaptation trigger" level initiates transgenerationally transmitted epigenome change? Hereto, we propose a theory which states that stress, or, more specifically, the energy cost of an individual's adaptation to stress, represents a viable candidate for the transgenerational transmission trigger of a given acquired trait. It has been reported recently that the higher lifetime entropy generation of a unit's body mass, the higher the entropy stress level (which is a measure of energy released by a unit's organ mass where k = heat) and the irreversibility within the organ, resulting in faster organ degradation and consequent health problems for the entire biological system.
We therefore suggest a new variable: "stress entropic load" actually reflecting the energetic cost of an individual's adaptation that may be used to estimate the probability of inducing transgenerational response and propose methods for its estimation

P13.45-S
Prenatal diagnosis of partial trisomy 14 and partial trisomy 18 due to a 3:1 segregation of maternal reciprocal translocation t(14;18)(q13;q12.2)

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A 30-year-old woman was a known carrier of a balanced translocation 46,XX, t(14;18)(q13;q12.2), ascertained prenatally 30 years ago, when her

mother performed amniocentesis because of advanced maternal age. Karyotyping was confirmed by FISH five years ago. The woman with a known balanced translocation between chromosomal bands 14q13 and 18q12.2 was referred for prenatal diagnosis by amniocentesis at 17 weeks of gestation. Cytogenetic analysis of the father's lymphocytes showed a normal male karyotype 46,XY. Their first baby had normal karyotype 46,XX, also prenatally observed. We present the case of prenatally diagnosed tertiary trisomy with supernumerary derivative chromosome 14, due to a 3:1 segregation of maternal reciprocal translocation. The fetal karyotype was 47,XX,+der(14)t(14;18)(q13;q12.2)mat, resulting in partial trisomy 14(pter-q13) and partial trisomy 18 (q12.2-qter). There was no evidence of fetal malformations by ultrasound examination. After the amniocentesis procedure, amniotic fluid has leaked and the mother miscarried. In reciprocal translocation carriers these chromosomes can arise in different segregation modes, such as alternate, adjacent-1, adjacent-2, 3:1 or 4:0, resulting in the formation of 32 possible zygotes with different chromosome complements. This case supports the thesis that the incidence of 3:1 segregation is higher in female than in male carriers, more often occurs when one of the derivative chromosomes is relatively small, and also suggests that translocation with acrocentric chromosomes tends to produced 3:1 segregation.

P13.46-M
Mosaic Uniparental Isodisomy (UPD) of 11p, presenting as a regular beta-thalassemia carrier.

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Recently we discovered three independent cases of "severe late onset β-thalassemia", all presenting with the mild phenotype of beta thalassemia minor up to adult age and developing a severe transfusion dependent phenotype in the third and fourth decade of life when a presumed homozygosity for the beta-thalassemia mutation is observed. Affymetrix and/or Illumina SNP-array analysis revealed incomplete homozygosity for SNP's along almost the entire short arm of chromosome 11 containing the beta-globin gene, indicating mosaicism for a partial uniparental isodisomy of chromosome 11p. Three patients were born asymptomatic as beta-thal carriers and developed a severe blood-transfusion dependent beta-thalassemia major at different ages and with different percentages of mosaicism. Recently we discovered another case showing a similar mosaic UPD of 11p, presenting as a regular beta-thalassemia carrier. The fourth patient however did not develop the clinical severity despite of an almost 50% mosaicism determined from the DNA isolated from leucocytes. The most probable mechanism seems clonal selection for hematopoietic stem cells containing the uniparental isodisomy for the mutant beta-globin gene during life, this may account for the progressive development of the disease. However, there seems to be no correlation between the percentage of mosaicism measured in the DNA isolated from the white cells and the severity of the clinical phenotype related to the expression in red cells, which strongly suggests hematopoietic tissue heterogeneity for the observed UPD containing cell lineages. This may have serious consequences for disease prediction and counseling, as this is largely dependent upon DNA isolated from leucocytes.

P13.47-S
Cryptic genomic imbalances and developmental delay and/or congenital defects in apparently balanced rearrangement carriers

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Apparently balanced chromosomal rearrangements (ABCRs) are associated with an abnormal phenotype in 6% of cases. It has been described that in over 40% of cases thie phenotype may be caused by cryptic genomic imbalances, both at the breakpoints (25%) and elsewhere in the genome (15%). However, cryptic genomic imbalances detectable by array-CGH have also been postulated as the underlying cause of developmental delay and/or congenital abnormalities (DD/MCA) in 10-15% of patients with normal karyotype. The aim of this study is to determine if copy number variants are in fact a major genetic defect associated with DD/MCA in ABCRs carriers,
We performed CGH-array studies in three groups of patients: G1.1: 21 ABCR carriers with DD/MCA; G1.2: 22 ABCR carriers with normal phenotype and G2: 45 cases with normal karyotype and DD/MCA. Similar number of pathogenic imbalances were detected in both groups of patients with DD/MCA, independently of their karyotype (5/21 of cases in G1.1 (24%) and 9/45 in G2 (20%)). Only one of the ABCR carriers (5% of cases) had an imbalance at the breakpoints.
Conclusion: Simple ABCR do not seem to confer an independent and significantly higher risk for DD/MCA associated with genomic imbalances,

P13.48-M
Patterns of X inactivation in abnormal X chromosome

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Background: X inactivation is a dose compensation mechanism which results in silencing majority of genes on one of the two X chromosomes in every somatic cell of human females. Early in embryonic development, cells inactivate all their X chromosomes except one. Once an X is chosen, it is stably inherited through subsequent somatic mitotic divisions. The process of X inactivation is under the control of X inactivation center. Purpose: Study of X inactivation patterns in cases with abnormal X chromosome and its correlation with patients phenotype. Methods: 15 selected patients having abnormalities of the X chromosome were subjected to Clinical examination, GTG banding, FISH technique to detect origin of some structural X abnormalities and Detection of X chromosome replication pattern (Late Replicating Chromatin) technique. Results: Cases were classified according to their karyotypes into three groups: Cases with numerical X abnormalities, Cases with iso X chromosome and Cases with other structural X abnormalities. In most of X aneuploidies, each cell has only one active early replicating X, while other extra or abnormal X chromosomes are inactivated late replicating. Regarding the balanced X;autosome translocation, there was a mosaic pattern; in majority of cells translocated X was late replicating inactive, while in few cells translocated X was early replicating active X chromosome. Conclusion: It has been found that at least 30 X-linked genes are expressed on the inactivated X chromosome. The varying degree of phenotypes within each syndrome occurs because the genes that escape X inactivation are expressed at varying degrees.

P13.49-S
Uncovering the oligomeric structure of the y+LAT1/4F2hc amino acid transporter

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y+LAT1 and 4F2hc are protein subunits that form a transporter complex for cationic amino acids in the basolateral membrane of epithelial cells, mainly in the small intestine and proximal kidney tubules. Mutations of y+LAT1, 56 of which are currently known, cause lysinuric protein intolerance (LPI, OMIM #222700), a rare metabolic disorder characterised by diminished intestinal absorption of the cationic amino acids lysine, arginine and ornithine and by severe loss of these amino acids into the urine. The more detailed structure of this transport complex has so far been unclear - it has remained unelucidated whether the complex is formed as a dimer or a tetramer of the subunits. What has been known, however, is that the y+LAT1 subunits cannot reach the plasma membrane without forming a complex with 4F2hc. We previously established fluorescence resonance energy transfer (FRET) microscopy and FRET-FACS as tools in studying the interactions of y+LAT1 and 4F2hc. We have now applied these techniques together with immunohistochemistry to the exploration of the heteromerisation status of the y+LAT1/4F2hc transporter complex. We discovered that when fused into fluorescent vectors and transfected into the HEK293 cells, the y+LAT1 proteins interact together in the presence as well as absence of 4F2hc. Our initial results therefore suggest that the holotransporter is a multimer of y+LAT1 and 4F2hc subunits.

P14.01-S
Technical aspects of ALK FISH test to improve therapy selection of lung cancer patients

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Introduction. ALK gene (2p23) rearrangement characterizes a subgroup of patients affected by lung adenocarcinoma who may benefit from the ALK-inhibitor crizotinib. ALK translocation is principally related to a small paracentric inversion. FISH with break-apart strategy is considered the gold standard to investigate ALK. Evidence based studies settled the presence of ≥15% cells with rearrangements as cut-off to classify patients as positive (ALK_FISH+). Recently, same Authors identified a subset of borderline patients that might benefit from therapy, wondering whether this cut-off could reflect a real biologic distinction between ALK-positive and ALK-negative tumors.
Materials & Methods. We investigated ALK gene status by FISH, using ALK LSI Dual-Color Break-Apart (Abbott) and ALK Split-Signal (DAKO) probes, in

243 lung adenocarcinomas collected in three Institutions. A series of standardized ALK-negative lung cancer cell lines (Abbott) were used as negative controls. A specific scoring system considering not only the splitting of the signals but also their distance was developed.
Results. We identified 12% ALK_FISH+ patients, with similar rate among the three Institution (13%, 12%, 9%). ALK_FISH+ cases showed: 53% inversion/translocation, 28% deletion and 18% both patterns. Concordance was observed between the different operators and between the two probes, both on patients and on the panel of negative controls. In this last, the cut-off obtained (by calculating M+3SD) was 14.9%.
Conclusions. Difficulties in interpretation of ALK FISH signals pattern might be bypassed using our detailed scoring system, that is reproducible among different operators, probes, and provide experimental evidence that 15% is a reasonable cut-off for patients selection.

P14.02-M
Allelic drop-out in large Iranian Brugada syndrome family revealed by new generation sequencing

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Background: Sanger sequencing is a gold standard of DNA diagnostic currently used for NGS validation. Nevertheless, it has its own limitations producing false results. The well-known mechanism of allelic dropout is the presence of SNPs in 3'-region of PCR primers. The early recognition of allelic dropout prevents diagnostic errors.
Materials and Methods: The DNA samples from Brugada syndrome Iranian family were extracted from peripheral blood. Sanger sequencing of SCN5A gene coding areas was performed for proband and relatives. Control re-sequencing via semiconductor PGM IonTorrent platform using Ampliseq primer pool encompassing coding area of 10 genes including SCN5A was performed for family members.
The control group comprised 100 ethnically matched healthy donors.
Results: Sanger sequencing has revealed a novel heterozygous genetic variant p.P1506S in exon 26 of SCN5A gene in proband. Surprisingly, his son carried p.P1506S variant in homozygous state. Carriage of p. P1506S variant in mother had been excluded by Sanger sequencing and PCR-RFLP analysis. We performed control re-sequencing via semiconductor PGM IonTorrent platform for proband and his son. NGS results showed a discrepancy with primary Sanger sequencing results, proving heterozygousity of p.P1506S variant in proband's son. Sanger re-sequencing using alternative oligonucleotide primers for exon 26 confirmed NGS outcome. Thus, SNP rs41315501 was identified in 3'-region of previously used forward primer. Prevalence of rs41315501 was assessed by PCR-RFLP in 200 chromosomes cohort and MAF (minor allele frequency) accounted 9,4%. SNP was detected in proband's wife and son in heterozygous state.
Conclusion: Currently Sanger sequencing is used for validation of NGS techniques. But vice versa, NGS technology can be applied for capillary sequencing quality control.

P14.03-S
Scanning Alpha Haemoglobin-Stabilizing Protein (AHSP) gene by High Resolution Melting Analysis

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Alpha-Haemoglobin Stabilizing Protein (AHSP) is involved in the stability, folding and binding of alpha globin to the hemoglobin complex, regulates the free alpha globin pool and has been associated with heterogeneity of thalassaemia phenotypes. AHSP is encoded by a relatively small gene and is a good candidate for High Resolution Melting Analysis (HRMA). The aim of this study was to develop a rapid, specific and sensitive HRMA approach for scanning AHSP gene. Alpha thalassaemia heterozygotes with the same underlying mutation (αPA1α/αα) but heterogeneity in haematological indices MCV and MCH and no iron deficiency were selected and divided in three subgroups. Ten normal samples were used as controls. AHSP gene was divided in small sized overlapping amplicons and PCR conditions were optimized prior to HRMA. Samples presenting distinct derivative plots were Sanger sequenced. Two variations were detected. The variation 12895 G>T (exon III) was detected in samples regardless group and may represent a neutral polymorphism as reported previously. The second variation detected, 12391 G>A, is located in an Oct-1 binding site and was also detected in samples from different sub-groups. These results indicate that HRM analysis can be used as a fast, cost effective, relatively simple and high-throughput amenable method for scanning AHSP gene. This approach can be incorporated in order to analyze higher number of samples and strengthen the characterisation of these changes as neutral or not polymorphisms in the Hellenic population. Moreover, detection of other variations may associate them with heterogeneity of alpha / beta thalassaemia phenotypes.

P14.04-M
NGS network for diagnostic of autoinflammatory diseases (AID)

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Hereditary autoinflammatory diseases (AID) are characterized by recurrent bouts of systemic inflammation caused by dysregulation of proteins of the innate immunity system. Identification of about 25 genes over the last 17 years rendered possible genetic diagnosis, a major tool to discriminate patients with close phenotype. This genetic heterogeneity and the rarity of these conditions motivated an International concerted action for the use of next generation sequencing for diagnosis of AID.
A survey among AID experts was conducted in 2013. Twenty laboratories declared willing to participate in this network and filled out a questionnaire. Results were as follows:
• Sequencing equipments varied across the laboratories. Illumina MiSeq was the major device (29%), followed by Lifetech PGM (25%), Illumina HiSeq (18%), Roche Junior (14%), other (14%).
• Sequencing results were analyzed with either a software from the platform, an in house pipeline, a collaboration (21% each), or a combination (37%).
• The number of AID genes included in the panels varied from the five most frequent to all published genes.
• Clinical expectations included ethics: develop new informed consent (53%) and guidelines (47%), and collaboration: sharing experience for variant interpretation (31%), access to patients and database (28%), harmonization of technology and reports (17%).
Through a close collaboration with clinicians, we could connect this project with two existing dedicated patient (Eurofever) and mutation (Infevers) registries. Integration of clinical and genetic data will help 1) elaborate a consensus for classification criteria and 2) identify new AID genes in orphan patients.

P14.05-S
Next Generation Sequencing approach to molecular diagnosis of autoinflammatory diseases: from gene panel design to variant call

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Auto-Inflammatory Disorders (AIDs) are monogenic diseases caused by primary dysfunctions of the innate immune system. However, molecular diagnosis performed by Sanger sequencing of known genes fails to detect mutations in around 86% of patients recruited to our Unit. Clinical misdiagnosis, mutations in untested gene regions, genetic heterogeneity and/or a complex mode of inheritance are all possible explanations.
The Next Generation Sequencing approach has been undertaken to improve mutations detection in AIDs. By using the Ion Ampliseq™ Designer (LifeTech) online tool, we designed a panel of 203 amplicons including the 121 exons of 11 genes already known to be involved in AID, for a total of 22Kbs. Eight samples can be sequenced in one Ion PGM™ 314-chip. The mean coverage has turned out to be 225X, with 93.4% of the target region covered >20X and 76,5% >100X. The analysis from FastQ to VCFs was carried out using three different workflows: i) Ion Torrent Alignment and Ion Reporter™ 4.0, specific for data generated by PGM ii) in-house pipeline based on free-tools like BWA and GATK, iii) CLC Bio software.
Focusing on three representative DNA samples already genotyped for the respective causative genes, Ion Reporter and CLC allowed to detect all the three expected mutations, while GATK did miss one of these. In order to maximize sensitivity and specificity for routinely use of the procedure in AIDs diagnosis, we will present data from 50 additional DNA samples carrying mutations at least at one of the 11 genes in the present panel.

P14.06-M
A robust approach for blind detection of balanced chromosomal rearrangements with whole-genome low-coverage sequencing

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Balanced chromosomal rearrangement (or balanced chromosome abnor-

mality, BCA) is a common chromosomal structural variation. Next-generation sequencing has been reported to detect BCA-associated breakpoints with the aid of karyotyping. However, the complications associated with this approach and the requirement for cytogenetics information has limited its application. Here, we provide a whole-genome low-coverage sequencing approach to detect BCA events independent of knowing the affected regions and with low false positives. First, six samples containing BCAs were used to establish a detection protocol and assess the efficacy of different library construction approaches. By clustering anomalous read pairs and filtering out the false-positive results with a control cohort and the concomitant mapping information, we could directly detect BCA events for each sample. Through optimizing the read depth, BCAs in all samples could be blindly detected with only 120 million read pairs per sample for data from a small-insert library and 30 million per sample for data from non-size-selected mate-pair library. This approach was further validated using another 13 samples that contained BCAs. Our approach advances the application of high-throughput whole-genome low-coverage analysis for robust BCA detection, especially for clinical samples, without the need for karyotyping.

P14.07-S
Using biotin-streptavidin interaction for binding plasmid to a cell-penetrating peptide

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Gene therapy strategies based on plasmid DNA are more preferable than viral-based gene delivery. Plasmids are more stable in vivo and known to be less immunogenic. Retroviral-based vectors integrate in actively transcribed genes that can lead to disruption of tumor suppressor genes. Gene delivery methods based on plasmid DNA do not cause mutagenesis and they do not integrate in chromosomes providing increased safety. Despite the potential advantages of using plasmid DNA, delivery issues with plasmid vectors exist. One of the ways of plasmid delivery into a cell can be biotin-streptavidin interaction of the plasmid to a cell-penetrating peptide (CPP). Streptavidin binds the small molecule biotin with femtomolar affinity. We have created a biotinylated pEGFP-N3 circular plasmid DNA to further its binding to the streptavidin-CPP fusion protein. Few thymines were replaced by biotinylated uracils in the plasmid. The presence of biotinylated uracils in purified plasmid was verified in restriction analysis followed by dot blot procedure. Streptavidin-conjugated alkaline phosphatase was used to detect the biotin-uracils. The ability to express green fluorescent protein was tested in Hela cell culture. We have created a genetic construct for synthesis of monovalent tetrameric streptavidin, which consists of one WT streptavidin and three inactive streptavidins. Each inactive streptavidin is fused to chain of four HIV TAT-peptides. The resulting chimeric protein binds one biotinylated circular plasmid DNA due to the biotin-streptavidin interaction. This structure can be used for gene therapy.

P14.08-M
Methyl-DNA detection: a performance comparison of four commercial kits

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Epigenetic alterations, including DNA methylation, play an important role in the regulation of gene expression. Several methods exist for evaluating DNA methylation, but bisulfite sequencing remains the gold standard by which base-pair resolution of CpG methylation is achieved. The main limitation of this method is its harshness to DNA and several commercial kits try to find a balance between reagent harshness and conversion efficiency. Our study compares four popular kits (Diagenode, Promega, Epigentek, Qiagen) regarding their conversion efficiency and degradation effect. We used in-vitro methylated and unmethylated forms of two λ-phage PCR products to create various DNA mixtures (spikes), including ratios 1:0, 1:1, 1:3 and 0:1 methylated to unmethylated DNA. These were bisulfite converted with all 4 kits and then PCR amplified producing templates for Sanger sequencing and NGS. Our Sanger sequencing results showed 100% conversion efficiency of cytosines across all kits and there was a correct trend of methylation status at CpG sites which reflected the expected ratios of the spikes. However, the method was limiting in displaying accurately the extent of methylation in each case. DNA degradation was compared between kits based on the efficiency of PCR amplification and it was shown that the Diagenode and Promega kits had the least degrading effect.
Our NGS results offered higher resolving power as expected, allowing accurate assessment of methylation ratios lower than 1:3. Our results indicate bisulfite-NGS applications are applicable for detecting small variations in methylation while our comparison shows which kit is the least harsh and the most efficient.

P14.09-S
Molecular testing of BRCA1&2 genes with NGS technology: a three-step approach

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The aim of our study was to enhance the throughput of mutation detection analysis in order to supply high sensitivity, fast and accurate data on BRCA1/2 genes' mutational status of selected breast and/or ovary cancer patients. Starting from June 2012 a panel of 8 selected BRCA1/2 pathogenic mutation–prone cases have been analysed on a GS 454 Junior platform (Roche) and 6 out of 8 pathogenic variants were successfully identified. One of the two unidentified variants was a single nucleotide insertion just adjacent to a homopolymer stretch of 8 Adenines in the coding region of BRCA2. The second unidentified variant was a deletion of the whole exon 14 of BRCA1 gene. The latter two unidentified pathogenic variants were revealed subsequently using the BRCA Homopolymer (BRCA HP) kit from MULTIPLICOM and MLPA analysis. We then decided to adopt a Three-step mutational screening approach. As a first step BRCA1 &2 amplicon libraries were generated with BRCA MASTR™ assay from Multiplicon, and sequenced on our GS 454 junior platform. Sequencing data are then analysed with Amplicon Variant Analyzer (AVA) software (Roche). The samples with no pathogenic variants identified are then involved in the second step which is the processing with BRCA HP kit in order to uncover insertions or deletions in homopolymeric coding regions of both genes. The third step is MLPA analysis to uncover large genomic rearrangements. So far 181 samples were analysed in our laboratory and 29 pathogenic variants were identified with our three step testing approach.

P14.10-M
Efficient sharing of BRCA1 and BRCA2 variant and phenotype data between diagnostic labs

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The Dutch and Belgian working group for Breast Cancer DNA Diagnostics (LOB) has decided to share over 7500 variants detected in the BRCA1 and BRCA2 genes in breast cancer families since 1997. For this, the data, almost evenly split among both genes, have been submitted to the LOVD 3 shared gene variant database installation (1,2). Previously, variants identified in Dutch and Flemish (Belgian) DNA diagnostic labs were collected yearly and stored in an Excel data file. Advantages of the LOVD system include: simple submission of new data in a standardized way, instant updates after curation, easy maintenance and automatic backups. Although most data are publicly accessible online, some data are shared by members only. Others can see whether such information is available (password protected file links), giving them the option to contact the submitter for further details. Members can contribute their opinions about variant classification, increasing its consistency, but being aware of potential misinterpretation they have reservations sharing this information. Data are stored variant-by-variant and connected to each individual patient and submitting diagnostic lab. Using existing LOVD functionality, users can perform queries per gene or individual, use other linked resources of interest, get genome browser views of the data and use web services to access variants stored in other gene variant databases. In addition, LOVD3 has a new access level, designated "collaborator", allowing submitters to share otherwise non-public data with other submitters, e.g., to share detailed phenotype information with other diagnostic labs only.
1) http://databases.lovd.nl/shared/genes/BRCA1
2) http://databases.lovd.nl/shared/genes/BRCA2

P14.11-S
Validation of a Next Generation Sequencing assay for BRCA1 and BRCA2

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Next Generation sequencing (NGS) is rapidly finding a place in routine diagnostics although, extensive validation of such platforms and the associated bioinformatics is urgently required before they reach equivalent confidence as Sanger sequencing. Routine testing of BRCA1 and BRCA2 for Breast Cancer predisposition has resulted in numerous positive and negative controls for the rigorous assessment of NGS. A further consideration when implementing a new technology for routine testing is it's suitability to the diagno-

stic laboratory which will be discussed.
This study examines approximately 400 samples referred for BRCA1 and BRCA2 testing in the context of familial breast cancer and compares the performance of NGS to Sanger sequencing. The approach used involved microfluidic PCR (Fluidigm Access Array), followed by NGS on an Illumina MiSeq instrument and subsequent bioinformatic analysis with NEXTGENe (SoftGenetics). A single assay is capable of performing 42 BRCA1 and BRCA2 screens in less than 5 days. The failure rate for the assay was 4% (17/399), primarily contributed by the initial two assays (N=94) with subsequent assays providing a 1.6% failure rate (5/305). The sensitivity of the assay compared to Sanger sequencing was estimated at 99.7%. Overlapping amplicon design was the most significant problem encountered with bioinformatic analysis, requiring several rounds of optimisation. Validation of the workflow consisted of ~400 samples distributed across retrospective (including a reproducibility component) and prospective arms as well as a blinded/inter laboratory sample assessment and a concurrent testing period. The validated assay has been accredited to international standards and is now in routine use.

P14.12-M
Initial evaluation of the cardiomyopathy-specific content of Illumina's TruSight ONE next generation sequencing assay

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Introduction: The recently launched TruSight ONE panel comprises 4812 genes and is meant to enable a one-for-all-mendelian-diseases test. Objective: To determine whether the cardiomyopathy-specific genes are covered sufficiently good enough to justify the use of TruSight ONE in clinical genetic testing. Subjects, Materials and Methods: In order to initially analyze control samples, DNA was obtained from three individuals presenting with conditions other than cardiomyopathy (CM). TruSight ONE DNA enrichment and next generation sequencing (NGS) was conducted according to the manufacturer's instructions using a MiSeq instrument. A proprietary NGS data processing and filtering pipeline was used. Results: A) Performance characteristics for 46 core CM genes: The fraction of bases covered with less than 20 reads varied between 2.3 and 5.4% (mean: 3.7%). The average read depth varied in the range of 84- to 147-fold (mean: 120-fold). A mean of 4 relevant variants (i.e. missense, nonsense or splicing) was observed (range: 2-8). B) Performance characteristics for a set of 288 genes annotated for cardiomyopathy by NCBI's Entrez Gene (including the 46 core CM genes): A mean fraction of low coverage bases of 4.7% was observed (range: 3.7-6.6%). The mean average read depth was 118-fold (range: 81-148-fold) and 15-30 (mean: 22) relevant variants were detected. Conclusions: The cardiomyopathy-specific genes could efficiently be analyzed using TruSight ONE and a MiSeq instrument. The sensitivity and specificity of the test will be determined and compared to that of Illumina's separate 46 gene cardiomyopathy assay.

P14.13-S
Computer-aided facial recognition of Cornelia de Lange Syndrome (CdLS): a follow-up study

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CdLS is a genetically heterogeneous disorder, exhibiting a wide phenotypic spectrum. Approximately 70% of the clinically diagnosed CdLS patients are confirmed for a cohesin-related gene mutation.
In "*Computer-aided facial recognition of Cornelia de Lange syndrome: a comparison to the recognition by human experts*" presented at the 2012 DSW workshop, the *FDNA*® technology successfully recognized facial dysmorphology associated with CdLS from 2D photos, producing results comparable with those of human experts.
In this study, we collected and tested blindly 18 photos of probands with a molecularly-confirmed or clinical diagnosis of CdLS and 10 with confirmed diagnosis of various non-CdLS syndromes (e.g., *kabuki, Aarskog, dubowitz, etc.*). For each photo, the system produced a score above (positive) or below (negative) a threshold, determined from the results of the original study.
4/5 probands with the NIPBL mutation (including 1 mosaic mutation), 3/4 with the SMC1L1 mutation and 5/9 clinically diagnosed by geneticists as CdLS-like, but carrying various genomic imbalances, received positive scores. 10/10 non-CdLS probands received a negative score. The 6 false-negatives were reported to have a mild phenotype or not to meet the clinical diagnostic criteria, of which 3 received a score well below the threshold and 3 a score immediately below the threshold, suggesting that the threshold may be skewed upwards and requires further adjustment to increase system's sensitivity from 67% to 83%, while maintaining specificity of 100%.
Results show consistency with experts' evaluation of the "classic" CdLS facial morphology, validating the system's ability to discern between CdLS's and other syndromes' phenotype.

P14.14-M
Profiling circulating miRNAs in plasma samples of celiac disease patients

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Celiac Disease (CeD; "gluten intolerance") is diagnosed by symptoms, detecting CeD-specific antibodies, and biopsy results. The only available therapy is a life-long gluten-free diet. Despite the availability of diagnostic tools, as many as 7 out of 8 adult CeD patients are either not or incorrectly diagnosed, highlighting the need for novel biomarkers.
Circulating miRNA profiles have been shown to be disease-specific, even disease-stage-specific, in patients with cancer or gastro-intestinal disease. We examined whether circulating miRNAs in plasma samples of CeD patients can be used as CeD biomarkers. By next generation sequencing we profiled miRNAs in 95 plasma samples from 12 CeD patients, 5 patients positive for CeD-associated antibodies and 5 control subjects from the Prevent CD cohort. In this cohort, newborns at risk for CeD were challenged with low levels of gluten between 4-6 months of age to induce gluten tolerance, and plasma samples were taken every 3 months until diagnosis. Comparing data from samples taken at diagnosis to samples taken at 3 months of age (first available sample), we found 62 miRNAs significantly differently expressed (FDR<0.05). Of those, 4 miRNAs were previously reported to be associated with CeD, 12 were implicated in other autoimmune diseases and 7 miRNAs have been implicated in immune signaling. One of these immune related miRNAs is miR-23b, which appears to be downregulated 2-fold in CeD patients at time of diagnosis. This miRNA is known to limit tissue inflammation. A downregulation of this miRNA would therefore contribute to the pro-inflammatory state in active CeD.

P14.15-S
Northern Lights Assay of cfDNA damage in body fluids

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Structural damage in cfDNA molecules in body fluids has been little studied. Such damage may reflect normal and abnormal cell turnover, genome instablity or exporsure to genotoxic agents. We analyzed cfDNA damage in plasma, urine and saliva. Standard methods of isolation of cfDNA in plasma and urine are based on inducing ssDNA with a chaotropic agent and selective cordination binding of lone pair electrons on guanine to silica. These methods were not usable. In contrast, we found that selective ion exchange chromatography allowed gentle isolation of DNA without inducing damage. Damage in isolated DNA was assessed with the Northern Lights Assay. This assay is based on Two-Dimensional Strandness-Dependent Electrophoresis (2D-SDE) in premade microgels. Each sample was run in duplicate i.e. uncut and cut with Mbo I, an enzyme which cuts both single- and double-stranded DNA. Single-stranded breaks, either nicks or gaps, were detected as horizontal streaks from uncut DNA molecules. Double-stranded breaks generated an arc in the gel. DNA molecules with interstrand crosslinks (ICL) migrated as an arc behind normal dsDNA molecules. DNA with intrastrand crosslinks and bulky adducts were bent and migrated in front of that arc. Single-stranded DNA molecules, too damaged for complementary strand binding, formed a diagonal line. Patterns of cfDNA in plasma of normal subjects showed an apoptosis pattern with single- and double-stranded breaks of nuclesomal fragments. cfDNA in urine showed composite patterns of apoptosis and nonspecifc degradation. The most extensive damage and variable patterns were seen in saliva including prominent single-stranded breaks.

P14.16-M
A retrospective view on External Quality Assessment of Charcot-Marie Tooth disease over 16 years

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Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous disorder of the peripheral nervous system. CMT1A is the most frequent autosomal dominantly inherited form caused by a 1.4-Mb tandem duplication in chromosome 17p12. In 1997 the first annual German external quality assessment (EQA) was performed for CMT1A. At this time the molecular genetic analysis was mainly based on 5 methods: RFLP Southern blotting, STR markers, PCR for junction fragments within the CMT1A-REP elements, PFGE and FISH. 10 laboratories in Germany participated. In the year 2000 the scheme was offered to a wider audience with 22 participating laboratories. Only one genotyping error occurred. Since 2008 the European Molecular Genetics Quality Network (EMQN) has organised the EQA scheme. In 2012 the number of participating laboratories increased to 66 from 22 countries (198 reports) representing countries from around the globe.
Methods like FISH, PFGE and Southern blotting have disapeared; PCR, qPCR and MLPA are now used. Next generation sequencing (NGS) allowing simultaneous sequence analysis as well as gene dosage determination is a future perspective.
Currently at least 60 genes are associated with disorders of the peripheral nervous system. Consequently the scheme scope has been extended to sequence analysis of GJB1 (CX32) (CMTX1). In 2012 again only 1 genotyping error occured. The efforts that have been made for an EQA in molecular genetic diagnosis for Charcot-Marie-Tooth disease demonstrate very good laboratory analytical performance. Nevertheless national laws affecting human genetics are different, thus harmonization in political terms (e. g. predictive testing) remains as important task.

P14.17-S
Comparative-High Resolution Melting - a novel method of simultaneous screening for small mutations and copy number variations

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Efficient and cost-effective screening for DNA sequence changes, both small mutations and copy number variations (CNVs), is a crucial aspect for routine genetic diagnostics as well as for basic research. In this study we present a development and evaluation of comparative high resolution melting (C-HRM), a new approach for the simultaneous screening of small DNA changes and gene CNVs. In contrast to other methods, relative quantification in C-HRM is based on the results obtained during the melting process and calculations of the melting peak height ratio in the multiplex reaction. Validation of the method was conducted on DNA samples from 50 individuals from Duchenne muscular dystrophy (DMD) families, 50 probands diagnosed with familial adenomatous polyposis and a control group of 36 women and 36 men. The results of analyses conducted on fragments of the DMD and APC genes correspond completely (100%) with the results of previous studies. C-HRM sensitivity in CNV detection was assessed through the analysis of mixed DNA samples with different proportions of a deletion carrier and wild type control. The results are presented as a linear regression with R2 of 0.9974 and imply the capability of the method to detect mosaics. C-HRM is an attractive and powerful alternative to other methods of point mutations and CNV detection with 100% accuracy in our studied group.

P14.18-M
New molecular technique to monitor minimal residual diseased in CML

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by the Philadelphia chromosome (Ph), resulting from the t(9;22)(q34;q11) balanced reciprocal translocation that generates the BCR-ABL1 fusion protein. The first line therapy of CML is Imatinib Mesylate, which targets BCR-ABL1 protein, inhibiting proliferation pathway. Residual leukemia is assessed by a sensitive molecular quantitative manner evaluating levels of BCR-ABL1 transcripts by real-time reverse transcriptase PCR (qRT-PCR). Undetectable levels of chimeric transcript, however, can reflect either an effective elimination of leukemia cells, or the presence of a quiescent leukemic stem cells transcriptionally silent. We developed a novel highly sensitive method to identify quiescent leukemic cells and possible candidates for discontinuation of therapy through quantitative real-time PCR (Q-PCR) based on the DNA. Detection of BCR-ABL1 fusion gene is critical for the diagnosis of chronic myeloid leukemia and to follow the disease progress in patients under therapy. We applied targeted next-generation sequencing (NGS) to resolve sequence breakpoints in BCR-ABL1 fusion gene in CML patients and K562 cell line. In conclusion, DNA genomic Q-PCR is a very sensitive and direct technique to identify quiescent leukemic cells and patients that could be possible candidates to stop Imatinib therapy. We identified junction sequences in all samples using Agilent SureSelect enrichment and Illumina paired-end sequencing.

P14.19-S
Rapid Serial PCR Instrument with High Speed Melting (HSM) Analysis

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We have developed a prototype instrument capable of rapid serial PCR/HSM, enabling multiple targets to be tested sequentially. The current system processes eight samples simultaneously and has a liquid handling system that delivers PCR reagents to a cartridge with microfluidic channels used for rapid PCR and HSM. A unique feature is that once PCR/HSM is completed (<12min per test), reagents for the next assay are then delivered, tested and analyzed.
We tested 100 blinded clinical blood samples (obtained from ARUP Laboratories) for *F2* (c.*97G>A), *F5* (c.1601G>A), and *MTHFR* (c.665C>T and 1286A>C) genotype using this instrument. The samples were also tested on a LightScanner®32 (Biofire™) using high resolution melting analysis (HRMA) assays designed by the University of Utah. Data from both instruments was interpreted using custom Melting Wizard software. Results show that all four tests with all 100 samples were genotyped accurately. Follow-up testing was required on 1.4% of the assays for a definitive genotype.
The prototype instrument described allows for extremely rapid PCR (each thermal cycle < 15 seconds) and HSM (melting rate up to 2°C/s) due to unique design of the microfluidic chip. The onboard liquid handling system allows multiple tests to be performed sequentially, and adding tests during the run. This method facilitates reflex testing, such as exon scanning followed by a reflexive genotyping assay, or a second reflexive genotyping assay. These features are better aligned with a workflow that requires multiple tests run on a single sample adding flexibility compared to current batch PCR workflows.

P14.20-M
Challenges in the interpretation of variants identified in autosomal dominant familial nonsyndromic congenital heart defects by targeted next-generation sequencing

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High throughput sequencing technologies enable efficient large-scale genetic analysis, dramatically accelerating the genetic research and diagnostics. Our study aims to determine the proportion of familial nonsyndromic congenital heart defects (CHD) that can be explained by pathogenic mutations in currently known CHD associated genes, by implementing next-generation sequencing technologies in genetic diagnosis for familial nonsyndromic CHD. Targeted resequencing of known cardiac genes was performed in 36 patients from 13 nonsyndromic CHD families, using either array-based or solution-based method to capture the coding regions of 57 genes associated with CHD. Following variant analysis and Sanger validation, we identified 6 functional deleterious mutations in 3 genes, explaining the defects in 6 families. The genetic heterogeneity of CHD and remarkable variability of expression make it challenging to interpret the large number of identified variants in patients. Thus, setting up a well-defined analysis pipeline is necessary to identify causative mutations. In conclusion, by targeted resequencing of known CHD associated genes in well selected nonsyndromic CHD families and cautious variant interpretation, likely causative mutations were identified in 6/13 (46%) families. Reduced penetrance and possibility of phenocopies complicate the interpretation. Targeted next-generation sequencing is a powerful tool in genetic testing of familial nonsyndromic CHD, however, variant interpretation remains a major challenge for the diagnostic application.

P14.21-S
Whole exome sequencing in congenital myopathies: two case reports

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Congenital myopathies are a heterogeneous group of genetic diseases characterized by hypotonia and muscle weakness. Many causative genes have been identified in the last decade, underlining the high genetic heterogeneity in these pathologies. Sanger molecular diagnosis is an expensive and time consuming step-by-step process. In addition some genes involved are very large and high demanding in terms of molecular diagnostics. Here we report on two "families-of-four" with congenital myopathy in which we performed whole exome sequencing approach (WES). The analysis was performed by deCODE Genetics and we analyzed the families applying candidate genes interrogation and autosomal recessive inheritance; identified variations were prioritized for the pathogenic prediction and the rare allele frequency under 1% of the population.
In the first family, with one affected child, we found a known pathogenic in-frame deletion of the isoprenoid synthase domain-containing protein gene (ISPD). Mutations in this gene are known to be associated to congenital muscular dystrophy phenotypes. In the other family, with two affected sibs, we identified a mutation in RYR1 gene. In both families a previous partial molecular diagnosis by Sanger, and not exploring all exons, was performed.
In these two cases WES identified mutations in two known genes "correcting" a non-exhaustive diagnostic approach. This is an example confirming the usefulness of next generation sequencing as an accurate molecular diagnostic approach for pathologies with high genetic heterogeneity and/or large genes.
The Neuromics EU project is acknowledged.

P14.22-M
Detection of Copy Number Variation by PCR under Limiting Conditions

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DNA copy number variation (CNV) is associated with genetic disorders, chromosomal rearrangements, and cancer. We present a simple closed-tube method based on high-resolution melting that is accurate enough to reliably determine all common CNV ratios. Target and reference regions of genomic DNA were PCR amplified together using a variety of reagent and cycling protocols. We found that the ratios of reference and copy number variants were maintained either by limiting PCR cycles, or by limiting quantities of dNTPs or Taq polymerase and allowing PCR to plateau. High-resolution melting analysis with the DNA binding dye LCGreen Plus was used to determine CNV ratios from the relative heights of the target melting peak after reference melting peaks were equalized. All common CNV ratios may be detected using any of the PCR limiting protocols. The most robust and simplest method uses limiting dNTP concentrations. CNV ratios differing by 10% could be distinguished when dNTPs were limited to 3.25 uM. Trisomy was easily and accurately typed using duplex PCR, and chromosome X and Y copy number variants were identified using triplex PCR. The method also identified large CFTR exon 2 and exon 3 heterozygous deletions that are difficult to detect using other approaches. This protocol is simple, closed-tube, fast, economical, and is more accurate and less susceptible to contamination than other methods. Assays are easy to design, limiting dNTPs is simple, and the results are stable and reliable. It may also have application to the analysis of gene expression via relative template quantification.

P14.23-S
Study of the molecular basis of cystic fibrosis by Next-Generation Sequencing

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Despite extensive genetic screening, 1-5% of cystic fibrosis (CF) patients still lack a definite molecular diagnosis. Today, next-generation sequencing (NGS), combined with target enrichment and multiplexing, is making increasingly affordable a sequencing-based approach to mutation detection in extended genomic regions. In this frame, we analyzed 24 selected CF patients by full CFTR gene resequencing (8 with known CFTR genotype, 11 with only one mutation identified, and 5 with no mutations after conventional screening). A custom capture SeqCapEZ Library (Roche) and an HiSeq 2000 platform (Illumina) were used. Multiplexing 6 samples in the capture phase and 12 in the sequencing step, we obtained a mean depth >1,000X, with a 98% coverage. An in-house developed pipeline was used for variant detection and annotation, which allowed the identification of all 14 previously known mutations. Moreover, 4 genetic lesions undetected by genetic screenings were found, including a large heterozygous deletion. Additionally, we found new intronic variants, whose possible role on RNA splicing was excluded by a combination of in-vivo and in-vitro analyses. In conclusion, NGS increased the percentage of genetically diagnosed patients, being able to disclose mutations escaped to "standard" mutational screening. Moreover, read alignment also allowed an immediate definition of large deletion breakpoints at the nucleotide level. Finally, even after this extensive sequencing strategy, 5 probands did not show any mutation in CFTR (nor in the SCNN1A, SCNN1B, and SCNN1G coding regions), suggesting the intriguing possibility of an additional locus for CF. These subjects are currently being processed for whole-exome sequencing analysis.

P14.24-M
Performance of the Luminex xTAG Cystic Fibrosis kit (EU)

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The xTAG® Cystic Fibrosis Kit (EU) [herein termed xTAG CFE] is a qualitative genotyping device used to simultaneously detect and identify a panel of mutations and variants in the Cystic Fibrosis transmembrane conductance regulator (CFTR) gene in human blood specimens and blood spots. The assay identifies the recommended ACMG/ACOG mutations and variations, as well as some of the world's most prevalent mutations. Additionally, xTAG CFE identifies mutations specific to Europe, such as 4016insT, L1065P and L1077P in the French and Italian populations, 2184insA in Central Europe, T338I in the Italian (Sardinia) population, 712-1G>T in the Spanish population, and E585X, R1066H, Q552X and G1244E in Southern Europe. DNA samples can be screened for 5 variants and up to 75 CFTR mutations, out of a total pool of 85 available mutations, allowing the user to customize CF testing to a particular region. The assay is comprised of a single multiplex polymerase chain reaction which is then used in three separate Allele Specific Primer Extension (ASPE) reactions (A, B, and C). The limit of detection of the assay is 2 ng/µL with an input genomic DNA range of 10 ng to 1.5 µg. Diagnostic accuracy was assessed for the xTAG CFE assay using the xTAG Cystic Fibrosis and bidirectional dideoxy-sequencing as comparator methods. Over four hundred samples were analyzed that included genomic DNA extracted from whole-blood specimens, dried bloodspots and Coriell samples. Sensitivity and specificity for the xTAG CFE assay across all mutations was 100%.

P14.25-S
Missing the forest for the trees: integrating traditional and molecular cytogenetics technology in hematological neoplasia

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This retrospective study investigates appropriate utilization of technology for cost effectiveness without compromising patient care in hematological malignancies. Bone marrow aspirates from 409 cases submitted for cytogenetics workup of myeloid disease were reviewed. 163 (40%: median age 60 years; M/F=1.6) had a combination of cytogenetics, FISH and SNP array testing. Based on morphology, they were classified as non-diagnostic (60), MDS (55), MPN (9), MDS/MPN (13) and AML (26). The mean turnaround time (day) was 17.4 (range 6-24) for cytogenetics, 7.0 (4-13) for FISH, and 15.0 (7-29) for array. Overall discrepancy between cytogenetics and FISH results was 14.1% (abnormal(+) cytogenetics/normal(-) FISH in 14/99 cases; P<0.0001). Discrepancy between cytogenetics and array results was 32.3% (+cytogenetics/-array in 8/65 cases; -cytogenetics/+array in 10/65 cases; +cytogenetics/+array in 3/65 cases; P=0.0020). There was 96% concordance for clinically relevant copy number changes between cytogenetics and array. Array detected cryptic deletion in 4% not seen by cytogenetics. However, low-level mosaicism for copy number changes was missed by array in 11% and 13% showed LOH/-cytogenetics; of these 5% were clinically

relevant. Therapy was initiated before release of results in 27%, after cytogenetics in 9%, after FISH in 3%, after array in 1% and supportive care in 60%. The preliminary data suggests a tier testing approach does not compromise patient care if tier testing is completed within 3-4 weeks of initial diagnosis. In presence of unfavorable prognostic abnormalities by cytogenetics, array testing may not be warranted. Based on this data, we would like to propose the optimal genetic testing algorithm.

P14.26-M
Real-time and Droplet PCR quantification for non-invasive determination of RHD incompatibility between mother and foetus

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The aim of this study was to compare two strategies of DNA quantification: Real-Time PCR (qPCR) and Droplet PCR (dPCR). The benefit of dPCR against qPCR is represented by absolute quantification of target nucleic acid molecules without the requirement of calibration curves. The methods were compared by quantification of standard nuclear DNA of known concentration. DNA was eightfold diluted (2-0.015 ng/ul) and twelve replicates were realized for each dilution. Concentrations were then measured as levels of amplicons of two genes, housekeeping gene GAPDH and human RHD gene (exon 10). The same genes were analyzed in plasma cell-free DNA and also in cell-free fetal DNA isolated from maternal plasma. Evaluation of these two methods' performance was based on several parameters, such as degree of linearity (R^2), accuracy, detection limit, etc. In case of standard nuclear DNA, both methods showed high accuracy and low detection limit, both genes were detected even in most diluted samples. High linearity degree (R^2 > 0.99) has been reached by both methods. The correct RHD status of tested women was immunologically verified. Regarding analysis of cell-free fetal DNA in RHD negative maternal plasma, dPCR failed against the qPCR to convincingly distinguish RHD positive samples from the negative ones. For the purpose of prenatal screening, the qPCR method would be probably the preferable alternative due to very low fetal DNA concentrations (about 0.002 ng/ul) in maternal plasma, which is under the detection limit of dPCR.
Supported by the Ministry of Health of the Czech Republic RVO VFN64165

P14.27-S
Development and evaluation of a gene panel for the diagnosis of monogenic epilepsies

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Next Generation Sequencing (NGS) is a great advance in the field of molecular genetics, especially for diseases with high genetic heterogeneity, allowing parallel testing of many disease-causing genes. It will lead to a better access of a larger number of patients to molecular diagnosis. In order to improve the diagnostic yield of genetic testing in patients with epileptic diseases, we developed a panel including 41 genes causing monogenic form of epilepsy or neurodevelopmental disorders frequently associated with seizures. Until now, only 13 of these genes have been currently screened by Sanger sequencing, on a sequentially basis. In the present study we compared two methods: the Haloplex technology (Agilent) and the SeqCap (Roche) technology. Sequencing was performed using an Ion Torrent PGM sequencer (Life Technologies). We performed NGS sequencing on DNA samples from 44 patients, including 38 patients in whom a (probably) causative mutation had been previously found by sequential Sanger sequencing in our routine practice, and 6 novel patients who had not been screened. DNA samples were anonymized and the first steps of the analysis were performed following a blind protocol. DNA from all the patients was analyzed with the Haloplex technology and only 24 of them were also analyzed with the SeqCap technology (they were sorted among the 44 patients). Data analysis will be performed with the NextGene Software (Softgenetics) and also with specific BWA-GATK pipeline based on the recommendations of BroadInsitute. Our analysis will include coverage, sensitivity and false positive rate as well as a cost evaluation.

P14.28-M
Scaling up whole-exome sequencing on Ion Proton using AmpliSeq

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The Ion Proton[a] and Ion PGM[a] (Life Technologies) technologies are used in the National Genomics Infrastructure - Sweden (NGI) to handle the different requests and needs for rapid massively parallel sequencing, (MPS).
Ion Proton[a] enables a rapid workflow for human whole exome sequencing. The PCR based whole exome capture (Ion Ampliseq Exome Kit) provides consistent high coverage across annotated exonic regions, with the PI[a] chip yielding 50-60.000 SNPs per sample, with ~98% of these overlapping variants reported in dbSNP. Sample preparation and sequencing is performed in less than two days. We are exploring the possibility to scale-up the throughput by loading two PI chips on one initialization and to combine 3 exomes per PI chip. With this set up it will be possible sequence up to 30 exomes per week on one Ion Proton[a].
The bioinformatics analysis has been streamlined using an in-house database system, based on R and MySQL, where all detected variants from all in house exome-sequencing runs are stored. This system allows for very efficient and fast filtering of SNPs or indels between any groups of samples and we currently have a success rate of almost 80% of finding disease-causing variants in small families or trios with rare Mendelian disorders.
Ion Proton[a] is also being used for clinical applications such as identification of fusion transcripts from cancer samples, mutation screening using panels of candidate genes. The sample preparation will be simplified and faster when the automated system, Ion Chef[a] is introduced and established in the workflow.

P14.29-S
Evaluation of three sequence capture platforms for whole exome sequencing

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Whole exome sequencing (WES) can be effective for identifying sequence variants. Here, we present a comprehensive comparison of the most recent next generation sequencing (NGS) exome enrichment methods of Agilent (SureSelect V5+UTR), NimbleGen (SeqCap V3+UTR), and Illumina (Nextera Expanded Exome). Exomes of six human DNAs were captured by these methods and sequenced at 100x depth of coverage on an Illumina HiSeq 2000 platform by four vendors. Read depth, % of coverage, GC bias, and number of detected single nucleotide variations (SNVs) and small indels were compared. To examine the methods' ability to identify SNVs and small indels, we analyzed heterozygous positions and small indels previously detected by Sanger sequencing (SS). For two DNAs, WES data were also compared to whole genome sequencing (WGS). SureSelect and NimbleGen demonstrated highest average read depth in target region. Considering ≥20x for read depth, SureSelect covered the largest proportion of its targeted bases. SureSelect and NimbleGen showed the highest average read depth as well as detected the highest number of SNVs and small indels in RefSeq. However, all SNVs identified by SS were accurately called by all three methods. Less consistent was the detection of small indels, where a substantial difference among the platforms and vendors was observed. In addition, all three methods showed high GC bias which was not seen in WGS. Our analysis indicated a considerable variability among exome enrichment methods, DNA sequencing laboratories, and even among DNA samples. Our data revealed that both SureSelect and NimbleGen performed better than Nextera.

P14.30-S
The importance of root cause analysis following an external quality assessment (EQA) to improve the quality and accuracy of a diagnostic service

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EQA is essential to verify the quality and accuracy of the diagnostic service. When new technologies emerge, EQAs need to be developed to verify the laboratory diagnostic validation process and enable benchmarking of their performance. When laboratories introduce new technologies diagnostically, most critical errors in EQAs are analytical. As laboratories gain more experience in the new technologies, the critical errors usually become limited to the interpretation of results.

EQA is intended to be educational so if a laboratory receives a poor performance, they are asked to review their results and perform an audit so they can identify the root cause of the error. If required, repeat EQA samples are made available to exclude the rare instances of a problem with a manufacturer's kit. In 2013, a thorough root cause analysis by one participating laboratory identified a potential problem with one manufacturer's probe. An independent verification by CEQAS using multiple FISH probes and arrays confirmed a 967kb deletion of 13q14.2q14.3. There is a discordant result between the microarray and FISH probes for one of the deletion breakpoints. As a consequence the manufacturer immediately withdrew the probe and replaced it with a new product to eliminate the likelihood of future false negative results. A second laboratory identified an internal problem with their fluorescent microscope filters which they replaced and then verified gave the correct results using validated EQA material.
This presentation will give examples of how different approaches to investigating a poor performance can result in different outcomes including changes to practice.

P14.31-S
Identification of point mutations and deletions in Fanconi anemia using a next generation sequencing approach

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Fanconi Anemia (FA) is a bone marrow failure disorder characterized by high clinical and genetic heterogeneity (at least 16 genes), which makes diagnosis complex and time-consuming. Next-generation sequencing technologies, such as the Ion PGM™ System (IPGM; Life Technologies), could improve the molecular genetic testing in FA. To test IPGM, we sequenced 30 DNA samples: 2 from controls and 28 from FA patients, whose mutations were previously identified by Sanger sequencing in 18 of them. According to the Ampliseq Designer software, the molecular target has a size of 74.2 kb covering 96% of the FA coding exons and their flanking regions. After exclusion of one sample for low coverage, we found that the coverage was higher than expected (>100X in 97% of reads per run) except for one sample that was excluded from the analysis. Then, comparing the IPGM and Sanger sequencing data, we asses sensitivity (100%), specificity (99%) and accuracy (100%). In addition to confirming all the mutations (31 alleles) identified by Sanger sequencing, this study allowed us to characterize another 18 out of the 54 FA alleles in our cohort. The remaining 5 are likely to be localized in genomic region not covered by the sequencing analyses. Moreover, quantitative analysis aimed at detecting copy number variations (CNVs) detected complete (n=1) and partial (n=7) deletions of FANCA, or entire removal (n=1) of FANCD2.All the CNVs were confirmed by SNP array or MLPA analysis. Our data suggest that IPGM is suitable for detection of point mutations and large deletion in FA.

P14.32-M
Detection of an extra fragment by PCR amplification of the FMR1 triplet repeat region in normal range alleles: can it be somatic instability?

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Genetic testing of Fragile X disorders is now performed with PCR-based methods which sensitivity and precision allow detection and accurate sizing of FMR1 alleles in the entire spectrum of the CGG repeat amplifications. These molecular tools, designed to analyze a region of DNA with intrinsic characteristics of instability, could lead to new observations that were out of reach of previous methods. We report the detection of a smaller and less represented fragment in PCR amplifications of the FMR1 repeat region in independent samples of unrelated individuals, three males with intellectual disability and one normal female, with otherwise normal Fragile X alleles and karyotype. A smaller fragment present in PCR reactions from different DNA extractions and samples of each identified individual, has been detected by three different Italian laboratories with different settings (primers, amplification, electrophoresis). In one of the cases both parents of the proband resulted to not carry the smaller fragment, thereby excluding a rare X-chromosome or autosomal polymorphism as a possible cause. The possibility of random somatic instability as a cause of such finding in our cases is challenged by the similar size (286-293 bp) and quantitative representation of the peak. The fact that the same result has been found in four unrelated subjects (three Italian and one Israeli) out of 2000 tested, suggests a frequency of about 1/500 individuals. Further characterization of the extra peak is underway to establish its sequence origin and better understand the nature of what could give new information on the Fragile X region.

P14.33-S
Development of a Next-Generation-Sequencing based framework for comprehensive genetic analysis of Neurogenetic disorders

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With the advance of sequencing technologies, the capacity to generate accurate genotype data greatly outpaces our ability to analyse it. To facilitate data intrepretation, a framework were developed for estimating the relative pathogenicity of variants. We used Tuberous Sclerosis, an autosomal dominant Neurogenetic disorder caused by mutations in either TSC1 or TSC2, to test our famework. As all kinds of mutations, including single nucleotide variants (SNVs), small insertions/deletions (indels), and large deletion/duplication mutations have been reproted in TSC1 and TSC2 scattering almost all along the 65 exons of the two genes without any obvious hotspot, the Neurogenetic disorders panel including 399 genes, in addition to TSC1 and TSC2, was used for the genetic analysis of 276 referred TSC, or probably-TSC patients. All identified variants of potential clinical interest were verified by Sanger sequencing or MLPA in probands and family members whenever available. 137 patients (49.6%) were found to carry known disease-causing mutations, 98 (35.5%) patients carried 93 unique highly-likely pathogenic variants. Accumulatively, this assay were able to identify the causative mutation in 85.1% (237/276) TSC patients. Of note, 23 large structural variants and 2 cases of somatic mosaicism were identified in our patients which might otherwise be neglected in sanger-sequencing based genetic testing. In conclusion, our framework can be readily used to analyze the gene variant profiles of TSC with high sensitivity, fidelity, throughput and speed. This framework lay the basis for the genetic testing of other complex Neurogenetic disorders.

P14.34-M
EGFR screening using Gold nanoprobes

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Targeted chemotherapy directed at epidermal growth factor receptor for treatment of non-small cell lung carcinoma patients has been put forward as an alternative to standard chemotherapy. However, the application of EGFR inhibitors therapy is only effective in cancers with mutated and overactive EGFR, requiring preliminary screening to detect EGFR mutations.
Typically, diagnostics procedures examine the profile of genetic alterations in tumours removed for biopsy in search for mutations that make them susceptible to treatment. Usually, DNA mutations are detected by PCR and confirmed by sequencing, which are quite expensive and time-consuming. Thus, the trend in molecular analysis is directed at increasing sensitivity rather the multi-loci analysis. Therefore, multiplexing and multiparallel strategies may become an alternative to directed analysis of whole spectra of mutations.
The LungCARD project aims the development and optimisation of a low cost microfluidic chip to assess EGFR mutations directly from immune-captured circulating tumour cells (CTCs). Here, we present the optimisation of a protocol for PCR amplification of EGFR exon 19 and 21 directly from single cells and the subsequent identification of mutations present via a gold nanoparticle based assay. This colorimetric assay is based on the non-cross-linking approach using a set of gold nanoprobes (thiol-functionalised gold nanoparticles) that are capable to specifically detect the EGFR variants. Furthermore, preliminary results from Ferguson plot analysis revealed that wild-type and mutated samples present distinct rfs and can be discriminated. The assay is optimised for reduced reaction volumes and short time, in order to be compatible with a microfluidic device.

P14.35-S
Development of a next generation sequencing panel to assess hereditary cancer risk that includes clinical diagnostic analysis of the BRCA1 and BRCA2 genes

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Assessment of hereditary breast and ovarian cancer risk should include germline sequencing of BRCA1 and BRCA2 as well as additional genes with known associations in breast/ovarian cancer patients. Sanger DNA sequencing has been the gold standard for molecular genetic analysis but next generation sequencing (NGS) platforms could provide another sequencing alternative. However, optimized assay design and validation are critical to maximize the analytical sensitivity and specificity of NGS assays and to ensure high quality interpretation for clinical decision making. We developed a 25-gene NGS hereditary cancer panel that uses RainDance PCR technology for high-throughput sample preparation, Illumina HiSeq and MiSeq NGS technologies, and commercially available and lab-developed informatic tools. Initial assessment of analytical sensitivity and specificity was performed by comparing BRCA1 and BRCA2. NGS was performed on 1864 anonymized patient samples, which had previously undergone BRCA1 and BRCA2 Sanger sequencing. Sanger sequencing identified 15,878 variants, of which 681 were unique and 482 were classified as disease-associated mutations. We identified 15,877 variants at an initial sensitivity of >99.99% for BRCA1 and BRCA2. One polymorphic variant was missed due to a variant under the primer. Sensitivity was subsequently optimized through process improvements. No additional variants were found by NGS, yielding a specificity of 100%. This preliminary analysis facilitated assay optimization and validation of all 25 genes in the NGS panel. This analysis indicates that a NGS gene panel designed to meet rigorous quality standards can be used to provide clinical sequencing results equivalent to those obtained from Sanger DNA sequencing analysis.

P14.36-M
In silico analysis of the effect on splicing of single nucleotide changes at exon-intron junctions is predictive of pathogenicity. A study on *MYBPC3* mutations.

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Hypertrophic cardiomyopathy (HCM) is one of the most common inherited cardiovascular disorder with an estimated prevalence of 1/500. About half of the cases are familial and mutations in *MYBPC3* are among the most frequent cause of HCM.
Here we report on 14 patients with HCM in which single nucleotide substitutions potentially affect splicing of the MYBPC3 gene. Twelve mutations are located either on the last nucleotide of an exon or within the consensus 3'- and 5'- splice site sequences, while two are situated outside these regions. Mutations at the exon-intron junctions can potentially affect splicing. However, only changes affecting the invariant AG-GT nucleotides are considered to be certainly pathogenic, while the rest require functional studies. Mutations were first analysed *in silico,* using a combination of 5 splice site prediction algorithms, integrated in the Alamut 2.0 Splicing prediction module. For 12 mutations splicing prediction analysis indicated an effect on splicing and for 2 there were no alterations. Subsequently, all mutations were analysed by cDNA sequence analysis and these results were compared to the prediction models. In all patients the results of sequence analysis were consistent with the predicted effect, namely exon skipping, intron inclusion, partial exon deletions and insertions of intronic sequences or no altered splicing at all. In conclusion, this study indicates that *in silico* splicing analysis can accurately and reliably predict the effect on splicing of mutations occurring at exon/intron junctions representing a helpful tool in DNA diagnostics for the assessment of the pathogenicity of a sequence variant.

P14.37-S
Genetic diagnosis of familial colorectal cancer: Detection of large rearrangements in the MMR genes by molecular combing

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Hereditary Non Polyposis Hereditary Colorectal Cancer (HNPCC) or Lynch syndrome is an autosomal dominantly inherited cancer susceptibility caused by germline mutations in one of the DNA mismatch repair (MMR) genes (MSH2, MLH1, PMS2 and MSH6). Although most of the mutations reported in these genes are point mutations, large genomic rearrangements in one of the MMR genes occur with a frequency varying from 5 to 20% depending on the population. Effective methods to identify these types of mutation should be integrated in current diagnostic procedure to obtain a more comprehensive genetic screening strategy. Molecular Combing is a powerful FISH-based technique for the direct visualization of single DNA molecules which allows exploration of the genome at high resolution in a single analysis. We are developing a novel HNPCC genetic test based on molecular combing, for which specific "genomic Morse codes" (GMC) have been designed for each MMR genes. These GMC have been validated on combed genomic DNA extracted from the cancer-derived cell lines LoVo and Sk-OV-3, which harbor MSH2 and MLH1 large deletion respectively, and from HNPCC patients. From these patients, large rearrangements corresponding to either deletions of one to several exons in one of the MMR genes and with sizes ranging from 4 kb to 53 kb or the paracentric inversion in the MSH2 gene have been detected. Importantly, the mutations identified by Molecular Combing confirm the results previously obtained by Southern Blot analysis on the same patients, with a resolution in the 1-2 kb range.

P14.38-M
When an allele drops out of amplification: whole exome sequencing unmasks a case of false-negative genetic diagnosis

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Introduction: Sudden cardiac death without an attributable cause after clinical diagnostic work-up, is defined as idiopathic ventricular fibrillation (IVF). Mutations in genes primarily involved in inherited arrhythmia syndromes underlie some cases of IVF.
Methods: An IVF patient underwent routine mutational analysis of 6 arrhythmia-susceptibility genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2* and *RYR2*) through direct sequencing. Afterwards, exome sequencing (Agilent SureSelect) was performed on the proband and his non-affected parents on an Illumina HiSeq2000 (minimum coverage 20x for 90% of bases, genotype quality score≥40). Identified variants were subjected to a 3-step filtering strategy (*de novo*, nonsynonymous, absent from dbSNP-1000Genomes).
Results: Traditional mutation scanning failed to identify a pathogenic variant. When exome sequencing was performed, a *RYR2* candidate variant was identified (c.12006G>T, p.M4002I) that however could not be validated by Sanger sequencing. This prompted further investigation and all factors contributing to either a stochastic sampling or a systematic error were evaluated. Previously published primer sequences were inspected. A SNP near the primer's 3' site (rs790889, MAF=0.426) was identified and hypothesized to cause the mutant allele to drop out. Targeted screening with redesigned primers confirmed the presence of the variant.
Conclusion: In routine diagnostics often laboratories use screening conditions reported through literature dated at a time when genetic variation information was not widely available. Allelic dropout or, more precisely, the occurrence of a null/false allele due to primer-target mismatch, constitutes an avoidable laboratory error. Our report highlights the need to periodically reevaluate even previously well established screening conditions.

P14.39-S
Pyrosequencing Assay Panel For Imatinib Resistance Mutations

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Background: Chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia (Ph) chromosome. Ph chromosome results from the translocation of *ABL* protoonkogene from chromosome 9 and *BCR* gene on chromosome 22, which leads to the formation of BCR-ABL chimeric gene with constitutive tyrosine kinase activity. Imatinib (Gleevec) is the first BCR-ABL tyrosine kinase inhibitor used in the treatment of CML. Second generation drugs such as nilotinib and dasatinib, are now considered as alternative treatments to imatinib. Point mutations the kinase domain of the BCR-ABL were detected from 40% to 90% of the Gleevec-resistance cases. These mutations disrupt the binding site of imatinib on the tyrosine kinase, resulting in a loss of sensitivity to the drugs. **Method:** We performed a pyrosequencing assay panel for the detection of eleven important mutations in BCR-ABL kinase domain, which are Y253H, Y253F, E255K, E255V, V299L, T315A, T315I, F317L, F317V, F359C, F359V. Plasmids coding for respective vari-

ants were used to study assay performance and precision. Totally 25 RNA samples of BCR-ABL positive patients were analyzed by pyrosequencing and samples, which had mutation were also analyzed with sanger sequencing. **Results:** Our assay allows flexible, fast and reliable detection of mutations in cDNA from both blood and bone marrow with high concordance to Sanger sequencing. **Conclusion:** Our pyrosequencing assay for imatinib-resistant mutations provides a reliable method for the detection of the mutant BCR-ABL transcripts and also provides a significant value for the follow up of CML patients on imatinib.

P14.40-M
Next generation tissue profiling

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Cancer develops as a consequence of genetic mutations, which results in deregulation of gene expression and/or proteins with aberrant functions. These changes often interfere with cellular processes such as survival and proliferation. The cancer cells evolve and are subjected to natural selection through interplay with its microenvironment. As is the case with other ecosystems, also the tissue microenvironment changes and responds to the populations in its niche and to external interference. Communication with other cells in the microenvironment will provide input signals that are interpreted by the malignant cells, and responded to, based on their altered genetic programs. Hence, the consequence of a mutation has to be viewed in the environmental context of each individual cell. The activity status of a protein or signaling pathway can be visualized with in situ Proximity Ligation Assays (in situ PLA) using a pair of antibodies equipped with DNA oligonucleotides (proximity probes) to target interacting proteins. Proximal binding of such probes template the creation of a circular DNA molecule, which is a surrogate marker for the interaction. We recently developed a multiplexed version of in situ PLA by introducing unique tags as identifiers in each different proximity probe. The combinatorial events generating an in situ PLA signal will harbor a set of identifier tags that will be unique for each protein interaction. By combining in situ PLA with padlock probes, analysis of signaling activity can be achieved together with genotyping expressed mRNA in fixed tissue sections, retaining the architectural information while providing single-molecule resolution.

P14.41-S
Quantification of donor/recipient chimerism in leukemia samples by digital PCR

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During leukemia treatment mixed chimerism occurs in which both recipient and donor cells are present in the bone marrow or peripheral blood after transplantation. Chimerism analysis is performed to monitor peripheral blood or bone marrow in the recipient after allogenic stem cell transplantation to monitor for leukemic relapse. Observation of increasing mixed chimerism after transplantation is associated with a higher risk of relapse in acute leukemia. Previously, a quantitative PCR (qPCR) technique, using insertion/deletion polymorphisms, was found to predict relapse in 88.2% vs. 44.4% of individuals analyzed by VNTR markers with a median anticipation period of 58 days and a sensitivity of 0.01% vs. 3%. Here we present results from research experiments performed to determine if a digital PCR (dPCR) method is able to predict relapse earlier and with greater accuracy than the qPCR method using retrospective leukemia samples. Research results showed that dPCR using the QuantStudio™ 3D Digital PCR System and the qPCR method yielded similar percent recipient chimerism values when recipient DNA was present above the 1% level. Furthermore, dPCR using the system was found to be more sensitive than the qPCR method based on the ability to detect the recipient DNA in a relapsed individual about 2 months earlier where the percent recipient chimerism was 0.2% or less. The false positive rate was close to the complete chimerism value of 0.01% for peripheral blood samples.

P14.42-M
Hotspot mutation and fusion transcript detection from the same non-small lung adenocarcinoma sample

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The presence of certain chromosomal rearrangements and the subsequent fusion gene derived from translocations has been implicated in a number of cancers. Hundreds of translocations have been described in the literature recently but the need to efficiently detect and further characterize these chromosomal translocations is growing exponentially. The two main methods to identify and monitor translocations, fluorescent *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) are challenging, labor intensive, the information obtained is limited, and sensitivity is rather low. Common sample types for these analyses are biopsies or small tumors, which are very limited in material making the downstream measurement of more than one analyte rather difficult; obtaining another biopsy, using a different section or splitting the sample can raise issues of tumor heterogeneity. The ability to study mutation status (DNA) as well as measuring fusion transcript expression (RNA) from the same sample is powerful because you're maximizing the information obtained from a single precious sample and eliminating any sample to sample variation. We isolated DNA and RNA from the same non-small lung adenocarcinoma sample without splitting or dividing the sample, and both mutation analysis, as well as fusion transcript detection was performed using the Ion Torrent PGM™ platform on the same Ion 318™ chip. Using 10ng of DNA and 10ng of RNA input, we applied the Ion AmpliSeq™ Colon and Lung Cancer panel to analyze over 500 COSMIC mutations in 22 genes and the Ion AmpliSeq™ RNA Lung Fusion panel to detect 40 different fusion transcripts.

P14.43-S
Development and verification of an Ion Ampliseq RNA gene fusion panel for lung cancer: an OncoNetwork collaborative research study

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Chromosomal translocations and corresponding gene fusions play an important role in carcinogenesis. The recent establishment of ALK, ROS1, RET and NTRK1 fusion transcripts as predictive biomarkers for lung cancer therapy has increased the need for a technology that could detect these biomarkers starting from very limited amounts of material. Here we describe the development and verification of an Ion AmpliSeq RNA fusion panel that can simultaneously detect ALK, RET, ROS1 and NTRK1 fusion transcripts in a single reaction. For the development of the panel we used previously characterized samples comprising 6 cancer cell lines and 62 FFPE lung tumor samples. Upon RNA isolation and amplification using the panel, samples were sequenced on the Ion PGM system. Initially, using serial dilutions of RNA from cell lines in normal RNA a limit of detection of 1% was established. Next, using the FFPE samples a fusion transcript was detected in 21 of the 24 known positive samples. Two of the non-concordant results were due to lack of representative tumor material. For the third, although no specific fusion transcript was identified, the internal control for ALK expression revealed the presence of an unknown ALK fusion transcript. Additionally, two new RET fusions were detected in samples not previously tested for RET translocations. These preliminary results are highly encouraging regarding the possibility of a reliable experimental protocol for the detection of fusion transcripts in the biological material routinely obtained for the diagnosis of lung cancer. An extensive verification is under way within the OncoNetwork.

P14.46-M
MicroRNA detection by real-time TaqMan® assays for translational research

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MicroRNAs (miRNAs) are small non-coding RNAs involved in the multilevel regulation of gene expression targeting a battery of mRNA genes. Researchers have discovered that miRNAs are efficacious biomarkers for the classification of tumors and prediction of outcome for many diseases because of their evolutionary conservation, unique expression signatures, relative stability, and abundance. There has been an increased interest in recent years for the identification of circulating miRNAs in serum, plasma, and other body fluids because it holds great promise for a non-invasive approach to molecular diagnostic and therapeutics. However, detection of miRNAs in these clinically relevant samples has been difficult, often requiring greater sensitivity. We have developed a new method for the detection and quantification of miRNAs that is highly specific and sensitive. The highly efficient

upstream chemistry allows synthesis of miRNA template library that is used in the downstream real-time TaqMan qPCR for miRNA-specific detection. The universality of template synthesis simplifies the workflow and provides the flexibility for scalable content (miRNA coverage). Whether applied to basic or translational research, profiling, screening, or validation, this new and robust method allows detection and quantification of miRNAs to address the unmet needs in workflow and sensitivity that exists today with next generation sequencing and other qPCR technologies, especially with clinical samples. Results show miRNA-specific amplification with a 7-log linear dynamic range and sensitivity of 60 copies input to meet the needs of translational research and clinical/diagnostic application.

P14.47-S
A new strategy for genetic testing of neurodegeneration with brain iron accumulation (NBIA) using amplicon multiplexing and next generation sequencing

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In the expanding field of neurodegeneration with brain iron accumulation (NBIA), the development of sensitive brain imaging played a major role to characterize iron-related damages in patients. In parallel, new genes have been identified allowing genotype-phenotype correlations. However, overlapping phenotypes and non-informative pedigrees are still sources of delayed diagnosis. For these reasons, it would be more rapid and cost-effective to propose a simultaneous analysis of the nine genes identified so far.
Here, we propose a Next Generation Sequencing solution, tested in our series. NBIA genes (ATP13A2, CP, C19orf12, DCAF17; FA2H, FTL, PANK2, PLA2G6 and WDR45) were included in a custom panel of 35 genes dedicated to our routine molecular diagnosis platform. Two librairies of amplicons were designed and built with the AmpliSeqTM technology. Sequencing was performed according to the semi-conductor technology on a PGM. The Torrent Suite (Life Technologies) and AlamutTM interface (Interactive Biosoftware) were used for the mapping of reads, the variant calling and the interpretation. Workflow analysis was validated on known FTL and PANK2 mutations previously identified using Sanger sequencing. DNA samples irrelevant for NBIA were used as control to avoid considering recurrent artefacts. In silico AmpliSeq design covered 99% of NBIA genes targeted regions, of which 98% were sequenced at least 40X. Preliminary results obtained from 3 patients showed a perfect match between NGS and Sanger sequencing results. Thirty patients with compatible symptoms and MRI signs of NBIA who were referred to our laboratory for genetic testing are currently screened.

P14.48-M
New generation sequencing dedicated to *CFTR* genotyping: comparison of two kits for multiplexed amplicons resequencing

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NGS is predicted to become the method of choice for fast screening of known mutations as well as resequencing of the *CFTR* gene. Our diagnosis laboratory, involved in cystic fibrosis testing, is currently developing this new strategy using a PGM (Life Technologies). The aim of this study was to define a NGS workflow for specific resequencing of the coding and flanking intron regions of the *CFTR* gene. We studied the feasability and analytical performance of two kits designed for amplicon library building and sequencing on PGM: the AmpliSeq™ CFTR kit from Life Technologies and the CFTR MASTR v2 assay (multiplicom). The latter was used according two different PGM protocols, 200 and 400 bp sequencing, due to the length of amplicons. These two kits and two protocols were compared using 20 patients DNA samples that have been previously sequenced by capillary electrophoresis using a 3500xL Dx Genetic Analyzer for CFTR genotyping (27 exons and exon-intron junctions). Gene rearrangements were searched using SALSA® MLPA® kit (MRC- Holland) or custom array CGH. Mutations were deletions, duplications, splicing variants and different combinations of T and TG tracts in intron 9. All expected mutations were identified by the two kits while it remained difficult to characterize intron 9 homopolymers. We discuss about the reliability of these protocols and of dedicated analysis pipeline in the context of cystic fibrosis diagnosis. Further improvements of software analysis pipelines would help to allow for automated CFTR mutation detection, particularly in repetitive and homopolymer sequence regions.

P14.49-S
The road to next generation molecular diagnostics, when NGS takes over Sanger sequencing

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In diagnostics, next generation sequencing (NGS) of fixed sequence panels of captured genes/exomes is being introduced. Here it was our aim to develop a flexible, cost-efficient and easily expandable NGS-based workflow.
For target enrichment, we opted for PCR. Primer design is performed using an in-house developed primer design software program (Primer XL), allowing uniform PCR conditions for >3000 amplicons of >200 different genes. The library preparation is done with a modified version of the Nextera XT Sample Prep (Illumina) protocol. Our approach allows pooling of up to 15000 amplicons from different genes and patients in a single MiSeq run (2x250bp), resulting in an average coverage of 500x, and guaranteeing a minimal coverage of >36x for 99% of the samples.
The sensitivity of the data analysis pipeline was evaluated with 387 different positive controls. For substitutions and indels < 15 bp, an overall sensitivity of 99,99% was obtained, comparable to Sanger sequencing. A lower sensitivity was observed for the identification of indels > 15 bp, a known shortcoming of short read sequencing. Detection of these seems dependent on the sequence context and can be performed with capillary electrophoresis. For analysis of the specificity, the variants detected in 61 patients in 8 different genes (n= 327) were investigated with Sanger sequencing. A positive predictive value of 98% was obtained.
This ISO15189 accredited flexible workflow allows a strong reduction of cost and turnaround time compared to Sanger sequencing. The hands-on time will be further reduced by automation of the upstream PCR set-up.

P14.50-M
Validation of a next generation sequencing approach for rapid and accurate CFTR mutations screening

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Cystic Fibrosis (CF) is one of the most frequent autosomal recessive disease among Caucasians (prevalence 1:2500); it depends on mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene that encodes a chloride exchanger. More than 1500 causative CFTR mutations have been identified, including missense, frameshift, splice site, nonsense and deletion mutations. Their accurate detection is important to improve the clinical management of CF patients and to correctly identify all CFTR carriers. Here, we report the use of a next generation sequencing screening for the identification of CFTR germline mutations. The study was performed on 60 subjects, including CF patients, CFTR carriers and controls, previously analyzed by traditional methods (reverse dot blot and Sanger sequencing). CFTR coding regions (including their flanking sites) were amplified using the CFTR MASTR Assay kit (Multiplicom) to obtain a library/sample. The subsequent sequencing reactions were performed with both the GS Junior System (Roche) and the MiSeq System (Illumina) allowing the simultaneous analysis respectively of 10 and 60 patients/run. Finally, the downstream data analysis was carried out through the SeqNext tool (JSI Medical Systems). All the features and performances of the Next Generation Sequencing technologies are discussed in the light of results obtained with the two used systems. Comparative sequence analysis highlighted that the proposed method is reliable, since all mutations previously identified were confirmed and the time of analysis is also markedly reduced. Our results assess the feasibility of a next generation sequencing approach for CFTR mutation detection to be included in a routine diagnostic workflow.

P14.51-S
Molecular analysis of mutations in the CFTR gene by Next Generation Sequencing

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Cystic fibrosis (CF) is the most common autosomal recessive disease in Cau-

casians, with an Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians, with an incidence of approximately 1 in 2500 and a carrier frequency of 1 in 25. The gene responsible for CF, named the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), encodes the cyclic adenosine monophosphate (cAMP)-dependent chloride channel found in the apical membrane of secretory epithelial cells. The known mutations of the CFTR gene are 1965 (Cystic Fibrosis Mutation Database). The massive sequencing for mutation analysis and copy number variations (CNVs) by Next Generation Sequencing (NGS) allows investigating at the second and third level with the scanning of all exons, the flanking regions and the search for deletions and/or insertions in a single diagnostic test.
To check the reliability of massive sequencing by NGS as many as 100 control cases provided by an European network, were analyzed. All of the mutations previously obtained by Sanger Sequencing were confirmed by NGS that was shown to be highly sensitivity and specific.
The massive sequencing offers the possibility to carry out a reliable and comprehensive analysis for all genetic variants in reduced time (16h) testing up to 20 cases-samples in a single run. The costs of genetic analysis are substantially decreased.

P14.52-M
Enabling high-throughput discovery of the RNA transcription landscape using a directional RNA workflow and a combinatorial multiplexing approach

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Massively parallel next generation cDNA sequencing (RNA-Seq), has allowed many advances in the characterization and quantification of transcriptomes, including the detection of non-canonical transcription start sites and termination sites, and identification of alternative splice isoforms, transcript mutations and edits. Additionally, the ability to obtain information on the originating strand is useful for reasons including for example: identification of antisense transcripts, determination of the transcribed strand of noncoding RNAs, and determination of expression levels of coding or noncoding overlapping transcripts. However, standard methods for sequencing RNA do not provide information on the DNA strand from which the RNA strand was transcribed, and methods for strand-specific library preparation can be inefficient and time-consuming. To address this challenge we developed a streamlined, low input method for Directional RNA-Sequencing that highly retains strand orientation information while maintaining even coverage of transcript expression. This method is based on second strand labeling and excision after adaptor ligation; allowing differential tagging of the first strand cDNA ends. We have also extended the utility of this method by developing additional adaptor and primers, including a dual barcoding approach for multiplexing up to 96 samples. As a result, we have enabled highly multiplexed, strand-specific mRNA sequencing, as well as whole transcriptome sequencing (Total RNA-seq) from ribosomal-depleted samples, enabling the discovery of a much broader picture of expression dynamics including discovery of antisense transcripts. This work presents a streamlined, fast solution for complete RNA sequencing, with high quality data that illustrates the complexity and diversity of the RNA transcription landscape.

P14.53-S
Highly efficient diagnostic testing in patients with hereditary hearing loss using Panel-based Next Generation Sequencing

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Genetic heterogeneity complicates the molecular diagnosis of hereditary hearing loss (HHL). Although a multitude of genes are attributed to HHL, patients are routinely tested only for mutations in GJB2, GJB3 and GJB6 (~5-10% of HHL). Thus, screening of all known hearing loss genes in parallel by high-throughput sequencing methods (next generation sequencing technology) is the most promising tool for a comprehensive detection of causative mutations, especially after negative testing for GJB2. Here we present the methodology of the 'hearing loss' diagnostic panel comprising 110 genes associated with non-syndromic and syndromic HL (Target enrichment, NGS library preparation and sequencing on the Illumina HiSeq2500 platform, bioinformatic analysis and medical evaluation). A group of 200 patients with profound hearing loss were analyzed in a clinical setting. Multiplexed samples were sequenced with high coverage per base and combined with bioinformatic analyses, single base substitutions, small deletions, and insertions in known genes of genetic hearing loss can reliably be detected. In conclusion, we have established a panel-based NGS pipeline which is a highly sensitive, fast and cost efficient tool for the genetic diagnostics of HHL. NGS-based mutation analysis allows us to detect causative mutations in >55% of HHL patients. These results imply consequences for counseling of patients and families and can also be the foundation for novel gene- or even mutation-specific treatment options in hearing loss.

P14.54-M
Technical challenges towards extended blood group genotyping by next-generation sequencing (NGS)

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Generating libraries by using the AmpliSeq™ strategy (Ion Torrent) for next-generation sequencing (NGS) is very convenient in terms of technical handling and time. By these means we designed primers and generated libraries to investigate exons, flanking introns and UTRs of 18 genes involved in 15 human blood systems, for a total of ~57 kilobases. After normalization by two different approaches, libraries were sequenced by the Ion PGM™ Sequencer (Ion Torrent). While all coding DNA sequences, except in *ABO*, exhibited a significant ~90% coverage, data analysis of genotypes in homologous genes (i.e., *RHD* and *RHCE*; *GYPA*, *GYPB*, and *GYPE*) resulted in discrepancy with what expected due to misassignment of sequencing data to target genes by analysis softwares. That prompted us to specifically PCR-amplify the exons in our genes of interest with gene-specific primers. PCR products were subsequently fragmented and sequences from homologous genes were labeled with different barcodes. All products were mixed with the AmpliSeq™-generated products before sequencing. Sequencing data were finally found to be in accordance with known genotypes. This work actually made the proof of principle that 1/ libraries generated by two different means can be combined and sequenced together, and 2/ gene-specific primers are required to accurately investigate genotypes in homologous pairs of genes. Meanwhile we also define conditions to reduce both intra- and intersample variability. Overall this study illustrates that NGS is applicable for blood group genotyping, and may be used in a near future for molecular diagnosis at the laboratory level.

P14.55-S
Cardiovascular NGS-Panel testing: design and first experiences

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Syndromes resulting in primary structural heart disease or arrhythmia are characterized by a large clinical variability and a significant genetic heterogeneity. In fact, in a diagnostic setting it is not uncommon to have more than 40 genes as potential targets for an individual patient. For patient care, this situation is often not satisfactory because a precise diagnosis of the underlying cause of the disease directly affects patient treatment and allows counseling on risks for relatives.
To address these issues we designed an enrichment panel covering 97 genes, including all genes currently associated with LQT, SQT, BrS, IVFA, ARVC, HCM, DCM, RCM and SUDS/SIDS. After targeted enrichment, the DNA is sequenced on an Illumina MiSeq and subjected to analysis employing our analysis pipeline. Our strategy includes an indication specific analysis with an in depth evaluation for highly penetrant genes with a prevalence of >5% where we complement regions with a coverage below 50x by Sanger sequencing. For the remaining genes we generate a screening report and usually achieve coverage above 50x for more than 95% of bases. Up to date we applied our cardiovascular NGS panel to more than 25 patients, which allowed us to establish a definite diagnosis in many of these cases. For example, we detected mutations in *MYBPC3* in 5 out of our 8 patients with suspected HCM. We will present a summary of interesting cases and our experiences with this interesting diagnostic tool.

P14.56-M
Is exome sequencing of single patients with intellectual disability an effective diagnostic strategy?

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Trio-sequencing can be used in all disorders, and has particularly proven its value in finding causes of intellectual disability (ID) or multiple congenital anomalies. In contrast, we investigated whether sequencing only the affected patient without parents is sufficient to find the causative mutation, leading to a considerable reduce in costs. In this study, we enrolled 36 patients with unexplained ID, and sequenced the exome. The exome sequences were analysed with a stringent post-sequencing annotation pipeline including an

ID gene panel of ~500 genes for filtering of the data. All remaining variants with a potential clinical consequence were validated by Sanger sequencing and tested in the parents for inheritance.
After variant filtering we noticed an average of 13 variants per patient (range 2 to 27) requiring further clinical interpretation. The majority of these variants were inherited from one of the parents. Hitherto, we identified 5 de novo mutations in 36 patients (14%).
Without exome sequencing the parents, a relatively high amount of potentially pathogenic variants remain. All these variants require clinical interpretation which is very time-consuming, while most of these variants were likely benign because they are inherited from one of the parents. With trio-analysis inherited variants can be filtered out suggesting that this strategy, at this moment, is more efficient in identifying the causative variant. In the future when databases are filled with more and more exome data and consequently with more rare benign variants, exome sequencing single patients will become a more realistic diagnostic approach.

P14.57-S
Detection of large rearrangements in the CFTR gene: Comparison between custom CGH array and NGS

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24 years after the discovery of the CFTR gene, more than 1900 anomalies are described, mainly single base-pair substitutions or micro-insertions/deletions, but many large rearrangements are also described.
Identification of mutations has important implications for genetic counselling, prenatal diagnosis, cascade screening in families, and for understanding the genotype-phenotype relationship.
Classical approaches for gene analysis aim either to look for point mutations or to identify large deletions or duplications, but no strategy allows, to date, to identify both types of mutations.
Since a few years, NGS technologies enable us to overcome the classical approaches of whole coding sequence sequencing at single nucleotide resolution.
The aim of this study is to validate NGS data analysis to detect large rearrangements in the CFTR gene.
23 DNA carrying known large deletions (n=20) or duplications (n=3) previously identified by CGH-array or qFM-PCR and two control samples, one complete deletion, and the other corresponding to the wild-type sequence of the gene were included in this study.
Analysis was conducted by SeqNext and Nextgene sofwares and by a simple spreadsheet by comparing the average depths obtained for each amplicon.
This method allowed us to confirm all the large deletions/duplications affecting more than one exon. Only one short deletion of 1 kb affecting exon 17b alone was not detected by both methods.
In conclusion, NGS technology should be the first technique that allows in a single step both the identification of point mutations and large rearrangement in a gene.

P14.58-M
The impact of NGS on gentic services: prioritization criteria and accountability

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Clinical use of Next Generation Sequences (NGS) technologies is appealing, but requires high accuracy, simple assays, small inexpensive instruments, flexible throughput, short run times and most importantly, robust data analysis as well as tools for biological interpretation of results.
We have launched a pilot project to assess the clinical value of NGS technology according to the following criteria: 1) phenotypes characterized by genetic heterogeneity; 2) accurate selection of patients in collaboration with a clinical team; 3) the informed consent of the patient to perform the test. We performed targeted NGS analysis on 38 selected patients with Hypertrophic cardiomyopathy (HCM) and Epileptic Encephalopathies (EE) using custom panels of candidate genes on PGM (Life Technologies).
Our study highlight important issues which have to be considered for the accountability of management and transmission of the results: 1) increased identification of new variants with uncertain pathogenic value affecting the expectance of patients, especially in predictive or presymptomatic testing; 2) incomplete representation and coverage of target regions lead to missing clinically relevant mutations. Accordingly, Sanger sequencing seems to be required to analyse specific genomic segment. 3) Our experience indicates that most of identified nonsynonymous missense coding variants are validated by standard sequencing techniques. On the other hand small indels are more frequently false positives or miscalled.
The study confirms the potential of NGS techniques, indicates caution in interpretation and validation of data and suggests a soft transition between classical methods and NGS approach in clinical application.

P14.59-S
Application of NextGeneDx, a validated NGS based procedure, in the clinical practice

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c We have developed a NGS based procedure, called NextGeneDx, with the main objective that it can be used for genetic diagnosis, by using specific designs targeted at specific clinical problems. High coverage and representativity (100%) guaranties a sensitivity and specificity comparable to Sanger sequencing. Validation has been performed by an extensive comparison between the results obtained with NextGeneDx and Sanger sequencing. Versatility and robustness of NextGeneDx allows to increasing gradually the number of genes and diseases analysed. Nine months after the validation of the whole procedure we have developed more than 46 NextGeneDx services for multigenic or genetically heterogeneous diseases including more than 180 genes. The application of this technology in the clinical practice shows a significant reduction of the global costs of sequencing, making accessible the diagnosis of multigenic and heterogeneous diseases, bringing to an increase in the detection of disease-causing mutations and, definitely, a higher number of patients with a genetic diagnosis. Cost effectiveness analyses, from DNA extraction to obtain the final report, bear an important reduction of the costs, especially that related with hands-on and turnaround time, without compromising the diagnostic accuracy of the analysis. In conclusion, NextGeneDx provides an analytical accuracy comparable to Sanger sequencing and permits the analysis of all of the coding region and adjacent intronic sequences including those refractory to other types of sequencing. Therefore, NextGeneDx is a cost effective approach for the genetic diagnosis of multigenic and genetically heterogeneous illnesses, with a negligible number of incidental findings or unsolicited genomic information.

P14.60-M
Noninvasive prenatal testing for fetal trisomies: a validation study using the SOLiD Wildfire platform

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Next-generation sequencing of cell-free DNA, isolated from plasma of pregnant women, is widely used for noninvasive prenatal testing (NIPT) for fetal trisomies. Recently, a new upgrade for the SOliD platform was released: the SOLiD Wildfire. The Wildfire has a simplified sample preparation protocol removing the laborious and expensive emulsion PCR step, an increased sequencing throughput, and the capability to use individual lanes and reuse unused flowchip lanes. Consequently, run time and costs are reduced significantly whilst obtaining equal numbers of mapped reads for statistical analysis. In this study, we aimed to validate the use of the SOLiD Wildfire for NIPT. In total, 154 samples were tested (between 11-20th weeks of gestation) including sixteen T21, ten T18 and four T13 (validated by karyotyping or QF-PCR in chorion villi or amniocytes). Cell-free DNA was extracted from 1 ml plasma and processed using a NIPT optimized library preparation and multiplexed (up to 16-plex). Libraries were sequenced on the Wildfire (35bp) targeting > 10 million uniquely mapped reads without mismatches per sample. The in-house developed pipeline CHROMATE (CHROMosomal Aneuploidy TEster) was used for GC-correction, read filtering, and statistical analysis to calculate Z-scores.To conclude, we demonstrate that the Wildfire can be used to reliably detect fetal trisomies and that results are robust between runs and under suboptimal conditions. Low costs, ease of use, decreased run time, and robustness underline the suitability of the Wildfire for cost-effective and rapid NIPT in clinical practice.

P14.61-S
Epigenetic strategies for non-invasive prenatal diagnosis: The power of the fetal methylome

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Epigenetic modifications have proven to play a significant role in cancer development as well as fetal development. Taking advantage of the knowledge acquired during the last decade, great interest has been shown worldwide in deciphering the fetal epigenome towards the development of methylation

based non-invasive prenatal diagnostic (NIPD) assays. We hereby highlight the different approaches implemented such as sodium bisulphite conversion, restriction enzyme digestion and methylated DNA immunoprecipitation, for the identification of differentially methylated regions (DMRs) between free fetal DNA found in maternal blood and DNA from maternal blood cells. Furthermore, we evaluate the use of selected DMRs identified towards the development of NIPD for fetal chromosomal aneuploidies. In addition, we perform a comparison analysis; we evaluate the performance of each assay and provide a comprehensive discussion on the potential use of different methylation-based technologies in retrieving the fetal methylome, with the aim to further expand the development of NIPD assays.

P14.62-M
Next-generation variant effect predictions and data integration

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The interpretation of variants flowing out of next-generation sequencing experiments is one of the largest bottlenecks for DNA diagnostics. Frequently used tools like PolyPhen and SIFT are not up to this task, and consequently there is an enormous need within the community for novel tools to reliably interpret the effects of polymorphisms. In response to the growing demand for high-quality variant effect predictions we present 3DM; a data integration & mutation prediction platform for protein families.
3DM relieves many of the burdens that researchers face in dealing with the growing amounts and complexity of biomedical data. For each protein family a large amount of information that is extracted from protein structures, alignments and scientific literature, among others, is available. All this information is integrated and validated, and can be analysed via a number of different methods and tools.
By intelligently combining all this heterogeneous information 3DM is able to provide state-of-the-art predictions about the effects of genetic variations.
Collaborative work with a number of the largest hospitals in the Netherlands has shown that our solutions represent a major step forward in helping researchers and clinicians making accurate assessments of the pathogenicity of their variants, both by providing predictions, as well as enabling them to quickly navigate to literature and other relevant data.

P14.63-S
The Israeli experience of the first 300 Panorama™ tests that use 19,488 single nucleotide polymorphisms (SNPs) followed by high-throughput sequencing for common trisomies risk assessment

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Background Cell free DNA (cfDNA) has emerged over the last year as an alternative for amniocenthesis for diagnosis of the common aneuploidies looking at trisomy 21, 13, 18, sex chromosomes and triploidy. **Methods** We present our experience of the first 300 Panorama™ tests sent from Israel. This method is based on massively multiplexed PCR amplification of cfDNA isolated from maternal plasma, targeting 19,488 SNPs, followed by high-throughput sequencing. The fetal fraction is determined. The SNP pattern of maternal DNA (from buffy coat) is compared to the SNP pattern of free DNA from maternal plasma, which contains maternal and fetal DNA. Paternal genomic samples, when available, were included in the analysis; in the absence of a paternal sample, the algorithm considers population allele frequencies. Combining the maximum likelihood ratio with a priori risk generates a risk score. **Results** The results of the first 300 sequential tests performed in Israel were analyzed. Fifteen samples necessitated redraw, two samples failed analysis. Four samples yielded high risk scores: two cases for trisomy 21, one for Kleifelter syndrome (KS) (47,XXY) and one for trisomy 18. Confirmation of both trisomy 21 and one KS were done by CVS or amniocenthesis. The suspected trisomy 18 is in the process of confirmation. There are no known false negative results. **Discussion** Panorama™ test is a reliable tool for identification of pregnancies at high risk for fetuses with the common aneuploidies with a high success rate. We recommend confirmation of the diagnosis for high risk scores pregnancies using invasive tests.

P14.64-M
Quality control procedure for assessing good manufacturing of a molecular diagnostics test in freeze-dried format

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Quality Control (QC) procedures are crucial in manufacturing of molecular diagnostics tests. The aim of this work is to describe QC procedures used to check properties of a freeze-dried PCR master mix for molecular diagnostics (STAT-NAT®, Sentinel CH. SpA).
A liquid bulk was prepared as follows: reaction buffer, dNTPs, $MgCl_2$, DNA polymerase, primers and probe for detection of human beta-globin gene, preservatives and stabilizers. Prior to freeze-dry, a PCR was performed with an aliquot of this mix to assure the functionality (Pre-Lyophilization control, Pre-Lyo). The mix bulk was then dispensed in aliquots of 25µL using a Freedom EVO 100 liquid handler (Tecan) and freeze-dried using an Epsilon 2-12D freeze-dryer (Martin Christ). A PCR was performed with lyophilized mix to check the functionality (Post-Lyophilization control, Post-Lyo). PCR performances were evaluated on a 7500 Real-time PCR System (Applied Biosystems). Moreover, to inspect the residual moisture in the lyophilized mix, an analysis using C20 Compact Karl Fischer Coulometer (Mettler Toledo) was performed.
Test performances were checked using as template different concentrations of human genomic DNA (hu gDNA; Roche). Similar threshold cycles in Pre- and Post-Lyo controls were obtained with a concentration ranging from 0.1 to 10 ng of hu gDNA. Moreover, Karl Fischer analysis after freeze-drying showed residual moisture content less than 5%.
The described QC procedure assures to control in an effective way the manufacturing of a freeze-dried molecular biology test in terms of performance evaluation and residual moisture content.

P14.65-S
Phenotype ontology driven gene panel construction in diagnostic next generation sequencing

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Technological advances in determination of human genetic sequence have significantly facilitated genetic diagnostics in human disorders at progressively diminishing costs. Due to interpretative and ethical challenges faced in medical reporting of findings in whole exome sequencing (WES), sequencing of arbitrarily defined gene panels is commonly endorsed in clinical practice. Such selection of clinical sequencing target, however, narrows the diagnostic survey to genes directly associated with proposed diagnosis, is not resistant to ambiguity in determination of patients' diagnosis and suffers from large disparity in definitions of gene panels between diagnostic centres.
The systematization of human phenotype annotation, notably with the Human Phenotype Ontology (HPO) project now offers a possibility for formalized dissection of human phenotypic traits and offers a possibility for straightforward, systematic and individualised definition of clinical sequencing target based on distinct patients phenotypic features. We therefore propose a novel approach to outlining the clinical sequencing target for diagnostic next generation sequencing, where patient's phenotype is first defined according to HPO nomenclature and according gene panel is then dynamically constructed based on known phenotype-gene associations. Proposed algorithm automatically scores genes based on their phenotypic compatibility with disease under evaluation and defines a tailored sequencing target based on specific phenotypic characterization of disease.
We demonstrate that such an approach allows for judicious extension of currently established sequencing panels while assuring comparable power to establish genetic etiology and controlling for issues in either WES or targeted panel sequencing approaches.

P14.66-M
A new paradigm for prenatal chromosome microarray testing: increased resolution without equivocal findings

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A novel prenatal chromosome microarray testing strategy has been developed, that moves away from size-based detection thresholds, towards a more clinically relevant analysis, providing higher resolution than G-banded chromosomes but avoiding the detection of imbalances of unclear prognosis that cause parental anxiety. All prenatal samples fulfilling our criteria for karyotype analysis (n=353) were tested by chromosome microarray; only copy number variants of established deletion/duplication syndrome regions and any other imbalance >3Mb were detected and reported. A retrospective full-resolution analysis of 249 of these samples was carried out to ascertain the performance of this testing strategy. Using our prenatal analysis, 28/353 (7.9%) samples were found to be abnormal. Of the remaining samples, 249 were anonymized and reanalyzed at full postnatal resolution; a further 46 regions of imbalance were detected in 44 of these traces (17.7%). None of these additional imbalances was of clear clinical significance. This prenatal chromosome microarray strategy therefore detected all CNVs of clear prognostic value. This strategy avoids the problems associated with interpre-

ting imbalances of uncertain prognosis and the parental anxiety that are a result of such findings.

P14.67-S
Determining the sensitivity of diagnostic methods to maternal cell contamination (MCC) in prenatal diagnosis (PND)

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Background: MCC is a potential risk factor for misdiagnosis in PND. The purpose of this study was to quantify the percentage of MCC that can be present in a fetal sample without compromising the fetal genotyping result in each of the different assays used for PND in this laboratory.
Method: Maternal DNA carrying X-linked and autosomal recessive disease was mixed with unaffected fetal DNA (prepared from dissected chorionic villi) in known proportions. Diagnostic methods examined included primer-extension assays with detection by MALDI-TOF mass spectrometry, gap-PCR, MLPA, long range PCR and Sanger sequencing. STR analysis (AmpFlSTR Identifiler kit, Life Technologies) is routinely used in our laboratory to assess MCC and was run in parallel with each assay to assess the theoretical versus the detectable MCC.
Results and discussion: An unacceptable level of MCC was defined as the percentage of MCC that results in an equivocal or incorrect genotype in the fetus. This percentage of detectable MCC was assay dependent, ranging from 1.0% with the gap-PCR, 10-15% for long range PCR, 15-20 % for Sanger Sequencing and 50% for MLPA. A correct genotyping result was obtained with the primer extension assay up to 20% MCC. STR analysis had a limit of detection of 1.0%, providing a useful tool for quantifying MCC. Results of this study have provided a basis for interpreting a PND result when MCC is present and advising requesting clinicians when repeat invasive procedure is warranted.

P14.68-M
Mutation screening in patients with PCD by a multi-gene panel and next generation sequencing technology

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Primary ciliary dyskinesia (PCD) is a rare, autosomal recessive genetic disorder characterized by defect in the action of the cilia lining the respiratory tract, fallopian tube and also of the flagella of sperm in males. The impaired ciliary function results in neonatal respiratory distress, chronic oto-sino-pulmonary disease, infertility, and organ laterality defects in approximately 50% of cases. Currently, the diagnosis of PCD involves the study of cilia morphology, motility and ultrastructure, nasal nitric oxide measurement and, more rarely, genetic analysis. This is mainly due to the high genetic heterogeneity of the disease which has been associated to mutations in 26 different genes.
This study aims to improve the genetic diagnosis of PCD that it is routinely limited to a subset of the most frequently mutated genes. This approach is expensive, time consuming and ineffective leaving many patients without a molecular diagnosis. Using the AmpliSeq technology we developed a panel which allows comprehensive, rapid and cheap analysis of the 26 PCD-causative genes. A total of 160Kb genomic sequence including all the protein coding sequences, splice sites and 5'-3'UTRs will be amplified in 1151 amplicons using two primer pools and sequenced in the Ion Torrent platform. Twenty PCD patients diagnosed according to the protocol of the European Respiratory Society Consensus Statement have been selected for a test study. Technical details, results and cost-effectiveness analysis will be presented.

P14.69-S
Public Health Genomic and genetic tests. Cost evaluation analysis and quality standards as relevant factors in health care planning

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Genomic era has improved a lot molecular diagnosis, but in the last five years the new revolution of next generation sequencing has open a new window not only on research but also on diagnostic testing.
The scientific framework and the laboratory activities are completely changed: the number of known disease genes has increased exponentially, automation is now applied, certification and accreditation procedures are in place in a growing number of laboratories.
Both public National Health systems and private laboratories or companies are facing with an increasing demand of genomic tests for the most common diseases. Correct information and awareness among public and all stakeholders are crucial.
Since it has not been clearly defined how many genomic tests have enough clinical utility, the investigation of their costs could be a way to establish a correct public health policy.
Activity-Based Costing is the methodology used to assigns the cost of each activity.
The systematic analysis of activities needed to perform genetics tests has identified a number of indicators to assess the workload for every professional who participates in the process of diagnosis and the proportion of material used for each activity. All these parameters have been incorporated into a software able to split all the lab costs (personnel, material and general costs) for each test provided in order to compare costs among different laboratories, to compare the performance of the same laboratory in subsequent years and to make a priority list of genetic/genomic tests to provide, taking into account costs/benefits data.

P14.70-M
Testing homopolymers in pyrosequencing-based next generation sequencing chemistry

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In the human exome, approximately 1.43 million homopolymers exist with the size of 4-mer and up. Majority (97%) of them are in the range of 4-mer to 6-mer. To detect indel mutations in homopolymers is of great importance in order to implement the corresponding sequencing system into the routine clinical practice. To test the analytical performance of the pyrosequencing-based GS Junior system in assessing homopolymer sequences, a series of plasmid vectors were generated using pcDNA3.1 as template. Altogether 12 clones were produced, 4-mer, 5-mer, and 6-mer homopolymers with all four nucleotides using site-directed mutagenesis. Mutations were confirmed using Sanger sequencing. For the test system, each homopolymer tract was tested using three pairs of PCR/sequencing primers to test the hypothesis whether the beginning of the sequencing reaction might provide the necessary signal-to-noise ratio to accurately test the size of the homopolymers. In the first, the forward, in the second, the reverse primer was located (i.e. the primer's 3' end) just next to the homopolymer to be analyzed, respectively. In the third, the homopolymer was in the middle of the sequenced product. Average of the correct genotypings were 95.8% in 4-mers, 87.4% in 5-mers, and 70.0% in 6-mers, respectively. Contrary to the low genotyping accuracy in 5-mers and 6-mers, acceptable settings could be found in both with a minimum of 97% in 5-mer and 91% in 6-mer tracts. In conclusion, we have developed a test system that can be used for the assessment of genotyping accuracy of next generation sequencing systems.

P14.71-S
Target Enrichment and Next-Generation Sequencing for Diagnostic Testing of RASopathies

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RASopathies are a group of clinically and genetically related disorders, including Noonan, LEOPARD CFC, Costello, Legius and neurofibromatosis-Noonan syndrome as well as NF1. These disorders result from mutations affecting the RAS-MAPK signaling pathway, which explains the clinical overlap within this group of syndromes. In routine diagnostics, RASopathy-associated genes are usually tested sequentially using Sanger sequencing until a likely causative mutation is identified, which is laborious, time consuming and expensive.
We have developed a cost-effective multi-gene sequencing panel for RASopathies using HaloPlex target enrichment, sample barcoding and sequencing with MiSeq. The panel targets approximately 40kb of the coding sequences of NF1, PTPN11, SOS1, CBL, BRAF, RAF1, SHOC2, MAP2K2, MAP2K1 , SPRED1, NRAS, HRAS and KRAS. Eleven RASopathy-individuals with previously Sanger-validated variants were sequenced. Bioinformatic analysis was performed using the commercial softwares; NextGENe and BENCHlab NGS, as well as an in-house bioinformatic pipeline for HaloPlex data. The results obtained from the two bioinformatic anlaysis pipelines were similar. The gene panel was highly specific; 93% of sequencing reads aligned to the targeted regions. The average read depth in region of interest (ROI) was >2000 and ~98% of targeted bases in ROI had at least 30x coverage. All known variants were identified, including a 4 bp deletion and a splice-site mutation in NF1. Conclusively, the RASopathy-multi-gene panel presented here enables rapid and cost-effective high-throughput screening of RASopathies. The results furthermore show that NextGENe and BENCHlab NGS are

suitable for analyzing HaloPlex data in clinical diagnostics. Data from screening of additional patients will be presented.

P14.72-M
Next Generation Sequencing: New approach for diagnosis of autosomal dominant Retinitis Pigmentosa patients

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Introduction: Retinitis Pigmentosa (RP) is an inherited disease clinically and genetically heterogeneous causing degeneration of photoreceptors. Autosomal dominant Retinitis Pigmentosa (adRP) accounts for 15-30% of cases and mutations in 24 known genes are responsible for 60% of them. The 40% of our cohort of 200 adRP Spanish patients has been characterized using classical tools: different methods of screening (Single Strand Conformation Polymorphism (SSCP), CG-clamped Denaturing Gradient Gel Electrophoresis (DGGE) and ADRP genotyping microarray (Asper Biotech)) followed by Sanger sequencing gene by gene. Patients and methods: 66 previously studied and not characterized adRP families, were analysed by Next Generation Sequencing (NGS) panel of 73 genes associate to RP and other Retinal dystrophies (RD). Sequence variants were detected using the sequence DNA capture Haloplex (Agilent) and sequencing with a Miseq platform (Ilumina). Bioinformatic analysis was performed with a specific DNA Nexus pipeline. Results: potentially pathogenic variants in known adRD genes were found in 42,4 % of families studied: SNRNP200 (7,6%), PRPF8 (6 %), BEST1 (3 %), PRPF31 (3 %), PRPH (3%), RP1 (3 %), CRX (1,5 %), FSCN2 (1,5 %), GUCA1B (1,5 %), IMPG1 (1,5 %), KCNJ13 (1,5 %), PROM1 (1,5 %), PRPF3 (1,5 %), RGR (1,5 %), RHO (1,5 %), RP2 (1,5 %), and TOPORS (1,5 %). Additionally 7,6% of cases displayed mutations in ABCA4, RPGR and USH2A allowing us a clinical or genetic reclassification. Conclusion: this new approach seems to be a fast and reliable tool to detect the disease-causing mutation for adRP patients.

P14.73-S
Visualization of *XIST* expression in a female with structural X chromosome abnormality: Single-cell analysis by three-color interphase RNA-FISH

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An X-inactivation (XIA) analysis in females is commonly performed by metaphase R-banding or methylation-specific PCR (M-PCR) technique. The late-replicating X-chromosome can be recognized under a microscope at the single cell level by R-banding using BrdU incorporation, but there are limitations: 1) Not so many metaphases can be observed, and 2) Only an abnormal X which has deletion or duplication more than 10Mb can be detected. M-PCR can detect random or non-random XIA pattern in cell population, but the parental samples are needed to identify which is normal or abnormal chromosome X. A subject was a female with an X-autosome balanced translocation; 46,X,t(X;19)(p21.1;q12), and having clinical manifestations of Duchenne muscular dystrophy. The breakpoint of the derivative X was onto the *DMD* gene. We selected three kinds of BAC clones and used simultaneously for our RNA-FISH study; a clone consistent with *XIST* gene for detecting inactivation chromosome X, a clone consistent with *UTX* gene which is escaping XIA as a reference probe for both normal and abnormal Xs, and a clone consistent with *HDHD1A* gene which is escaping XIA for distinguishing between normal and abnormal Xs of this subject. We successfully visualized *XIST* gene expression at the single cell level in this subject. Normal X was inactivated in 67 cells, abnormal X was inactivated in 5 cells, and unclear XIA pattern was in 28 cells. This RNA-FISH results were in concordance with the phenotype of this subject, as an affected female of X-linked disorder.

P14.74-M
Quality control for SureSelect Strand-Specific RNA library preparation protocol using the Agilent 2200 TapeStation system

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Transcriptomics reveal global changes in gene expression that may contribute to the pathogenesis of a particular disease or help drive a fundamental biological process. RNA sequencing (RNA-Seq) has emerged as a promising and rapidly growing method for studying and characterizing cellular transcripts at single base pair resolution. Among different approaches for generating libraries for transcriptomics study, strand-specific library preparation method has added advantage over other methods. The Agilent SureSelect strand-specific RNA library preparation kit generates libraries with specific adaptors ligated to each strand enabling the identity of the DNA template strand of origin to be retained in downstream sequencing and data analysis. The high throughput deep-sequencing based RNA-seq studies generate more data compared to RT-qPCR and array based methods yet they are expensive and time consuming study. Proper quality control (QC) steps within the library preparation process are therefore crucial to ensure successful sequencing. The Agilent 2200 TapeStation system offers an easy to use automated electrophoresis system with rapid analysis time as well as flexible sample throughput capabilities. Here, we compare the performance and capabilities of the RNA and D1000 ScreenTape assays for use on the Agilent 2200 TapeStation system with the respective assays run on the 2100 Bioanalyzer system. The results from the QC of the starting total RNA and final sequencing libraries generated from Agilent's SureSelect strand-specific RNA library preparation kit reveal that the RNA and D1000 ScreenTape assays are a match to the visual and quantitative results obtained with the RNA 6000 Nano and DNA 1000 Kits.

P14.75-S
Expanded carrier screening tests currently on the commercial market

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There has been a recent rise in the advertising of preconception genetic carrier screening tests directly to consumers via internet. We analyzed the offers with particular focus on comprehensiveness, clinical validity and utility of the genetic tests proposed.
We gathered data from the websites of Counsyl, InheriGen, Pathway genomics and Recombine in December 2013. Of about 1300 recessively inherited diseases listed in OMIM (causative gene known), current offer includes a range of 73 to 206 for a total of 286 diseases of which 30 are included in the panels of all providers and 92 in at least three providers. According to the ORPHANET catalogue, 31% of screened diseases are more frequent than 1/100,000 in the general population. On the other hand, there are relatively frequent recessively inherited diseases (e.g. Duchenne muscular dystrophy, Friedreich ataxia) not covered by any of the screening tests. 52% of screened diseases manifest neonatally, 28% during childhood and 3% in adult life(e.g. Hemochromatosis), while the onset of the others is variable. 34% can be treated if diagnosed neonatally. On average, companies test for 4% (2,2% to 7%) of all known mutations on a particular gene (HGMD).
Currently, commercial companies offer screening for around 20% of known recessively inherited diseases. The great diversity of genetic diseases screened suggests a lack of clear inclusion criteria. Although advertised as pan-ethnic, all tests include population specific diseases which are extremely rare in the general population. Because of allelic heterogeneity and relatively poor mutation coverage the tests' sensitivity pan-ethnically is unknown.

P14.76-M
Detection of rare variations in a targeted genomic region in a population by NGS analysis using pooled DNAs

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It is important to find novel rare variations in a targeted region in some populations to get next resources for elucidation of 'missing heritability'. We developed a method to survey variations including low-frequent variations in a targeted region in a specific population by next-generation sequencing (NGS) analysis using pooled DNAs.
We present examples to trace variations and to estimate each frequency in a targeted region in Japanese and Okinawan people.
Targeted regions were amplified from each genomic DNA of 100 or 200 individuals by LA-PCR. After measuring the quantity and quality of each amplicon, equal amount of amplicon was mixed and amplicons for 100 or 200 individuals were pooled. Then, pooled targeted region was analysed using a NGS platform. After mapping of reads to the reference, SNPs and indels were called and the frequency was estimated by count rate of the reads. Then, we also performed NGS analysis for LA-PCR products from pre-pooled genomic DNAs. Next, we confirmed each variation and calculated each frequency in

the population by PCR-RFLP, allele specific PCR or direct sequencing in each individual.
Allelic frequency estimated by the NGS analysis using both pooled DNAs almost correlated the real frequency calculated by the individual analysis. For sensitivity to detection of allelic frequency, less than 0.5% of allele in the population could be detected.

P14.77-S
Increasing the detection rate of genome-wide high resolution SNP array analysis by using the right follow-up test procedures in constitutional genome diagnostics

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We routinely perform genome-wide high resolution SNP array analysis as the first-line diagnostic test for patients with intellectual disability and/or congenital anomalies and prenatally in case of structural ultrasound anomalies or intra uterine foetal death and a normal QF-PCR test result. So far, a total of 9,471 patient and 3,348 parental samples have been tested by SNP array in our diagnostic laboratory. A (potentially) causative copy number variant (CNV) was detected in almost 27% of the patients and analysis of SNP genotypes revealed one or more significant stretches of homozygosity in an additional 8% of patients. Follow-up testing by either gene mutation analysis or patient-parent trio information analysis subsequently led to the respective identification of pathogenic mutations in recessive disease genes or uniparental disomies (UPD), thereby increasing the diagnostic yield with at least 1%. Using the SNP genotype information also improved the detection of mosaic imbalances and enabled us to detect clinically relevant, mosaic, copy neutral changes of homozygosity in four patients. A mosaic finding (CNV, aneuploidy or allelic imbalance) was detected in a further 26 patient samples and 10 parental samples, resulting in a dramatically increased recurrence risk for these parents. The percentage of mosaicism often differed between tissues samples of mesodermal, ectodermal or endodermal origin from each of these individuals. Genome-wide high resolution SNP array analysis is a suitable and particularly effective technique in genome diagnostics to reliably detect in a single test CNVs, UPDs and mosaic imbalances as well as for homozygosity pre-screening.

P14.78-M
Classifying variations as de novo without available paternal DNA using SNP-array

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Inheritance of a variation is often used as a guide for diagnostic classification in microarray analysis. If a variation is inherited from a normal parent, morbidity is less likely, compared to a variation that has appeared de novo. Unfortunately paternal DNA is frequently not available for this analysis. Therefore many variations are never properly classified and the morbidity of the variation remains unknown. To overcome this problem we have incorporated a workflow that determines whether a variation is located on the maternal or the paternal allele in the patient. The workflow requires SNP-array analysis to be done on maternal and patient DNA samples. If a variation is located on the maternal allele in the patient and the variation is not found in the maternal DNA sample, then the variation can be classified as de novo. Utilizing this workflow enhances the diagnostic value of variations found by SNP-array, when paternal DNA is not available.

P14.79-S
EGFR and KRAS mutational profiling in fresh non-small cell lung cancer (NSCLC) cells

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Knowledge of tumor mutational status has become a priority for effective NSCLC-tailored treatment. NSCLC diagnosis is more often reached through biopsy; thus, there is a clear need to implement for routine tumor molecular profiling on small cytological samples. This work aims to screen and compare the EGFR and KRAS mutational prevalence in fresh tumor cells and in corresponding routinely processed samples derived from trans-thoracic fine-needle aspiration. The latter currently represents the most appropriate diagnostic procedure in case of peripheral lesions, such as adenocarcinomas, which account for almost 40 % of all NSCLCs and for the highest EGFR mutational rates.
Two hundred and forty-four patients carrying peripheral lung masses underwent CT-guided aspiration. The obtained material was split, and a part was addressed to conventional histopathological analysis while the remaining one was stored at −20 °C. In case of confirmation of adenocarcinoma, tumor genomic DNA was extracted from both fresh and fixed material, and EGFR and KRAS sequencing was performed.
We identified 136 adenocarcinomas; from 134, we could recover enough material for the study. A full match was demonstrated between EGFR/KRAS mutational prevalences through the two approaches tested. We found EGFR mutations in 13 patients (9.7 %); 7 were females and 11 never or former smokers. KRAS mutations occurred in 20 (14.9 %) patients. EGFR and KRAS mutations were mutually exclusive.
Mutational screening on fresh cancer cells is an achievable, safe and cost-effective procedure which might allow routinely tumor molecular profiling as powerful integration of conventional histopathological analysis.

P14.80-M
Identification of structural variation in whole exome sequencing and whole genome data

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Whole exome sequencing (WES) allows the detection of a wide range of variant types. With one single test it is possible to detect small variants (SNVs), and structural variation. Whilst the role of copy number variants (CNVs) in intellectual disability (ID) is well known, the role of CNVs in other diseases is less well studied, although it has been suggested that CNVs can be found in up to 10% of deafness patients. We have performed read depth WES CNV analysis on 600 patients across 5 heterogeneous disorders including ID, deafness, blindness, metabolic disorders, movement disorders. This analysis revealed several clinically relevant CNVs exerting a dominant effect, as well as CNVs unmasking a recessive mutation that lead to pathogenic compound heterozygous events. The cohort of 310 ID patients had previously screened negative for CNV microarray analysis as well as WES SNV analysis. Nonetheless clinically relevant CNVs were identified in 2% of patients. Systematic screening of the remaining patient groups identified CNVs in ~4% of individuals, including in *COL6A1*, *EYS* and deletions in *USH2A*. To further examine pathogenic structural variation, we performed whole genome sequencing on 50 patient-parent trios. In 7 patients we identified pathogenic *large* variants, including two single exon deletions, a tandem duplication, an inter-chromosomal duplication and one complex inversion/duplication/deletion event. Discordant reads provided positional information for duplicated sequences identifying an in-frame gene fusion. These results show that structural variation is not only an important cause of neurodevelopmental diseases but also a much broader range of genetic diseases.

P14.81-S
Targeted exome sequencing as a molecular diagnostic tool for skeletal dysplasias

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Skeletal dysplasias comprise a large group of more than 450 clinically distinct and genetically heterogeneous diseases associated with mutations in more than 300 genes. Clinical and radiological findings are used to diagnose these diseases. However, due to the genetic heterogeneity of these disorders, the diagnostic process is complex. With the advances in molecular genetics and the elucidation of the molecular basis of these diseases, molecular tests have become useful in the diagnosis of these dysplasias. Since many different genes have been associated with these disorders, standard diagnostic approaches using Sanger sequencing can be expensive and time consuming. To overcome these limitations, we used targeted exome sequencing and designed an 1.4Mb panel for simultaneous testing of more than 4800 exons in 309 genes involved in skeletal dysplasias, and 28 genomic regions which when deleted are associated with these diseases. DNA from 96 individuals with previous clinical diagnosis of skeletal dysplasia was sequenced in 8 multiplexed runs using an Illumina MiSeq sequencer. Reproducibility was tested by repeating the entire procedure for three patients. NGS of the captured exons resulted in an average coverage of 140X. Causative mutations were characterized in 13 patients so far including de-novo mutations in 7 cases. Analysis of the rest of the data is ongoing. Confirmation with Sanger sequencing was performed for these variants and all were confirmed. Our NGS panel provides a fast, accurate and cost-effective molecular diagnostic tool and can assist in the diagnosis of genetically heterogeneous diseases like skeletal diseases.

P14.82-M
Molecular diagnosis of hereditary spastic paraplegia: comparison of enrichment strategies for targeted next-generation sequencing

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Hereditary spastic paraplegia (SPG) is a group of neurodegenerative disorders with considerable phenotypic heterogeneity, and mutations in >70 genes involved to date. This makes conventional molecular testing arduous. We decided to develop a targeted next generation sequencing kit for diagnosis purpose.
We tested two enrichment approaches of 10 SPG genes: one using amplicons (Illumina, TruSeq Custom Amplicon - TSCA) on 48 patients, and the other using capture (Roche NimbleGen, SeqCap EZ) on 60 patients. Sequencing was performed on the MiSeq (Illumina). Coverage and variant distribution were evaluated using the Genomics Workbench software (CLC Bio).
After read mapping on whole genome (Hg19), the mean coverage per patient varied from 444X (sd 98X) for SeqCap EZ, to 587X (sd 127X) for TSCA. However, the percent of regions with minimal coverage <30X was <15% with SeqCap EZ, and decreased to <5% when the patient mean coverage reached 600X. With TSCA, this value was stable (15-20%).
We then compared the variants. Those reported in dbSNP137 were similar on both strategies. Concerning variants unknown in dbSNP137, 16-20 variants with TSCA vs 0-3 with SeqCap EZ were obtained per sample. This was explained by an enrichment of variants in some amplicons in TSCA suggesting false positives due to Taq polymerase errors.
SeqCap EZ was finally chosen to enrich our targets. We designed a new panel with 74 SPG genes, which gave <0.7% of regions with minimal coverage <30X. The challenge is now to interpret the variants discovered in our growing cohort of patient with unsolved HSP.

P14.83-S
Development of a specific and sensitive High Resolution Melting Analysis for detection of beta globin gene mutations

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Beta-thalassemia is one of most common autosomal recessive disorders worldwide. The high prevalence (8%) and heterogeneity in molecular level, observed in Greece, makes necessary the development of a reliable, cost effective and rapid scanning method for β globin gene analysis, easily adapted to a routine laboratory.
Here we describe the development of a beta-thalassaemia specific High Resolution Melting Analysis (HRMA) approach, in order to identify point mutations and small insertions or deletions of the beta globin (HBB) gene and substitute previously applied techniques (DGGE analysis, Sanger sequencing).
Specific sets of primers were designed to cover eight overlapping regions of the HBB gene containing the hitherto reported mutations of the Hellenic population. PCR conditions were identical for all amplicons, permitting thus multiplexing. Amplicons cover part of the HBB promoter region and the CDS region, except from the central part of IVS-II where practically no mutations has been reported up to now.
Initially, 150 previously genotyped samples were analysed. Different mutations produced distinct derivative plots when subtracted from the reference curve of a wild type control, resulting in 100% accurate mutation identification. In addition, HRMA analysis of 48 undefined samples totally matched subsequent diagnosis by either Sanger sequencing or DGGE analysis.
The described HRMA assay represents a rapid, simple, cost-effective and highly feasible strategy for identifying effectively underlying mutations of beta-thalassemia. The reported method is amenable to high-throughput systems and except from being highly specific and sensitive, does not require any post-PCR analysis.

P14.84-M
Optimization of an imaging cytometry protocol to determine the absolute mean corpuscular haemoglobin F in F-erythrocytes

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Around birth, a shift from γ- to β-globin gene expression causes a switch from foetal haemoglobin (HbF) to adult haemoglobin (HbA). Residual amounts of HbF are synthesized throughout life F-erythrocytes. Increased HbF levels ameliorate symptoms of β-haemoglobinopathies.
Krüppel-like factor 1 (KLF1) plays a central role in the developmental globin gene switch; carriers of KLF1 mutations have hereditary persistence of foetal haemoglobin (HPFH) and show variably elevated (10-40%) HbF. This variation may be due to differential expression of other modifier genes. The KLF1 interactome can be further defined by identifying potential molecular targets and observing their expression at the cellular level in comparison with HbF expression and distribution in F-cells.
The mean corpuscular HbF (MCHbF) is normally estimated by dividing the amount of HbF, determined by HPLC, by the number of F-cells, obtained by flow cytometry. Since HbF may be unequally distributed among F-cells, an imaging cytometry protocol enables quantification of HbF per F-cell by fluorescent emission measurements. Protocols presented in the literature were found lacking in efficacy, mostly due to inefficient fixation and high degree of autofluorescence. A new intracellular anti-HbF antibody-labelling technique was thus optimized for use with the Nikon Eclipse T*i* inverted fluorescence microscope. This technique resulted in images of superior quality from which the MCHbF could be determined for each F-cell observed. This protocol will be used to study the association of *KLF1* and other genes, obtained through NGS data analyses, with MCHbF in normal and HPFH individuals, to identify new therapeutic targets for β-haemoglobinopathies.

P14.85-S
Development and verification of an Ion AmpliSeq™ *TP53* Panel

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A large amount of data is available on the functional impact of missense mutations in TP53 and on mutation patterns in many different cancers. TP53 direct sequencing is a time-consuming method with limitations in detection level. Here we describe the development and verification of a Next Generation Sequencing method based on Ion AmpliSeq™ technology. The panel covers TP53 coding regions comprehensively using optimized primers to minimize off-target sequencing. It is comprised of 24 primer pairs across 2 pools requiring only 20 ng of DNA. The design of short amplicons of 125 to 175 bp permits the amplification of DNA starting from formalin-fixed paraffin embedded material. We verified the panel on 30 breast cancer samples previously characterized with Sanger sequencing. Sequencing reactions were carried out with the Ion PGM™ Sequencer on Ion 318™ chip. The average % of mapped reads on target was of 96,8% and average depth of coverage 20,386X. Loading 10 samples on an Ion 318™ chip 95% of the sequenced amplicons showed coverage higher than 500X. Data analysis was performed on Ion Reporter™ Software 4.0 using optimized TP53-panel workflows. We detected all the expected somatic mutations including single base substitutions, insertions and deletion and only a complex duplication of 18bp was not detected. The overall analytical sensitivity was 95,23%. The same results were obtained using 32 samples on an Ion 318™ chip. These preliminary data demonstrate that the TP53 Ion AmpliSeq™ panel workflow and Ion Reporter™ Software analysis solution meets the requirements of clinical research laboratories.

P14.86-M
Toward rapid identification of coding fusions and structural rearrangements in cancer genomes: Multiple Myeloma First

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Karyotype and FISH have been standard diagnostic tools in monitoring response to treatment and disease progression in hematological disorders, including multiple myeloma, for over 40 years. However, with the availability of array and NGS technologies in most clinical diagnostic laboratory settings, it is time to consider evaluated the use of more modern methods in diagnosing plasma cell dyscrasias. Although we routinely run RNA-Seq, SNP-CN arrays and a deep sequencing cancer panel as a cost effective workflow for clinical trials, we find the validation of translocations and structural rearrangement by PCR and/or FISH cumbersome. More recently, we have identified the BioNano Genomics Irys System which utilizes nanochannel technology and high resolution imaging for whole genome mapping of translocations and copy number abnormalities. As proof of concept, we have used the Irys system to analyze the multiple myeloma cell line, KMS11, and will demonstrate the ultility, speed and accuracy in detecting structural re-

arrangements in this line compared to whole genome sequencing, RNA-seq and SNP-CN datasets. We will also demonstrate the use of the system with unknown CD138+ enriched bone marrow samples from consented patients currently on clinical trials compared to FISH, G-band karyotype and SNP-CN arrays. We are confident that these data demonstrate that the Irys system is a disruptive innovation with broad applicability to genome research and refinement of normal variation and disease.

P14.87-S
Mutation analysis of triple negative breast cancer patients using next generation sequencing

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Despite the intense research in the field of breast cancer, this disease still remains the second most common cancer in women worldwide, being the first cause of death in women in Romania. Among the different subtypes of breast cancer, triple negative breast cancer (TNBC) needs a special attention due to its limitations in therapy and to its aggressiveness. Several studies have tried to identify different new mutation both in oncogenes and tumor suppressor gene in order to explain these limitations of TNBC treatment. The present study was aimed at the identification of mutations in 46 genes involved in cancer in 31 patients with TNBC operated at the Institute of Oncology "Prof. Dr. I. Chiricuta", Cluj-Napoca between 2006-2007, using Next Generation Sequencing. We used FFPE tissue samples which were sequenced using the Ion Torrent Personal Genome Machine and the Ion Reporter 1.6 software for data analysis. After data analysis we obtained 103 mutations in 34 genes of the 46 studied. The clinical assessment of the identified mutations showed that three mutations were benign, one was likely benign, 42 were likely pathogenic, 28 were pathogenic and 29 had no assessment. This study also identified KDR, TP53, PIK3CA, FGFR3 and FGFR2 genes as being the most frequently mutated genes. Our results show that TNBC has specific mutations leading to resistance to therapy and poor outcome of these patients.

P14.88-M
Four Decade Old Mummified Umbilicus Making Retrospective Molecular Diagnosis of Ornithine Carbamoyltransferase Deficiency

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In inborn errors of metabolism, sampling and preservation of viable specimen is critical in reaching the correct diagnosis. Utility of preserved umbilical cords have been demonstrated in several occasions, but it remains unclear how long the mummified umbilicus can preserve genomic DNA that tolerates sequence-based genetic analysis. We report a Japanese family of successive early deaths presumably due to clinically diagnosed ornithine carbamoyltransferase deficiency. The exact molecular pathology, c. 394T>C (p. S132P), was retrospectively delineated using DNA extracted from the mummified umbilicus that had been kept in a keepsake wooden box at home for four decades after the deaths of affected individuals. This observation illustrates the striking stability of DNA preserved in mummified umbilicus, and supports an efficient way of genetic sample preservation. From cultural standpoint, preservation of mummified umbilical cord is a unique but common tradition in Japan for at least several centuries. The exact molecular diagnosis of this X-linked inborn error of metabolism of the urea cycle had an significant impact on the affected family. Hemizygous female carriers are at risk of hyperammonemic crisis, and this disorder is treatable with supplemental dietary interventions. Since naturally mummified umbilicus provides a highly effective way of genetic sample preservation, utilization such material should be considered in the retrospective genetic investigation, particularly in patients with south east Asian cultural backgrounds.

P14.89-S
Direct trans-differentiation of skin fibroblasts for functional testing of unclassified variants

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Introduction Unclassified variants (UVs) are a common finding in DNA diagnostic testing. The introduction of next generation sequencing, allowing the simultaneous analysis of multiple genes, has enhanced the problem of variants with unknown clinical significance. For in vitro functional testing of UVs we need cells that express the specific gene. **Aim** The aim of this study is to use direct trans-differentiation of skin fibroblasts into smooth muscle cell (SMC) phenotype in order to study the effect of UVs in genes that are involved in susceptibility to thoracic aortic aneurysms. **Methods** We used culture media with horse serum and TGFβ1 to induce trans-differentiation of patients' fibroblasts into SMCs. Gene expression was tested with RT-PCR. Splice errors were studied on cDNA. Contraction of the cells was studied on collagen matrix. **Results** Cells with SMC phenotype, derived from fibroblasts from patients with UV's in MYH11 were compared to controls. The differentiated cells expressed SMC markers, including MYH11, confirming successful differentiation. An intronic mutation in MYH11, c.3879+2dup, in a patient with TAA and type B dissection with a positive family history for aortic dissection, does not affect the canonical splice donor site, but was shown in transdifferentiated cells from the patient to result in the use of a cryptic splice site in exon 29, resulting in an in frame deletion of 78 bases. The contraction of the patient derived cells was disturbed compared to control cells. **Conclusion** Trans-differentiation of skin fibroblasts towards SMC phenotype is a powerful tool for the functional analysis of UVs.

P14.90-M
Targeted re-sequencing and USH syndrome: a robust and accurate protocol overcoming the problem of genetic heterogeneity and leading to the discovery of several new mutations

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Usher Syndrome (USH) is characterized by phenotypic and allelic heterogeneity requiring a powerful and reliable molecular diagnosis. Thus, a targeted re-sequencing (TS) panel of the 10 USH genes has been developed. It covers 96% of the targeted regions being characterized by 872 amplicons using Ion Torrent™ technology (Life Technologies). This protocol has been applied to the analysis of 30 USH cases divided as follows: 16 with already known mutations were used for a validation step which demonstrated 100% accuracy, while the remaining 14 were analyzed at diagnostic level. The combination of systematic data analysis using an established bioinformatics pipeline followed by Sanger sequencing confirmation and segregation analysis led to the identification of 15 novel variants (1 in *USH2A*, 4 in *PCDH15,* 1 in *MYO7A*, 3 in *CDH23*, 4 in *GPR98*, 2 in *USH1G*). All these variants were predicted as pathogenic using several *in silico* predictor tools such as Mutation Taster, Polyphen-2, SIFT and others. Five known pathogenetic mutations were also detected. Overall, these 20 alleles explain 12 patients, one is heterozygous for a mutated allele and the second is lacking, and the last is completely negative (a new case of genetic heterogeneity or the presence of mutations in regions not yet analyzed of the USH genes?). In term of molecular epidemiology, USH2A characterizes 12 patients (40%) MYO7A 6 (20%), CDH23 4 (13%), PCDH15 3 (10%), GPR98 2 cases, USH1C and USH1G one each. In conclusion, this methodology clearly provided a reliable strategy for routine gene diagnosis of USH.

P14.91-S
p.Asp59Gly in the RAB40AL gene is a common allelic variant: no evidence for a causal relationship with Martin-Probst syndrome

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Two adjacent substitutions A to G (rs145606134) and C to A (rs138133927), both affecting codon 59 leading to a missense change p.Asp59Gly (GAC>GGA) in the RAB40AL gene, have been recently reported as a first association of the RAB40AL gene with a human disorder and as the molecular cause of Martin-Probst syndrome (MPS) (Bedoyan et al. 2012). This rare X-linked disorder is characterized by progressive sensorineural hearing loss, cognitive impairment, facial dysmorphism, variable renal and genitourinary abnormalities and late onset pancytopenia.
The p.Asp59Gly amino acid change resulting from the same two DNA substitutions was unexpectedly identified in our 53-year-old male proband,

who was diagnosed for a genetic cause of isolated optic atrophy using whole exome sequencing (HiSeq 1500 and TruSeq Exome Enrichment Kit, Illumina). Interestingly, the patient did not present any symptoms of MPS. Trying to further verify the pathogenicity of the RAB40AL variants, we screened a set of control DNA samples representative of the background population of Central Poland (n=788, female:male ratio 1:1) using allele-specific PCR and confirmed suspicious variants by Sanger sequencing (Applied Biosystems). The RAB40AL variants were found in 18 out of 788 subjects. It corresponds to an allele frequency of almost 2.3%. Results of our study have demonstrated that the p.Asp59Gly amino acid change in RAB40AL gene is present at a high prevalence in the general population that is typical for common polymorphisms. Our data questions the role of p.Asp59Gly as a disease causing-change for MPS.

P14.92-M
Comparative transcriptome and genome analysis down to the sequence level for individual cells

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Cell heterogeneity plays a central role in biological phenomena during normal development or disease (e.g., cancer development). As gene regulation is a fundamental process, the analysis of transcript profiles and genomes of single cells to dissect phenotypic variability is of key interest to scientists.
Deep genome and transcriptome analysis of small biological samples using next-generation sequencing (NGS) is often limited by the small amount of sample available (6 pg gDNA and 0.5 pg mRNA/human cell). We developed two new methods for whole genome amplification (WGA) and whole transcriptome amplification (WTA), based on Multiple Displacement Amplification (MDA) technology, from small samples down to just a single cell.
Parallel WGA and WTA reactions begin with lysis of 25–1000 cells from the same sample. Both reactions include a ligation and multiple displacement amplification (MDA) reaction. This results in amplification products (WGA-DNA, WTA-cDNA) of the highest comparability and can be used for comparative studies of the genome and transcriptome from the same sample.
The second method (WTA) amplifies RNA from 1–1000 cells directly and includes an efficient lysis, cDNA synthesis, and amplification strategy that results in minimal bias and errors. gDNA is effectively removed to prevent false-positive results.
We amplified a variety of human cells and checked the resulting genome and transcriptome using NGS or qPCR methods. Discussed are experiments on cell-to-cell variation, GC content in comparison to genomic DNA, percentage and consistency of protein coding transcripts, genome coverage with respective error rates, and genome-wide real-time PCR analysis.

P14.93-S
3Gb-Test: Introduction of Whole Genome Sequencing as diagnostic application

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3Gb-TEST a two year CSA-EU-FP7 project, gathers information on the gaps and needs for implementing Whole Genome Sequencing (WGS) into the diagnostic service. The aim is to prepare a detailed implementation plan or roadmap for the transition to the future of genetic testing WGS included where appropriate and applicable.
As the cost of sequencing is decreasing and the knowledge of our genome is increasing we expect that this transition to the future will take off within the next 5 years. 3Gb-TEST will define needs and gaps using questionnaires, expert meetings and literature to shed light on and obtain insights in the following topics:
- "Wet lab" innovations
- The bioinformatics
- Clinical interpretation
- Personalized medicine and pharmacogenomics.
- Quality issues and External Quality Assessment
- The clinical utility and cost effectiveness

The 3Gb-TEST project brings stakeholders together and disseminates information with respect to the desirable and undesirable developments. The Consortium will inform the healthcare community and make recommendations to the European Commission, the European Society of Human Genetics, and national organizations relevant to this field. The roadmap will address all afore mentioned topics and prepare an implementation plan for the transition to the future of genetic testing in which WGS is embedded. Substantial investments may be required and the logistic restructuring of genetic services will be addressed. Monitoring and harmonization at the European level of all developments is therefore of the utmost importance.
3Gb-TEST is actively getting in contact with the genetics community and will present the initial results and opinions at the ESHG conference.

P14.95-S
Design and evaluation of two novel methods for detection of ATP7B gene mutations

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Wilson's disease is a rare autosomal recessive disorder of copper metabolism (OMIM#27790) leading to copper accumulation in the liver, brain, kidneys and cornea. It is caused by mutations in the ATP7B gene with a carrier frequency of approximately 1.2%.
We report the design and evaluation of two different screening methods for the detection of ATP7B gene mutations which could be used in conjunction in order to minimize the cost and time for genotyping.
1) A specific mutation detection assay by dry-reagent dipstick biosensors applied for the 10 commonest mutations in the Greek population (H1069Q, R969Q, Q289X, 2530delA, L936X, 2299insC, I1148T, 845delT, 1708-1G>A, X1466R) covering 80% of disease chromosomes. Fragments flanking the 10 ATP7B mutations are amplified by multiplex-PCR, followed by multiplex primer extension (PEXT) reaction using allele-specific primers. The primer extension products are simultaneously detected by visual multi-allele dipstick type DNA biosensor using anti-biotin conjugated gold nanoparticles. Optimization studies on the efficiency and specificity of the PEXT reaction were performed.
2) A gene scanning protocol using High Resolution Melting (HRM) analysis, designed to analyze all coding ATP7B regions.
The first method was evaluated by analyzing 50 samples of known genotypes (confirmed by sequencing) along with 50 blind samples. The results were fully concordant with reference methods. HRM was evaluated by testing 50 known genotypes and 100 blind samples.
The proposed methods are simple, rapid, do not require purification of the PCR products and could be particularly useful in diagnostic laboratories. The benefits and drawbacks of each will be discussed.

P14.96-M
EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013

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The molecular diagnosis of Y-chromosomal microdeletions is a common routine genetic test that is part of the diagnostic workup of azoospermic and severe oligozoospermic men. Since 1999, the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) have been actively involved in supporting the improvement of the quality of the diagnostic assays by publication of the laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions and by offering external quality assessment trials. We aim to present an overview on clinical novelties related to the Y chromosome and provide an update on the results of the quality control programme. The original basic diagnostic testing protocol based on two multiplex PCRs remains fully valid and appropriate for accurate diagnosis of complete AZF deletions requiring only minor modification in populations with a specific Y chromosome background. However, in light of novel data on genotype-phenotype correlations, the extension analysis for the AZFa and AZFb deletions is now routinely recommended. Novel methods and kits with excessively high number of markers do not improve the sensitivity of the test, may even complicate the interpretation of the results and are not recommended. The routine screening for gr/gr deletion, a significant risk factor for impaired sperm production, is under debate and eventually it can be performed in selected populations. Annual participation in an external quality control programme is strongly encouraged. The 12-year experience with the EMQN/EAA scheme has shown a steep decline in diagnostic (genotyping) error rate and a simultaneous improvement on reporting practice.

P14.97-S
Introduction of a targeted next generation sequencing gene panel for familial cancer in DNA diagnostics and genetic counselling

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Aim: Our aim was to develop a targeted next generation sequencing (NGS) gene panel that enables to analyze large numbers of genes in patients with familial cancer at relatively low cost.
Methods: A panel of 70 known tumour syndrome genes based on Agilent Sure Select Target Enrichment® for simultaneous mutation detection was developed and validated in two series of twelve patients for variants of the MMR or the BRCA genes previously identified through Sanger sequencing. In an additional set of twelve patients all genes were analyzed anonymously. The samples were sequenced using 151 base pair paired-end reads on an Illumina MiSeq® sequencer and analyzed using Softgenetics' NextGENe® and Cartagenia's Benchlab NGS® software. Within the NGS panel, three virtual non-overlapping gene subpanels were designed, based on the levels of preventive options and strength of risk information for the panel genes. In genetic counselling the genes to be tested were discussed as the 3 subpanels rather than individually. For diagnostic purposes NGS with the targeted panel was performed on 75 patients, in whom a pathogenic mutation in the MMR and BRCA genes has been excluded. Only results from chosen subpanels were reported to patients.
Results and Conclusion: In the validation all pathogenic mutations, unclassified variants and neutral polymorphisms detected previously were identified by the NGS panel. In addition, 40 novel variants could all be confirmed by Sanger sequencing. The results of the first 75 patients will be presented. Genetic counselling was adopted to discuss genes as groups rather than as individual genes.

P15.01-S
Swedegene: Genome-wide association studies of adverse drug reactions

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Objective: Swedegene (www.swedegene.se) is searching for genetic and clinical factors that predispose patients to adverse drug reactions (ADRs). The aim is to minimise the risks of ADRs by finding biomarkers that enable selection of the right drug for a patient. **Method:** We are assembling a biobank of patients with selected ADRs that are identified from the nationwide ADR reporting system or referred directly from collaborating physicians. Consenting patients are interviewed concerning medical and drug history, and supplementary information is collected from medical records. A blood sample for genomic testing is drawn and kept until a sufficient number of cases are available for genome-wide analyses. We are collecting generalised ADRs, e.g. liver reactions, severe skin reactions and agranulocytosis, and drug-specific ADRs, e.g. angioedema and cough related to ACE-inhibitors or angiotensin receptor blockers and statin-induced myopathy. We have 5000 unrelated controls with genome-wide data from the Swedish Twin Registry. From this cohort it is possible to select *treated* controls, i.e. twins that have been prescribed a drug without being diagnosed with the ADR in question. **Results:** Swedegene started in 2010 and currently has >1600 cases with DNA and complete phenotypes. We have promising, but inconclusive, genome-wide results for three of the ADR diagnoses. **Conclusion:** Serious ADRs are too rare to study in a single country, even using a nationwide ADR register. It is essential to collaborate internationally to obtain enough samples. We invite new collaborators interested in sampling cases with the aim to investigate genetic and clinical risk factors for ADRs.

P15.02-M
Pharmacogenomics and definition of genetic profiles as predictors of blood pressure response to antihypertensive drugs

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OBJECTIVE: In hypertensive population only 1/3 of patients reaches blood pressure (BP) targets with the main classes of antihypertensive drugs, with a heterogeneous BP response.
METHODS: We enrolled newly discovered and never treated (naïve) HT patients with BP office >140/90 and <160/110 mmHg. SNPs were genotyped by 128 SNP array on TaqMan OpenArray system, in genes for renal transport of sodium, the dopaminergic and RAAS system, vasodilation, growth factors. Eligible patients were treated with Perindopril 4 mg or Hydrochlorothiazide (HCTZ) 12.5 mg. Genetic associations with General Linear Model and chi-squared; logistic regression analysis for responder/non responder comparison after one-month therapy.
RESULTS: We derived Genetic profiles for systolic BP (SBP) response to HCTZ or Perindopril as a specific combination of variants selected by pathway-based algorithms associated to greater response to these drugs. Carriers of HCTZ genetic profile (215 HT) displayed a SBP fall of -18.21 mmHg compared to those not having the profile (-5.56 mmHg), with an effect size of -12.6 mmHg (p<0.00001, sensitivity 41%, specificity 96%, PPV 86%, Negative Predictive Value (NPV) 75%). Perindopril genetic profile (149 HT). was associated to a SBP fall of -16.3 mmHg compared to all the others (-4.7 mmHg), with an effect size of -11.6 mmHg (p<0.00001, 94% specificity, 33% sensitivity, 72% PPV, 74% NPV).
CONCLUSIONS: We developed two pathway-based algorithms for creating genetic profiles in response to HCTZ and Perindopril. We are currently employing these genetic profiles to a priori choose the first drug in naïve HT patients, as innovative methodology for treating essential HT.

P15.03-S
The APOB insertion/deletion polymorphism (rs17240441) influences the postprandial triacylglycerol and insulin response in healthy Caucasian adults - insights from the DISRUPT cohort

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The concept of personalized medicine is now being extended to the field of nutrigenetics with the ambition of giving personalised/stratified dietary advice with greater efficacy in health promotion and disease prevention. To this end, we investigated the impact of 18 polymorphisms (previously implicated in lipid metabolism) on postprandial lipid, glucose and insulin responses in up to 262 healthy adults. The participants consumed a standard sequential mixed test meal, which included a test breakfast (0 min; 49g fat) and lunch (330 min; 29g fat). Blood was collected at baseline (0 min) and on 11 subsequent occasions until 480 min after the test breakfast. Plasma total (TC), low density lipoprotein (LDL-C) and high density (HDL-C) cholesterol, triacylglycerol, insulin and glucose was determined. There was a significant impact of APOB insertion/deletion polymorphism (rs17240441) on fasting TC (P=0.003), LDL-C (P=0.003), HDL-C (P=0.0004), triacylglycerol (P=0.003) and insulin (P=0.003) with higher concentrations in the deletion allele carriers. A significantly higher area under the time response curve was evident for the triacylglycerol (P=1x10-6) and insulin (P=0.006) response in the deletion allele carriers (n=93) relative to the insertion/insertion homozygotes (n=52).Our findings indicate that the APOB polymorphism is likely to be an important genetic determinant of the large inter-individual variability in the postprandial response to dietary fat intake. Greater understanding of how APOB gene influences postprandial lipaemia will advance the prospects for personalised nutrition, where the deletion allele carriers may benefit from personalized dietary strategies to reduce the marked lipaemia in response to meal ingestion.

P15.04-M
rs37973 in GLCCI1 is associated with asthma treatment response

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Asthma treatment response is highly variable and pharmacogenetic markers that predict treatment response would be one step closer to personalized treatment. Polymorphisms in *GLCCI1* could be associated with asthma treatment response. We genotyped for rs37973 in *GLCCI1* in 208 adult asthma patients treated with inhaled corticosteroids (ICS). Change in %FEV1 was analysed after 3 months and after at least 3 years of treatment. Treatment success was defined as good when FEV1 decreased less than 30 ml/year. After 3 months of treatment, change of %FEV1 was higher in patients with GG genotype than in patients with AG+AA genotype (p = 0.049), and this genotype dependent difference was only evident and even higher in non-smokers (p = 0.037). Similar results were found after at least 3 years of treatment when all patients were analysed (p = 0.041) and in non-smokers (p = 0.034). Even though, no differences in treatment success (good vs. poor response) were observed when analysing the entire group of patients, treatment success was highly influenced by genotype and smoking status.

GG genotype was overrepresented in non-smokers with good response (p = 0.030) and on contrast, AA genotype in smokers with good response (p = 0.015). There were no differences between smokers and non-smokers when they were not stratified according to genotype. Our results showed that treatment response to ICS is associated with rs37973 in *GLCCI1*, but smoking has great influence on genotype dependent treatment response and further studies are warranted to elucidate the role of *GLCCI1* in treatment response.

P15.05-S

Influence of single nucleotide polymorphisms on deferasirox C_{trough} levels and effectiveness.

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Deferasirox (DFX) is the only once-daily oral chelator for first-line therapy of blood transfusion-related chronic iron overload. DFX pharmacokinetic has been related with response to therapy. This drug is metabolized in liver by UDP-glucuronyltransferase (*UGT*) *1A1* and *1A3*, by cytochrome-*P450* (*CYP*) *1A1*, *1A2* and *2D6* enzymes, and it is eliminated via biliary-enteric circulation through multidrug resistance protein 2 (*MRP2*).
Our aim was to evaluate DFX plasma concentrations according to single nucleotide polymorphisms (SNPs) in genes involved in this drug metabolism and elimination, in a cohort of non paediatric β-thalassemic patients. Further aim was to define a plasma concentration cut-off value predicting an adequate response to therapy.
DFX concentrations were determined from plasma samples obtained at the end of dosing interval (C_{trough}) using an HPLC-UV method. Allelic discrimination for SNPs in *UGT1A1, UGT1A3, CYP1A1, CYP1A2, CYP2D6, MRP2* and *BCRP1* genes was performed by real-time PCR.
DFX C_{trough} levels were significantly influenced by *UGT1A1C>T* (rs887829) [p=0.045], *CYP1A1C>A* (s2606345) [p=0.017], *CYP1A2A>C* (rs762551) [p=0.014], *CYP1A2C>T* (rs2470890) [p=0.004] and *MRP2G>A* (rs2273697) [p=0.032] SNPs. According to Chirnomas and Galanello efficacy definitions, a DFX plasma cut-off value of 20,000 ng/mL was identified (ROC curve, p=0.008). A logistic regression analysis was performed to determine factors able to predict this value: both *CYP1A1 C>A* rs2606345 AA (p=0.017) and *CYP1A2 C>T* rs2470890 TT (p=0.037) genotypes may forecast drug concentrations below 20,000 ng/mL, suggesting a negative predictive role of therapy efficacy.
Our data, the first obtained in non paediatric patients, suggest the feasibility of a pharmacogenetic-based DFX dose personalization.

P15.06-M

ADRB1 and ADBR2 gene polymorphisms and the ocular hypotensive response to topical betaxolol in healthy Mexican subjects

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Background The β adrenergic receptors (ADRB) are expressed in the ciliary body and trabecular meshwork, structures involved in aqueous humor production and outflow, respectively. ADRB are members of the adrenergic family of G-protein-coupled receptors. Topic β blockers have a good local and systemic tolerance; they reduce the aqueous humor production and eye strain blocking the ADRB of the ciliary body and interfering with adenylate cyclase. However, the ocular hypotensive response is not the same in all patients and could be mediated by the polymorphisms of the ADRB genes. Material and Methods Seventy two healthy subjects were studied after treatment with topical betaxolol in both eyes. We analyzed ADRB1 and ADRB2 gene polymorphisms by PCR and automated DNA sequencing. Results There was statistically significant difference between baseline IOP and final IOP of both eyes (Baseline IOP 16.2±1.2 - Follow-up IOP 13.6±2.0 (mean difference -2.5±1.3, P<0.001). Gly389 had a higher baseline IOP than Arg389 (17.0±1.2 mmHg vs. 16.0±1.2 mmHg; P=0.02) and conversely Arg389 had a greater magnitude of response than Gly389 to betaxolol therapy (-2.9±1.1 mmHg vs -0.7±0.4 mmHg; P< 0.001). Gln27 had a higher response than Glu27 (-2.7±1.3 mmHg vs -1.9±1.0 p=0.02) Conclusion Arg389 polymorphism of the ADRB1 gene and Gln27 polymorphism of the ADRB2 gene were associated with the hypotensive response to topic betaxolol in healthy Mexican volunteers.

P15.07-S

The impact of CYP2C19, CYP3A4, CYP2B6, ABCB1, ITGB3 and PON1 gene variants on the antiplatelet effect of clopidogrel in Turkish patients with acute coronary syndrome syndrome

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Clopidogrel is an effective inhibitor of platelet aggregation due to its selective and irreversible blockade of the P2Y12 receptor on platelet cell membranes. Antiplatelet treatment with clopidogrel and aspirin is a recommended procedure for the reduction of stent thrombosis and a vital strategy for patients undergoing percutaneous coronary interventions. It has been demonstrated that clopidogrel does not exert an antiplatelet effect in a certain proportion of patients in many studies, known as clopidogrel resistance.
In order to research the exact mechanism of clopidogrel resistance, we aimed to determine whether there is the impact of CYP2C19, CYP3A4, CYP2B6, ABCB1, ITGB3 and PON1 gene variants on the antiplatelet effect of clopidogrel in Turkish patients. We evaluated on 223 Turkish patients with acute coronary syndrome underwent percutaneous coronary intervention with stent implantation. Platelet reactivity (PRU) and % inhibition were measured with VerifyNow P2Y12 assay in blood samples collected from patients that took a standard dose of clopidogrel (75 mg/day) for at least 7 days. 12 genetic variants were genotyped using the Sequenom MassARRAY system.
The PRU and % inhibition values of the genotypes were compared statistically. The CYP2C19*2 (G636A) single nucleotide polymorphism was associated with a reduced antiplatelet response (p<0.001). Conversely, the CYP2C19*17 (C806T) single nucleotide polymorphism was associated with an enhanced antiplatelet effect (p=0.025). There was no statistically significant difference between the PRU and % inhibition values of the other genetic variant genotypes.
Our findings suggest that clopidogrel resistance might be triggered or decreased by the haplotypes of CYP2C19*2 and CYP2C19*17 variants.

P15.08-M

miR-155 and other miRNAs expression pattern in blood of breast cancer patients and healthy individuals carrying BRCA1 mutation

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miR-155 is oncogenic microRNA upregulated in many tumors including breast cancer (BC). As potential circulating biomarker, expression pattern of miR-155 can be determined from blood and may discriminate BC patients from healthy individuals, report on treatment efficacy and further prognosis. Development of BC may be a consequence of BRCA1 tumor suppressor gene mutations involved in DNA damage response and repair. BRCA1 epigenetically controls miR-155 expression and its pro-cancerous potential. In this study, we evaluated expression levels of miR-155 in circulation of individuals carrying BRCA1 mutations with no disease manifestation as well as in BRCA1 BC patients after tumor resection and therapy.
We determined miR-155 expression in lymphocytes and plasma from peripheral blood. To amplify mature form, we used qRT-PCR with 1 RT and 2 qPCR specifically designed primers. Similarly, we determined expression levels of miR-17, miR-18a, miR-21 and miR-27a, also known to be involved in BC pathogenesis.
Compared to healthy controls, individuals carrying BRCA1 mutations with no disease manifestation displayed an increased miR-155 expression in plasma, but not in lymphocytes. In BRCA1 BC patients, we observed significantly elevated miR-155 expression in both, separated lymphocytes and plasma, a phenomenon that persisted after tumor resection and therapy. Other studied miRNAs were also deregulated.
Our results point to epigenetic control of miR-155 by BRCA1 in wide time scale. Accurate identification of miRNAs from peripheral blood represents the key point of development of non-invasive blood-based detection platforms for BC diagnosis, staging, therapy monitoring and prognosis.
Supported by European fond for regional development ITMS 26240220074/OPVaV-2011/4.2/07-SORO.

P15.09-S
CYP1A2 gene non-coding region polymorphisms in Roma and Hungarian population samples

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CYP1A2 enzyme contributes to biotransformation of wide range of therapeutically important drugs, including caffeine, clopidogrel, clozapine, warfarin, procarcinogens and endogenous substrates. The purpose of this study was to determine and describe the pharmacogenetic profile and interethnic differences of variants of CYP1A2 gene between Roma and Hungarian population. From genomic DNA, 404 Roma and 396 Hungarian healthy subjects were genotyped for two non-coding variants of CYP1A2, namely -163C>A (*1F) and
-3860G>A (*1C). Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique was applied. The minor allele frequency for CYP1A2*1C variant was significantly different between Hungarian and Roma samples (2.02% vs. 0%, p<0.001). The AA homozygous genotype was not detectable. For CYP1A2*1F polymorphism we found a remarkable differences in presence of AA genotype in Roma population compared to Hungarians (31.9% vs. 49.5%, p<0.001) and in minor allele frequency (56.9% vs. 68.6%, p=0.025). The following CYP1A2 genotypes were identified in Roma and Hungarian samples, respectively: *1A/*1A (18.1% vs. 12.4%), *1A/*1F (50% vs. 36.9%), *1F/*1F (31.9% vs. 46.7%). In Hungarian population we found the *1C/*1F genotype (4.04%), but it was not present in Roma subjects. In conclusion, analysis of distribution of CYP1A2 gene variants revealed further pharmacogenetic differences between Roma and Hungarian population samples. Hungarians have higher chance for rapid metabolism of CYP1A2 substrates, intensified procarcinogen activation and thereby elevated risk for cancers.
This research was supported by TÁMOP-4.2.3-12/1/KONV-2012-0028.

P15.10-M
CYP2C9*8 frequency distribution in Puerto Ricans: Implications for warfarin dosing

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Warfarin is an oral anticoagulant that requires individual monitoring since serious adverse events are common. CYP2C9 encodes for the enzyme mainly responsible of S-warfarin's metabolism. Polymorphisms in CYP2C9 have been previously found to be associated with observed warfarin dose variability in different populations, but not in Caribbean Hispanics. Caribbean Hispanics originated as a result of a complex admixture among Caucasians, Africans and Amerindians ancestors-a characteristic that should be considered for warfarin management. The rare loss-of-function CYP2C9*8 allelic variant is reportedly more prevalent among individuals with African heritage. Since Puerto Ricans has a significant contribution of African ancestry in their genetic backgrounds, this cross-sectional study was aimed to determine the frequency of CYP2C9*8 in a cohort of 150 Puerto Rican patients undergoing warfarin therapy. DNA specimens were extracted and genotyped for the CYP2C9*8 using a PCR-based Taqman genotyping assay. We found 3 heterozygous for the CYP2C9*8 variant in our study cohort, corresponding to a minor allele frequency of 1% (95%CI: 0.0026-0.031). The observed frequency met Hardy-Weinberg equilibrium. Allele frequency in our cohort was found to be significantly lower than that from a previous report in African-Americans (0.01 versus 0.047, respectively, p=0.045 by two-tailed z-test), with a carrier frequency of 1 in 50 (Puerto Ricans) versus 1 in 11 (African-Americans). Due to the CYP2C9*8 prevalence found among Puerto Ricans, we concluded that this variant should be included in any pharmacogenetic-guided algorithm for warfarin dose predictions in this population.
Approved by University of Puerto Rico, Medical Sciences Campus Institutional Review Board protocol A4070109.

P15.11-S
Extrapyramidal Disorders while Schizophrenia Therapy - analysis of Cyp2D6*4 allele Influence

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Introduction
A lot of clinical investigations of the schizophrenia therapy reveal the dependence of medicament concentration on the individual metabolism peculiarities. The latter depend to a greater degree on the CYP2D6*4 allelic state of the person. The discrepancy between the metabolic status of the patient and definite antipsychotic drug choice and dosage results in various side effects, among which the extrapyramidal disorders (EPD) are the most invalidating.
Methods
The CYP2D6*4 genotype (rs3892097) was revealed for 211 patients suffering from schizophrenia that were cured with various antipsychotic drugs: Fluphenazine, Trifluoperazine, Haloperidol, Flupentixol, Zuclopenthixol, Sulpiride, Risperidone. PCR with specific primers was used for allele estimation. All the patients from Republican Research and Practice Center for Mental Health where cured with definite (one) medicine for 2 weeks and then examined once for the presence of (EPD) with the help of Extrapyramidal Symptoms Rating Scale (ESRS). The analysis of the results was carried out with the WinPepi package of statistical programs for epidemiology.
Results
In the process of pharmacotherapy the patients fell into two groups: those with (99) or without (112) (EPD). The influence of CYP2D6*4 A allele as a risk factor for the EPD development was proved for Fluphenazine (P =0,001), Flupentixol (0,0001). Conclusion We proved the importance of CYP2D6*4 genotyping for the cohort of the patients we studied that were cured with special antipsychotic medication. The elaboration of informative clinical approaches to the adequate drug choice as well as their dosage must take into account the patient's CYP2D6 genotype.

P15.12-M
Towards personalized medicine in Type 2 Diabetes (T2DM): a genetic risk score for the response to the first line treatment in T2DM, metformin

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T2DM is a common disease characterized by high blood glucose levels. The goal of T2DM treatment is lowering the blood glucose levels and the prevention of complications. For this a stepwise treatment approach is used in which metformin is the first treatment step. Unfortunately the glycaemic response to metformin is highly variable between individuals with a large proportion of patients unable to reach the treatment target, defined as glycated hemoglobin (HbA1c)<53mmol. Genetic factors appeared to be involved in the variability in metformin treatment response (h^2=0.36). At the moment only one GWAS (n=1024) for metformin treatment response has been published (Zhou et al) and this revealed a genome wide significant locus near the *ATM* gene, however, eleven other loci (MAF>0.05) reached borderline significance ($p<10^{-5}$). In this study we measured these SNPs in Dutch T2DM patients treated with metformin monotherapy (n=600) and we generated a genetic risk score of the five SNPs showing a directionally consistent association in our study (Odds Ratios (OR) for achieving treatment success ranged between 0.69 and 0.84). In a logistic regression analysis with baseline HbA1c, eGFR and metformin dose as covariates having more risk alleles lowered the OR for achieving treatment success (ORs for 2/3/4, 5/6, 7/8 or 9/10 risk alleles were 1.0, 0.54, 0.45 and 0.30 resp. p=0.027).
In conclusion, using a cohort of patients with T2DM, we showed that a genetic risk score was associated with metformin treatment response. Our results are a first step towards the introduction of pharmacogenetics in T2DM treatment.

P15.13-S
Variant Server in a box

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Many groups have implemented annotation and analysis pipelines to asses pathogenicity of large numbers of variants observed in next-generation sequencing clinical and research assays. Despite all these efforts only 50-75% of patients receive a negative or uncertain diagnostic report because a small set of time-tested data sources and predictive tools were used and the etiology of many diseases is not known. However, there are hundreds of tools and databases emerging as well as meta-databases and –services that have information for millions of variants. Moreover, there are many more resources such as drug targets, cellular pathways, tissue specific expression, quantitative trait loci, lab tests and model organism data.
Cumulative cost of identification, download, try-out, and quality validation of all these tools and database is huge and even if new tools can be integrated, it is hard to navigate all new information without smart filtering and/or visualization tools, user interfaces for which are not cheap and difficult to create. To enable rapid evaluation of new annotation resources, their

combination in pipelines, and user interfacing in challenging clinical NGS diagnostics, we have created MOLGENIS Variant Service, an open-source web application that can be installed as virtual machine, on local servers, as shared resource, and in a cloud.
We envision an NGS data exploration app as well as a sharing platform for unified data formats and pipelines, gold standard data sets, well-curated reference knowledge-bases, and optimal user interfaces, results of which can disseminate into research institutes, clinical software companies and individual labs.

P15.14-M
Personal genomics in Greece: An overview of available direct-to-consumer genomic services and the relevant legal framework

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The aim of this study is to provide an overview of the DTC genomic services available in Greece and the legal framework within which they operate.
Based on literature review, a questionnaire that was distributed in a genetics conference in Greece and in-depth interviews with human geneticists in Greece, we assess the landscape of the DTC genomic testing market and highlight possible particularities of Greek consumers.
Furthermore, we identify the existing legal framework regarding DTC genetic testing. Our interest is not limited only to issues such as consumer protection laws, lab quality accreditation and the provision of genetic counseling. We also explore the role of medical specialties and their respective legal responsibilities in the Greek context, since for example the specialty of Clinical Geneticist does not exist. We also explore the legal authority of the National Organization of Medicines (E.O.F.) regarding the approval of genetic tests and specific issues relating to paternity tests. We identify gaps in the current regulatory scheme and conclude with recommendations for a more comprehensive legal framework.

P15.15-S
2020 vision and beyond - educating tomorrow's clinicians for the genomic era

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Genomic technology is opening-out the clinical applications of DNA from the preserve of clinical geneticists in single-gene disorders, to integration of genomic information in all clinical care by clinicians in all fields. In the UK, research underpinning this, is spearheaded by current genome sequencing initiatives including GeL and PGP (both 100K genomes), worldwide 1K (now 2.5K), vertebrate 10K, DDD 12K, and cancer 25K.
Consequently, genomic education requires basic generic training for all, plus tailored higher level focus in each specialty, rooted in present practical application, and preparing for the future. The education must be a continuum from medical student to trainee to autonomous clinician, and will require curricular and CPD learning outcomes (LOs), learning resources (LRs) and equipped educators for each level and specialty. Clinical geneticists may need wider genomic training for laboratory interface and educational roles (a recent snap survey of an 'interested expert' audience found only 21% correctly answered a question based on simple molecular report notation and interpretation); but each specialty will need genomic champions as clinical and educational leads.
Key to this is setting genomic LOs and CPD competencies, and identifying and creating appropriate accreditable LRs. At NGGEC, through working with professional groups and educators, we have developed 'genomics' LOs for medical students and trainees, and generic 'genomic' CPD competencies, and are in discussion with educational bodies regarding finalisation and implementation. An audit and evaluation of current on-line genomic LRs will be presented including our own website and e-learning modules, and our view for further resources.

P15.16-M
Functional annotation of Estrogen Receptor binding sites in the light of the 1000 Genomes Project

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Understanding the person-to-person variability in diseases by using the unprecedented amount of available genomic data may be approached as never before. We propose to investigate the impact of individual genetic variations in breast cancers. We performed a meta-analysis of all the publicly available ER ChIP-Seq datasets in four ER positive cell lines: MCF7, T-47D (breast cancer derived) cell lines and ECC1, Ishikawa (endometrium cancer derived) cell lines. The number of peaks in intergenic regions is roughly 50 to 55% while depending on the cell type 30 to 40% of the peaks are localized in introns. Some of the genes may have several intronic binding sites. Intronic binding sites are most likely to regulate their own genes, therefore based on these statistics and the individual assignment of the binding sites to their genomic locations, we have identified a set of genes that have ER binding sites in their introns. The number of SNP-s available in the dbSNP database for the identified intronic binding sites of the identified genes is relatively high, that is more than one thousand. Based on our investigations a significant number of SNP-s could influence directly the estrogen dependent regulation of relevant target genes.

P15.17-S
Circulating levels of FSH in men are genetically determined: study on the combined effect of polymorphisms in the FSHR and FSHB genes

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Recent study have shown that polymorphisms in the FSHR and FSHB genes can modulate circulating levels of FSH. FSHR variants Asn680Ser and c. -29 G>A have been extensively studied in women, while FSHB variant -211 G>T seems to influence male serum FSH levels. There are no studies on the combined effect of these three polymorphism. We have studies 365 subjects: 39 azoospermic, 177 oligozoospermic and 149 normozoospermic. We evaluated seminal parameters; hormone levels; testicular volume; FSHR and FSHB polymorphisms. FSHB polymorphism -211 G>T was found significantly associated to FSH levels. FSHR polymorphism -29 G>A and Asn680-Ser alone, are not associated to different concentration of FSH. Combined analysis of the three polymorphisms again highlights that the major determinant in FSH levels is -211 G>T polymorphism, but shows also that this effect is modulated by -29 G>A polymorphism, as subjects with -211 GG/-29 GG genotype have higher FSH level. Total sperm count and testicular volume is also modulated by the genotype: Homozygotes -211 TT are invariably azoo-oligozoospermic with a reduced testicular volume and FSH <8 UI/L. Combined effect of Asn680Ser is negligible. This is the first combined study on the influence of FSHB and FSHR polymorphisms on male reproduction and shows that -211 G>T FSHB polymorphism plays an important role, only slightly modulated by FSHR polymorphisms, on FSH levels, sperm count and testicular volume. FSHB -211 G>T influences transcriptional FSH gene activity, thus causing an isolated FSH deficiency with azoospermia and thus represents the best pharmacogenetic marker to FSH treatment.

P15.18-M
Evaluation of microarray gene expression profiling as response to zearalenone exposure on normal intestinal epithelial cell

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Zearalenone (ZEA) is an estrogenic secondary fungal metabolite produced by several Fusarium species. Several studies presented the cytotoxic or effect of this mycotoxin, but the specific mechanism of action of ZEA still remains unidentified. In order to decipher the molecular changes that occurred during the exposure ZEA in IPEC cells, we assessed the impact of a single dose on gene expression profile at 24 h posttreatment. 10 μM ZEA was proved to have no effect on cell viability (as displays MTT and xCELLigence data), but the microarray expression profiling data for this dose was lead to the identification of 790 genes overexpressed and 1164 downregulated, considering a fold change of □-1.5or□1.5 with a p-value of <0.05. Gene Ontology (GO) analysis of expression microarray data was done to identify the key processed altered. Some of these gene class associations as represented by GO terms are as would be predicted (cell proliferation and differentiation, apoptosis or cell cycle), while others are unexpected, like the class of the cell adhesion molecules or cellular invasion. These primarily processes altered are usefully to predict the negative impact of this toxin, by generating an interaction network analysis for the significant statistic genes. The effects of ZEA are much more complex as we observed from the bioinformatics analysis of the genes list associated with critical disease pathways for the case of a single non-cytotoxic dose of ZEA, being the first step for the acquisition of genetic alteration, particularly in the case of mycotoxin co-contamination or continuous exposure.

P15.19-S
Taking gene-based diet in real life: a prospective study.

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Increased knowledge in the field of nutrigenetics led to the development of different genetic risk scores and dietary interventions. We have decided to test the efficacy of a gene-based diet (gdiet from www.genex.me) on 191 obese (BMI>25) attending the same nutritional center. Participants were randomly divided in two groups (87 test and 104 controls). For both groups a standard nutritional plan was defined subtracting 600 calories from individual need. DNA from the test group was analyzed for 19 genes known to impact on different metabolic areas and taste. Diet was modulated according to individual genetic profile (i.e. people with a non-favorable lipid metabolism profile were given less lipids in their diet) without varying the overall amount of calories. Physical activities plans were personalized in the same way in the two groups. No significant differences in age, sex and BMI distribution were present in the two groups. Follow-up took place every six months for 2y and showed, in both groups, BMI loss over time and similar compliance. Very interestingly, people in the test group (gene-based diet) lost 33% more weight than controls corresponding to 0.47 BMI points for the square root of time (-1.36 in the control group vs -1.83 in the test group, $p=1.8x10^{-8}$). Similarly, the percentage of lean mass increased more in the test group (+6.2%) than controls (+5.3%) ($p=2x10^{-4}$).
In conclusion, present findings indicate that a gene-based diet might be more effective in helping people loosing weight as compared to standard nutritional plans.

P15.20-M
Urine-derived human renal progenitor cultures for modeling of genetic kidney disorders in subject studied by Next Generation Sequencing

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The advent of high-throughput sequencing has fostered the identification of novel causative genes of kidney disorders, and has allowed the continuous discovery of genetic variants of unknown significance often raising the problem of the functional testing of their pathogenic role. However, emerging evidence implicates that influence of the genomic background of the patient, as well as epigenetic modifications are critical in determining the clinical phenotype. Renal progenitor cell (RPC) cultures obtained from the affected patient may represent an ideal alternative for personalized disease modeling. Since loss of renal cells in urine naturally occurs in patients, urine may represent a potential RPC source.
In this study, we selected and amplified RPC cultures from the urine of patients with renal disease, and we evaluated the possibility to use these cells for modeling of genetic kidney disorders. Urin-RPC were obtained from five children affected by Nephrotic Syndrome carrying mutations in genes encoding for podocyte cytoskeleton proteins, identified thought Next Generation Sequencing, as well as from children without genetic alterations (five). The first cells exhibited altered synthesis of mutated proteins, abnormal cytoskeleton structure and functional abnormalities; by contrast, the second ones showed normal phenotype, structure and function.
The development of functional assays with Urin-RPC could serve as a fundamental step for rapid testing of putative pathogenic mutations. In particular, this tool can provide an essential support for the clinical diagnosis of nephrotic syndrome in patients carrying variants of uncertain significance and provide information to optimize an affected individual's personalized medical care.

P15.21-S
The MedSeq and BabySeq Projects: Clinical trials that explore the impact of genomic sequencing in adults and newborns

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Whole exome and whole genome sequencing (genomic sequencing or GS) is becoming increasingly available and used in medical care. We are conducting the NIH-funded MedSeq and BabySeq Projects to examine the implementation and outcomes of GS in the practice of medicine.
In the MedSeq Project, we are enrolling adult patients and their physicians in both primary care and cardiology settings. In the BabySeq Project, we are preparing to enroll newborn infants and their physicians from the neonatal intensive care unit and the newborn well nursery. In both projects patients are randomized to receive standard of care with or without GS. Physicians and patients are followed through surveys, interviews and medical record review to examine the behavioral and clinical impacts of GS results.
In the GS arms of both projects, GS is conducted and interpreted in CLIA-approved molecular laboratories and the results, including all medically relevant unexpected findings, are being provided in a digestible report to the physicians. Reports summarize and present pathogenic variants in rare dominant conditions, variants indicating carrier status for rare recessive conditions, pharmacogenomics variants for commonly used medications and blood group antigens. Interviews, standardized scales and ongoing reviews of medical records are being used to track physician actions, patient (or parent) behaviors and attitudes, health outcomes and health care costs. The MedSeq and BabySeq Projects will help to illuminate the benefits, risks and limitations of GS in the medical care of both adults and infants.

P15.22-M
Single Nucleotide Polymorphisms of the Glucocorticoid-Receptor Gene Influence the Outcome of Cardiac Surgery

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Cardiac surgery triggers systemic inflammatory response which is associated with postoperative morbidity and mortality. Glucocorticoid effects are mediated by glucocorticoid receptors (GRs) for which a number of common single nucleotide polymorphisms (SNPs) exist that influence GR sensitivity to cortisol. We selected three common SNPs of the GR gene that are known to affect GR sensitivity and analyzed these data in relation to early outcome variables in patients undergoing cardiac surgery. We tested the effects of the following GR-SNPs: rs4123247 (BcII, increased cortisol sensitivity), rs33388 (cortisol hypersensitivity) and rs10052957 (FPB5, cortisol resistance). All study personal was blinded with regard to the patients` genotype. Study endpoints (primary outcome variables) were the required dosages of hydrocortisone during surgery and in the Intensive Care Unit. The study population consisted of 95 patients. Homozygous carriers of alleles associated with increased GR sensitivity (Bcll *G, n=10 and rs33388 *G, n=25) required significantly lower dosages of hydrocortisone in the Intensive Care Unit than non-carriers of the respective alleles. Homozygous individuals (n=6) for the TT-allele of the FPB5-SNP required significantly higher dosages of hydrocortisone during surgery and in the Intensive Care Unit than heterozygous carriers. They also needed significantly higher norepinephrinemax and epinephrinemax dosages in the Intensive Care Unit to achieve hemodynamic stability and showed a significantly longer duration of Intensive Care Unit therapy than heterozygous or non-carriers of the T-allele. In summary, hydrocortisone dosages administered according to evidence based algorithm to cardiac patients during and after cardiac surgery are influenced by SNPs in the GR.

P15.23-S
Genome-wide association study of chemotherapeutic agent-induced severe neutropenia/leucopenia for patients in Biobank Japan

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Chemotherapeutic agents are notoriously known to have narrow therapeutic range that often results in life-threatening toxicity. Hence, it is of clinically important to identify patients who are at high risk for severe toxicity to certain chemotherapy through pharmacogenomics approaches. In this study, we carried out 17 genome-wide association studies (GWAS) with 13 122 cancer patients recruited from the Biobank Japan, who received different chemotherapy regimens, including cyclophosphamide- and platinum-based (cisplatin and carboplatin), anthracycline-based (doxorubicin and epirubicin), and antimetabolite-based (5-fluorouracil and gemcitabine) treatment, antimicrotubule agents (paclitaxel and docetaxel), and topoisomerase inhibitors (camptothecin and etoposide), as well as combination therapy with paclitaxel and carboplatin, to identify genetic variants that are associated with the risk of severe neutropenia/leucopenia in the Japanese population. In addition, weighted genetic risk score analysis was performed to evaluate the cumulative effects of common genetic variants associated with chemotherapeutic agents-induced severe neutropenia/leucopenia. Instead of illustrating all 17 GWAS, we will utilize the result from GWAS of paclitaxel-carboplatin combined therapy for further explanation. Although we failed to identify genetic variants that surpassed the genome-wide significance level

($P<5.0x10^{-8}$) through GWAS, probably due to insufficient statistical power and complex clinical features, we were able to shortlist some of the suggestive associated loci. The current study is at the relatively preliminary stage, but does highlight the complexity and problematic issues associated with retrospective pharmacogenomics studies. However, we hope that verification of these genetic variants through local and international collaborations could improve the clinical outcome for cancer patients.

P15.25-S

Preventive genetic testing for large spectrum of monogenic disorders using microarray technology in Russian population

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According to current statistics around 5% of newborns have hereditary disorder or inborn defect. Our work shows that this number is higher in reality. Often ill child is born in couple without any health problems. Nowadays the most affordable way to detect carrier status in such couples is using microarray technology. The content of such screening panels should be ethnically optimized.

To spread preventive personalized approaches in medical practice in 2013 the microarray "Ethnogene" was developed based on APEX technology. It allows detecting 60 monogenic and 4 multifactor disorders. Its content is adapted to characteristics of Russian population.

9.5% of 213 genotyped patients were diagnosed to have monogenic disease (table 1). 60% appeared to be heterozygous carriers of 1-3 mutations (table 2).

Thus using of microarray "Ethnogene" helps to reveal carrier status among couples planning pregnancy and to understand real frequencies of monogenic disorders in general population of Russia. Early detection of disorders in asymptomatic patients allows preventing severe complications by prescribing pathogenic therapy.

Table 1.

Frequency of detected monogenic disorders in cohort of 213 persons genotyped by microarray Ethnogene

Disorder	Gene	Mutation	Genotype	Frequency, %
Muscular dystrophy, limb-girdle, type 2A	*CAPN3*	c.550delA (Thr184fs)	Del/Del (fs/fs)	0.5%
Ichthyosis vulgaris	*FLG*	c.1501C>T (Arg501ter)	C/T (Arg/ter)	0.9%
		c.2282del4	N/Del	1.5%
		Compound heterozygous state for c.1501C>T (Arg501ter) and c.2282del4	Arg/ter; N/Del	0.5%
Galactosemia, duarte variant	*GALT*	c.-119delGATC	Del/Del	2.3%
Hemochromatosis, type 1	*HFE*	c.187C>G (His63Asp)	G/G (Asp/Asp)	1.4%
		Compound heterozygous state for c.187C>G (His63Asp) and c.845G>A (Cys282Tyr)	His/Asp; Cys/Tyr	1.4%
Familial Mediterranean fever	*MEFV*	c.442G>C (Glu148Gln)	C/C (Gln/Gln)	0.5%
Leber optic atrophy	*MTCYB*	m.15257G>A (Asp171Asn)	A (Asn)	0.5%
Total				**9.5%**

Table 2.

Frequency of heterozygous state for recessive disorders in 213 persons genotyped by microarray Ethnogene

Disorder	Gene	Mutation	Frequency, %
Stargardt disease 1	*ABCR*	c.2588G>C (Gly863Ala)	3.4%
		c.3113C>T (Ala1038Val)	
		c.5882G>A (Gly1961Glu)	
Wilson disease	*ATP7B*	c.3402delC (Ala1135fs)	0.5%
Muscular dystrophy, limb-girdle, type 2A	*CAPN3*	c.550delA (Thr184fs)	0.5%
Cystic fibrosis	*CFTR*	c.1518delCTT (Phe508 del)	2.5%
		c.3587C>G (Ser1196ter)	
		c.621+1G>T	
		dele2,3 21080bp	
Myotonia congenita, recessive	*CLCN1*	c.1437_1450del14 (Ile479fs)	5.3%
		c.2680C>T (Arg894ter)	
Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation	*DARS2*	c.455G>T (Cys152Phe)	0.5%
Smith-Lemli-Opitz syndrome	*DHCR7*	c.452G>A (Trp151ter)	0.5%
Glycogen storage disease II	*GAA*	IVS1AS-13t>g	0.5%
Galactosemia, duarte variant	*GALT*	c.-119delGATC	8%
Galactosemia	*GALT*	c.1430A>G (Gln188Arg)	1%
		c.855G>T (Lys285Asn)	
Deafness, autosomal recessive	*GJB2*	c.35delG	0.5%
Hemochromatosis, type 1	*HFE*	c.187C>G (His63Asp)	32.3%
		c.845G>A (Cys282Tyr)	
Epidermolysis bullosa, junctional, Herlitz type	*LAMB3*	c.1903C>T (Lys635ter)	0.5%
Familial Mediterranean fever	*MEFV*	c.2282G>A (Arg761His)	1.5%
		c.442G>C (Glu148Gln)	
Phenylketonuria	*PAH*	c.1208C>T (Ala403Val)	1.5%
		c.1222C>T (Arg408Trp)	
Mitochondrial DNA depletion syndrome 4B (MNGIE type)	*POLG*	c.752C>T (Thr251Ile)	0.5%
Hyperphenylalaninemia, BH4-deficient, A	*PTS*	c.216T>A (Asn72Lys)	1%
Alpha-1-antitrypsin deficiency	*SERPINA1*	c.1096G>A (Glu342Lys) c.863A>T (Glu264Val)	1.5%

P15.26-M

Assessing the role of known MS genetic variants in the familial aggregation of MS and other autoimmune diseases

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Background: The contribution of MS loci on familial aggregation for MS or other autoimmune diseases is still unclear.

Objective: The aim is to investigate the role of MS associated genetic variants in explaining the familial aggregation for MS or other autoimmune diseases, by comparing sporadic and familial MS cases of Italian origin.

Methods: genetic and familial aggregation data were available on 572 MS patients (474 sporadic cases and 98 familial cases). A weighted genetic risk score (wGRS), based on 106 SNPs found to be associated to MS according to the Immunochip project, was calculated.

Results: we did not observe a difference in terms of wGRS between sporadic and familial cases of MS (10.67±0.038 vs 10.62±0.093). However, when assessing the impact of the known MS loci on the aggregation for MS and other autoimmune diseases, we observed that there is a statistically significant difference between sporadic and familial cases (10.71±0.046 vs 10.56±0.066, $p<0.05$). Moreover, it appears that males with other cases of MS or autoimmune diseases in the family have a higher wGRS compared to females (10.81±0.120 vs 10.45±0.078, $p=0.02$).

Conclusions: our data suggest that known MS genetic variants may have a role in explaining the aggregation for MS and other autoimmune diseases; this is in agreement with the fact that most of these loci are involved in immunological functions. When stratifying by gender, it appears that the genetic variants have a stronger impact in males, suggesting that probably in females other factors are involved, like hormones or other environmental causes.

P15.27-S

A pharmacogemonic study of long-term response to Interferon-beta treatment in patients with Multiple Sclerosis

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Background: Previous pharmacogenomic studies conducted in Interferon-beta (IFN)-treated Multiple Sclerosis (MS) patients explored the influence of genetic variants on the short-term response to the drug. The aim of this study is to perform a genome-wide association study (GWAS) of long-term response to Interferon-beta observed using a follow-up period of 4 years after drug start.

Methods: The GWAS study was conducted using the Omni-express Illumi-

na® array on a cohort of 329 IFN-beta treated MS patients followed for 4 years of treatment. We used three different outcome measures of response to treatment: a disability criterion, measured as the increase of ≥1 point at Expanded Disability Status Scale (EDSS), and two composite criteria, which take into account measures of disability progression and relapse occurrence (≥2), on which separate GWAS analyses have been performed.
Results: No genome-wide significant association signal was detected. When using a cut-off p-value of 10^{-4}, 42 SNPs emerged as associated according to the disability criterion, while 28 and 41 SNPs for the two composite criteria. A modest overlap (11.8%) was observed among associations across three phenotypes.
Conclusions: Our study showed new targets potentially involved in the modulation of the response to IFN-beta treatment. An in silico replication analyses on identified targets is planned on an independent cohort of treated MS patients.

P15.28-M
Genetic polymorphisms influence the response to adalimumab in Crohn disease patients

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Biological therapy using fully humanized monoclonal antibodies against TNF-α (adalimumab) is efficiently used to induce and maintain remission in around 70% of Crohn disease (CD) patients not responding to standard treatment or developing adverse drug effects to corticosteroids. We investigated if single nucleotide polymorphisms (SNPs) contributing to CD risk could help to predict the response to adalimumab (ADA) treatment in CD patients. We used IBDQ index and biological biomarkers (CRP levels) to monitor therapy response after 4, 12, 20 and 30 weeks after first treatment with adalimumab. The strongest association between CRP levels and treatment with ADA was found for ATG16L1 SNP rs10210302. After 12 weeks of treatment 85% of patients with CT or TT genotype in rs10210302 had a positive response (drop of CRP to normal levels or by more than 25%) to treatment with ADA compared to 37,5% of patients with CC genotype (p=8,11E-04). We also found significant associations between SNP rs10512734 located near PTGER4 gene and ADA treatment response measured with both, IBDQ index and biological response measured with CRP. Average increase in IBDQ index (delta IBDQ) after 12 weeks of treatment was higher in the group of patients with GG genotype for SNP rs10512734 (47,6) compared to those with AA or AG genotype (17,4, p=7,06E-03). Additional SNPs in 6 out of 33 tested CD associated genes (CASP-9, IL27,C11orf30, CCNY, IL13) showed suggestive association with ADA response. Our results suggest ADA response in CD patients in partially genetically predisposed by SNPs in CD risk genes.

P15.29-S
AlleleTyper™ Software: a flexible application for mapping SNP genotype and CNV data patterns to pharmacogenomic allele nomenclature

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Pharmacogenomic (PGx) studies require genetic testing of individuals for multiple variants in drug metabolism enzyme (DME) and transporter genes. For phenotype interpretation purposes, genotyping results must be translated to star (*) allele nomenclature. Star alleles are gene level haplotype patterns that are associated with protein activity levels. Genetic variants within a haplotype can include SNPs, InDels, and copy number variations (CNVs). Knowing the combination of variants within a given haplotype, and the diploid content in an individual, is of key importance for studying drug metabolism, response and adverse reactions. To facilitate the translation of results for individuals genotyped in studies using TaqMan® SNP and DME Genotyping Assays and TaqMan® Copy Number Assays, we developed a web-based flexible software tool called AlleleTyper™. This software maps sample genotyping data to genetic pattern information translation tables to star allele or other nomenclature. User-defined monoallelic translation tables containing haplotype genetic information, for the gene variants tested in a study, are automatically converted by the software to biallelic translators containing diploid genetic patterns. AlleleTyper™ matches the sample genotypes in results files from TaqMan® Genotyper Software and/or CopyCaller® Software to the patterns in the biallelic translator and reports the star allele genotypes determined for each sample. A review of the software workflow and features will be presented, along with data analysis examples. AlleleTyper™ Software greatly facilitates PGx study data analysis. It can also be used for other genotyping applications that require translation of data from multiple TaqMan assays, including triallelic SNP data analysis and blood genotyping.

P15.30-M
ABCB1 haplotype construction in a pharmacogenetic study of cyclosporine treatment response in Greek patients with psoriasis

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Psoriasis is a chronic, inflammatory skin disorder affecting 2-3% of the population worldwide. While there are a large number of effective modalities for treating psoriasis, response to therapy varies among patients which could be due to genetic factors. Cyclosporine is considered to be a cost-effective first-line systemic therapy for psoriasis, however the response rate is around 60-70%. The aim of the present study, which is based on a Greek multi-centre collaboration, was to target ABCB1, which encodes for P-glycoprotein, by selecting polymorphisms that could influence the absorption and disposition of P-glycoprotein substrate drugs like cyclosporine. In detail, T-129C (rs3213619), G1199A (rs2229109), C1236T (rs1128503), G2677T (rs2032582) and C3435T (rs1045642) polymorphisms were selected as candidate markers of response to cyclosporine treatment after 3 months of therapy and genotyped in 84 psoriasis patients under cyclosporine therapy. Fifty-two patients (62%) were defined as responders (ΔPASI ≥75%) and thirty-two (38%) as non-responders (ΔPASI ≤50%). Single-SNP and haplotype construction showed that the haplotype for marker C3435T could account for the prediction of ~20% of the non-responders to cyclosporine therapy. Notwithstanding the importance of this finding, there is a remaining 80% to be identified, which could be illuminated by systems network analysis, whereby additional pharmacogenetic targets that may be involved in cyclosporine transport, processing or metabolism are identified. Overall, systems biology coupled with experimental validation of specific markers in large independent cohorts could lead in the creation of a molecular algorithm for the prognosis of psoriasis patients' response to cyclosporine as well as for other therapies.

P15.31-S
Identification of a new late radiotherapy toxicity locus through a three stage genome wide association study

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Increasing evidence supports the role of genetic variants in the development of radio-induced toxicity. Therefore, we performed a three-stage genome wide association study that involved a Spanish cohort of 741 prostate cancer patients treated with radiotherapy to identify new susceptibility loci. The replication cohorts consisted of 633 prostate cancer patients from the UK, and 368 prostate cancer patients from a North-American Caucasian cohort.
Standardized total average toxicity (STAT) scores were derived from individual toxicity endpoints to assess overall acute and late toxicities. Association tests of genotype with STAT-acute or STAT-late scores were performed with linear regression. Residuals from multivariate linear regression models, including associated non-genetic covariates, were calculated for each patient to quantify the toxicity not accounted for by the available non-genetic covariates. To obtain per allele ORs by logistic regression, patients were dichotomized as ≤ or > than 1 standard deviation of the acute and late residuals.
Seven and 42 loci were associated (P-value≤10-5) with overall acute and late toxicity, respectively. Only one locus associated with overall late toxicity, 2q24.1 (STATlate P-value =6.85x10-9; OR=6.67, 95%CI: 2.25-19.80) was replicated in the UK cohort (STATlate P-value=2.08x10-4, OR=6.17, 95%CI: 2.25-16.95; meta-analysis P-value=4.16x10-10). The inclusion of the third

cohort gave a meta-analysis P-value=4.64x10-11.
Although it is biologically possible that this locus is involved in the regeneration of muscle previously damaged by radiotherapy, future efforts are needed to identify the causal variant underlying the observed association and determine the molecular mechanisms involved.

P15.32-M
Genetic determinants of methotrexate toxicity in Tunisian patients with rheumatoid arthritis: a study of polymorphisms involved in the folate pathway of methotrexate

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Methotrexate (MTX) is an anti-rheumatic drug used in the treatment of rheumatoid arthritis (RA). However, side events are present in 40% of the patients.
The aim of this study was to determine the impact of genetic polymorphisms of 5, 10-methylenetetrahydrofolate reductase (MTHFR C677T and A1298C), Dihydrofolate reductase (DHFR 19-base pair deletion allele), thymidylate synthase (TYMS 2R→3R), methionine synthase (MTR A2756G) and methionine synthase reductase (MTRR A66G) in a group of 143 Tunisian RA patients and evaluate its association with MTX toxicity.
Patients who experienced MTX adverse events were defined "with toxicity", those who did not, as "without toxicity". Genotyping was performed using PCR and PCR-RFLP method. Demographic and clinical characteristics were obtained and MTX-related adverse effects were recorded. Allele and genotype association were performed using chi2 test , genotype relative risk (GRR) and Odds ratio (OR). The regression logistic was also used to investigate the correlation between patient characteristics (MTX dose, duration of treatment, disease duration, age, sex, route of MTX administration) and toxicity.
The analysis highlighted a significant genotypic association of MTHFR C677T polymorphism with increased MTX toxicity [p=0.004], and the strongest association was shown in the T/T genotype [p=0.006]. However, The MTHFR A1298C , DHFR 19-base pair deletion allele and MTR A2756G polymorphisms were not associated with increased MTX toxicity. While TYMS 2R→3R polymorphism had a protective effect on overall MTX toxicity [p=0.038]
Moreover, our results revealed a positive correlation of both dose and route of administration of MTX with toxicity in RA patients (p=0.027; p= 0.004) respectively.

P15.33-S
CYP1A1 variant in Roma population samples

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Polycythemia vera is a rare bone marrow disorder that leads to an abnormal raising in the number of red blood cells, although the numbers of white blood cells and platelets are also on high levels. In the background of the disease a gene mutation called JAK2V617F can be found, but the cause of this and other possible disorder-causing mutations is undiscovered. Our goal was to examine the rs1048943 polymorphism located in CYP1A1 gene in 90 Roma (Gipsy) with polycythemia vera versus 95 Roma individuals without this disease. Genotypes were determined with real-time-PCR method, SPSS 20.0 statistical program was applied for evaluating the results. The preliminary data showed 6.11% G allele frequency in Romas with polycythemia vera, which it was found to be 3.70% in non-affected Roma controls. The CYP1A1 1384 A/G mutation has never been analyzed in Roma population before, but further extended studies are required to test the clinical relevance of CYP1A1 in different populations.

P15.34-M
Full resequencing of TRAF3IP2 gene in Mozambican patients with SJS/TEN induced by Nevirapine treatment: a pharmacogenetics study

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Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor, widely prescribed for type 1 human immunodeficiency virus infection. A small proportion of individuals treated with NVP experience very severe cutaneous adverse events, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN).
In the last years TRAF3IP2 gene variants were associated with susceptibility to psoriasis, psoriatic arthritis, systemic lupus erythematosus and cutaneous manifestations in inflammatory bowel disease. We hypothesized that this gene, involved in immune response and in NF-KB activation, could be also implicated in the SJS/TEN susceptibility.
To verify our hypothesis we performed a full resequencing of TRAF3IP2 gene in a population of patients treated with NVP. Twenty-seven patients with NVP-induced SJS/TEN and 78 controls, all from Mozambique, were enrolled. All ten exons of TRAF3IP2 gene, including the intron/exon boundaries, were analyzed by direct sequencing. We performed a case/control association analysis and a multivariate logistic analysis.
We identified 8 exonic and 3 intronic variants. We did not find any novel variations. The case/control association analysis highlighted an association between the rs76228616 SNP in exon 2 and the SJS/TEN susceptibility. In particular the variant allele (C) was more present in SJS/TEN patients than in controls, resulting significantly associated with a higher risk to develop SJS/TEN (P=0.012 and OR=3.65 [95% CI 1.33-10.01]). A multivariate analysis by logistic regression confirmed the significant involvement of TRAF3IP2 (rs76228616) in the susceptibility to SJS/TEN (P=0.027). Of course, further studies on larger samples and replications in other African populations are necessary to confirm our results.

P15.35-S
Potentially Functional Single Nucleotide Polymorphisms (pfSNPs) associated with Response to Fluorouracil in Metastatic Colorectal Cancer Patients

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Colorectal cancer (CRC) is amongst the top three most commonly diagnosed cancer in the world. Majority of these patients require treatment for metastatic CRC since a third are at stage IV during diagnosis and another third of the 'curatively' resected patients (Stages I-III) will relapse. 5-fluorouracil is a common drug used for the treatment of CRC but the response rate to this drug either alone or in combination is less than 40%. Developing a reliable biomarker that can predict response can facilitate the appropriate tailoring of treatment for these patients.
Here we report a novel potentially functional Single Nucleotide Polymorphism (pfSNP) approach to identify SNPs predictive of response to 5-FU in Chinese metastatic colorectal cancer (CRC) patients. 1547 pfSNPs and one variable number tandem repeat (VNTR) in 139 genes in 5-FU drug (both PK and PD pathway) and colorectal cancer disease pathways were examined in 2 groups of CRC patients. Shrinkage of liver metastasis measured by RECIST criteria was used as the clinical end point. We identified a total of 9 novel pfSNPs including 4 non-responder-specific pfSNPs with potential functional significance that may be able to distinguish non-responders from responders to 5-FU. These may thus serve as good biomarkers for response to 5-FU.

P15.36-M
Semiconductor Next Generation Sequencing (Ion Torrent) of the ABCB1, CYP3A5, and CYP3A4 genes in kidney transplanted patients treated with Tacrolimus

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Tacrolimus (Tac; FK-506) is an immunosuppressive drug used to avoid the rejection of solid organs. Tac has a narrow therapeutic range and a high interindividual variability in dose-requirements. Previous studies have linked common CYP3A4-3A5 and ABCB1 (MDR-1) polymorphisms to Tac dose requirements (CYP3A5*3, CYP3A4*1B, CYP3A4*22, C3435T). Our aim was to identify new rare CYP3A5, CYP3A4 and ABCB1 variants that could influence Tac dose through massive parallel sequencing with the Ion Torrent PGM. We created three pools of 75 patients who differed in Tac dosage. The coding exons of the 3 genes were amplified in only two tubes with a custom Ampliseq, and the three pool reactions bar-coded, library amplified, and sequenced in a semiconductor PGM-318 array. These pooling + multiplex approach would facilitate the rapid screening of the three genes at a low cost and with minimum labor requirements. We identified several rare variants in CYP3A5 and CYP3A4 (P405T in CYP3A5, and S195P and I193S CYP3A4 new, non reported). These missense changes could affect protein function. No missense ABCB1 variants were found.
In conclusion, we identified new variants in CYP3A5 and CYP3A4 that could have an effect on Tac dose requirements. Our NGS-PGM procedure would help to uncover the variation in these genes at a population scale.

P15.37-S
TGF-B1 -509C>T polymorphism and response to montelukast in childhood asthma

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Transforming growth factor-beta 1 (TGF-B1) is a key mediator in asthma airway inflammation and remodelling. Leukotriene receptor antagonists are among the most prescribed drugs for asthma management. They decrease the level of inflammation in asthmatic airways and inhibit the expression of the TGF-B1. The aim of this study was to investigate the influence of the most common polymorphism in the TGF-B1 gene, -509C>T, on the response to montelukast in childhood asthma. Response to montelukast was tested in an ex vivo experiment using induced sputum cells from six children (five girls and a boy, age 7-18 years) previously genotyped for TGF-B1 -509C>T polymorphism. The cells isolated from 1mL of induced sputum were incubated with and without montelukast (50nmol) and the levels of TGF-B1 were measured by a commercial enzyme-linked immunosorbent assay kit. In all six cases after the treatment with montelukast a decreased TGF-B1 levels in sputum were observed. Although the differences were not statistically significant, the decrease in TGF-B1 levels was different in carriers of different genotypes: CC - 738±107pg/mL, CT - 1439±616pg/mL, and TT - 397±187pg/mL. Although preliminary, the results of this study indicate that the TGF-B1 gene promoter polymorphisms may influence response to leukotriene receptor antagonists in childhood asthma. This finding should be confirmed in a larger cohort of patients and the effect of other polymorphisms should also be investigated. Ex vivo stimulation of sputum cells may be further developed into a test for assessment of individual response to asthma medications and potentially used as personalized medicine tool.

P15.38-M
Development of personalised therapeutics for lattice corneal dystrophy type I

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Personalised medicine offers the prospect of treating genetic conditions using novel molecular methods, and our current research seeks to establish a viable treatment for Lattice Corneal Dystrophy Type I (LCDI) using mutation-specific short-interfering RNAs (siRNAs).
Corneal dystrophies comprise a group of debilitating hereditary disorders. Most of these conditions are inherited autosomal dominantly and caused by deleterious missense mutations. To date our group has focused on two corneal disorders; Meesmann's Epithelial Corneal Dystrophy (MECD) and Lattice Corneal Dystrophy type I (LCDI), which are caused by mutations in the *KRT12* and *TGFBI* genes, respectively. The primary focus of this research was to develop a lead siRNA targeting the TGFBI-Arg124Cys mutation, the most common cause of LCDI.
A panel of 19 mutation specific siRNAs was assessed to select lead candidate siRNAs using a high-throughput dual-luciferase reporter assay and pyrosequencing to determine the lead siRNA. Potency and allele specificity of the lead siRNA was assessed in corneal epithelial cells isolated from a patient with a TGFBI-Arg124Cys mutation, using pyrosequencing, qRT-PCR and a TGFBI ELISA. Treatment with this siRNA resulted in a 44% reduction ($p<0.01$) of the endogenous Arg124Cys allele in this *ex vivo* model of LCDI, and was without effect on the wild-type allele, demonstrating allele specificity. This research confirms the potential of siRNA therapeutics as a personalised medicine approach for the management of heritable TGFBI-associated corneal dystrophies and opens the prospect for the translation of this technique to the treatment of other corneal dystrophies.

P15.39-S
Search for potential causal variants in transcription factor binding sites using a database integrating SNPs and human genome information

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Human genetic variation underlies a majority of phenotypic differences between individuals, including susceptibility to disease.
Genome wide association studies (GWAS) have identified many susceptibility loci/SNPs for complex traits and diseases. The greatest challenge in the 'post-GWAS' era is to understand the functional consequences of these loci. When SNPs occur within a gene or in a regulatory region, they may play a direct role in disease by affecting the gene function. Therefore SNPs may help to predict an individual response to certain drugs or the risk of developing particular diseases. SNPs are found everywhere and those associated to complex diseases are found preferentially in non-coding regions. The recognition and the binding of transcription factors (TF) to specific consensus sequences in the genome are crucial for a successful transcriptional regulation in cells. Therefore, we thought to predict and identify potential causal variants that can alter the binding sites of TF using a database integrating SNPs and human genome information. To this frame, we applied this approach looking for variants altering the Activator Protein 1 (AP1) binding site and we identified 7518 SNPs capturing approximately 4500 genes throughout the whole human DNA sequence. In order to screen for candidates genes to be further analysed with functional studies, predicted results were grouped and analysed with a large number of bioinformatics tools. Overall, these findings will give new insights into the value of SNPs in AP1 consensus sequence and it will provide an innovative approach that potentially yields additional new candidates genes for several disorders.

P16.01-S
Searching for circulating epigenetic biomarkers of Alzheimer's disease

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Increasing evidence points to a possible contribution of folate metabolism in modulating the methylation profile and the expression of disease-related genes in complex diseases, such as Alzheimer's disease (AD). Impaired folate metabolism could result in altered DNA methylation and expression of genes involved in AD pathogenesis.
Indeed, taking into account the inaccessibility of brain DNA samples until death, there is increasing interest in searching for peripheral epigenetic biomarkers of the disease.
In this regard we obtained peripheral blood DNA of 25 AD patients, 25 individuals with Mild Cognitive Impairment (MCI), and 25 matched controls and searched for changes in DNA methylation of the promoter/first exon of genes involved in DNA methylation: DNMT1, DNMT3B and MTHFR, and in amyloid beta production: BACE1 and PSEN1.
DNA methylation analyses were performed by means of Methylation Sensitive-High Resolution Melting (MS-HRM) technique. Moreover, we searched for correlation between the methylation levels of each of the studied genes and circulating levels of homocysteine, folate and vitamin B12, all involved in one-carbon metabolism the key pathway for DNA methylation reactions.
The MTHFR gene showed an inter-individual variability in methylation profiles, and we observed a significant inverse correlation between plasma homocysteine levels and the methylation status of the MTHFR gene. To further address the link between one-carbon metabolism and DNA methylation profiles, we are currently assessing global peripheral DNA methylation biomarkers by means of the analysis of long interspersed nuclear elements-1 (LINE-1).
The study was supported by the Italian Ministry of Health (GR-2009-1606229; F.C. Principal Investigator).

P16.02-M
Not all reference gene annotation is created equal

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Having a good quality geneset is essential for the interpretation of functional genomic, functional transcriptomic, and variation data. The GENCODE geneset represents the reference human gene annotation for the ENCODE project and is produced by merging manual annotation and automated Ensembl gene predictions with extensive computational and experimental QC and validation. Using different reference genesets will inevitably give divergent results and the absence, truncation or misannotation of a gene, exon, or alternatively spliced (AS) transcript may hinder analysis. We will highlight some significant differences between the GENCODE and NCBI Reference Sequence Database (RefSeq) genesets. Specifically, we will discuss divergence in the annotation of alternative splicing (where GENCODE protein-coding loci have a mean of 7.6 AS transcripts while RefSeq only have 2.1), long non-coding RNAs (GENCODE 2.5 fold more genes and 3.7 fold more transcripts), pseudogenes (GENCODE 10% more loci), genomic coverage of annotated exons (GENCODE 1.7 fold greater coverage), degree of manual curation (GENCODE 4.5 fold more manually curated transcripts), experimental validation, and functionally descriptive biotypes. We will detail the continued extension and refinement of the GENCODE geneset, including the integration of RNAseq, CAGE, polyAseq, ribosome profiling and epigenomic data, to

identify novel loci, define 5' and 3' transcript boundaries and identify novel translation initiation sites. Finally, we will explain our use of RNAseq data to determine the expression level of all GENCODE transcripts, allowing us to present a reduced, but biologically meaningful, set of transcripts including e.g. only those that are highly expressed or expressed in a particular tissue.

P16.03-S
Jannovar: A Java library for Exome Annotation

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Transcript-based annotation and pedigree analysis are two basic steps in the computational analysis of whole-exome sequencing experiments in genetic diagnostics and disease-gene discovery projects. Here, we present Jannovar, a stand-alone Java application as well as a Java library designed to be used in larger software frameworks for exome and genome analysis. Jannovar uses an interval tree to speed up the identification of all transcripts affected by a given variant, and provides HGVS-compliant annotations both for variants affecting coding sequences and splice junctions as well as UTR sequences and non-coding RNA transcripts. Jannovar can also perform family-based pedigree analysis with VCF files with data from members of a family segregating a Mendelian disorder. Using a desktop computer, Jannovar requires a few seconds to annotate a typical VCF file with exome data.

P16.04-M
Comparison of GWAS and EWAS results to identify loci and genes of interest in asthma

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Asthma is a complex trait with a large phenotypic diversity for which more than 300 genes have already been associated. In order to better define the heritability of the disease, "omics" technologies were used in several studies to explore genetic and epigenetic factors. The large amount of results obtained with these methods comes with complications for identifying true genes of interest. This study aims to compare genome-wide association studies (GWASs) and epigenome-wide association studies (EWASs) of asthma and related phenotypes (atopy and allergic asthma) using a well-described asthma familial collection in order to identify loci and genes of interest. GWASs and EWASs were performed on DNA samples extracted from whole blood and EWASs were also performed on DNA extracted from isolated eosinophils. GWASs were analyzed using a quasi-likelihood score test performed using the MQLS program and EWASs data were compared using a Mann-Whitney analysis implemented in the GenomeStudio software by Illumina. Analyses allow identifying 2 common loci between EWAS and GWAS analyses for asthma phenotype, 5 common loci for atopy and 3 for allergic asthma. Among them 1 principal locus (including 5 CpG sites or more) was identified in asthma (17q21.2) and 2 principal loci in atopy (5q31.1 and 6p22.1). The three loci identified have already been associated with asthma in previous studies. Analyses are in progress in order to compare common genes associated in both "omics" methods. These results highlight the potential of the combination of "omics" analyses in order to identify potential loci of interest in complex traits.

P16.05-S
DNA methylation signature of IL1R1 and IL1R2 genes in asthma

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We have previously reported an association between interleukin 1 receptor (*IL1R*) type 1 and 2 polymorphims and asthma and related phenotypes. We have also reported *IL1R2* mRNA level differences in lung tissue of asthmatic individuals in comparison to controls. According to these results, this study aims to assess whether DNA methylation levels at both loci are also associated with asthma and allergic asthma and correlate with their mRNA levels. We measured leucocyte's DNA methylation levels at *IL1R1* and *IL1R2* gene loci for 93 individuals using bis-pyrosequencing. mRNA levels were quantified using qRT-PCR in a subsample of 37 individuals. Individuals with asthma had higher DNA methylation levels at both *IL1R1* and *IL1R2* gene loci in comparison to controls (without history of asthma and/or atopy) (15.9±4.1 vs 13.0±2.9, p=0.02 and 48.6±13.1 vs 40.8±10.8, p=0.04 respectively). Higher methylation levels were also observed in individuals with allergic asthma at two *IL1R2* CpG sites (48.4±13.4 vs 40.8±10.8, p=0.03 and 32.0±9.4 vs 25.5±8.3, p=0.02). Finally, *IL1R2* DNA methylation levels were negatively correlated with *IL1R2* mRNA levels in our subsample of 37 participants (r=-0.45, *p*=0.007). This study highlights that *IL1R1* and *IL1R2* have a specific epigenetic signature in the context of allergic asthma and that differences in *IL1R2* gene expression might be related to DNA methylation level changes.

P16.06-M
Methylation analysis and molecular diagnostics of Beckwith-Wiedemann syndrome in 1000 subjects

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Beckwith-Wiedemann syndrome (BWS), a congenital overgrowth disorder with variable expressivity, results from disordered expression and/or function of imprinted genes at chromosome 11p15.5. There are no generally agreed clinical diagnostic criteria, with molecular studies commonly performed to confirm diagnosis. In particular, methylation status analysis at two 11p15.5 imprinting control centres (IC1 and IC2) detects up to 80% of cases. In order to evaluate the relationship between the clinical presentation of suspected BWS and IC1/IC2 methylation abnormalities we reviewed the results of >1000 referrals for diagnostic testing.
507/1091 (46.5%) referrals had a positive diagnostic test. The rate of a positive diagnostic test increased with increasing numbers of clinical features. Previously reported genotype-phenotype correlations with paternal uniparental disomy, IC1, and IC2 epimutation groups were confirmed and potential novel associations detected. Predictive values of previously described clinical diagnostic criteria were compared, and although there were differences in sensitivity and specificity, receiver operating characteristic (ROC) analysis demonstrated that these were not optimal in predicting 11p15.5 methylation abnormalities. Using logistic regression, we identified clinical features with the best predictive value for a positive methylation abnormality. We developed a weighted scoring system (sensitivity - 75.9% and specificity - 81.8%) to prioritise patients presenting with the most common features of BWS, and ROC analysis demonstrated superior performance (area under the curve - 0.85; 95% CI: 0.83-0.87) compared to previous criteria.
We suggest that this novel tool will facilitate selection of patients with suspected BWS for routine diagnostic testing and so improve the diagnosis of the disorder.

P16.07-S
Genome-wide methylation analysis on epimutated BWS patients for detection of known and novel imprinted loci multilocus defects

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Loss of imprinting (LOI) through methylation loss (LOM) or gain (GOM) may result in imprinting disorders (IDs). Beckwith Wiedemann syndrome is a paradigmatic ID, which arises from dysregulated expression of imprinted growth regulatory genes at 11p15.5. We pursued a genome-wide methylation study to investigate our cohort of IC2 hypomethylated BWS patients for the occurrence of Multilocus Methylation Defects (MMD) at imprinted loci. To this purpose we selected from a cohort of 171 patients with clinical presentation in the spectrum of Beckwith-Wiedemann syndrome carrying an 11p15.5 epimutation, 18 cases hypomethylated at IC2: 14 with a complete phenotype, 2 with isolated hemihyperplasia (IH) and two couples of monozygous twins with discordant phenotype, but concordant for the IC2 defect in blood tissue. The Processing of genomic DNAs from peripheral blood on an Infinium Human Methylation 450K BeadChip Kit Array (Illumina)showed that 7/16 (44%) of IC2 epimutated BWS and 2/2 IH patients

had multilocus defects. These comprised hypermethylation at GNAS DMRs and hypomethylation at GNAS, PLAGL1, DIRAS3, FAM50B and ZNF331 loci, confirming the view of a network of imprinting deregulated genes. MMD were detected in blood and saliva from both affected twin members, but complementary pyrosequencing and MS-MLPA analyses showed that methylation defects were absent/attenuated in the DNA from saliva in the unaffected co-twins. This findings will enhance clinical practice and epigenotype phenotype correlations. In conclusion 450K genome approach appears a reliable technique to profiling the methylation status of all known imprinted loci and unravel their recurrent simultaneous deregulation Supported by 2009MBHZPR_003(to LL).

P16.08-M
Comprehensive methylation profiling in Beckwith-Wiedemann syndrome and Silver-Russell syndrome

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The investigation of human imprinting disorders has provided important insights into the role of genomic imprinting in normal health and development. Beckwith-Wiedemann syndrome (BWS) is a congenital overgrowth disorder associated with abnormal function of 11p15.5 imprinted genes. The most common cause of BWS is loss of methylation (epimutation) at the imprinting centre 2 (IC2/KvDMR1). We and others have found that a subgroup of BWS patients also harbour epimutations at other imprinting centres (ICs) outside of 11p15.5. This multiple epimutation (ME+) phenotype has been associated with assisted reproductive technologies births though the clinical significance of these additional epimutations has not been clearly defined. Another human imprinting disorder, Silver-Russell syndrome (SRS) is also linked to the 11p15.5 imprinted gene cluster but, in contrast to BWS, is characterized by pre- and postnatal growth retardation and, most commonly, epimutations (loss of paternal allele methylation) at IC1. In order to comprehensively define potential ME+ epigenotypes in BWS and SRS patients we undertook methylation profiling in 67 and 22 patients respectively with the Illumina 450k methylation BeadChip. Analysis of methylation status at 37 imprinted differentially methylated regions (DMRs) (31 well characterized known DMRs (kDMRs) and 6 recently reported novel DMRs (nDMRs) (PMID:24402520)). The most frequently affected non-11p15.5 kDMRs in ME+ BWS patients were on chromosome 1, 6 and 15. In addition, epimutations at nDMRs located on chromosome 8 and 22 were frequent. Further analysis of the ME+ epigenotype patterns and clinical correlates in BWS and SRS patients will be presented in detail.

P16.09-S
Highlander: variant filtering made easier

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The field of human genetics is being revolutionized by exome and genome sequencing. A massive amount of data is being produced at ever-increasing rates. Targeted exome sequencing can be completed in a few days using NGS, allowing for new variant discovery in a matter of weeks. The technology generates considerable numbers of false positives, and the differentiation of sequencing errors from true mutations is not a straightforward task. Moreover, the identification of changes-of-interest from amongst tens of thousands of variants requires annotation drawn from various sources, as well as advanced filtering capabilities. We have developed Highlander, a Java software coupled to a MySQL database, in order to centralize all variant data and annotations from the lab, and to provide powerful filtering tools that are easily accessible to the biologist. Data can be generated by any NGS machine (such as Life Technologies' Solid or Ion Torrent, or Illumina's HiSeq) and most variant callers (such as Life Technologies' LifeScope or Broad Institute's GATK). Variant calls are annotated using DBNSFP and SnpEff, then imported into the database. The Highlander GUI easily allows for complex queries to this database, using shortcuts for certain standard criteria such as "sample-specific variants", "variants common to specific samples" or "combined-heterozygous genes". Users can then browse through query results using sorting, masking and highlighting of information.

P16.10-M
Somaticaller: a somatic and post-zygotic mutation detection software from DNA-seq data

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The ability to detect low-level genetic variants in heterogeneous populations of cells is necessary for identifying postzygotic or somatic mutations underlying human diseases such as mosaic birth defects involving the skin. Existing bioinformatics methods for detecting such variations have been mainly developed for cancer genomics and are usually based on the analysis of paired samples. Furthermore, they have limited sensitivity in detecting certain types of variants such as insertions/deletions. SomatiCaller was developed to systematically detect low-level variants in next-generation DNA sequencing data. It consists in browsing aligned sequence data (BAM files) of pairs or trios (i.e. index case and parents) to systematically identify positions with candidate variants. Statistical tests are first performed between different samples to measure the sample's independence of a candidate position for a given variation. In a second time, allelic ratios from all candidate sites are compared to a series of negative controls by Student's t-test to discriminate true positive variants from probable sequencing or alignment errors. Applied to targeted deep sequencing of a gene associated with mosaic overgrowth syndromes (PIK3CA), SomatiCaller led to identification of variants with allelic fractions as low as 0.01. Experiments from trio-based exome sequencing data demonstrated its ability to readily detect variants with allelic fractions as low as 0.05. Compared to standard variant callers, SomatiCaller showed increased sensitivity for genetic variants present in less than 10% of reads. Potential applications of this tool are numerous in the growing field of the genetics of mosaic diseases, both for research and molecular diagnosis purposes.

P16.11-S
An extensive, interactive variant-centered annotation browser

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Genome-wide association studies and, more recently, next-generation sequencing studies have created a huge amount of genetic associations with hundreds of common human traits. A still unsolved problem in this context is the correct annotation of the gene(s) linked to the mostly small effects observed for genetic variants. The current catalog of genomic annotations is multi-layered and highly complex, the annotation of variants on the other hand is often conducted using very basic heuristics. We try to even out this imbalance by integrating many different genome-wide annotation datasets into a user-friendly web-accessible resource. Our collection of tools combines linkage disequilibrium data, genetic associations, gene annotations, expression data and several layers of regulatory annotations. Besides evidence-based prediction of variant-to-gene projections, our resource provides data browsing and retrieval, as well as publication-ready plotting and an interactive variant-centered genome browser.

P16.12-M
Molecular diagnosis of bladder cancer by detecting gene p16INK4a from body fluids by methylation-specific polymerase chain reaction (MSP)

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Background: Carcinoma of the urinary bladder is the 5th leading causes of death worldwide. Hypermethylation of the CpG islands of gene promoter is one of the earliest and most frequent epigenetic alterations leading to cancer as well as in its development. The purpose of our study was to investigate if hypermethylation of gene $p16^{INK4a}$ can be used as a serum biomarker for early detection of bladder cancer. **Materials and methods:** Using the methylation-specific polymerase chain reaction (MS-PCR) method, we analyzed the methylation status of gene $p16^{INK4a}$ from serum and its matched tumor in 42 bladder cancer patients, and 35 samples from cancer-free individuals, as controls. Genomic DNA was extracted from serum and tissue samples. **Results:** Hypermethylation of gene $p16^{INK4a}$ was found in 38 (90.5%) of the 42 bladder cancer patients, and in only 2 (5.8%) of the 35 cancer-free individuals. Hypermethylation status of gene p^{INK4a} was found in both serum and its matched bladder biopsy sample. **Conclusion:** We conclude that hypermethylation of gene p^{INK4a} is involved in early bladder carcinogenesis and therefore might be used as a noninvasive serum biomarker for early detection of bladder cancer.

P16.13-S
Comprehensive analysis of abnormal methylation of the genes encoding extracellular matrix proteins in breast cancer

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Extracellular matrix plays a significant role in tumor development. Our study focuses on the epigenetic regulation of 12 laminin-encoding genes (*LAMA1, LAMA2, LAMA3A, LAMA3B, LAMA4, LAMA5, LAMB1, LAMB2, LAMB3, LAMC1, LAMC2, LAMC3*), 8 genes of integrins (*ITGA1, ITGA2, ITGA3, ITGA4, ITGA6, ITGA7, ITGA9, ITGB1*), 2 nidogen genes (*NID1,NID2*), 2 genes of the cadherin family(*CDH2,CDH3*) and the dystroglycan gene *DAG1*.We have surveyed 109 samples of breast cancer, 109 paired adjacent nonmalignant samples, 6 samples of normal mammary gland from autopsy and 6 samples of breast cancer cell lines for aberrant promoter methylation of all 25 genes.
Promoters of 11 genes (*LAMA1, LAMA2, LAMB1, ITGA1, ITGA4, ITGA7, ITGA9, NID1, NID2, CDH2, CDH3)* have demonstrated abnormal methylation in 1,9% to 42% samples of breast cancer and/or adjacent tissues. Our results may be important for understanding of the dramatic changes in the extracellular matrix during tumor growth and development.
Extracellular matrix proteins genes abnormally methylated in breast cancer

Gene symbol	Methylation in breast cancer and/or adjacent nonmalignant samples (%)	Methylation in normal mammary gland from autopsy (%)	Presence(+) / absence(-) of methylation in breast cancer cell lines					
			ZR	MCF7	T47D	BT	HBL	HS
LAMA1	35	0	-	-	+	+	-	-
LAMA2	42	0	+	+	+	+	+	-
LAMB1	29	0	+	+	+	+	-	-
ITGA1	16	0	-	-	-	-	-	-
ITGA4	21	0	+	+	+	+	-	-
ITGA7	1,9	0	-	-	-	+	-	-
ITGA9	37	0	+	+	+	+	+	-
NID1	36,8	0	+	+	+	-	-	+
NID2	31,5	0	-	+	+	+	-	-
CDH2	5,4	0	-	-	-	-	-	-
CDH3	27	0	+	+	+	+	+	-

P16.14-M
Comprehensive transcriptome profiling of breast cancers

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Breast cancer is one of the most frequent malignancies among women and is a major cause of cancer-related mortality, despite advances in early detection and treatment. Breast cancer is a heterogeneous disease with a poorly defined genetic landscape, which poses a major challenge in diagnosis and treatment. Recent reports have described an intricate interplay among diverse RNA species, including protein-coding mRNAs and ncRNAs (long ncRNAs, pseudogenes, miRNAs and circular RNAs) which are also implicated in numerous diseases such as cancer.
By massively RNA-sequencing, we obtained 7.8 billion reads from 55 healthy and tumour breast tissues belonging to Basal, HER2-positive and Luminal breast cancers, with or without hereditary predisposition. At the beginning, we aim to define their comprehensive digital transcriptome. In order to analyse the transcriptomes in specific tumour cells, we used Laser Capture Microdissection system. Furthermore, we performed a RiboZero-based rRNA depletion in order to focus the sequencing effort on the non-ribosomal portion of the total RNA. Comparative transcriptomic analyses highlighted differentially expressed transcripts, between the different breast cancer groups, identifying transcripts which may be possible modulator RNAs.
This global transcriptomic profiling can illustrate the intricate internal mechanism of the transcriptome at a very high resolution, allowing us to explore the distinct nature of these breast cancer subtypes, and could provide a new inventory of diagnostic and therapeutic targets.

P16.15-S
Genetics of Cardiomyopathies: In-silico Analysis of Variants in Cardiomyopathies-Associated Genes

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Cardiomyopathies are genetically heterogeneous group of diseases of the myocardium. Mutations in multiple genes have been associated with cardiomyopathy and many cases of cardiomyopathy have a genetic component. Genetic factors may be responsible for 30-50% of cases of dilated cardiomyopathy (DCM), and about 90% of cases of Hypertrophic cardiomyopathy (HCM) are familial. As clinical genetic testing is rapidly emerging with a principal rationale of identifying at-risk asymptomatic relatives, knowledge of predisposing loci can guide clinical surveillance for disease onset, thereby enhancing preventive and treatment interventions.
We collected over 6000 genetic variants from over 80 genes with known associations with cardiomyopathies, and queried the 1000 Genomes Project database for exonic variants in the genes. We analyzed and compared the rate of missense variation in the two datasets using a window bin of 50bp. Though most variants are rare (78.7%), and 58.4% (2751/4708) being private, 25.7% (655/2544) of missense variants from 1000 Genomes Project database are predicted to be pathogenic. Of the overlapping variants, 20.4% (42/206) and 42.2% (87/206) are known and predicted pathogenic variations respectively. We identified 104 regions distributed among 15 genes, including *LMNA, MYH7, TNNT2*, and *MYBPC3,* with high density of pathogenic variants and no or extremely low variant in the population data. Majority of the identified loci overlap protein domains that play critical roles.
The identified regions with high density of pathogenic missense variants are likely hotspots that could predispose to cardiomyopathies, hence no or extremely rare variations have been reported in them in the population data.

P16.16-M
Complex Chromosomal Rearrangements In B-cell Lymphoma: Evidence Of Chromothripsis?

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Genomic instability is a well-known hallmark of cancer. Recent genome sequencing studies have identified a novel phenomenon called chromothripsis in which complex genomic rearrangements are thought to be derived from a single catastrophic event rather than by several incremental steps. While chromothripsis is well documented in solid tumors and leukemias, chromothripsis in lymphoma is rarely reported. We report a case of possible chromothripsis in a patient presenting with a thyroid mass suspicious for diffuse large B-cell lymphoma. Chromosome analysis showed a complex karyotype with multiple rearrangements, including a translocation of chromosomes 3 and 7 involving the BCL6 gene region, chromosomes 14, 7 and 22 with involvement of the IGH gene region and an unbalanced structural rearrangement involving chromosomes 8 and 18 involving the BCL2 gene region.. The karyotype was interpreted as 51~56,XX,+X,+2,t(3;7)(q29;p11.2),der(7)t(3;7)t(14;7;22)(q32;p11.2;q12),+der(7)t(14;7;22),der(8)t(8;18)(p12;q21),+der(9)t(5;9)(q13;q22),+13,der(14)t(14;7;22),+21,+1~4r[cp20]. FISH analysis confirmed the BCL6 gene rearrangement, IGH gene rearrangement/extra copies of IGH and 3 copies of BCL2. Array comparative genomic hybridization studies with Cytochip 60K custom oligo array showed multiple complex copy number variations including a previously unidentified chromosome 12 abnormality. However, array analysis did not reveal any imbalance involving the BCL6, BCL2 or IGH gene regions whose rearrangements were observed by FISH, thus suggesting that these rearrangements are balanced in nature. Our patient's genomic abnormalities show characteristics suggestive of chromothripsis and provides initial evidence that chromothripsis is not confined to solid tumors, but can also be seen in B-cell lymphomas with well characterized one or two-step lymphomagenesis.

P16.17-S
Describing chromothripsis using HGVS sequence variation nomenclature, suggested extensions

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Large chromosomal rearrangements are traditionally described using ISCN nomenclature based on chromosomal banding patterns (1). Due to the limited number of translocation breakpoint sequences identified the sequence variation nomenclature guidelines of Human Genome Variation Society (HGVS, http://www.hgvs.org/mutnomen), which are mainly focused on simple variants, did not require specific rules for detailed description of genetic rearrangements. This changed with the introduction of new technologies allowing rapid discovery of breakpoint sequences from complex structural rearrangements including translocations and chromothripsis. The description of such complex variants challenges the existing guidelines. Previously, we have proposed new HGVS rules for detailed translocation descriptions (2). Here, we suggest extending the HGVS nomenclature guidelines to facilitate unambiguous descriptions of chromothripsis. The main feature of these descriptions is that the precise combination and order of chromosomal fragments can be derived easily. The suggested format should provide sufficient flexibility and consistency limiting alternative interpretations and ambiguous descriptions. The new rules can be combined with those proposed previously for complex changes, which included: i) nesting to support description of changes within inversions and duplications, ii) composite changes to support concatenation of inserted sequences (3). We have applied the rules in practice by describing an instance of stably inherited chromothripsis. The specifications should allow easy implementation in

sequence variant nomenclature checkers (e.g. Mutalyzer, https://Mutalyzer.nl).

1) ISCN (2013). 2013. An International System for Human Cytogenetics Nomenclature. Shaffer LG, McGowan-Jordan J, Schmid M (eds). Basel: Karger.
2) http://www.hgvs.org/mutnomen/SVtrans_HGVS2013_PT.pdf
3) Taschner PE, den Dunnen JT. Hum Mutat. 32:507-511 (2011).

P16.18-M

Concordance of methods for CNV detection from whole-exome sequencing as compared with microarray: 100 sample autism cohort

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Copy number variants have been implicated as drivers of many developmental disorders, including autism spectrum disorder. The platform of choice to detect genome-wide CNVs has traditionally been microarray (including SNP arrays that can also detect copy neutral LOH regions); however, since samples have often undergone sequencing to discover pathogenic sequence variants, it is desirable to exploit these data to also detect CNVs. Various methods have been proposed to detect CNVs from NGS data, but little information is available which compare the success of these approaches to orthogonal methods. Using a large data set of constitutional samples from an autism cohort that have been subjected to both whole-exome sequencing (WES) and to a genome-wide SNP microarray, we compare the ability to detect CNVs between these platforms. Several algorithms for CNV detection from the WES data are examined, and the results are compared with an established HMM-based method for detection of CNVs from the microarray platform.

P16.19-S

De Novo Assembly and Structural Variation Discovery in Complex Genomes Using Extremely Long Single-Molecule Imaging

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De novo genome assemblies using only short read data are generally incomplete and highly fragmented due to the intractable complexity found in most genomes. This complexity, consisting mainly of large duplications and repetitive regions, hinders sequence assembly and subsequent comparative analyses. As a result of the remaining limitations of DNA sequencing and analysis technologies, it is not feasible to create similarly high quality assemblies of individuals to detect and interpret the many types of structural variation that are refractory to high throughput or short-read technologies.
We present a single molecule genome analysis system (Irys) based on NanoChannel Array technology that linearizes extremely long DNA molecules for direct observation. This high-throughput platform automates the imaging of single molecules of genomic DNA hundreds of kilobases in size to measure sufficient sequence uniqueness for unambiguous assembly of complex genomes. High-resolution genome maps assembled de novo preserve long-range structural information necessary for structural variation detection and assembly applications. We have used Irys genome mapping for the assembly and characterization of several genomes, including human, plant, fungi, and bacteria.
In addition to describing the technology and analysis approaches useful for dissecting complex genomes, we demonstrate results from several genomes, where genome maps span remaining reference gaps, identify known and novel structural variants (including balanced rearrangements) and phase variation within haplotype blocks. We also resolve and measure long tandem repeat regions that are likely impossible to assemble by other methods.

P16.20-M

Understanding complex gene-environment interactions leading to impaired DNA methylation in colorectal cancer

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We recently applied Methylation Sensitive-High Resolution Melting (MS-HRM) technique to evaluate the methylation levels of APC, MGMT, hMLH1, RASSF1A and CDKN2A genes in over 100 colorectal cancer (CRC) samples and 80 adjacent healthy mucosa specimens (Coppedè F et al. Epigenetics 2014, DOI: 10.4161/epi.27956).
Our analysis revealed a correlation between hMLH1 methylation, a marker of the CIMP high phenotype, with low folate levels, tumor location, increasing age, female gender, high number of methylated genes, and the TYMS 1494 6bp ins/del polymorphism. Increasing age correlated also with MGMT methylation levels in CRC, and both tumor stage and the MTR 2756A>G polymorphism with RASSF1A methylation.
The APC gene resulted frequently methylated in both CRC tissues and healthy adjacent mucosa specimens. APC methylation levels correlated with the TYMS 1494 6bp ins/del polymorphism in CRC samples and with both increasing age and the MTR 2756A>G polymorphism in healthy mucosa.
In order to shed some light on complex gene-environment interactions leading to impaired DNA methylation in CRC we are currently elaborating those data by means of artificial neural networks (ANNs) searching for correlation with lifestyle factors evaluated by means of a detailed questionnaire on lifestyles and dietary habits filled in by each CRC patient. ANNs are able to understand non-linear relationships among studied variables and to highlight through a graph the complexity of connections among the studied variables.
The study was supported by Istituto Toscano Tumori (ITT).

P16.21-S

Copy Number Calling for Gene Panels with Next-Generation Sequencing (NGS)

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Detection of copy number variation (CNV) from NGS data is an important mechanism for understanding and identifying genetic factors in constitutional diseases and cancer. Our evaluation of existing public algorithms revealed that they did not reliably detect short length CNVs from NGS gene panel data. A new CNV detection algorithm is introduced in SureCallv2.0 software for target enriched NGS data generated from SureSelect and Haloplex. This algorithm is able to predict breakpoints using the low genomic coverage of small panels. This feature makes it different from other algorithms based on read depth that require adequate genomic coverage to normalize the data and model copy number of different regions. A diploid reference is used to compute log ratio values from the normalized read depths with respect to the sample that minimizes the need to make additional corrections with respect to GC content and mappability. Reference and sample can either be run together or taken from different sequencing experiments. An internal read depth normalization step handles the difference in the number of sequencing reads between sample and reference. The resolution of detection is that of a single interval where the interval boundaries are defined from the baits or amplicons that cover the regions of interest in the genes. Aberrant intervals are labeled as significant after applying an iterative method that optimizes the tradeoff between least-squares fit of the data and the penalty caused by break points. An adaptive interval sizing feature allows for the detection of sub-exon copy number changes.

P16.22-M

Quantification of FCGR3B gene CNVs using Digital PCR highlights tandem repeat copies in Rheumatoid Arthritis patients

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Introduction: Copy number variation (CNV) was more and more investigated in complex diseases. We analysed CNVs of Fc fragment of IgG, low affinity IIIb, receptor (FCGR3B), a candidate gene in Rheumatoid Arthritis (RA). Droplet® Digital PCR was used to quantify CNVs and to identify tandem repeat. Patients and Methods: Thirty-eight RA trio families (one patient with two parents) and seven control samples (with known copy number) were genotyped using Droplet® Digital PCR (ddPCR, BioRad), which performs an absolute quantification using up to 20,000 reactions for one sample. Digestion reactions were done in the reaction mix for each sample with several concentrations of endonuclease FokI enzyme, in order to separate replicated copies in tandem on the same chromosome. Results: CNVs quantification showed 1 (9.65%), 2 (83.33%) or 3 (7.02%) copies. Comparison of results from undigested and digested DNAs (2U of enzyme with 33ng of DNA) showed a difference in copy number (CN) estimation for samples with tandem copies. The CN value obtained without digestion was under estimated in comparison with digestion. However, when one copy of the gene was located on each chromosome, the CN estimation was not different between the two protocols. Conclusion: Use of ddPCR with digested and undigested DNAs allowed CNVs quantification for FCGR3B. We also highlighted recombination mechanisms (tandem copies) leading to a better genotype characterization. Further investigation of FCGR3B CNVs and their transmission in 200 trio families will be performed, to better understand the impact of these candidate gene variations in RA.

P16.23-S
Variable R.Msp1 fragmentation in genomic DNA due to DNA hypomethylation in CRF patients with MTHFR C677Tgene polymorphism: from genetics to epigenetics

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Background and Objective: The role of inflammation, hyperhomocysteinemia, familial genetic markers and epimutations remains incompletely understood in chronic renal failure(CRF). DNA methylation is a post-replicative modification mechanism that is strongly involved in the physiological control of epimutations and gene expression. In the current study it was aimed to find out the possible role of epigenetic alterations in renal failure due to functional MTHFR deficiency in CRF patients that requiring long-term haemodialysis. Method: Current cohort includes 228 CRF patients and 212 healthy individuals from the same population. The MTHFR C677T SNP was genotyped by real-time PCR analysis and genomic DNA fragmentation sizes were correlated for wild, heterozygous and homozygous mutated CRF patients after methyl marker cognate enzyme of R.Msp1 digestion. Fragments were compared by Scion Image histogram plot analysis. Results: Increased T allele frequency was detected in CRF patients when compared to the health individuals from the same population. MTHFR 677TT (homozygous) genotype was found 6.1% and the T allele frequency 2.5-fold increased in CRF when compared with control group. Distinct global DNA methyl patterns that showed variable R.Msp1 fragmentations were also detected in current MTHFR gene mutated CRF patients when compare to the wild CRF patient. Conclusions: The current results indicate that individuals with germ-line MTHFR C677T mutations have a risk for CRF pathogenesis due to the reduced enzyme activity and global DNA hypomethylation. Results needs to be confirmed with a larger scale of sample size.

P16.24-M
DNA methylation changes at the 24-dehydrocholesterol reductase gene are associated with High Density Lipoprotein serum levels

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DNA methylation modifications may occur in response to a wide variety of exposures, including diet and metabolic status. In the context of the EPICOR study aimed at identifying relationships between DNA methylation status and myocardial infarction risk (292 cases, 292 controls from the EPIC-Italy cohort), we evaluated the association of lipidic profiles to genome-wide DNA methylation in a sample of 277 subjects (153 cases, 124 controls) not treated for diabetes nor under cholesterol/blood pressure-lowering treatments. DNA methylation was assessed with the Illumina HumanMethylation450 BeadChip, and data analysed according to standard procedures (MethyLumi, Bioconductor), correcting for gender, BMI, season and centre of recruitment, estimated white blood cells percentage, chip position and batch. At a linear regression analysis, we found an interesting association between a CpG site in the promoter region of the 24-dehydrocholesterol reductase gene (*DHCR24*) and serum HDL levels (effect size $1.2*10^{-3}$, $p=3.93*10^{-5}$). The result was strengthened and overcome the Bonferroni threshold ($1.03*10^{-7}$) when including in the analysis further 186 EPIC-Italy subjects belonging to other on-going studies (N_{TOT}=463, effect size $6.7*10^{-3}$, $p=2.52*10^{-9}$). Among the CpG beadchip probes in the *DHCR24* gene region, only one is clearly and significantly associated with HDL levels, making it a putative biomarker of HDL serum levels. Interestingly enough, recent papers report an anti-inflammatory effect of HDLs in vascular endothelial cells through the upregulation of *DHCR24* expression. Our preliminary findings are thus worthy of further investigation and may contribute to better describe the regulation of *DHCR24* in the HDL mediated inhibition of vascular inflammation.

P16.25-S
DNA methyltransferases and acute inflammatory pain

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Pain is the most unpleasant symptom of illness, which is mediated by a variety agents released from local inflammatory cells. Recent studies points to the involvement of epigenetic mechanisms both in the development and maintenance of pain states. One of the most fundamental epigenetic marks is the methylation of cytosine residues in DNA, catalyzed by DNA-methyltransferase enzymes. The aim of the present study was to analyze the changes of DNMT1, DNMT3a and DNMT3b in the trigeminal ganglia (TG) neurons during the maintenance phase of pain states and their modulation by NSAID.
The Mustard oil (10%) was applied to four- month-age rats to induce acute inflammatory pain and responses to mechanical and heat stimuli were assessed. All animal studies conformed to the Guidelines of International Association for the study of Pain regarding investigations. The levels of DNMT1, DNMT3a and DNMT3b were measured in nuclear extracts of Trigeminal ganglia (TGs) neurons in study and control groups. DNMTs assay kits were used to measure the amount of DNA- methyltransferases.
We found that the Mustard oil-evoked pain increased the levels of DNMT3a and DNMT3b, while it has no significant effect on levels of DNMT1 in the same regions compare to control sample. Previous administration of the NSAID revials reduced levels of DNMT3a and DNMT3b.
This study provides the evidence that DNA methylation plays a role in development of pain in animal models. This has important implications for understanding the mechanisms involved transition from acute to persistent pain and for pain therapy.

P16.26-M
SNPs identification in duchenne muscular dystrophy female carriers as biomarkers to discriminate symptomatic/asymptomatic phenotypes

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Duchenne muscular dystrophy female carriers are clinically asymptomatic. Nevertheless a small group, defined as manifesting carriers, can develop symptoms, varying from a mild muscle weakness to a DMD-like phenotype. Manifesting carriers may also present cardiac involvement (dilated cardiomyopathy), either alone or in addition to the muscle weakness. Lack of relationship between X-inactivation, transcription balancing and asymptomatic/symptomatic phenotype was recently observed. To date, the molecular mechanism underlying the clinical heterogeneity in female carriers is unknown.
In this study, the combined approach of high-troughput technologies and novel statistical analysis methods has allowed us to identify a group of SNPs which could discriminate the symptomatic/asymptomatic phenotypes.
We performed Whole Exome Sequencing and RNA-seq analysis (Illumina GAIIe, Agilent Sure Select enrichment) on a symptomatic DMD carrier. The interrogation of the NGS output for a list of 883 genes correlated with the dystrophin pathway (MedScan Pathway Study, Ariadne Genomics), resulted in 29 candidated SNPs: 17 in coding regions, 4 in the 3'UTR and 8 in non coding regions.
These selected SNPs have been investigated in two cohorts of carriers: 18 symptomatic (muscle weakness) and 16 asymptomatic (family history of dystrophinopathy or mild myopathic signs, but absence of muscle weakness).
Novel statistical approaches on selected SNPs based on multidimensional scaling and membership probability, allowed us to identify three SNPs in 3'UTR region which could discriminate the symptomatic/asymptomatic phenotypes.
Finally, the combined approaches of NGS and novel statistical analysis could be a very useful approach for the investigation of small cohorts of patients affected by rare diseases.

P16.27-S
Effects of Duplex-specific nuclease on human cells expression profiling using RNA-seq

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RNA-seq is a next-generation sequencing method able to characterize thoroughly gene expression profiles and to perform very accurate differential gene expression analysis. High expression of some genes could both influ-

ence the estimate of relative expression of less expressed genes, as well as reduce the estimate accuracy of gene expression levels of low expressed genes. Therefore, it would be convenient to apply a method able to reduce excessively abundant mRNA transcripts. A cDNA depletion method for the most represented transcripts called duplex-specific nuclease (DSN) has been effectively used in several studies. Three different cell types were studied: blood, monocytes and keratinocytes. From each cell type two RNA-seq libraries were produced (untreated libraries), for a total of 6 libraries. A part of each library was then treated with DSN, producing a total of 6 DSN treated libraries. Overall, 12 RNA-seq libraries (6 untreated and 6 treated) were prepared.
In blood tissue the most abundant transcripts in both DSN-treated and DSN-untreated samples corresponded to globins (HBA2, HBA1 and HBB), accounting for ~70% of total transcripts. In this sample maximum effects of DSN treatment was observed. Transcripts found to be the most expressed in monocyte sample were ~10 times smaller than globins expression in blood sample. Gene expression of keratinocyte libraries was not influenced by DSN treatment.
DSN treatment has been able to strongly reduce globins expression. DSN treatment also reduced, even if with a smaller effect, the most expressed genes in monocyte sample.

P16.28-M
Effects of the culture condition and chromatin remodeling agents on the epigenetics of organotypic cultures

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Alterations of the epigenetic pathways are established as hallmarks of tumorigenesis, together with genetic/genomic variations. Tumor epigenetic alterations are of increasing relevance to clinical practice, because they are important "druggable" targets for cancer therapy using chromatin remodeling agents (CRAs). New evidences highlight the relevance of microenvironment on the epigenetics and the need to use culture models that preserve the tissue morphology, to better understand the mode of action of CRAs. We studied the epigenetic response induced by culture condition and CRAs treatment, in a preclinical model based on organotypic culture from normal and neoplastic lung specimens, that preserves ex vivo the original tissue microenvironment and morphology. We assessed the expression pattern of histone deacetylases (HDACs) and methylation profile of "long interspersed nuclear elements" LINE1s and a panel of tumor suppressor genes. We observed a different behavior of the organotypic culture respect to that reported for other ex vivo models. Interestingly, culture induced an overall increase of LINE1s methylation, whereas CRAs caused LINE1s demethylation. Differently, culture and CRAs induced opposite effects on the genes: the samples responded specifically, showing demethylation for some promoters and increased methylation for others. Moreover, we noted that the culture caused alterations in the HDACs expression pattern.
These overall data reveal the importance of the maintaining the cells in their original organ architecture to study the mode of action of the CRAs, and suggest that CRAs do not work only in a non-specific way, as previously thought, in particular on the gene promoters.

P16.29-S
Bioinformatic approaches for next generation sequencing variant calling in matched sensitive-resistant ovarian tumor pairs

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Introduction Epithelial ovarian cancer (EOC), is generally sensitive to first line platinum based therapy, however more than 80% of patients experience relapse within 18 months and become resistant to subsequent lines, until the disease becomes incurable. Despite recent advances, the mechanisms underlying drug resistance in EOC have yet to be clearly identified. Transcrptional profiling, performed in a cohort of patients from which matched biopsies were taken at primary surgery (PS-O) when tumor was naïve to chemotherapy and at time of relapse (SCR) when the tumor was resistant, identified the EMT pathway as a key player in relapse (Marchini et al., 2013). Here we describe the development of computational approaches to identify somatic variants using targeted DNA resequencing with an Illumina MiSeq on our cohort of SCR and PS-O samples.
Methods We improved an existing community developed bioinformatics pipeline: bcbio-nextgen. After an examination of existing programs, we added support for tumor samples in the pipeline using three best-practice somatic variant callers (MuTect, VarScan2, and FreeBayes). The pipeline was tested on a a high performance cluster computing platform (Cloud4CARE project).
Results The method was initially tested on a reduced data set from the Cancer Genome Atlas, then run on the complete data set of matched PS-O - SCR samples. Variants identified by the pipeline were correctly discriminated as germline or somatic, and confirmed by external validation.
Conclusions Our results suggest that our bioinformatic approach is sensitive, robust, reproducible and viable for analysis of matched EOC samples.

P16.30-M
Benign copy number variations in the human exome: focusing on intellectual disability genes

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The human exome seems to represent a dynamic system in terms of interindividual variability. However, due to the lack of widely accepted criteria for determining copy number variation (CNV) pathogenic value, the creation of a map depicting the wide spectrum of benign CNV in the human exome is hindered. To make a step forward on the road to the map of human exome variations, we have evaluated 151 unrelated individuals using high-resolution CNV analysis by Affymetrix 2.7M SNP-array and an original bioinformatics technology. CNV affecting exons of intellectual disability genes were associated with X-linked mental retardation, X-linked dominant conditions and autosomal dominant intellectual disability. Exome CNV affecting X-linked mental retardation genes in males were as follows: duplication of one OPHN1 exon (3 individuals) and four DLG3 exons (3 individuals), duplication of twenty six L1CAM exons (7 individuals). Benign intragenic CNV affecting X-linked dominant genes in females were associated with duplication of twelve SMC1A exons (Cornelia de Lange syndrome 2) in 8 individuals and duplication of six PORCN exons (Focal dermal hypoplasia) in 3 individuals. Benign exome variations affecting genes associated with autosomal dominant intellectual disability were associated with deletion of one SMARCA2 exon (3 individuals) and duplication of one TSC1 exon (12 individuals), KANSL1 duplication (18 individuals) and deletion of two NHEJ1 exons (5 individuals). Our data demonstrate that benign exome CNV affecting intellectual disability genes are relatively common in humans representing a pitfall for molecular diagnosis of genomic and single-gene disorders. Supported by the Russian Federation President Grant (MD-4401.2013.7).

P16.31-S
The Utility of Clinical Diagnostic Exome Sequencing in Adults with Suspected Genetic Disorders

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Clinical diagnostic exome (CDE) sequencing is being increasingly used in the clinical management mostly of young children with diagnoses or phenotypes which do not allow a clear presumptive clinical diagnosis. CDE is offered either as a trio or proband only study depending on the laboratory and availability of other family members. To date, the positive yield of these studies, mostly in children, ranged from 25-32% with 5-7% of cases showing a de novo variant identifiable only on a trio based study. gDNA from seven adult patients seen in our adult genetics clinic who presented respectively with spontaneous carotid artery dissection, skin laxity, adult onset progressive neurodegenerative disease of unknown etiology, severe familial osteoporosis, recurrent aseptic meningitis, lower limb weakness and peripheral neuropathy, and non-alcoholic steato-hepatitis (NASH) with autoimmune phenomena was subjected to clinical diagnostic exome sequencing (5 trios and 2 probands only). Deleterious or likely deleterious variants were identified in COL3A1, GJB5 and GJB2, VPS35 genes which led to the diagnosis of Ehlers-Danlos Syndrome type IV (vascular type), Cutis laxa, and Parkinson disease 17 in the first three patients. Additionally, medically significant variants, not related to the presenting symptoms were identified in the first two patients. Variants of unknown significance were identified in two patients

with osteoporosis and aseptic meningitis while the remaining two exomes were negative. We conclude that CDE is a clinically helpful tool both for the diagnosis and management of adults with complex phenotypes and that it should be considered early in the evaluation of such individuals.

P16.32-M
A bioinformatics pipeline to identify candidate disease-causing variants from exome sequencing data

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In a three year pilot study we sequence around 900 exomes of patients suffering from various genetic disorders. In these patients the disease causing variants are unknown: either known disease-related genes have initially been found to be unaffected, or the cause of the disease is generally unclear. Depending on the expected mode of inheritance either patient/parent trios or only the patients are sequenced.
From the total set of single nucleotide variants (SNVs) and small indels, we remove common polymorphisms and calling artifacts by comparison to the 1000 Genomes database and an in-house control database. From the remaining variants we identify a candidate set based on the assumed mode of inheritance, which are then prioritized based on the predicted functional effect of the variant and the tolerance of the gene to coding mutations. In addition, network analysis of the candidate genes together with a list of genes known to be associated with the particular disease is performed to identify the most promising candidate(s) which then undergo functional evaluation.
We found a rare homozygous mutation in the COQ6 gene in a nephrotic syndrome patient. A homozygous mutation in the same gene was reported earlier to be associated with nephrotic syndrome and sensorineural deafness. Since the patient also suffers from severe hearing impairment, it is highly likely that the candidate variant is the cause of the symptoms. This case emphasizes the efficiency of our filtering and prioritization strategy.

P16.33-S
Implementation of an IT-platform for the multicenter analysis and clinical annotation of exomes of 250 children with intellectual disability

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The introduction of exome and whole genome sequencing into clinical diagnostics requires new structures for sequencing and data analysis. In principle, data analysis in this context is as simple as comparing a list of variants identified in a patient with a comprehensive list of disease causing variants. In practice, this is currently limited by the substantial number of DNA variants with false annotation or uncertain significance.
We set up an IT environment that supports central sequencing and automated primary data analysis and subsequently provides the results via a web interface to researchers and geneticists for manual curation, annotation and experimental validation.
As a pilot project, three cooperating diagnostic teams are investigating 250 trios consisting of patients with severe to mild ID and their healthy parents for which high coverage exome sequences have been generated.
Variant data including Pindel and CNV calls are generated by an analysis pipeline and stored in a database. Preliminary analysis revealed 1.7 de novo non-synonymous coding and canonical splice site mutations per patient. 20% of the investigated cases carried a mutation in genes already known to cause ID. In addition, evidence for novel ID candidate genes is being generated from the detection of SNVs and indels in specific genes from known microdeletion regions.
In summary, we show that exome sequencing in a multicenter setting with an appropriate IT environment can efficiently be used to generate clinical diagnoses by integrating the advantage of standardized central sequencing and distributed evaluation of the resulting data in the clinical context.

P16.34-M
Unbiased functional clustering of gene variants with a phenotypic-linkage network

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Groupwise functional analysis of gene variants is becoming standard in next-generation sequencing studies. As the function of many genes is unknown and their classification to pathways is scant, functional associations between genes are often inferred from large-scale omics data. Such data types - including protein-protein interactions and gene co-expression networks - are used to examine the interrelations of the implicated genes. Statistical significance is assessed by comparing the interconnectedness of the mutated genes with that of random gene sets. However, interconnectedness can be affected by confounding bias, potentially resulting in false positive findings. We show that genes implicated through *de novo* sequence variants are biased in their coding sequence length and longer genes tend to cluster together, which leads to exaggerated p-values in functional studies; we present here a method that addresses these bias. To discern molecular pathways relevant to complex disease, we have inferred functional associations between human genes from diverse data types and assessed them with a novel phenotype-based method. Examining the functional association between *de novo* gene variants, we control for the heretofore unexplored confounding bias in coding-sequence length. We test different data types and networks and find that the disease-associated genes cluster more significantly in an integrated phenotypic-linkage network than in other gene networks. We present a tool of superior power to identify functional associations among genes mutated in the same disease even after accounting for significant sequencing study bias and demonstrate the suitability of this method to functionally subcluster gene variants underlying a complex disorder.

P16.35-S
VarElect: phenotype-based variation prioritizer in GeneCards

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Next generation sequencing has provided the scientific community with a key technology for deciphering the genetic cause of human diseases. Typical whole exome sequences depict ~25,000 non-reference coding variants; only a few signify disease. As a rule, one relies on criteria such as rarity in the general population, predicted damage, and evolutionary conservation in the encoded protein and segregation in more than one affected individual, to shorten the potential gene list. We have constructed the VarElect tool for phenotype-dependent variant prioritization, leveraging the rich information and scoring mechanisms of GeneCards and MalaCards, the human gene and disease compendiums, to enable the zooming in on a handful or even just one candidate gene. Its algorithm affords inferring direct as well as indirect links between genes and phenotypes, matching the provided keywords best describing the disease and its symptoms. An example of an indirect GeneCards-based inference of GeneA to Phenotype PhenX is when the disease is found to be linked to GeneB, which in turn shares a pathway with GeneA. Such gene-to-gene relations are also formed (among others) by interaction networks, paralogy relations, domain-sharing, and mutual publications. MalaCards, in turn, allows one to produce a comprehensive phenotype search expression by utilizing this database's built-in information about diseases, their relationships, and their underlying symptoms. Thus VarElect provides a robust algorithm for ranking genes within a short list, and pointing out their likelihood to be related to a disease, which will prove indispensable in future clinical applications.

P16.36-M
Enacting ethics in genomic medicine

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Background WGS/WES translation from research to a clinical setting requires a governance framework that addresses: • informed consent for clinical and research genetic testing • management of findings • storage of clinical and genetic data Development of a protocol for governance review has catalysed local policy formulation in this rapidly developing field which accommodates clinical and research regulatory regimes, the interests of researchers, clinicians, patients and families, and diverse approaches to the risk and handling of incidental findings. **Methods** Extensive discussions were held between researchers, clinicians, genetic counsellors, clinical scientists and ethicists to gauge expectations and areas of potential divergence of interests. Reviews were undertaken of current bioethics literature. European and US guidelines and existing WES/WGS patient consent documents

were used as the basis for discussion and development. Final patient documents were reviewed by patient groups. **Results** Multidisciplinary discussions have resulted in a protocol which anticipates uncertainties in results and interpretation. A phased approach to consent will be taken, in which patients can elect to undergo a process which begins with current clinical testing, progresses to a targeted analysis of WES/WGS data and ends with analysis of the full sequence. All findings will be subject to clinical verification and a multidisciplinary committee will determine whether and how incidental findings relating to serious and clinically actionable diseases should be reported according to consent. **Future Work** Will involve monitoring and evaluation of the consent process, MDT decision process, participant engagement and qualitative research into participant understanding, attitudes and preferences concerning WGS/WGS.

P16.37-S
Genome-wide DNA methylation analysis identifies novel differentially methylated regions in patients with imprinting disorders

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Genomic imprinting is the regulation of gene expression by parent of origin. Imprinting is maintained by epigenetic mechanisms including parent of origin-specific DNA methylation, and its disruption leads to imprinting disorders affecting growth, metabolism and predisposition to cancer. Eight 'classical' imprinting disorders are known, linked to (epi)mutations of specific loci. However, some individuals have methylation anomalies affecting multiple imprinted loci (HIL) throughout the genome. Here we used epigenomewide analysis to investigate aberrant DNA methylation in HIL patients.
We used the Illumina Infinium Human Methylation450 BeadChip array to assay genomic DNA methylation at ~475,000 sites in ten HIL patients with two clinical presentations (Beckwith-Wiedemann syndrome and neonatal diabetes). We developed a novel informatic pipeline capable of small sample number analysis, and statistical criteria to quantify DNA methylation.
The pipeline robustly detected hypomethylation at known imprinted loci, and 25 further candidate imprinted regions (nine shared between patient groups) including one in the Down syndrome critical region (WRB) and another previously associated with bipolar disorder (PPIEL). Targeted analysis of three candidate regions (NHP2L1, WRB and PPIEL) confirmed allelic expression, methylation patterns consistent with allelic maternal methylation, and frequent hypomethylation among HIL patients.
This study has identified new candidate imprinted genes, and shown a remarkable epigenetic similarities between patients with different imprinting syndromes. Our informatic methods make possible epigenomic profiling of small groups or even individual patients, and have potential to expand our understanding of epigenetic regulation in health and disease.

P16.38-M
Genomic imprinting defects: role of cis-acting elements and trans-acting factors

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Gamete-of-origin dependent gene expression, namely genomic imprinting, is controlled by allele-specific epigenetic modifications of Imprinting Control Regions (ICRs). Either hypo- or hyper- DNA methylation abnormalities abolishing the epigenetic asymmetry at the ICRs result in altered gene expression and disease. DNA methylation changes are accompanied by changes in histone modifications and long-range protein interactions. ICR epigenetic alterations result from either mutations acting in cis or lesions affecting factors acting in trans, but in many cases their cause is unknown. A large cluster of imprinted genes lies on a conserved region of chromosome 11p15.5 and is associated with the growth disorders Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS). By studying several mutations affecting the IGF2/H19 ICR, we recently observed that the penetrance of the BWS phenotype correlated with the intensity of ICR hypermethylation and that this is dependent on the affinity of the mutant allele for CTCF. We also implicated CDKN1C imprinting defects in prenatal growth restriction by demonstrating a deletion of the CDKN1C ICR in a case of severe familial intra-uterine growth restriction (IUGR). Finally, we found that in mouse embryonic stem cells (ESCs) the ZFP57/KAP1 complex is required to maintain CpG methylation and histone H3K9 trimethylation by specifically interacting with a methylated target motif that is enriched at the ICRs. The role of novel cis-acting elements and trans-acting factors in the origin of the imprinting defects is being investigated further by high-throughput analysis of ZFP57 binding in human and mouse cells cells and 11p15.5- and exome-targeted resequencing of patient DNAs.

P16.39-S
PECAS: Prokaryotic and Eukaryotic Classical Analysis of Secretome

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Secretome analysis is a topic of study in different fields such as biomarkers identification for cancer development, neurobiology, stem cells, exosomes and hormonal regulation in relation to tyroid disorders[1]. Next-Generation Sequencing (NGS) is currently the main source of raw data at genomics research but tools for secretome analyses out of such data are lacking. Here, we present the web server PECAS (Prokaryotic and Eukaryotic Classical Analysis of Secretomes) that provides a well established prediction tool on secreted proteins starting from multiple data sources, allowing users to retain most sensible raw data files. These goals are achieved through a well established pipeline on secreted proteins prediction by screening different features for each of the evaluated proteins: (i) location of the predicted N-terminal signal peptide (SP), (ii) detection of the presence and location of SP cleavage sites in amino acid sequences, (iii) presence of a maximum of one transmembrane domain and (iv), glycosylphosphatidylinositol membrane anchoring. Other analysis like Gene-Ontology enrichment has also been implemented as an optional complement to the prediction steps. It is to be highlighted that it is the first tool designed to perform secretome analysis on data derived from NGS technologies. Moreover, the whole process is carried out through a user-friendly interface in a single submission step, which displays a series of downloadable graphical results and a table with the features predicted. All in all, we expect this tool will aid to decipher straightforwardly clue biological questions as emphasized recently[1].
[1] Greening,.&Simpson. (Eds.) (2013) Biochim. Biophys. Acta 1834(11): 2225-2462

P16.40-M
Human genome resources in Ensembl

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Ensembl provides up-to-date annotation for the human genome including comprehensive updates for all of our resources whenever a new assembly is released. Here we present all the available and upcoming human genome resources in Ensembl.
In December 2013, the Genome Reference Consortium released the latest version of the human assembly GRCh38. A fully annotated version of this assembly will be part of Ensembl v76 scheduled for summer 2014. Before the full release, preliminary Ensembl annotation based on GRCh38 will be available at http://pre.ensembl.org. This preview site, available in spring 2014, includes alignments of human-specific cDNA, protein and EST sequence data as well as the current GENCODE gene set, based on GRCh37.p13, projected onto the new assembly alongside variation data.
For the full release, the GENCODE gene set will be reassessed and rebuilt from the component Ensembl and Havana annotation sets. The complete set of variation data, regulatory features and all comparative data such as sequence alignments will be recomputed for the new assembly. HumanBodyMap 2.0 RNASeq data will also be reanalysed and remapped. In addition to the primary assembly, the new human genome also contains 35 „assembly units", with a total of 261 alternative loci that encompass all fix and novel patches to the GRCh37 assembly. Ensembl fully annotates all of these alternative sequence regions.
Moving a research project to a new version of the human genome assembly is a major undertaking and so we are committed to supporting the previous GRCh37.p13 annotation resources for use by the scientific community.

P16.41-S
Full deconvolution of clonal populations in recurrent hematological cancer using gaussian mixture model

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We performed Whole Exome Sequencing on five samples collected in eight years during the disease history of in a single patient with hematological cancer (Acute Lymphoblastic Leukemia, Remission and therapy-related Myelodysplastic Syndrome at three different time-points). We identified a joint set of 1201 variants that were present at least in one stage of the disease, for which we calculated allele frequencies corrected by the estimate of tumor purity. These were used to train a set of Gaussian Mixture Models (GMM) allowing for increasing number of classes. We selected the best mo-

del using Akaike Information Content criterion and assigned each mutation to a specific GMM clone. We calculated mutational signatures of each GMM clone according to the procedure proposed by Alexandrov (Nature, 2013) and estimated distance between them. We identified a single clone common to all relapses but not LLA, we built parent/child relationships with other clones using distance among signatures. Our analysis reveals a branching evolution of clones that putatively diverge in response to the clinical treatment. We show GMM is an effective and unbiased technique that can be applied to deconvolve clonal populations in cancer data only using allele frequencies. Clones can be then characterized by their mutational landscape, this information is sufficient to build clonal relationships when studying the evolution of the disease.

P16.42-M
H3M2: Detection of Runs of Homozygosity from whole-exome sequencing data

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Runs of homozygosity (ROH) can be defined as sizable chromosomal stretches of homozygous genotypes, ranging in length from tens of kilobases to megabases. ROHs can be relevant for population and medical genetics, playing a role in predisposition to both rare and common disorders. ROHs are commonly detected by SNP microarrays, but attempts have been made to use Whole Exome Sequencing (WES) data. Currently available methods developed for the analysis of uniformly spaced SNP-array maps do not fit easily the sparse and non uniform distribution of the WES target design. To meet the need of an approach specifically tailored to WES data we developed H3M2, an original algorithm based on Heterogeneous Hidden Markov Model that incorporates inter-marker distances to detect ROH from Whole Exome Sequencing (WES) data. We evaluated the performance of H3M2 to correctly identify ROHs on synthetic chromosomes and examined its accuracy in detecting ROHs of different length (short, medium and long) from real 1000 genomes project data. H3M2 turned out to be more accurate than GERMLINE and PLINK, two state-of-the-art algorithms, especially in the detection of short and medium ROHs. H3M2 is freely available at https://sourceforge.net/projects/h3m2/.

P16.43-S
Hepatitis C Virus genome variability analysis from high-throughput pyrosequencing data

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High-throughput pyrosequencing enables discovery of rare Hepatitis C Virus (HCV) variants and estimation of viral diversity within a host, which may play an important role in understanding patient's response to personalized therapy. The aim of our study was to estimate intra-host genomic variation of HCV 1b in naïve and Pegylated Interferon-Ribavirin treated patients. Serum viral RNA from 11 patients was reverstranscribed and amplified to produce 7.5 kb-long amplicons that were random-fragmented. A sequencing library was generated by specific adaptor-ligation and analyzed using Roche 454 Titanium chemistry and GS FLX platform. Data were analyzed using software published by The Broad Institute. Reads from each patient were assembled into contigs (Vicuna), oriented and frameshift-corrected (V-Fat). Raw-reads were corrected for pyrosequencing errors (RC454) and sequence variants were called using V-Phaser 2.0 and analysed with V-Profiler. The HCV 1b Con1 isolate was used as control sample. The sequencing run resulted in over 1 million passed filter reads with an average length of 397 bases and 30.81 quality score. Sustained viral response was associated to lower number of non-synonymous mutations within the initial viral population compared to non-responders. Also, NS2 mutation frequency seems higher in non-responding patients. High-throughput pyrosequencing is an effective tool for assessing intra-host variability of HCV genome, able to reveal differences in genomic variation of viral communities from patients with diverse response to therapy. Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number 88/2012, PN-II-PT-PCCA-2011-3.2 (GO).

P16.44-M
Systems biology approaches to the search for disease genes in Hirschsprung's disease

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The availability of new methodologies of high performance has revolutionized our ability to discovery. However, the high resolution of such technologies can become a double-edged sword. Reduced sample sizes available in rare diseases are often an obstacle for the detection of candidate genes or the study of their molecular basis. The limitations of the approaches based on isolated genes can be overcome using systems biology approaches.
This study shows as a combination of pathway-based analysis (PBA) and network analysis allowed to discover four new loci (RASGEF1A and IQGAP2, DLC1 CHRNA7) related to signalling and migration processes associated with the disease, which were then validated in a cohort of 106 independent trios.
The further study of an international cohort of 162 HSCR trios allowed us to confirm the molecular bases of disease using PBA. We found a significant association of processes related to signalling and its regulation as well as formation of the enteric nervous system. Although the genes associated with HSCR varied in different populations, the functions and interaction of affected networks of proteins were always the same, which reflects the complexity of the disease. This methodology of prioritization of candidate genes can be extrapolated to any technology of high performance (WES, RNA-seq, etc.).

P16.45-S
The impact of antisense transcription on epigenetic signals

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Several histone post-translational modifications are preferentially located in the promoter region of genes and are associated to the promoter activity. It is debated whether the histone modifications regulate the gene expression i.e., the histone code hypothesis or if they are put there as a consequence of transcription.
In an earlier study we observed that the histone 3 acetylation signal upstream of the transcriptional start site (TSS) was lower in unidirectional compared to bidirectional genes. Following this observation, we hypothesized that the transcribed region is modified during transcription and that the observed upstream signal is caused by transcription in the opposite direction from the TSS. Promoters directing transcription in both directions are frequent in the genome, which would explain the common occurrence of upstream signals.
Here we identified bidirectional or unidirectional genes based on TSS identified from cap analysis of gene expression and database searches. We compared histone modification signals between these two classes of genes across several cell types. We have found significant differences for well-known histone modifications, e.g. H3K4me3, H3K9ac and H3K27ac for which the upstream signal is higher in the bidirectional genes. Furthermore, we have compared transcription factor bindings between bidirectional and unidirectional genes and found examples of differences in their prevalence and position relative to the TSS.
In conclusion, our results support the model of histone modifications occurring in transcribed regions and thus being a consequence of transcription. In addition, we have identified transcription factors which may be involved in the direction of transcription initiation.

P16.46-M
Gene expression profile of human amniotic stem cells xenotransplantated in a sheep achilles tendon defect model

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Amniotic derived cells are ideal seed cells for regenerative medicine proto-

cols since they conjugate a remarkable plasticity to safety properties. This study investigated the role exerted by human amniotic cells during the process of tendon healing, evaluating by microarray technique, the presence of transcriptome variations in human amniotic epithelial (hAECs) and mesenchymal (hAMCs) stem cells when xenotransplanted into the preclinical ovine model of tendon defect, compared to freshly isolated ones. This analysis allowed to understand in which way, after transplantation, hAECs and hAMCs transcripts have been affected.
Functional analysis of the affected transcripts revealed that the main biological functions involved are: Cell Death and Survival, Cellular Growth and Proliferation, Inflammatory Response, Cellular Function and Maintenance, Skeletal and Muscular System Development and Function and Connective Tissue Development and Function.
These results show that hAECs and hAMCs after xenotranplantation produce a modulation of the genes involved in their survival, and in the inflammatory response. Intriguingly, it has been observed also the up-expression of the transcripts related to the connective tissue development and function as COL11A1 which is involved in the synthesis of collagen type I, the most expressed protein in the tendon. In conclusion, the present study demonstrates that human amniotic derived cells have a direct involvement in new tendon matrix remodeling and tissue regeneration. Altogether these results strongly support the idea that human amniotic cells could be effectively proposed as a prompt stem cell-based therapy that does not require any preliminary in vitro differentiation or any genomic transfection.

P16.47-S

Genome-wide characterization of imprinted methylation in humans: Implication for patients with multi-locus methylation defects

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Differential methylation between the two alleles of a gene has been observed at imprinted regions, where the methylation of one allele occurs on a parent-of-origin basis, the inactive X-chromosome in females, and at those loci whose methylation is driven by genetic variants. We have extensively characterized imprinted methylation in a substantial range of normal human tissues, reciprocal genome-wide uniparental disomies and hydatidiform moles, using a combination of whole genome bisulphite sequencing and high-density methylation microarrays. This approach allowed us to define methylation profiles at known imprinted domains at base-pair resolution, as well as identifying 24 novel loci harbouring parent-of-origin methylation, 18 of which are restricted to the placenta. We observe that the extent of imprinted differentially methylated regions (DMRs) is extremely similar between tissues, with the exception of the placenta. Further we profiled all imprinted DMRs in sperm and embryonic stem cells derived from parthenogenetically-activated oocytes, individual blastomeres and blastocysts to identifying primary DMRs and reveal the extent of reprograming during pre-implantation development. These results have important implications for individuals with imprinting disorders with underlying multi-locus methylation defects, including Transient Neonatal Diabetes Mellitus patients with ZFP57 mutations.

P16.48-M

Computational pipeline to analyze genomic variants with respect to clinical phenotypes by mining literature. Study of genomic regions related to intellectual disability

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Whole exome sequencing (WES) in clinical diagnostics is used as a tool to identify genomic variants in a patient's genome that may possibly give rise to a particular disorder. Variant interpretation partially relies on consulting databases like OMIM and DECIPHER containing known genomic variants causative of the specific disorders and databases of common genomic variants such as dbSNP.
Novel variants identified by WES have to be characterized with respect to a particular clinical context in order to improve the diagnostics. The functional effect of the variant can be assessed by programs such as PolyPhen, SIFT and Evolutionary Rate Profiling. Cross-referencing of novel variants with genomic databases and literature can be challenging mostly because these variants and their impact on the associated genes causing the manifestation of the phenotype of interest are poorly characterized.
We present a computational exome analysis pipeline based on the ABI SOLiD Lifescope platform. The novel added feature in our pipeline is augmenting the ANNOVAR variant annotations by links to genes strongly connected to the disease phenotypes through the MeSH terms. These connections are deduced computationally by utilizing MEDLINE database and gene ontology annotations of the genes.
The utility of our approach is demonstrated by analyzing genomic regions linked to the intellectual disability phenotype identified in Lithuanian patients.
The research leading to these results has received funding from Lithuanian-Swiss cooperation programme to reduce economic and social disparities within the enlarged European Union under project agreement No CH-3-ŠMM-01/04.

P16.49-S

IntSplice: A tool to predict the effect on pre-mRNA splicing of intronic nucleotide substitutions

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Precise spatiotemporal regulation of splicing is mediated by splicing *cis*-elements on pre-mRNA. The next generation sequencers disclose a large amount of single nucleotide variations (SNVs) in the human genome. Tools to analyze the effects of SNVs on the protein functions have been recently published PolyPhen-2 and SIFT. SNVs affecting intronic *cis*-elements potentially compromise splicing, but no dedicated tool has been available. We thus sought for a prediction model. According to the effect size analysis of each nucleotide at intronic positions -50 to -3 on alternative splicing, we extracted 34 parameters that possibly dictated the strength of splicing signals. We also partitioned all the alternative 3' splice sites in the human ENSEMBL annotation into 500 categories and generated a neural network model. The model predicted the efficiency of alternative splicing events with a correlation coefficient greater than 0.8 for a validation dataset. The model discriminated splicing consequences of intronic single nucleotide variations in dbSNP134 and the Human Gene Mutation Database. We next compared the ENSEMBL-based model with models deduced from RNA-seq of normal human brain, cerebral cortex, heart, liver, skeletal muscle, and colon, and found that a colon-based model yielded the best discrimination. We created a web service program, IntSplice. IntSplice is the first tool for predicting splicing consequences of nucleotide variations at intronic positions -50 to -3.

P16.50-M

Genome-wide methylation analysis in Klinefelter syndrome

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Background: Epigenetic changes such as DNA methylation have been proposed to play a role in human disorders such as psychiatric, autoimmune and metabolic diseases. Klinefelter syndrome (KS) is associated with an increase risk of these disorders, however no study to date have investigated global methylation changes in patients with KS. The aim of this study is to investigate the global methylation pattern in KS. We hypothesize that the methylation pattern is changed in KS and that the methylome changes will explain phenotypic characteristics present in KS. **Methods:** We performed genome-wide DNA methylation analysis on blood leucocytes from 73 patients with KS and 73 age- and gender-matched controls using the Illumina Infinium Human Methylation 450K BeadChip. **Results:** 70.525 CpG-sites covering over 15.000 genes were found to be differentially methylated in patients with KS compared to the age-matched controls. Among these 61.567 were on autosomal chromosomes, 8903 were on the X-chromosome and 55 were on Y-chromosome. One of the genes with promotor associated differentially methylated CpG sites is the NSD1 gene which is involved in the androgen receptor (AR) transactivation. Other genes possibly involved in the phenotype of KS and differentially methylated were ABI3BP, APOB, C1orf59, CACYBP, DPPA5,GABRG1, HOXA4, LRRC61, NLRP2, PEX10, RPLP1, RFPL2, SDHAF1, SPEG. **Conclusions:**For the first time we show that KS is associated with pervasive genome-wide methylation changes, changes which could play a role in the clinical phenotype seen with KS.

P16.51-S
Locus Reference Genomic (LRG) records: reference resource for the reporting of clinically relevant sequence variants

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A Locus Reference Genomic record (LRG) is a manually curated resource designed specifically for the reporting of clinically relevant variants. An LRG provides a stable and non-versioned genomic DNA sequence for a region of the human genome, along with transcripts used as reference standards and their protein products. These sequences are selected in consultation with the diagnostic and research communities, locus specific database curators, and mutation consortia. Once an LRG is made public, this core sequence content will not change. However, associated metadata, such as genome mapping information, annotation of additional transcripts, overlapping genes and legacy exon numbering systems, is regularly updated to reflect current knowledge on the LRG locus.
The LRG's stable nature enables unambiguous reporting of variants using stable identifiers (e.g. "t1", "t2" for transcripts, and "p1", "p2" for proteins) and HGVS nomenclature. Variant reporting is possible in LRG genomic DNA (e.g. LRG_1:g.8463G>C), mRNA (e.g. LRG_1t1:c.572G>C), non-coding RNA (e.g. LRG_163t1:n.71a>g) or protein (e.g. LRG_1p1:p.Gly191Ala) coordinates. Variants can also be submitted to public databases through the LRG submission process outlined on the website (http://www.lrg-sequence.org/). LRGs can be viewed in Ensembl (http://www.ensembl.org/) and NCBI (http://www.ncbi.nlm.nih.gov/) genome browsers.
Over 800 LRGs have been requested, of which over 400 are public. The intent is to create an LRG for every locus with clinical implications. Widespread use of this resource will ensure consistent variant reporting over time. LRGs are created jointly by the NCBI and EBI (http://www.ebi.ac.uk/). All LRG records are available on the LRG website, which also provides the complete LRG specification.

P16.52-M
Whole blood DNA methylation changes are associated to malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) is a rare and aggressive tumor strongly associated with asbestos exposure. Its onset is usually 30-40 years after the first exposure, and it is characterized by a poor prognosis with a median survival of 12 months. Alterations in DNA methylation have been reported in several cancers, and are becoming an established hallmark of tumor. MPM is frequently associated with genetic mutations but also epigenetic changes leading to gene expression modifications. The identification of MPM-specific epigenetic markers in peripheral blood might be a useful methodology for defining biomarkers for potential early detection and may define methylation changes due to asbestos exposure. We conducted an epigenome-wide analysis (>450K CpG sites) on DNA from whole blood cells of 129 MPM cases and 127 controls to evaluate differences in methylation profiles. The sample population was randomly split into two sets: training and test set. In the training set 60 differentially methylated regions (DMRs) between cases and controls, adjusting for gender, age, asbestos exposure, and white blood cells percentage were found (FDR adjusted p< 0.01). Using a cluster algorithm we validated the DMRs prediction performance in the test set (AUC=0.7625). We found significant enrichment for genes involved in leukocyte trans-endothelial migration, natural killer cell mediated cytotoxicity and cell adhesion molecules pathways. Moreover we identified several genes belonging to the inflammation pathways and related to the cancer progression. Our results suggested that methylation status in whole blood DNA might provide a useful biomarker for potential MPM early detection.

P16.53-S
Melan-OMICS: whole-exome and transcriptome sequencing to dissect molecular complexity of cutaneous malignant melanoma

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Cutaneous melanoma is the most fatal skin cancer and, although some effective molecular therapies exist, novel targets and drugs are still needed. To provide new insights for novel targets discovery, we performed an extensive characterization by next-generation sequencing (NGS) of a collection of melanoma cell lines derived from metastatic cases. Samples were profiled by whole-exome sequencing (WES) and RNA-sequencing using Illumina technology. Starting from WES data, we developed a bioinformatics pipeline to catalogue 2,172 novel mutations affecting genes in melanoma key pathways targeted by current therapies (MAPK and glutamate pathways) as well as genes never described for melanoma [Cifola, 2013]. Moreover, WES data were used to perform copy number alteration (CNA) analysis using a novel software developed by us, called Excavator, which is very sensitive and precise in DNA copies estimation even in situations of great sample heterogeneity [Magi, 2013]. CNA results were concordant with 250K SNP Array data and used to explore CN state of mutated genes. To collect and share these results, we created a Melanoma Exome Database (https://155.253.6.64/MExDB/). On the same samples, we also carried out RNA-sequencing and performed both a traditional gene expression analysis and more sophisticated structural evaluations. Focusing on fusion transcripts, we identified 72 putative events generated by either inter-chromosomal translocations or intra-chromosomal rearrangements, recently defined "conjoined genes" and representing an additional gene regulation mechanism. Globally, NGS proved to be extremely powerful to dissect cancer complexity at both genomic and transcriptomic levels, and to identify novel potential targets for personalized treatment of cutaneous melanoma.

P16.54-M
Integrated sequence analysis pipeline provides one-stop solution for identifying disease-causing mutations

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Next-generation sequencing has greatly accelerated the search for disease-causing defects, but even for experts the implementation of data analysis can be a major challenge. To facilitate the data processing in a clinical setting, we have developed a novel Medical Re-sequencing Analysis Pipeline (MERAP). MERAP assesses the quality of sequencing, and has optimized capacity for calling variants, including Single Nucleotide Variant, insertion and deletion, Copy Number Variation, and other structural variants. MERAP identifies polymorphic and known causal variants by filtering against public-domain databases, and flags non-synonymous and splice-site changes. MERAP uses a logistic model to estimate the causal likelihood of a given missense variant. MERAP considers the relevant information such as phenotype and interaction with known disease-causing genes. MERAP compares favorably with GATK, one of the widely used tools, because of its higher sensitivity for detecting indels, its easy installation, and its economical use of computational resources. Upon testing more than 1,500 individuals with mutations in known and novel disease genes, MERAP proved highly reliable, as illustrated here for 5 families with disease-causing variants, including a novel ANKS1A mutation identified in a patient with autosomal recessive non-syndromic intellectual disability. We believe that the clinical implementation of MERAP will expedite the diagnostic process of many disease-causing defects.

P16.55-S
Epigenome-wide analysis identified highly significant age-related DNA methylation changes

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Aging is associated with an increased risk for many complex diseases. DNA methylation represents one of the most promising biomarkers of aging. To evaluate the effect of age on DNA methylation, we examined the methylation levels of more than 450K CpG sites in 206 cases and 206 matched controls belonging to the Italian section of the EPIC cohort. EPIC healthy volunteers were recruited between 1994-98 and followed up for myocardial infarction and other diseases. For the CpG methylation level assessment on blood DNA we used the Illumina HumanMethylation450 BeadChip. Data were analyzed according to standard procedures (MethyLumi, Bioconductor). All analyses were corrected for sex, BMI, season, center of recruitment, cellular subtypes

and batch effect. Linear regression analysis showed that 10,000 CpG sites were significantly associated with age after Bonferroni correction (p<10-7). Positive correlation with age was found for 5,687 CpG sites (57%), whereas 4,313 CpG sites (43%) were negatively correlated with age. The top four significant loci (p<10-28) were located in the CpG islands of ELOVL2 and FHL2 genes. Methylation levels of the 4 CpG sites were positively correlated with increasing age, according to previously published results. These results confirm that DNA methylation alterations occur during aging and that ELOVL2 and FHL2 genes could be used as biomarker of aging. Moreover, the methylation differences associated with age could help in understanding molecular mechanisms that underlie the development of age-related diseases.

P16.56-M
Whole genome methylation analysis in idiopathic generalized epilepsies

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Genetic factors play a predominant role in the etiology of common idiopathic generalized epilepsy(IGE) syndromes. An increased rate of maternal inheritance and excess number of affected females implicate involvement of epigenetic effects in the etiology of IGE syndromes.
In this study, we have performed a whole genome methylation analysis for 15 parent-offspring trios with vertical inheritance of the IGE trait. DNA samples were subjected to bisulphite conversion prior to IlluminaHuman Methylation 450K Beadarray application. Beadchips were scanned through Illumina iScan platform. We used a comprehensive R-Bioconductor package, namely „RnBeadsV0.99.11“, which implements an analysis workflow that can be used for paired and unpaired differential methylation analysis.
After data normalization, quality control, filtering and methylation profiling steps, differential methylation analysis revealed 239 genes with p-values smaller than 0.05. As an alternative approach, we have run a pathway-based analysis to see if genes with differential methylation values map to common pathways. This analysis with PANOGA software revealed various pathways particularly with synaptic and immunological functions.
Analysis of high throughput methylation data has proven to be challenging where various pipelines with alternative normalization and filtering methods should be applied. Two approaches presented herein have resulted in identification of new epilepsy related genes and pathways that could as well be applied to analysis of other trio based methylation studies.

P16.57-S
Molecular characterization of a de novo mutation in a pediatric patient with isolated ectopia lentis as a diagnostic criterion for Marfan syndrome

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Marfan syndrome is an inherited multisystem disorder that affects the connective tissue. The typical onset is in adulthood and often occurs in children with isolated clinical signs. The revised Ghent criteria allow to confirm the diagnosis if ectopia lentis is in concomitance with a mutation in the FBN1 gene definitely associated with Marfan syndrome. Our patient (5 years old) presents a mutation (p. C154Y) never reported in the literature, hence the difficulty in establishing a diagnosis. We perform molecular dynamic simulation to investigate how this mutation affects the protein stability. Analysis was carried out on the mutated form of the N-terminal domain of the human fibrillin-1 (pdb code 2M74) . Structural analyses indicates that mutation perturb the secondary structure of the protein, which reduce the folding stability of the system. The C154Y mutation disrupts the C154-C166 disulfide bridge and consequently the two antiparallel beta sheets are impaired. In conclusion, the mutation leads to a destabilization of the native folding of the protein, and therefore a probably reduction of the physiological activity. The patient is monitored in follow-up because it has all probability of developing the Marfan syndrome. This study emphasizes the utility to characterize a mutation through molecular modeling in all late-onset diseases in order to correct follow up of patients.

P16.58-M
Prediction of LHX1-target genes relevant to Müllerian aplasia

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LHX1 is a putative transcription factor widely distributed among vertebrates. In mice, *Lhx1* acts as a high-hierarchy regulator during urogenital development, as female *Lhx1*-null mutants lack Müllerian derivatives. Deletions and point mutations affecting *LHX1* have been found in female patients with Müllerian aplasia (MA). However, the low recurrence of these alterations indicates that this disorder is multifactorial, and few reports using high-resolution genome mapping techniques have been published. Hence, the identification of candidate genes for MA demands deeper analysis. In order to identify candidate genes for MA, we used a bioinformatic approach to predict LHX1-target genes, and the results were contrasted against the set of genes with copy-number alterations compiled from the microarray reports on MA patients. Starting from 4 documented *Lhx1* regulation targets in *Xenopus* and their homologs in 5 other species, a highly conserved 50-nt signature was identified (e-value $1.34e^{-36}$). Several known transcription factor-binding sites were contained in such signature, including those of HOXA5 and HNFB1, which have been previously associated to MA. The refined motif was further searched against the human genome promoter regions, and 45 putative target-genes of LHX1 were identified. Of these, 38 genes are expressed in uterine tissues, 19 during embryogenesis, and 15 in both conditions. Two genes, *NPHP1* and *PIAS3,* were found deleted in MA patients. In conclusion, our approach will be useful to prioritize genes relevant to Müllerian development and may help to establish future validation protocols.

P16.59-S
Age at onset and disease severity in primary progressive multiple sclerosis: a genome-wide association study, pathway and network analysis

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Background: the mechanisms underlying the accumulation of neurological disability during the progressive phase of Multiple Sclerosis (MS) are currently unresolved. It is hypothesized that a complex polygenic background could regulate the clinical expression of progressive MS, in particular of the rare primary progressive course (PPMS). Aim: to look for common genetic variants associated to age at onset (AAO) and severity of PPMS, the latter measured as Multiple Sclerosis Severity Score (MSSS). Methods: 451 PPMS patients of Italian origin were genotyped for 296,589 SNPs using Illumina® OmniExpress and Human660-Quad chips, and allelic association with AAO and MSSS was studied; a protein-interaction based pathway and network analysis was performed using the following tools/databases: VEGAS, GTEx eQTL-browser, STRINGV9.05, WebGestalt, GeneCodis, GO, KEGG, MetaCoreTM. Results: no single association signal exceeded genome-wide significance in both AAO and MSSS analyses. We observed a replication at nominal level for the SNP rs758944A (p=1.15*10-3; beta=0.76), previously identified as suggestively associated to MSSS in MS patients (IMSGC,2011). Nominally associated loci to AAO (n=950) were enriched for chemokine signaling (adjusted-p=9*10-4) and oxidative phosphorylation (adjusted-p=9*10-4), while for MSSS, 845 loci were mainly associated to leukocyte trans-endothelial migration (adjusted-p=2.4*10-3) and B-cell receptor activity (adjusted-p=2.2*10-3). The genes p53 and CREB1 were identified as central hubs in network analysis. Conclusions: despite no major-effect signals were detected in our GWAS, our data suggest that common low-risk genetic variants acting mainly in the context of immune processes could play a significant role in modifying the clinical severity of PPMS. These observations could have interesting therapeutic implications.

P16.60-M
Genetic and epigenetic biomarkers of thymomas in Myasthenia Gravis

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Thymomas, epithelial neoplasms of the thymus, are present in about 10-20% of the patients affected by Myasthenia Gravis (MG), an autoimmune disease characterized in almost 80% of the cases by auto-antibodies against the nicotinic acetylcholine receptor (AChRAb). Changes in DNA methylation might contribute to the risk of thymomas but the role of epigenetics in MG and MG-associated thymomas is still not clarified.
Polymorphisms in one-carbon metabolism genes, the key pathway for nu-

cleotide synthesis and DNA methylation, have been often linked to aberrant DNA methylation and risk of various types of cancer. We investigated four polymorphisms in genes of this pathway, namely methylenetetrahydrofolate reductase (MTHFR) 677C>T, thymidylate synthase (TYMS) 28 bp repeats, DNA methyltransferase (DNMT3B) -149C>T and DNMT3B -579 G>T, in 110 AChRAb+ MG patients with thymoma (64 females and 46 males, mean age 56.1±13.1 years) and 429 matched healthy controls (276 females and 153 males, mean age 61.4±16.8 years). No difference in allele and genotype frequencies was observed between patients and controls in MTHFR and TYMS gene polymorphisms but an increased frequency of both the DNMT3B -579TT genotype (P = 0.03) and of the combined DNMT3B -579TT/-149CT genotype (P = 0.01) was observed in the total cohort of thymoma patients with respect to controls. After gender stratification both associations resulted significant in males. We are currently evaluating the methylation levels of the promoters of tumour-related genes, such as CDKN2A, hMLH1, and MGMT, in thymoma cells of MG patients, searching for correlation between DNA methylation levels and DNMT3B polymorphisms.

P16.61-S
Behind the scenes: the hidden challenges of exome sequencing in consanguineous populations

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Our research aims to identify recessive mutations in the endogamous Irish Traveller population using exome sequencing. Through our studies, we have identified issues that may complicate identification of disease mutations in consanguineous populations: (a) increased number of candidate variants due to higher than expected levels of genome homozygosity, (b) the effect of cryptic relatedness on the use of population-matched controls and (c) increased phenotypic variability due to increased likelihood of multiple disorders in the same individual.
Homozygosity levels in patients from traditionally endogamous populations are often much higher than the predicted levels from first-cousin marriages in non-endogamous populations. This results in a large number of novel/rare homozygous variants per patient exome. Population-matched controls are very useful in this situation to distinguish benign from pathogenic variants. However, due to Traveller culture, key pedigree information is often not forthcoming. Therefore, there is an increased risk of cryptic relatedness between the patients and the controls, which can lead to the erroneous filtering out of pathogenic variants.
Inter- and intra-familial variability in patients with the same disease mutation may be due to variable expressivity. However, in families from endogamous populations there is the increased possibility of patients having more than one recessive disorder, thus distorting the phenotype. When faced with phenotypic variability, we found it useful to analyse each patient's phenotype and exome individually and have identified patients with up to three different recessive disorders using this approach.
Our findings support the need for customised approaches to exome sequencing in families from endogamous populations.

P16.62-M
Similarity metrics can be used to avoid multiple entries of a single individual in databases of genomic sequence variants

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Removing frequently detected variants is one of the most effective approaches to reduce the number of candidate mutations in the data analysis of next-generation sequencing studies. The incidence of a
rare disorder in a population serves as an upper bound for the allele frequency or genotype frequency that can be used as a filter for dominant or recessive disorders. However, the frequentist inference
requires that genotypes of a single individual are represented in the database only once. With many and decentralized data submitters the risks increases that samples of the same individual are sequenced multiple times and are contributed independently under different pseudonyms. We developed a metric that computes the distances to reference samples of the 1000 genomes project. The distance profile of a sample is a unique signature that may be used to assess whether a
list of sequence variants has already been submitted. We show that this distance signature is highly specific for a sample but still error tolerant. This allows the identification of replicates from different enrichment procedures, sequencing platforms and bioinformatics pipelines. Furthermore the distance signature of a sample provides also a possibility to identify a pseudonymized sample without using the sequencing variants itself and might help to protect medically sensitive patient information.

P16.63-S
A new PCR primer design tool for Sanger sequencing confirmation

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High data quality and accuracy are recognized characteristics of Sanger resequencing projects and are primary reasons that next generation sequencing projects compliment their results by capillary electrophoresis data validation. We have developed an on-line tool called Primer Designer™ to streamline the NGS-to-Sanger sequencing workflow by taking the laborious task of PCR primer design out of the hands of the researcher by providing pre-designed assays for the human exome. The primer design tool has been created to enable scientists using next generation sequencing to quickly confirm variants discovered in their work by providing the means to quikkly search, order and receive suitable pre-designed PCR primers for Sanger sequencing.
Using the Primer Designer™ tool to design M13-tailed and non-tailed PCR primers for Sanger sequencing we will demonstrate validation of 28-variants across 24-amplicons and 19-genes using the BDD, BDTv1.1 and BDTv3.1 sequencing chemistries on the 3500xl Genetic Analyzer capillary electrophoresis platform.

P16.64-M
Towards integrative family analysis on OMICs data for individual patient diagnostics

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During recent years generating OMICs data became cheaper and faster. Notably DNA sequencing benefited from this change, becoming more popular and frequently used, specially in the domain of diagnostics. As became apparent, the data from DNA sequencing does often not allow for conclusive diagnostics, as it only shows one aspect of what contributes to the patients phenotype. The combination of OMICs data like genomics, transcriptomics and proteomics presents itself as a solution to this problem, especially in the context of family analysis which helps to identify interesting features. While many methods have been developed combining different OMICs sources to create more complete and comprehensive analyses of a patients genotype, doing family analysis in the context of OMICs data with an user friendly visualization of the data is still explored very little. We present our vision of a comprehensive, user friendly application which concentrates on several key features of OMICs data for comparative analysis. Our vision concentrates on family analysis of three key features. Individual SNP comparison on genomic data, gene expression comparison with transcriptomic data and protein variation from proteomics data. While those features are well understood individually, our vision is to create an easy to use tool to combine those three data sources based of GensearchNGS, an application that already provides the required genomics tools and allows for visualization of the analysis and underlying data. Examples will pertain to genetic disorders in myopathies, where we recently could show some progress regarding functional understanding and therapy (Walter et al., 2013)

P16.65-S
Transcription as a key determinant of the DNA methylation landscape in oocytes

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DNA methylation in gametes, particularly in oocytes, marks a subset of genes for appropriate expression in the next generation, as in the case of imprinted genes. Imprinting errors have been associated with assisted reproduction technologies (ART): because ART may involve manipulation of oocytes when methylation may be most vulnerable, it is important to understand the mechanisms of methylation establishment. In oocytes, methylated CpG islands (CGIs) often localise within transcription units, as in the case of the differentially methylated regions of imprinted genes; moreover, methylation has been described as being predominantly over gene bodies. These observations suggest that methylation is governed by the activity of promoters induced during oocyte development.
To investigate the fraction of the methylome linked to transcription, we integrated genome-wide methylation maps with deep RNA-seq from different stages of oocyte development. This revealed that most hypermethylated domains precisely match active transcription units; this is true especially where transcription units do not match standard genome annotation because of the existence of unannotated upstream transcription start sites (TSS)

in oocytes. Conversely, most hypomethylated domains are transcriptionally silent. We also conclude that methylated CGIs are predominantly intragenic, while unmethylated CGIs are intergenic or at active TSSs. To test the association functionally, we investigated the imprinted gene *Zac1*; deletion of its oocyte-specific promoter led to absence of DNA methylation across its entire gene body.
These results suggest that perturbations in transcriptional regulation during oogenesis could have profound effects on the oocyte methylome and may provide a link between aberrant methylation and ART.

P16.66-M
The influence of genetics on personality development

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Personality is known as hereditary to a certain extent. In this work we attempt to classify personality traits as binary traits based on genetic information only. For this we used the 60-item NEO-FFI and over 8 million SNPs from 6655 Dutch participants. For feature selection we performed a genome-wide association for each personality trait in a five-fold cross validation setup. All SNPs with a p-value <0.01 were chosen as predictors for a given fold and a given personality trait, amounting to approximately 2,500 associated SNPs for each trait. An artificial neural network was trained with the SNPs as input and the personality scores as output. We found it possible to classify a person's personality to the two sides of the scale significantly better than random. The results of this study prove in a novel way that genetics have an influence on personality. The next step is to identify, which genes these SNPs belong to, which hopefully will lead to a greater understanding of the processes involved in personality development and the onset of personality disorders.

P16.67-S
Analysis of GSTP1 promoter hypermethylation in urine samples of Bulgarian prostate cancer patients and controls

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Introduction: Prostate caner (PC) is one of the most commonly diagnosed cancer in men of the developed countries. The epigenetic modifications in PC are frequent and extensively studied. One potential noninvasive biomarker in urine is GSTP1 DNA methylation.
Materials and Methods: Using HRM technology we have analyzed GSTP1 promoter hypermethylation in urine samples of 64 PC patients, 26 controls with benign prostatic hyperplasia (BPH) and 20 young asymptomatic men (YAM). Our goal was to determine the diagnostic accuracy and correlations of this biomarker with clinicopathological characteristics in Bulgarian PC patients.
Results: Methylation was found in 70.31% of the patients, in 65.38% of the BPH controls and 15% of the YAM. Our results demonstrated that there was not significant difference of GSTP1 methylation among patients and BPH controls but among patients and YAM (p=0.000016). The ability of the biomarker to distinguish patients from BPH controls was evaluated. Serum PSA levels outperformed GSTP1 with estimated AUCs in ROC curve analysis 0.745 and 0.525 respectively. Methylation of GSTP1 correlates with age (Pearson's correlation coefficient 0.225, p =0.018) but not with Gleason score, tumor stage and PSA.
Conclusions: In our study the GSTP1 promoter hypermethylation in urine did not prove to be sensitive and specific diagnostic biomarker for PC and probably it is associated with age. Further investigations are needed to confirm these observations.
Acknowledgements: This work was supported by Infrastructural Grant: DUNK01/2/2009 funded by National Science Fund, Ministry of Education and Science, Bulgaria

P16.69-S
Solving the cold case - A diagnostic and clinical trial framework for WGS based rare disease discovery.

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The number of rare monogenic diseases is estimated to be >5000. For half of these the underlying genes are unknown (McKusik V.A., 2011). An increasing proportion of common diseases, such as schizophrenia or autism, previously thought to be due to complex multifactorial inheritance, are now thought to represent a heterogeneous collection of rare monogenic disorders (Mitchell KJ, Porteous DJ, 2011), the large majority of which is still unknown. For the efficient investigation of genetic mutations next generation sequencing (NGS) technology has revolutionized molecular diagnostics. Its big advantage is the ability to sequence enormous amounts of nucleic acids in a short time at an affordable cost. However, the benefits come with a number of challenges like NGS data management, quality control, mapping, variant calling and their annotation. We have developed a mutation screening and decision support for analysis of data generated by most common sequencing platforms: Illumina, 454, Ion Torrent, and Sanger Sequencing. The system furthermore allows the clinician to identify causal mutations through NGS data analysis and suggests which regions need to be validated with ABI Sanger sequencing. This makes it much easier to the scientist and the clinician to find causative Mutations. Samples of multiple patients can be processed and their mutations can be compared with ease. Keeping all gathered information of patients together grants for traceability and enables re-use and re-analysis of existing data. For questions where a single causative mutation is to be found in millions of other mutations, comprehensive data management and data analysis is essential.

P16.70-M
From NGS back to Sanger Sequencing: Connecting and Synchronizing NGS and CE Variant Files with the VR Toolkit

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Whole exome or panel sequencing projects performed by next generation sequencing technologies typically reveal a large number of variants which may require verification by an orthogonal method. To that end, Sanger sequencing is the method of choice since it is accurate, affordable and easy to perform. To facilitate the re-sequencing of any exon in the human genome we have recently made available to the scientific community a free to use tool called Primer Designer™ . The tool provides the designs for over 350.000 PCR primer pairs that cover 99% of all exons in the human genome. Amplicons generated with these primers can be readily sequenced using the BigDye® Direct sequencing kit on the Genetic Analyzer capillary electrophoresis platforms. For variant identification the sequencing files are analyzed with Applied Biosystems Variant Reporter® software which requires the import of a text file with a reference sequence for alignment and comparison. Here we show the utility and workflow of a new on-line tool called VR toolkit that generates a reference file from Primer Designer -derived PCR amplicons that contains the chromosomal coordinates. Use of this annotated reference file in Variant Reporter allows the generation of an output file that can be compared and matched to a variant call file (vcf) from an NGS instrument. The VR toolkit enables the connection and synchronization between NGS data and traditional Sanger sequencing data analyzed with Variant Reporter software and should be of benefit for all researchers seeking to validate NGS data by Sanger sequencing.

P16.71-S
Analysis of Genomic Loci in Single Cells on the Fluidigm® C1™ Single-Cell Auto Prep System Without Whole Genome Amplification

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Genomic DNA in single cells can be analyzed using whole genome amplification (WGA) on the Fluidigm C_1 Single-Cell Auto Prep System, followed by next-generation sequencing [1]. This approach is invaluable when screening for variants, for studying *de novo* mutations, as well as for looking for defined variants. We present an alternative to WGA for looking at predefined variants, with a simpler workflow, completed in less than eight hours with about three hours of hands-on time.
We isolated single GM12752 B-lymphocyte cells, extracted genomic DNA and preamplified [2, 3] 96 specific loci on the C_1 System, using a pool of 96 genotyping assays (all heterozygous) that amplify SNP loci on 22 human chromosomes. The products were directly analyzed on a 96.96 Dynamic Array™ IFC (integrated fluidic circuit), and the overall allelic dropout rate was found to be very low (≈4% for single alleles).
The approach described here can quickly provide insight into specific genes or loci from single cells. We believe this technique will find its applications in single-cell genotyping, variant detection, targeted sequencing, and, potentially, copy number variation.

P16.72-M
The influence of structural variants on alternative splicing

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Structural variants (SVs) impact tissue transcriptome by modifying the level and timing of expression of genes that localize within and on their flanks. We used mouse inbred strains to extensively gauge the influence of structural variants on the transcriptome complexity and regulation. We generated extensive RNA-seq data from mice liver and brain and intersected them with the Mouse Genomes project catalog of SVs encompassing insertions, inversions, deletions and copy number gains to assess simultaneously the impact of genome structural changes on both gene expression and alternative splicing.
While large SVs directly impact transcript expression levels, smaller SVs significantly influence splicing diversity in several manners. When lying in an exon, SVs significantly favor the emergence of alternative splicing through usage of multiple donor sites regardless of the maintenance or disruption of the open reading-frame. Conversely, a deletion or insertion within an intron significantly increases the number of alternative splice acceptor sites of the surrounding exons. Finally, exons that lie just upstream or downstream of a SV-containing intron are significantly more often skipped out by splicing. These impacts are independent from the SV size suggesting that it is a property of the rearrangement per se rather than the consequence of a change of size of the intron or exon. Interestingly, when several SVs are embedded within an exon or intron they appear to have a cumulative effect on splicing events.
We show that SVs do impact tissue transcriptome on a global scale by altering its complexity and diversity through alternative splicing.

P16.73-S
Rapid Detection of Large Structural Variations in a Human Genome Using Nanochannel Genome Mapping Technology

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Large structural variations (SVs) are less common than SNPs and indels in the population but collectively account for a significant fraction of genetic polymorphism and diseases. Base pair differences arising from SVs are on a much higher order (>100 fold) than point mutations, however, none of the existing prevailing methods can comprehensively and effectively detect them. To address these challenges, we first applied a high-throughput, cost-effective genome mapping technology using long single molecule (>150kb) to discover genome wide SVs and structure differences in the YH genome. We detected 278 large SVs (>10 kb), of which 251 of 278 (90%) are retrospectively supported by multiple orthogonal methods such as whole genome or fosmid end sequencing (200/278) and historical evidence contained in the DGV database (51/78). To further investigate the SVs that couldn't be validated by sequencing based tests, we found that 71 out 78 (91%) intersected with repeat elements, often the blind spot of re-sequencing and de novo assembly methods.. More than 70% of detected SVs are insertion events, known to be difficult to detect by sequencing. In this study, genome mapping also provides valuable information for complex regions (MHC, KIR, TRB/TRA, IGH/IGL et.al) with haplotypes.
In addition, for the first time, with long single molecule labeling patterns, inserted exogenous viral sequence and locations can be mapped on a whole genome scale important for understanding virus induced oncogenesis.
Nanochannel based genome mapping make it now feasible and cost effective to conduct large population-based comprehensive SV studies efficiently on a single platform.

P16.74-M
Analysis and correction of crosstalk effects in pathway analysis

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Identifying the pathways that are significantly impacted in a given condition is a crucial step in understanding the underlying biological phenomena. All approaches currently available for this purpose calculate a p-value that aims to quantify the significance of the involvement of each pathway in the given phenotype. These p-values were previously thought to be independent. Here we show that this is not the case, and that many pathways can considerably affect each other's p-values through a ``crosstalk'' phenomenon. Although it is intuitive that various pathways could influence each other, the presence and extent of this phenomenon have not been rigorously studied and, most importantly, there is no currently available technique able to quantify the amount of such crosstalk. Here, we show that all three major categories of pathway analysis methods (enrichment analysis, functional class scoring, and topology-based methods) are severely influenced by crosstalk phenomena. Using real pathways and data, we show that in some cases pathways with significant p-values are not biologically meaningful, and that some biologically meaningful pathways with non-significant p-values become statistically significant when the crosstalk effects of other pathways are removed.
We describe a technique able to detect, quantify, and correct crosstalk effects, as well as identify independent functional modules. We assessed this novel approach on data from four real experiments coming from three phenotypes involving two species. This method is expected to allow a better understanding of individual experiment results, as well as a more refined definition of the existing signaling pathways for specific phenotypes.

P16.75-S
EVA: A Completely New Variation Resource at EMBL-EBI

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European Variation Archive (EVA) is a new resource from EMBL-EBI aimed at accepting submissions of and providing access to all types of genetic variation data for all species. To this end, EVA works with partner databases, such as dbSNP, to guarantee free global access to all this genetic variation data. Where available, data submitted to EVA, mainly in VCF format, are closely linked with supporting BAM files in the ENA database. The web portal of EVA aims to provide a dynamic and visually interactive set of queries and filters based on HTML5. Users can browse and explore our study or variation catalogue, visualize variations and alignments via our genome browser tool or search for a gene or disease topic. Access to EVA variation data is also provided in a programmatic way by using RESTful web services for a variety of applications, such as annotation pipelines. Performance and functionality are also important goals. EVA backend datastore is being developed with most advanced computing technologies to scale up to PetaBytes of data whilst still being responsive. Some other functionalities developed include data mining and visualisation in order to allow direct access to variation data from a number of entry points including gene, disease, genomic location, variation type and consequence. Technology implementations are only as valuable as the use cases they serve. EVA also aims to be an important resource for clinical, evolutionary and systems biology researchers by linking and combining regulatory information, pathways and protein-protein interaction data from different resources of EMBL-EBI.

P16.76-M
Whole-exome sequencing reveals novel variants for migraine in Taiwan

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Migraine is a common neurological disorder characterized by recurrent disabling attacks of headache and that affects roughly 14% of the population. Family and twin studies suggest significant genetic basis of migraine. Previous studies have identified several genetic variants by different approaches such as linkage, candidate gene and genome-wide association studies, however, the results remains inconsistency. In order to identify genetic variants for migraine in Taiwan, we performed whole exome sequencing on thirteen individuals from two tetra and one penta migraine families. Except for the father, all subjects in the family are affected. All the three pedigree were compatible with autosomal dominant inheritance. Among these subjects, a total of 3,271,336 variants were identified and an average of 269,865 variants was detected in each individual. Of which 14 variants were identified to be shared by all affected individuals but not by three unaffected fathers after filtering procedures. The variants we identified were mapped to four gene s (FRAS1, ABLIM3, FOXD4L5, and BAGE2) and three intergenic regions (1p22.2, 4q13.3, and Xq11.1). Eight variants clustered in ABLIM3 were compatible with autosomal recessive inheritance. One novel single nucleotide polymorphism in FOXD4L5 gene was detected and was compatible with autosomal dominant inheritance. Our results reveal several novel gene variants for migraine and further studies are required to confirm this finding.

P16.77-S

Epigenetic mechanisms potentially influencing gene expression in a rare form of familial Wilms Tumour

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Wilms Tumour is the most prevalent type of pediatric kidney cancer. Most occurrences are sporadic, however approximately 5% of cases are hereditary; one such familial case has been identified in Atlantic Canada. None of the mutations that are currently known to cause Wilms Tumour are present in this family. Although our research to date has yielded no obvious causative mutation, whole-genome sequencing and RNAseq expression data have led to a long list of genes that could potentially be involved. The need to prioritize this list, coupled with the clinical observation that age of onset differed depending on whether the disease was inherited maternally or paternally, have led us to consider epigenetic modifications. We have started by considering DNA methylation. Methylation occurs on cytosine residues of CpG islands, and can cause gene inactivation by hindering access of transcriptional machinery to the promoter regions of genes. Therefore, gene transcription can be inhibited through hyper-methylation or gene expression may be promoted by hypo-methylation. We have assessed the methylation status of three genes (HDAC5, IGF2BP1, and POU6F2) in two patient and three control kidney samples using a methylation digest assay followed by qPCR. POU6F2 methylation was significantly decreased in both patient samples, which correlates with the increased expression we observed through RNA-seq. However, the percent methylation varied between digest replicates, so we are currently attempting to confirm the results using bisulphite conversion. Next, we will assess the methylation status of other candidate genes and will also investigate another epigenetic mechanism, histone acetylation.

P17.01-S

Genetic relationship of European Roma people and eight ethnic groups from the Caucasus area which suggest Romas probably admixed with during their migration

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Historical and genetic studies have suggested that Roma people migrated from India about 1500 years ago and settled in Europe. We investigated the genetic connection of Central European Romas to populations they could be connected or admixed with during their migration through the Caucasus. Population samples were from Abhkasians, Armenians, Chechens, Kumyks, Kurds, Nogais, North-Ossetians and Tadjiks living in the Caucasus area. We used publicly available Caucasus datasets and our Roma samples genotyped on Affymetrix Genome-wide Human SNP Array 6.0 chip. We also used Stanford-HGDP, HapMap Phase 3 and Indian datasets in order to get a better perspective about population relationships. Applying linkage disequilibrium based pruning method, our datasets contained 88781 SNPs, respectively. Using advanced algorithms which are capable of processing large autosomal SNP datasets, we investigated genetic connection of these populations. In order to infer the structure and relationship of populations, principal component analysis (PCA) and ADMIXTURE analysis were applied. PCA results show that Romas, compared to Central European and Indian populations, have more common genetic elements with the 8 investigated populations of the Caucasus area. Roma samples were most closely to Tadjiks and Nogais, while the others clustered farther from Romas, approximately in the same distance. ADMIXTURE analysis supports PCA results and shows that the eight populations from Caucasus contain also more Indian ancestry than Central Europeans. The data may also suggest that despite the short interval of contact they still admixed or populations of the Caucasus also have the same strong Ancestral North Eurasian origin as Romas.
This research was supported by TÁMOP-4.2.3-12/1/KONV-2012-0028.

P17.02-M

Study of the influence of HLA alleles on the premature biological aging

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Objective:. There is growing evidence of a premature biological aging in many autoimmune diseases. So far, the different genetics factors involved on premature aging have not been established. The decline of thymus function is a measurement of biological aging and can be determined by measuring sjTRECs concentration (signal joint excision circles produced during T-cell development). Our aim was to investigate the potential contribution of the major HLA II alleles associated with autoimmunity on the premature biological aging. **Methods:**. A total of 670 healthy individuals were included in the current study. The sjTRECs concentration was determined by qPCR in duplo per individual using sjTRECs primers and probe as well as albumin as a reference gene (dCTvalue albumin-dCT value sjTRECs). The different HLA alleles were determined by imputation based on Immunochip data using a Type 1 Diabetes Genetics Consortium (T1DGC, 5,225 individuals) as a reference panel. **Results:**. We found a statistically significant lower sjTRECs concentration in male compared with females (p=2.04*10-9). In addition, we found a lower sjTRECs concentration in males carrying *HLA-DQA1*01:02/DQB1*06* haplotype, compared with *HLA-DQA1*01:02/DQB1*06* negative male individuals (p=0.01) regardless of the carried *HLA-DRB1* allele (either *HLA-DRB1*15:01* or *HLA-DRB1*13:02*), although the difference does not remain significant after corrections for multiple testing. **Conclusion:** Our results indicate that gender affect thymic output and HLA alleles do not seem to play a key role on the speed of thymic involution and senescence of T-cells.

P17.03-S

A genome-wide association study of asthma in spanish: results from the discovery stage

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Asthma is a chronic inflammatory disease of the airways, associated with genetic and environmental factors. Despite the heritability of asthma-related traits, suggesting an important genetic contribution in disease susceptibility, a number of genome-wide association studies (GWAS) have only revealed a handful of firm susceptibility genes to explain the architecture of this disease. In order to reveal new *loci*, here we leverage the distinctive genetic features of the Spanish population, which harbors 4-20% North African admixture on average, and the information provided by the 1000 Genomes Project (1KGP), by performing a two-stage GWAS with unrelated subjects. We have analyzed over 6.5 million common variants of the genome (MAF>5%, Rsq>0.3) in 380 physician-diagnosed asthmatics, from the Genetics of Asthma (GOA) study, and 552 population-based controls, using the Axiom Genome-Wide CEU1 array and imputation in 1KGP. Our findings provide evidence for enrichment of genes from the cytokine-receptor interaction (FDR=0.006) and the WNT-signaling (FDR=0.04) canonical pathways. We have prioritized 21 *loci* associated at $p \leq 5.0E\text{-}05$ significance, some of them nearby firm susceptibility genes (e.g. *IL1RL1-IL18R1*, *HLA*, and *IL33-PTPRD*), while the remaining 76% have not been previously associated with asthma traits in previous GWAS. These results are being validated in independent Spanish samples from 795 cases and 1436 controls.
Supported by the Health Institute "Carlos III" (FIS PI11/00623, FI11/00074 and FI12/00493), by the European Regional Development Funds, "A way of making Europe" from the European Union, and by Fundación Ramón Areces. We thank SAII (Servicio de Apoyo Informático a la Investigación, ULL) for the HPC support.

P17.04-M

Childhood growth patterns linked to grandmothers' smoking in pregnancy

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Emerging evidence indicates that population variation in growth and development is influenced by the early life experience of parents and ancestors. For example, paternal smoking in mid childhood is associated with excess fat mass (5-10kg) in his sons by age 17ys (Northstone et al 2014). Here we use smoking in pregnancy by either grandmother as the exposure to test for transgenerational effects on growth. The Avon Longitudinal Study

of Parents and Children (ALSPAC) has information on prenatal exposure of father (n=9677) and mother (n=12,707) with detailed follow up (including DXA scans) on their children to age 17ys .
With *non-smoking* mothers, those with prenatal exposure themselves had larger sons at birth, who went on the have increased lean mass and grip strength by 17ys; no effects in daughters. By contrast, with non-smoking mothers, *paternal* prenatal exposure showed no effects at birth but increased growth (weight, BMI, lean and bone mass) in both sons and daughters by 17ys; the daughters additionally had increased height and fat mass.
With *smoking* mothers prenatally exposed themselves, there was no effect on sons at birth or later, whilst daughters had later decreases in height, weight, and fat, lean and bone mass. The only transgenerational effect with smoking mothers and *paternal* prenatal exposure was decreased head circumference at birth in sons which was associated with reduced IQ at 8ys. Taken together, these data evoke the transgenerational plasticity theory of evolved human life history strategy. Interaction analysis with imprinted genes PHLDA2 and INS VNTR/IGF2 are underway.

P17.06-M
Ancient mtDNA diversity in Bulgaria

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Background: The study of the genetic origin of the Bulgarian population helps for determination of population evolution and furthers our understanding of changes in the gene pool in space and time. We try to present the formation of our population based on available ancient mitochondrial DNA data.
Materials and methods: Using the main criteria for working with ancient mtDNA we analyzed 122 ancient samples dating from III Millennium B.C. to VIII - X Century A.D. from different regions of Bulgaria. We've performed several steps of amplification of overlapping fragments which cover the first hypervariable segment (HVS I) of mtDNA. The amplified fragments were cloned by using specific competent cells (E.coli), followed by sequencing of 360bp of HVS I.
Results: Preliminary results from phylogenetic analysis of 20 ancient samples have shown 18 independent haplotypes. Four of the samples are dating from III Millennium B.C. and 16 of them are dating from VIII - X Century A.D, which coincides with the First Bulgarian state. From the16 samples dating VIII - X Century A.D eleven have European origin, two - Western Eurasian origin and three- unknown origin. From the 4 samples dating III Millennium B.C. one is with European origin, one with Western Eurasian, one with controversial origin- Western Eurasian or East Asia and one is with unknown origin.
Conclusion: This research presents first original data on ancient and medieval mtDNA samples from individuals who inhabited in time the current Bulgarian territories.

P17.07-S
The ancient genomic DNA (anDNA) and analysis of genetic risk factors related to autoimmune rheumatic diseases HLA linked

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The mountain areas of L'Aquila city, such as Barete and Rocca di Cambio are characterized by environmental conditions (low temperature and humidity) that facilitate the preservation of the humans remains and anDNA: this factor explains the often tempering of a number of paleoanthropological findings. The migratory events surely resulted in the disappearance of several genes as well as the introduction of foreign alleles, among which some responsible for the development of autoimmune diseases HLA-restricted. In this work we show some preliminary genetic data generated by applying different bone anDNA extraction protocols, innovative and/or suitably modified from commercially available kits for forensic analysis. The immunogenetic assays have showed the positivity for HLA gene HLA-Cw3 (an increased frequency of HLA-Cw3 and HLA-Dw4 is tipically observed in Rheumatoid Arthritis). This work has laid the foundation for the design of protocols for extraction and PCR reaction improved and optimized, to be applied to paleogenetics and paleopathology samples on which to assess genetic risk factors related to autoimmune rheumatic diseases „HLA-Linked".
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This work was partially funded (2013) to A. Poma by ANCE Associazione Nazionale Costruttori Edili L'Aquila

P17.08-M
Association between Azoospermia Factor c (AZFc) rearrangements and Y chromosome lineages

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Complete deletions of Azoospermia Factor c (AZFc) region are common genetic cause of male infertility, while the contribution of partial deletions and duplications to spermatogenic impairment is still controversial. Some studies suggested that Y chromosome background affects the formation of AZFc rearrangements and contributes to spermatogenic failure. The aim of our study was to investigate the association of AZFc rearrangements and Y chromosomal lineages among 474 men from R. Macedonia (328 Macedonians, 110 Albanians and 36 of other ethnicity). AZFc rearrangements were determined by STS markers and gene dosage analysis. Y lineages were assigned using multiplex SNaPshot of 26 Y-SNPs and fluorescent PCR of 17 Y-STRs. Five different AZFc rearrangements (b2/b4, gr/gr and b2/b3 deletions and b2/b3 and b2/b4 or gr/gr duplications) and 18 different haplogroups were detected, of which five [E1b1b, I2a1(xI2a1a), R1a, R1b and J2b] were present in 85% of the studied men. The presence of any AZFc rearrangement was positively associated with R1a (p=3.53x10-19) and negatively with R1b (p=5.88x10-5) and J2b (p=8x10-3) haplogroups. Most of the b2/b4 duplications were found on R1a, with a higher frequency among Albanians than Macedonians. The b2/b4 and gr/gr deletions were detected on different haplogroups with gr/gr present almost exclusively among Macedonians. B2/b3 deletion was present in four men with E1b1b and in the only two men with N haplogroup. In conclusion, determination of both AZFc rearrangements and Y chromosome lineages in ethnically matched populations may improve our understanding of their role on male infertility.

P17.09-S
Association BDNF Val66Met genotype and personality traits in healthy female subjects

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Brain-Derived Neurotrophic Factor (BDNF), a member of the nerve-growth-factor family, plays an important role in the regulation of plasticity synaptic and seems to be involved in the expression of personality traits.
The aim of this study was to understand the effects of the BDNF Val66Met functional genetic variant on several personality dimensions assessed using self-reported instruments. We administered the Big-Five-Questionnaire (BFQ-2) and the Temperament and Character Inventory-Revised (TCI-R) for the measure of personality; the Emotional Intelligence Scale (EIS) and the Emotional Quotient Inventory (EQ-I) for the measure of Emotional Intelligence construct; a new instrument for the measure of Fluid Intelligence construct (CAT-FIT). We tested the possible interactions between these variables on a cohort of 154 healthy female students recruited at the G. d'Annunzio University, Chieti. A significant and positive correlation has been observed between the BFQ-2 Agreeableness score and the BDNF 66Met carriers compared with the BDNF Val66Val genotype ($r = .17$; $p < .05$). In addition, an association has been evidenced between BDNF 66Met carriers and the TCI-R Reward Dependence subscale ($r = -.20$; $p < .05$); furthermore, the BDNF 66Met carriers was significantly associated with the TCI-R Self-Trascendence subscale ($r = .17$; $p < .05$). No significant association was demonstrated for the BDNF polymorphism and for any of the constructs analyzed. Our findings support the association between Agreeableness, Dependence and Self-Transcendence personality traits and the BDNF 66Met variant in female healthy subjects.

P17.10-M

Radboud Biobank: a central facility for prospective clinical biobanking in the Radboud university medical center, Nijmegen

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Biobanking is crucial for science-based health care solutions in the 21st century. In order to improve the diagnosis, prevention and treatment of complex, multifactorial disorders and diseases a better understanding is needed of the underlying genetic and environmental pathways. Therefore, there is a growing need for large-scale biobanks for biomedical research.
The Radboud university medical center is building a central biobank facility for disease-based biobanks to optimize the use of biomaterial. The Radboud Biobank encompasses biomaterial and their descriptions (patient, disease-specific and phenotypic data) and subsequent data (genotypic data, microarray gene expressions).
For all patient groups included in the Radboud Biobank different samples types are being stored, however, DNA is stored for all groups. The isolation and storage of DNA-samples takes place at the department of Human Genetics of the Radboudumc. An automated sample flow for sample handling, storage and retrieval of all DNA-samples is present . DNA is stored in an automated -20 storage system. This system will assure sample security over the long term, sample integrity and efficient and quality based sample management .
At the moment DNA-samples are available of, among others, the following patient groups: individuals with a confirmed form/an increased risk of colorectal cancer, patients with a cerebrovascular infarct/cerebral hemorrhage/venous thrombosis, or with rheumatoid arthritis/arthrosis, neurodegenerative disorders, type 2 Diabetes Mellitus, Crohn's disease/ulcerative colitis, leukemia/multiple myeloma/lymphoma and related disorders, chronic (progressive) renal failure and patients who have had a cerebrovascular vascular accident (18-50 years). There are also DNA-samples available of healthy individuals.

P17.11-S

Establishing of national birth defects registry in Thailand

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Background: Deaths attributed to birth defects are a major cause of infant and under-five mortality as well as a lifetime disability among those who survive. In Thailand, birth defects contribute to 21% of neonatal deaths. In 2012, Queen Sirikit National Institute of Child Health has initiated a Birth Defects Registry to capture birth defects among newborn infants. **Methods:** The birth defects data come from 4 mainly sources: National Birth Registry Database; National Health Security Office's reimbursement database; Online Birth Defect Registry Database designed to capture new cases detected later; and birth defects data from 20 participated hospitals. All data are linked by unique 13-digit national identification number and International Classification of Diseases (ICD)-10 codes. This registry includes 19 common structural birth defects conditions and pilots in 20 hospitals. The registry is hospital-based, hybrid reporting system, including only live births whose information will be collected up to 1 year of age. **Results:** During the first year of implementation, 20 hospitals from 16 provinces participated. A total of 3,696 infants were diagnosed as having congenital anomalies among 67,813 live births. The prevalence rates (per 1,000 live births) of major anomalies were 26.12. The 5 most common birth defects were congenital heart defects, limb anomalies, cleft lip/cleft palate, Down syndrome and congenital hydrocephalus respectively. **Conclusion:** Information obtained from the birth defect surveillance is the essential in the planning for effective intervention. We suggest that this program should be integrated in the existing public health system to ensure sustainability.

P17.12-M

Results of exome sequencing in familial patients with non-HHT Brain Arteriovenous Malformations

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Background: Brain arteriovenous malformations (BAVMs) are a tangle of poorly formed blood vessels. Patients with Hereditary Hemorrhagic Telangiectasia (HHT), a disease caused by mutations in *ALK1*, *ENG* or *SMAD4*, often have BAVMs. We sought to identify novel functional variants that may contribute to non-HHT BAVMs.
Methods: Exome sequencing was performed on 5 unrelated families (8 non-HHT BAVM samples, 2 BAVM-free controls). Illumina Truseq exome capture kit was used for exome capture and sequencing was performed on the Illumina HiSeq2000. Reads were mapped to the reference human genome with (BWA). Variants were called using (GATK) and annotated using (Annovar). Cases were screened for variants reported in the HHT Mutation Database http://arup.utah.edu/database/HHT. Potentially pathogenic variants were kept and variants present in the 2 controls,1000 Genomes Project, ESP6500, dbSNP137 and segmental duplications were filtered out. Variants with unknown prediction of functional impact or predicted deleterious were included. Variants shared between affected siblings were kept.
Results: On average, we obtained 4.3GB of mappable sequence data; target coverage of 69x and 43,339 variants per individual. No subjects had variants reported in the HHT Mutation Database. 23 genes had unique novel variants in at least 2 families. *TMEM87B* and *SHISA7* had novel variants in three families. *NACAD*, *KRT75* and *IGFN1* had novel variants in 4 families.
Conclusions: We identified several genes bearing novel potentially pathogenic variants in multiple families in pathways relevant to BAVM. Experiments are needed to confirm these findings and determine the potential role of these genes in BAVM pathogenesis.

P17.13-S

Variation in mutation spectrum partly explains regional differences in the breast cancer risk of female *BRCA* mutation carriers in the Netherlands

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Background: Previously, relatively high cancer risks were observed in BRCA2 mutation carriers (BRCA2 carriers) over 60 in the Northern Netherlands. We aimed to quantify these regional differences in the breast cancer risk, and analyzed whether they could be explained by mutation spectrum or population background risk. **Methods:** This consecutive cohort study included all known BRCA1/2 carriers in the Northern Netherlands (N = 1,050). Carrier and general reference populations were: BRCA1/2 carriers in the rest of the Netherlands (N = 2,013) and the general population in both regions. Regional differences were assessed with hazard ratios (HR) and odds ratios (OR). HRs were adjusted for birth year and mutation spectrum. **Results:** All BRCA1 carriers and BRCA2 carriers under age 60 had a significantly lower breast cancer risk in the Northern Netherlands, HR were 0.66 and 0.64, respectively. Above age 60, the breast cancer risk in BRCA2 carriers in the Northern Netherlands was higher than in the rest of the Netherlands (HR = 3.99, 95%CI 1.11-14.35). Adjustment for mutational spectrum changed the HRs for BRCA1, BRCA2 <60 and BRCA2 ^{3}60 years by -3%, +32% and +11%, respectively. There was no difference in background breast cancer incidence between the two regions (OR = 1.03, 95%CI 0.97-1.09). **Conclusions:** Differences in mutation spectrum only partly explain the regional differences in breast cancer risk in BRCA2 carriers, and for an even smaller part in BRCA1 carriers. **Impact:** The increased risk in BRCA2 carriers over 60 may warrant extension of intensive breast screening beyond age 60.

P17.14-M

Immunochip SNP effect on candidate gene expression in Celiac Disease

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Celiac disease (CD) is an immune mediated disorder caused by intolerance to ingested gluten that develops in genetically susceptible individuals. The major genetic risk factor is HLA but it is not sufficient to explain all genetic susceptibility. GWAS and IMMUNOCHIP identified 39 non-HLA regions associated with CD, and a long list of candidate genes mapping these regions has been proposed. We aimed to analyze the influence of the associated SNPs in proposed candidate gene expression in the intestinal mucosa of active/treated CD patients and controls. 14 control individuals and 9 sample pairs from the disease group with genotype and expression data were included in

this study. Merlin 1.1.2 was used for the association test, performed independently in each of the studied groups in order to avoid false associations due to duplicated genotypes in CD sample pairs. Genotype effect of SNPs in the expression of many genes was found, but in none of them was the candidate gene located under the association peak of its putative SNP affected. We analyzed the genomic area around the associated SNPs searching in online data bases (Haploreg, Ensembl and UCSC) to find possible common regulatory elements that could be altering the expression of genes far away. Findings include open chromatin regions, novel protein coding sequences, novel anti-sense processed transcripts, processed pseudogenes, microRNAs, novel LincRNAs and some altered motifs in the SNPs showing regulatory power. Further functional studies must be done to determine if those elements could be real players in the development of CD.

P17.15-S
Gaining access to large European population cohorts through the transnational biobanking infrastructure BBMRI-LPC

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Large Prospective Cohorts are essential in studies of disease etiology. After collecting biospecimens and extensive lifestyle information, the prospective cohorts follow the initially healthy study participants for years until disease occur. This gives investigators the opportunity to study biomarkers and other exposure prior to disease onset, an essential feature for evaluating pre-diagnostic biomarkers and etiological factors. However, accessing the biospecimens and data across borders is costly and sometimes hindered by ethical and legal regulations. To overcome these issues and to make the large European prospective cohorts on human health and disease more accessible for research, an EU-funded biobanking network infrastructure BBMRI-LPC (Biobanking and Biomolecular Resources Research Infrastructure - Large Prospective Cohorts) project was established in 2013.
BBMRI-LPC involves 30 partners from 17 different countries including universities, research centres and private companies offering their expertise in science, technology, ethical and legal aspects. The partnering biobanks include 20 large cohorts from which high quality biospecimens and data are available. BBMRI-LPC offers funding and administrative support for access to these cohorts to research investigators throughout Europe and EU associated states, in an open call followed by competitive, peer-reviewed selection. The 1st scientific call entertains study proposals in the field of common chronic diseases including cardiovascular disease, Type II diabetes and cancer, and is open from 15 May till 15 July 2014. The poster will present the specifics of the call, which offers a unique opportunity for scientists to carry out innovative research projects by EU-funded access to the unprecedented collection of European cohorts.

P17.16-M
Risk assessment of chronic kidney disease based on linear mixed models in Korean populations

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Recent genome-wide association studies (GWASs) have found multiple single-nucleotide polymorphisms (SNPs) associated with chronic kidney disease (CKD); however, these variants explained only a small fraction of genetic variation. The linear mixed models (LMM) have been proposed to control for population stratification and other confounding factors in GWAS. In this study, we developed models combining the effects of genetic and non-genetic risk factors to predict the risk of CKD in Korean populations. We conducted a GWAS using LMM and estimated the heritability of CKD using a set of 491,680 tag SNPs (r^2<0.8) selected from 4,721,497 SNPs being directly genotyped or imputed based on the 1000 Genomes data after adjustment for age and gender in 943 patients with CKD and 950 healthy controls. The narrow sense heritability of CKD was 0.23 (P<0.05). We created genetic and non-genetics risk scores and validated their predictability in two independent studies. The best fit model contains five non-genetic factors: age, gender, body mass index, triglycerides, and total cholesterol/ high-density lipoprotein cholesterol ratio (Hosmer-Lemeshow X^2 test, P_{model}=0.021). Although the most significant variant, rs35645016 located in the RB1CC1 gene, did not reach genome-wide significance (OR=1.1, P=1.7×10^{-6}), the predictability of non-genetic model was improved from 61% to 78% by considering the effects of 49 CKD-associated tagSNPs (P<1×10^{-4}). These models further validated in two independent studies (e.g. AUC, 72 to 77; $P_{difference}$<0.0001). Incorporating genetic information in a model of conventional risk factors may lead to improved predictability of developing CKD.

P17.17-S
Genetic adaptation of the human circadian clock to day-length latitudinal variations

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The temporal coordination of biological processes into daily cycles is a common feature of most living organisms. Day/night cycles represent a major circadian synchronizing signal and vary widely with latitude. In humans disruption of circadian rhythms is a common feature in psychiatric diseases including schizophrenia, bipolar disorder, depression and autism. We applied an approach that analyses spatial correlations between genetic variation and environmental factors. We exploited genotype data from 52 human populations distributed worldwide and determined the annual maximal variation in day-length (Δphotoperiod), this latter used as a measure of selective pressure. With this approach we analyzed 5 independent sets of genes involved in circadian regulation or in sleep homeostasis. For all sets a significant enrichment of variants showing signals of photoperiod-driven selection was detected. Analysis of population genetic differentiation (FST) confirmed strong spatial signatures of natural selection at photoperiod-selected variants. Also, tests based on haplotype homozygosity indicated that a significantly high proportion of photoperiod-selected SNPs underwent selective sweeps in non-African populations. Finally, using variants identified in genome-wide association studies we determine that a significantly high proportion of risk SNPs for schizophrenia and/or bipolar disorder correlates with Δphotoperiod. Notably, many risk variants showing signals of photoperiod-driven selection map to genes with strong evidence of involvement in circadian rhythm regulation. Thus, human populations adapted to life at different latitudes by tuning their circadian clock systems. This process also involved risk variants for neuropsychiatric conditions. Data herein suggest possible genetic modulators for chronotherapies and candidates for interaction analysis with photoperiod-related environmental variables.

P17.18-M
Using mean inbreeding coefficient to estimate total pathogenic allele frequency in autosomal recessive disorders results in a biased estimate of this frequency

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Some years ago we suggested to estimate total pathogenic allele frequency (q) of autosomal recessive disorders from the proportion of compound heterozygotes among affected patients with consanguineous parents. The method works well when all parental couples in the sample have the same value of F (provided F>0). The effect of combining data with different F's, was never examined, although taking a weighted average was already reported in literature. While testing the use of simulation as a method to examine the accuracy of estimating q, it was discovered by one of us that taking a mean value for F would always overestimate q. Here we illustrate the impact of using an average F in a hypothetical data set consisting of two equally sized populations, one with an F=0 and the other with a variable F (1/16, 1/64/ 1/256 and 1/1024). It is assumed that 4 different pathogenic alleles are involved, with relative frequencies 0.4, 0.3, 0.2 and 0.1. The table shows the resulting biased estimates for two different values of the true q.

		F			
		1/16	1/64	1/256	1/1024
True q	0.01	0.0419	0.0178	0.0119	0.0105
	0.02	0.0516	0.0278	0.0219	0.0205

Using mean inbreeding coefficient to estimate total pathogenic allele frequency clearly overestimates true q. The same applies when combining samples with different F's, all of which are >0. One possible solution is to estimate q for each subset with a particular F separately, and then take the weighted average of the estimated q's.

P17.19-S
Pre-Processing Mining in Congenital Malformation Database

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The increase in healthcare data volume causes great difficulties in extracting useful information for decision support. Traditional manual data analysis has become inadequate, and methods for efficient computer-based analysis indispensable. To satisfy this need, medical informatics may use the technologies developed in the new interdisciplinary field of knowledge discovery in databases (KDD), encompassing statistical, pattern recognition, machine learning, and visualization tools to support the analysis of data and the dis-

covery of regularities that are encoded within the data. DM is the search for new, valuable, and nontrivial information in large volumes of data.The Registry of Congenital Malformations in Maracaibo, Venezuela is a study for the clinical and epidemiological This paper presents a future proposal for a study of Congenital malformation monitoring program in Maracaibo, Venezuela with techniques for Data Mining (DM) and discuss how we carry out knowledge discovery on the Congenital Malformation database (CMDB) of the ECLAMC (F01) The aim of this paper is to demonstrate that the techniques of pre-processing dates are the focus of DM processes and we will present step–by step the CRISP-DM standard approach to help the physician explore their CMDB

In summary, DM algorithms can be applied using the prepared data. The adequacy of data preparation often determines whether this data mining is successful or not speciality in Congenital Malformation database.

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P17.20-M

High frequency of hypoketotic hypoglycemia associated CPT1A mutation in Northeast Siberia caused by positive selection

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Siberian populations represent a unique case for the study of human adaptation to the extreme cold environment. Our previous selection scans based on genome-wide genotype data highlighted a 3Mbp region on chromosome 11 with 79 genes as the strongest candidate region under positive selection in Northeast Siberians. However, it was not possible to distinguish which gene specifically was causing the signal. Here, using whole genome high coverage sequence data and additional neutrality tests, we identify the most likely causative mutation of this selection signal in the CPT1A gene which is a key regulator of long-chain fatty acid oxidation. We find a non-synonymous mutation (P479L) at >80% frequency in the coastal Northeast Siberian populations while it is absent in other Siberian populations and public genomic datasets. The only other known ethnic groups with this mutation are Canadian and Greenland Natives among whom the P479L mutation has been reported in association with hypoketotic hypoglycaemia and high infant mortality. The mutation is also present in the genome of the Paleo-Eskimo from Greenland suggesting that the mutation is at least 4KYA. The localised high frequency and associated haplotype homozygosity of the P479L mutation in the Arctic region supports the hypothesis that its selective advantage is either due to specific dietary or thermoregulation associated adaptation.

P17.21-S

Genome-wide association study and functional characterization of a locus associated with CRIPTO serum levels in Cilento isolates

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CRIPTO, the founding member of the EGF-CFC genes, plays an essential role in embryo development and cancer progression. Very little is known about the variability of serum levels of CRIPTO in humans and the genetic contribution underlying this variability remains still unknown. We performed a GWAS of CRIPTO serum levels in two isolated villages from Cilento area in South Italy. The most associated SNPs (p-value<10-8) were all located on the chromosome 3p22.1-3p14.3, around the CRIPTO gene. Performing a conditional analysis for the best signal (rs3806702, p-value=1.03*10-159) no loci remain associated at genome-wide significance, while many SNPs were associated at 5*10-8<p-value<1*10-4, twenty of those were replicated in an independent sample. Genes closest to the replicated SNPs were included in a unique network involved in cellular development, death and survival. The replicated loci explain the 89.8% of the CRIPTO variance, with the 84.9% explained by the most associated SNP. Among the top associated SNPs, the rs112481213 variant (p-value=1.53*10-158), located within the 5′UTR of the CRIPTO gene, was predicted to create a binding site for AP-1 transcription factor. To assess a functional role of this SNP, allele effect was tested on the transcriptional activity of luciferase in a cell system: the A allele was more efficient in driving transcription than the T allele, demonstrating that rs112481213 is a functional regulatory element of CRIPTO transcription. Further, EMSA experiments demonstrated that the A allele acts creating a AP-1 consensus binding site. Further investigation is warranted to detect association between the functional SNP and diseases susceptibility.

P17.22-M

Twelve-year experience in CFTR gene mutation screening at Sant'Anna General Hospital, Como Italy

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Cystic Fibrosis (CF) is the most frequent severe inherited disease among Caucasians, having prevalence of 1:3000 and a carrier frequency of 1:27 in Italy. Three mutations in CFTR gene (F508del, N1303K, G542X) represent approximately 60% of mutated alleles, while a multitude of additional mutations exhibit a low prevalence. In addition, the distribution of individual mutations varies throughout the country and several mutations are peculiar to specific regional areas, i.e, T338I is typical in Sardinia (15.1% of mutated alleles), 2183AA->G and R1162X are frequent in Northeastern Italy (8% each). We now present data on CF genetic testing in Como, a 0.6 million population regional area of Lombardia (Italy), for which mutation prevalence information is limited.

From 2002 to 2013, our Unit of Genetics analyzed 3369 individuals for CFTR mutations, using reverse dot blot-based commercial diagnostic kits with a detection rate ranging from 75% to 85%. The laboratory successfully accomplished National Health system (ISS)-sponsored external quality control program since 2003. Most of the individuals were tested for CF in the context of clinical examinations for infertility or as partners of known carriers. Additional cases represented symptomatic patients with suspected cystic fibrosis or prenatal diagnosis testing.We detected 147 mutated alleles (4.3% CF carrier frequency). Frequency of individual mutations were F508del (40.1%), G542X (11.6%), N1303K (8.8%), 2789+5G->A (6.8%), 2183AA->G (6.1%). Additional 16 mutations comprised 26.6% of mutated alleles. A survey of mutations in relation with medical indications (including male infertility) and compared to literature data on different Italian areas will be presented.

P17.23-S

Polymorphism A2756G at methionine synthase (MTR) and the maternal risk of Down syndrome: evidence from a meta-analysis of case-control studies

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A2756G is a common polymorphism related to the methionine synthase (MTR). Since it has been suggested a role for the folate pathway in chromosome 21 nondisjunction, many studies have demonstrated a relationship between *MTR* A2756G and Down syndrome (DS). However, the data from these studies have shown conflicting results. Therefore, we performed this meta-analysis to derive a precise estimation of this association. Studies were searched from PubMed up to January, 2014 and were eligible if they included case mothers (DSM) that gave birth to at least one child with DS, and controls mothers (CM) that have given birth to children without abnormalities. Pooled odds ratios (ORs) with 95% confidence intervals were estimated by fixed or random effects models. Heterogeneity among studies was evaluated using Q test and I^2 statistic. Publication bias was examined by a Begg's (funnel plot) and Egger's tests. Sensitivity analysis were performed by using allelic, dominant, recessive and codominant genetic models, Hardy-Weinberg equilibrium (HWE) and ethnicity. Eight case-control studies (987 DSM and 1,334 CM) were included. No association evidence has been found, neither in the overall analysis, nor in the stratified analysis by ethnicity (ORs with $P > 0.05$). The shape of funnel plots was symmetrical in almost all genetic models. There is no evidence of heterogeneity, the genotype distributions of the controls of all studies were consistent with HWE and sensitivity analysis indicated robustness of our results. Taken together, our meta-analysis suggested that *MTR* A2756G polymorphism did not contribute as an independent risk factor of DS.

P17.24-M

FFBSKAT: fast family-based sequence kernel association test

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Background: The kernel machine-based regression is an efficient approach to region-based association analysis aimed at identification of rare genetic variants. However, it is computationally complex. The running time of kernel-based association analysis becomes especially long for samples with genetic (sub)structures, thus increasing the need to develop new and effective methods, algorithms, and software packages.
Results: We have developed a new R-package called Fast Family-Based Sequence Kernel Association Test (FFBSKAT) for analysis of quantitative traits in samples of related individuals. This software implements a score-based variance component test to assess the association of a given set of single nucleotide polymorphisms with a continuous phenotype. In the development of FFBSKAT, we applied several analytical and algorithmic improvements to speed up the analysis without any loss of accuracy.
We compared the performance of our software with that of two existing software for family-based sequence kernel association testing, namely, ASKAT and famSKAT, using the Genetic Analysis Workshop 17 family sample. Results demonstrate that FFBSKAT is several times faster than other available programs, while being similarly accurate. With respect to the available analysis modes, we combined the advantages of both ASKAT and famSKAT and added new options to empower FFBSKAT users.
Conclusion: The FFBSKAT package provides fast, accurate, and easy-to-use method to perform kernel machine-based regression association analysis of quantitative traits in samples of related individuals. The FFBSKAT package, along with its manual, is available for free download at http://mga.bionet.nsc.ru/soft/FFBSKAT/
This work was supported by RFBR grant №13-04-00272-a.

P17.25-S

Exome sequencing revealing Nunavik Inuit specific variants in genes regulate lipid metabolism

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Nunavik comprises the northern part of Quebec, with 90% of its habitants being Inuit. The modern Inuit of Nunavik came from the Thule people from coastal Alaska and traveled eastward along the Arctic tree line and reached Nunavik around 1500 AD as they became the ancestors of current Inuit residents. In this study, we performed exome sequencing on 113 Nunavik Inuit, using Agilent SureSelect V4 capture kit and Illumina HiSeq platform. Standardized data processing is used to extract all exonic variants. We performed preliminary analysis in order to identify Inuit specific novel or rare nonsynonymous variants. The analysis yielded 62 protein changing variants which have allele frequency over 0.5 in Inuit while less than 0.01 in other populations. Among these, a variant p.P479L in the Carnitine Palmitoyltransferase 1A (CPT1A) gene is the most prominent one with allele frequency 0.94. This variant is absent in non-Inuit populations, which frequency also appears to be the highest in Nunavik when compared to other Inuit populations. Further analysis revealed Nunavik Inuit also have significantly higher mutation burden in other carnitine acyltransferase family genes compared to non-Inuit populations such as Asian and Caucasian. The specific genetic profile of Nunavik Inuit with higher number and frequency of damaging variants in genes regulate lipid metabolism such as carnitine acyltransferase genes may be associated with the adaptation to their special diet and living environment in the extreme cold climate. Understanding the role of Inuit unique variant(s) is very important to the study of Inuit special health care.

P17.26-M

GWAS and candidate gene analysis highlight many novel loci associated to food preferences

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Food preferences are the first factor driving food choice, nutrition and ultimately diet-related diseases. To understand the genetic component of food preferences we used two phase approaches: PHASE1: association of bitter taste receptors and coffee liking and PHASE 2: two-step GWAS of 42 different food likings.
PHASE1 revealed a significant association between coffee liking and H212R variant of TAS2R43 gene. Moreover, this variant is also associated with different thresholds in caffeine perception.
For PHASE 2 2311 Italian subjects were used for the discovery step while 1755 from Europe and Central Asia for replication. Association analysis revealed 17 independent GWAS significant replicated loci/genes (combined $p<5x10^{-8}$). Briefly Artichokes (CHSY3, LOC100128714 and CCRN4L); Bacon (CNTN5 5.93E-09); Broccoli (KIF2B, RYBP 4.50E-09); Coffee (FIBIN); Chicory (CSMD1 2.56E-09); Dark Chocolate (DFNA5); Blue Cheese (TCF7L18.81E-09); Ice Cream (IRX4); Liver (RNU6−66); Oil or Butter on Bread (BPNT1 3.62E-10); Orange Juice (FARS2); Plain Yogurt (IGLV4-60); White Wine (HLA-DOA) and Mushrooms (C9orf123).
Regarding possible effects on metabolic traits we found that rs12994253 (TCF7L1) is associated to lower Blue Cheese liking and lower BMI (p=0.0048) while rs2530184 (KIF2B) is associated to lower Broccoli liking and higher total cholesterol (p=0.0042)
None of the identified genes belong to taste or olfactory receptors thus highlighting new genes and pathways. 13 out of 17 loci show a non-additive inheritance highlighting the need of considering alternative genetic models in GWAS.
Our results represent a first step towards unraveling the genetic bases of food liking, and in understanding the genetics of human nutrition in general.

P17.27-S

FTO gene rs1421085 polymorphism is associated with obesity and metabolic syndrome in the Turkish adults

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Background and aim: Common polymorphisms in the fat mass and obesity-associated gene (FTO) have shown strong association with obesity, metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) in several populations. In the present study, we explored the association of FTO rs1421085 gene polymorphism with obesity, MetS, T2DM and other biochemical parameters in a larger cross-sectional population-based random sample of Turkish Adults (TARF Study). Methods: Genotyping was performed using the Taqman Sistem (ABI PRISM 7900 HT) in 1977 adult individuals (mean age 50.1±12.0; 48.3% male). Results: The frequency of the FTO risk-allele (C) was 0.42, and the genotype distribution was in Hardy-Weinberg equilibrium. Not women but men, carriers of C allele of the rs1421085 polymorphism were at increased risk for the presence of MetS (OR = 1.52; 95% CI: 1.13 to 2.04), elevated insulin level (8.31 ± 1.04, p=0.013), adjusted for age, smoking status, alcohol consumption, and physical activity. Logistic regression analysis demonstrated a significantly increased likelihood for obesity in women rs1421085 C allele carriers (OR = 1.56; 95% CI: 1.16 to 2.10), after adjustment for age, cigarette smoking, alcohol usage and physical activity, diabetes mellitus and menopausal status. The rs1421085 polymorphism was not associated with T2DM in both gender. Conclusion: Our findings indicate that the rs1421085 polymorhism in the FTO gene contribute to obesity and MetS in the Turkish adults depending on gender.

P17.29-S

Genomic description of the Generation Scotland Cohort: a large family base genetic study

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Generation Scotland's Scottish Family Health Study (GS:SFHS) includes over 24,000 participants from across Scotland with records for health-related traits and environmental covariates, 10,000 genotyped for ~700K SNPs. The cohort represents an important resource for the study of complex traits and diseases. We have analysed the genomic structure of GS:SFHS as a preliminary step towards choosing appropriate subsets of individuals and statistical techniques for future studies. Initially we merged the GS:SFHS data with 1092 individuals of diverse ancestries from the 1000 Genomes project and estimated genomic relationships using the ~700K SNPs. A Principal Component Analysis on the resulting relationships facilitated identification of a group of 70 individuals of likely Italian ancestry and a number of individuals with African or Asian ancestry. We characterised the amount

of genetic introgression and were able to differentiate between individuals with a few small exogenous regions in their genome, and those with long exogenous haplotypes covering a large part of the genome. We found that the pattern of homozygosity was very similar to that of other European populations and identified an individual carrying a chromosome 1 uniparental disomy. Overall, there is very limited evidence for geographic differentiation or stratification of the GS:SFHS sample within Scotland. These findings provide a genomic perspective on the history of the Scottish population, and have implications for further analyses, such as studying the contributions of common and rare variants to trait heritabilities and evaluation of genomic and phenotypic prediction of disease.

P17.30-M
Identification of mutations in the GLI2 gene in Combined Pituitary Hormone Deficiency (CPHD) in Italian patients

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The GLI2 transcription factor is a major effector protein of the sonic hedgehog pathway and it is suggested to play a key role in pituitary development. *GLI2* mutations cause holoprosencephaly or holoprosencephaly-like features. In some cases heterozygous *GLI2* mutations have been associated with hypopituitarism without other anomalies. However *GLI2* is not routinely screened in pituitary hormone disorders. The aim of this study was to determine the frequency of *GLI2* mutations in patients with combined pituitary hormone deficiency (CPHD) in Italian patients that resulted negative for mutations in other causative genes (*PIT1, PROP1, HEXS1, LHX3,LHX4*).
In this analysis, we screened the entire gene in 75 CPHD patients. Primers were specifically designed for the 13 coding exons that were analysed by direct sequencing in 18 separate fragments. We identified two novel missense mutations: c.731A>T in exon5 leading to the amino acid substitution p.Asp244Val and c.1157C>T in exon7 resulting in the change p.Pro386Leu. These two variants were absent in about 13.000 individual present in the Exome Variant Server. Both these mutations were predicted as probably damaging using the online tool Polyphen v2, with a high score (1.00 for Asp244Val and 0.923 for p.Pro386Leu). They fall within the NH2 terminal repressor domain and an *in vitro* functional analysis will help to clarify their significance.
These preliminary results are encouraging and we are planning to increase the number CPHD patients and extend the analysis also to Isolated Growth Hormone Deficiency (IGHD).

P17.31-S
A Genome-wide Association Analysis of Lipid traits in a sample of indigenous population from Mexico

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Given the high prevalence of diferent forms of dyslipidemia in Mexican population, in contrast to European populations, it has been suggested that genetic susceptibility in this population is probably related to its indigenous component as a result of adaptive processes related to energy savings. It is therefore of great importance to identify the genetic variation of the native Mexican population. In this study, 319 individuals from four indigenous populations (174, 70, 25 and 50 from Nahua, Maya, Totonac and Zapotec, respectively) were included. Genotyping was performed using Affymetrix SNP 6.0 microarray. The analysis included a local ancestry estimation to remove segments infered to be of European origin and mixed linear models using EMMAX, adjusted for age, gender and two principal components. Two steps of quality control were performed, before and after the European segments were removed. Log-transformed values of tryglecides (TG), total cholesterol (TC) and HDL cholesterol (HDL) were analyzed. By removing segments of European origin in the analysis we identify three suggestive signals, one for each trait. For HDL, TC and TG, an association was found in intergenic regions on chromosome 11 (p≤9.5x10-6), chromosome 21 (p≤8.8x10-5) and chromosome 2 (p≤6.1x10-5), respectively. In the three cases, the risk allele frequency is 20%, 16% and 10% higher in Indigenuos population than in European population. This regions have not been identified in previous GWAS studies thus it is important to replicate these results to confirm the involvement of these new regions.

P17.32-M
Selecting significant SNPs in Genome-wide Association Studies using a global False Discovery Rate

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Standard methods for assessing significance in Genome-wide Association Studies (GWAS) are generally too stringent as they are based on controlling the Family-Wise Error Rate. The FDR (False Discovery Rate) approach provides a more realistic and less stringent alternative. However, controlling the FDR for correlated entities is difficult without making specific assumptions on the correlation structure that may not be realistic. In this study, we define an FDR-like criterion (global-FDR) for assessing statistical significance of SNPs in GWAS accounting for correlation (Linkage Disequilibrium) between neighboring SNPs. The criterion extends the local-FDR criterion of Efron and ensures control of the False Discovery Proportion (FDP) under arbitrary correlation structure. We develop a fast and easily scalable Gibbs-type iterative algorithm to approximate the posterior class membership probabilities that are otherwise hard to compute in general. Unlike the non-parametric estimation approach used in local-FDR, we use a parametric three-group normal mixture model for SNP Z-scores with a known correlation matrix. Using simulations under different correlation scenarios, we found that the global-FDR approach performs significantly better than local-FDR, both in terms of power and accurate control of the type-I error (FDP). We also considered a sequential procedure to select the most strongly associated SNPs from independent (non-redundant) regions of the genome discarding correlated surrogates in the same LD region. We assessed the sensitivity and specificity of our single-step and sequential SNP-selection strategies. The methods were also validated against known associated regions using published GWAS data from the Collaborative Association Study of Psoriasis.

P17.33-S
A genome-wide association study identifies two susceptibility loci for non-syndromic cleft lip with or without cleft palate in the Polish population

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Non-syndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common congenital anomalies, with a complex and not yet fully elucidated etiology. Therefore, we conducted a genome wide association study (GWAS) for NSCL/P in a Polish population-based cohort consisting of 288 oral cleft cases and 576 controls. GWAS was carried out with the Illumina Human OmniExpressExome BeadChip technology. We confirmed the association between the previously identified 8q24.21 locus and the risk of NSCL/P. The most significant nucleotide variant in this 330-kb region (rs17242358) had a p-value of 3.21E-09. Under assumption of a dominant and recessive models, the calculated Odds Ratios for rs17242358 were 2.28 (95%CI: 1.70 - 3.06, p = 2.53E-08) and 2.99 (95%CI: 1.60 - 5.59, p = 3.32E-04), respectively. In addition, we found a novel cleft-associated region at chromosome 22q12.3, with a p value of 1.22E-08 for the most significant polymorphism (rs210804). The dominant model OR for the rs210804 variant was 8.20 (95%CI: 3.51 - 19.13, p = 1.19E-08). Four other chromosomal regions 6p12.3 (*DEFB112*), 22q12.3 (*MYH9*), 6p24.3 and 11q13.4 (*POLD3*) were associated with the NSCL/P risk at close to genome wide significance level (p values of 5.12E-08, 3.26E-07, 3.40E-07 and 8.15E-07, respectively). To confirm our GWAS findings further, larger sample size studies in different populations are needed.
Supported by grant no. 2012/07/B/NZ2/00115 from the Polish Ministry of Science and Higher Education.

P17.34-M
Family-Control analysis based on Hamming distance for prioritizing candidate sequence variants

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Linkage analysis has been used to narrow down disease loci for autosomal dominant (AD) traits. However, small pedigrees are underpowered for significant linkage results. We developed a novel method to detect disease loci in pedigrees.

For a set of basepairs flanking a candidate locus, we calculate Hamming Distance Ratio (HDR, proportion of basepairs differing between two individuals) for all pairs of individuals (an affected family member and 41 control individuals) and distinguish pairs containing the affected (group 1) from those that do not (group 2). We assess the difference in distribution of HDR values between the two groups by the Kolmogorov-Smirnov statistic at sets of variants 10KB to 100KB around each of ~600 candidate variants. In two hypertrophic cardiomyopathy families, known pathogenic mutations were previously detected: c.173G>A (p.R58Q) in MYL2 gene (rs104894369, family A) and c.746G>A (p.R249Q) in MYH7 gene (rs3218713, family B). We combine results over the 10 regions with suitable test statistics evaluated in permutation analyses and are able to narrow down known disease variants as small as 5% (rank as small as 29 out of 606 candidates, p=0.0001).
In a negative control, a restrictive cardiomyopathy pedigree with de novo mutation in the TNNI3 gene in one affected sibling, TNNI3 mutation was ranked 221th out of 631 variants.
Our new statistical method for prioritizing disease region by Hamming distance between affected family member and unrelated controls will be useful for small AD pedigrees and for differentiating AD and de novo mutations.

P17.35-S
Mitochondrial DNA variation analysis in historical provinces of Romania

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Since the Middle Ages, Romanian population lived in three distinct provinces Wallachia, Moldavia and Transylvania until the 19th century. Over the centuries, their territories were repeatedly invaded by different peoples and subjected to external political influences resulting in demographic changes that could affected the genetic structure of populations. We performed mitochondrial DNA analysis in order to visualize the relationships between Romanian and other populations Europe based on HVSI and HVS II mtDNA sequence data using Sanger sequencing. We presented here a large scale mtDNA analysis of 612 Romanians from these historical provinces of Romania. Multidimensional scaling (MDS) plot was constructed from the pairwise Fst values. The results showed that present day Romanians in all provinces share their maternal ancestry with both eastern/central European and Balkan populations. The three populations of Romania analyzed here exhibit slightly different mtDNA lineage compositions, mainly consisting of the haplogroups H, U, J, T, K, N and W, with significant frequency differences corresponding for H, U and W haplogroups. H haplogroup accounts for 47% in Moldavia, 35 % in Wallachia and 33 % in Transylvania. Overall, this study provides a first comprehensive analysis of mtDNA genome variation in Romania revealing the existence of different degrees of provincial differences of haplogroup frequencies.

P17.36-M
Heritability of age related cognition

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The role of genetic and environmental factors in specific cognitive abilities in the elderly is poorly understood. Here we estimated their contributions to variation in the cognitive domains memory, executive function and fine motor skills and tested the genetic correlations between these domains.
The study was conducted in the Austrian Stroke Prevention Study (ASPS), a population-based cohort study (n=479, mean age=64.6 years, 54.9% women) and in the ASPS-Family including relatives of the ASPS participants (n=376, 177 families, mean age=63.6 years, 60.1% women). The neuropsychological test battery included Bäumler's Lern-und Gedächtnistest, Trail Making B, Digit Span Backward, the Wisconsin Card Sorting and the Perdue Pegboard Test. Heritability was estimated by variance component analyses using SOLAR and by SNP genotypes using GCTA. Bivariate heritability was computed using SOLAR. We adjusted for age, gender, education and APOE.
Heritability of memory, executive function and fine motor skills using family structure were 60, 41 and 59%, and 11, 23 and 29% using SNP genotypes. Genetic and environmental correlations between memory and executive function were 53% and 20%, between memory and fine motor skills 41% and 14%, respectively. We found no genetic correlation between executive function and fine motor skills, the environmental correlation was 37%.
There is a substantial heritability of memory and fine motor skills and a moderate of executive functions. Half of the heritability of executive function and fine motor skills can be explained by common SNP. Our results support shared genetic factors for executive function and memory as well as fine motor skills.

P17.37-S
Involvement of HMGA2 (High-Mobility Group A2) in Idiopathic Short Stature (ISS).

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Several lines of evidences point to *HMGA2* as a candidate gene in ISS: i) independent GWAS identified some SNPs on chromosome 12q14 in the vicinity of *HMGA2* (rs1042725, rs7968682 and rs7968902) as one of the major determinants of height, ii) microdeletions on 12q14 have been identified in syndromic patients with short stature as common feature. Among these, one patient with only severe growth retardation carried the smallest deletion encompassing *HMGA2.* The aim of this study was to investigate the involvement of *HMGA2* in ISS through the search for mutations/deletions and perform an association study between the *HMGA2* SNPs and ISS. One hundred-four patients (48 males and 56 females) with height ranging from -3,8 and -2 SDS were analyzed by direct sequencing and MLPA. None of the patients carried mutation/deletion in the coding sequences and intron/exon boundaries. The same 104 patients and 330 normal stature matched controls were analyzed in an association study with rs7968682. The allele frequency was significantly different between patients and controls ($p=4x10^{-2}$). When the genotypes were considered the TT genotype showed an even stronger association ($p=6x10^{-3}$). In conclusion, our cohort of ISS patients did not show any pathological mutation in *HMGA2*, suggesting that high penetrance mutations are not a frequent cause of ISS. However our preliminary results suggest that *HMGA2* might be involved in the susceptibility to ISS. We are thus planning to replicate the data in an independent cohort and analyze the tag SNPs surrounding *HMGA2* to identify the variations directly responsible for the association.

P17.38-M
Thioredoxin reductase 1 (TXNRD1) gene variability influences quality of aging and longevity

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Oxidative stress is a major determinant of human aging and a common hallmark of age-related diseases. A protective role against free radicals accumulation has been shown for Thioredoxin (Trx) system and in particular thioredoxin-reductase TrxR, a key selenoprotein antioxidant enzyme, able to reduce Trx and other substrates, detoxifying cells from oxidative injuries. An effect of this antioxidant system in human aging can be hypothesized from the association reported between TrxR gene (TXNRD1) with late-life survival in a Northern-European nonagenarian cohort (Soerensen et al, 2013). Using a tagging approach, we investigated the association of 14 SNPs with longevity and quality of aging, by analyzing their variability in relation to markers of functional (Activities of Daily Living, ADL; Hand Grip, HG; Walking speed, WS) and cognitive (Mini Mental State Examination, MMSE) status, in a Southern-Italian elderly cohort (626 subjects, age range 65-104 years). The work confirms the association of TXNRD1 gene with human survival, with two intronic SNPs, rs7310505 and rs4964728, showing association with longevity ($p<0.04$). Furthermore, three other SNPs, two intronic (rs7962423, rs10861203) and one located in the promoter region (rs1128446), were significantly associated with ADL, HG and WS ($p<0.05$). Haplotypic analyses confirmed the single-SNP results. Moreover, bioinformatic analyses indicated the associated SNPs as putative regulatory sites, whose function should be further experimentally investigated.
On the whole, this study confirms a role of Trx antioxidant system on human age-related functional decline and longevity, possibly mediated by modulation of oxidative stress.

P17.39-S
Genetic analysis of thyroid peroxidase (TPO) gene in patients whose hypothyroidism was found in adulthood in West Bengal, India

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Recent research has revealed that genetic defects due to mutation in the Thyroid Peroxidase (TPO) gene can lead to thyroid dysfunction in the population. We aimed to study the association between genetic defects in TPO

gene and patients with hypothyroidism found in adult age. Two hundred consecutive treatment naive hypothyroid patients (age ≥ 18 years) (cases) who were negative for anti TPO antibody and their corresponding sex and age matched two hundred normal individuals (controls) were enrolled. The 17 exonic regions of the TPO gene were amplified and sequenced directly. We identified 6 different previously known single nucleotide polymorphisms (SNPs) and 2 novel deletions in TPO gene. Two of the six SNPs revealed a significant association with hypothyroidism; Thr725Pro (rs732609) and Asp666Asp (rs1126797). The c.2173C allele of the Thr725Pro in TPO showed a significant association among hypothyroid patients compared to controls (p = 0.01; Odds ratio=1.45; 95% CI: 1.09–1.92) suggesting it to be a potential risk allele toward disease predisposition. Analysis of genotype frequencies of the polymorphism between the two groups demonstrated CC as a potential risk genotype (p = 0.006; Odds ratio=1.95; 95% CI: 1.2–3.15) for the disease while another SNP Asp666Asp (c.1998T allele) showed protectiveness towards the disease (p = 0.006; Odds ratio = 0.67; 95%CI: 0.50-0.89). To our knowledge, this is first study reporting the role of TPO gene with hypothyroidism in a population of Asian Indian origin. The study threw up the possibility of TPO gene polymorphisms as a possible pathogenetic mechanism of hypothyroidism.

P17.40-M
Genes involved in interleukin-1 receptor type II (IL1R2) activities are associated with asthma related phenotypes

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We previously demonstrated that *IL1R2* (Interleukin-1-receptor-type-II) is overexpressed in bronchial biopsies of allergic asthmatic individuals and we also associated it with atopy (n>5500). This study aims to test for the effect of SNPs and interactions between 182 SNPs belonging to nine genes involved in *IL1R2* activities on asthma related phenotypes in the Saguenay-Lac-St-Jean familial asthma collection (SLSJ) and in French families of the Epidemiological study on the Genetics and Environment of Asthma (EGEA)). Single SNP analysis was performed using Family-Based Association Tests software (FBAT). In order to correct for multiple tests, we used a critical P-value threshold equal to $2x10^{-4}$ that took into account the number of independent SNPs and independent phenotypes. Interactions between SNPs were tested using Unphased software. SLSJ and EGEA results were combined through meta-analysis using the Stouffer's Z-score method. No SNP reached the critical threshold for significance but suggestive association were observed. The rs3732131 SNP in *IL1R1* was associated with asthma (p=0.0004), atopy (p=0.0005) and allergic asthma (p=0.002). We also found associations between three SNPs in *ERAP1* and allergic asthma ($0.0006 \leq p \geq 0.001$). Regarding SNPxSNP interactions, four interactions were found in SLSJ at $p<1.0X10^{-4}$ and one of those was replicated in EGEA for atopy (p=0.002, combined $p=3.62X10^{-7}$) between rs2241343 of *IL1RAP* and rs10208708 of *IL1R1*). The observed SNP-associations in the combined SLSJ and EGEA samples and the SNPxSNP interaction suggest that genes involved in the IL1R2 activities play a role in asthma and, especially in its allergic component.

P17.41-S
The frequency of SHOX mutations in patients with mild short stature is comparable to the frequency detected in severe Idiopathic Short Stature

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Mutations within the pseudo-autosomal Short stature Homeobox gene (*SHOX*, Xp22.33 and Yp11.3) and the downstream enhancer are among the few known causes of Idiopathic Short Stature (ISS). Routine diagnostic testing for the genetic alterations in this gene is carried out in ISS patients, selected with strict criteria regarding the phenotype, who generally show a good response to GH treatment. We analysed 217 ISS patients by sequencing and MLPA to screen for deletion/duplication in the coding sequence and the downstream enhancer region. Among these patients, 90 were severe short stature with a height SDS ranging from -3.9 to -2.0 and 127 patients had a milder phenotype with a height SDS ranging from -1.9 to -0.9. The age at diagnosis was 8.6±3.9 and did not differ significantly between the two groups. Mutations were identified in 7 patients (7.7%) with the most severe phenotype and in 7 patients (5.5%) with mild short stature. There was no significant difference in the mutation number, types and the extension of the detected deletions between the two groups. All the available relatives of the probands were analysed for segregation analysis. Consequently to the high recombination frequency within PAR1 region we observed several *SHOX* mutations moving from the Y to X chromosome and vice versa. This must be taken into consideration in genetic counselling. Notably, this study revealed the presence of SHOX mutations in individuals with mild short stature with a frequency comparable to that observed in ISS selected with the stringent classical criteria.

P17.42-M
Insights into the genetic variability of Italian population inferred from 2.5M single-nucleotide polymorphisms

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Aim: This study aims to characterize the genetic variability within Italian population and the level of inter-population variation between the Mediterranean basin and the Italian peninsula.
Methods: A total of 314 samples, collected from the Italian peninsula and Sardinia island were genotyped, with more than 2,370,000 single-nucleotide polymorphisms investigated. Newly acquired data were analyzed together with the already available genome-wide data sets of individuals of the Mediterranean basin. Several analyses have been performed to investigate intra e inter-population variability.
Results: The analysis highlighted that Italy is one of the genetically most diverse region within Southern Europe. These results may be due to the country's complex demographic history, to the large longitudinal extension of the territory and because of its position as a sort of natural bridge in the center of the Mediterranean basin.
Conclusion: We confirmed the high genetic diversity of some areas within Italy, such as Sardinia, and of other populations located along boundaries of the Italian territory.
We showed that the current genetic landscape of the Italian Peninsula may be explained by isolation-by-distance rather than a barrier to gene flow and may also reflect a genetic exchange for the Southern Italian populations between the 2 coasts of the Mediterranean basin.

P17.43-S
Familial Hypercholesterolemia screening in Patients with Coronary Artery Disease

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Background: Familial hypercholesterolemia (FH) is an autosomal-dominant disease that leads to markedly elevated low-density-lipoprotein cholesterol (LDLC) levels and high risk for premature coronary-artery disease (CAD). Despite well-established criteria for clinical diagnosis, it is estimated that in most European countries only around 5% of FH are identified. One hypothesis is that FH is simply overlooked in the large number of unselected CAD patients. Moreover, variable LDLC-levels and small family sizes may hamper the clinical diagnosis. Around 90% of known FH disease-causing mutations are found in the LDLR gene. Hence, systematic molecular-genetic screening of the LDLR gene in patients with premature CAD may improve diagnosis and allow preventive treatment of mutation carriers.
Aim: To evaluate the frequency of FH, we screened LDLR gene for deleterious mutations in 256 unselected CAD patients with disease manifestation before the age of 50 years.
Methods and Results: We filtered for rare (<1% 1000G) and non-synonymous variants. Around 6% of the screened CAD patients carried deleterious variants in the LDLR gene. The 10 variants were confirmed using Sanger-sequencing and validated by co-segregation. Nine are reported to cause FH. One of the reported variants (rs45508991) was found in six unrelated CAD patients. This variant shows incomplete co-segregation and is also found in internal control samples. Some of the identified LDLR-variants were also found in family members without a known manifestation of CAD but elevated LDLC levels.
Conclusion: These results underline the need for molecular-genetic screening to diagnose FH and to allow timely preventive treatment.

P17.44-M

Study homozygosity disequilibrium in human genome using the whole-genome sequencing data

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Homozygosity disequilibrium (HD), a non-random sizable run of homozygosity in the human genome, has been found related to the evolution of populations and able to confer susceptibility to diseases. In this study, we characterized HD in global populations based on the whole-genome sequencing data of 1,092 individuals from 14 populations of the 1000 Genomes Project. The whole-genome homozygosity intensity was estimated by using LOHAS (Yang et al, Genetic Epidemiology, 2011). Using the homozygosity intensity we identified common genomic regions undergoing HD. We found a high proportion of regions of HD to be population-specific but also identified functionally important regions of HD shared by multiple populations. Genetic differentiation of global populations can be characterized using the patterns of HD. In summary, this large-scale whole-genome sequencing study derives the distribution of HD in the human genome and proves that HD carries genetic information of human population important for studying genetic background. The information also provides important clues for the differential disease prevalence and drug responses in populations.

P17.45-S

A signal near FRMD4A is associated with lower extremity arterial disease in patients with type 2 diabetes in GoDARTS

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Lower extremity arterial disease (LEAD) is a common macrovascular complication of type 2 diabetes (T2D). Twin studies of ankle brachial index (ABI), the main diagnostic criterion of LEAD, estimate the heritability of ABI at ~30%. Two genome wide signals have been identified for LEAD near CHRNA3 and 9p21.3 irrespective of diabetes status. The aim of this study was to identify genetic determinants of LEAD in patients with T2D. LEAD cases were patients with T2D and an ABI < 0.9 or ABI>1.3 and or mid-thigh to mid-foot amputations and or corrective procedures related to LEAD and or prescriptions for medication used to treat claudication. Controls were patients with T2D free of LEAD, coronary artery disease and ischaemic stroke. Allelic effects of 5,882,833 SNPs estimated from 1223 LEAD cases and 5638 LEAD free controls were combined in fixed-effects 1000G meta-analysis. A signal represented by rs72780858 near FRMD4A reached genome wide significance (p=3.7E-8). SNPs in FRMD4A have been associated with nicotine dependence which may influence smoking status. Smoking is known to increases the risk of LEAD 10 fold. Other suggestive signals are located near genes that contain genome wide significant hits for risk factors related to LEAD: rs271946 (p=8.1E-6) near PCSK1 (T2D); rs34562 (p=8.2E-6) near EFNA5 (Coronary artery disease) and rs75417257 (p=1.2E-6) near CCSER1 (Glomerular filtration rate and albumin excretion rate). This is the first study of the genetic determinants of LEAD in patients with T2D and several signals including one at genome wide significance were identified.

P17.47-S

Molecular screening of major thalassemia mutations observed in 2012-2013 in eastern Sicily

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In this study we evaluated the incidence of alpha, beta and delta thalassemia in the province of Messina, in the past two years.
236 people were analyzed (Molecular Genetics Laboratory of Genetics and Immunology Pediatric UOC), after healthy carrier screening of I and II level of Thalassemia by the diagnostic procedure that provides for the CBC, the determination of the hemoglobin fractions and the assessment of iron status. The phenotypic framework (classical and non classical), who had hematological parameters indicative of mutations at the level of alpha clusters and/or non-alpha-globin, were studied by molecular analysis of related genes. This investigation has shown that in the alpha globin gene, the $-\alpha^{3.7}/\alpha\alpha$ (genotype frequency 13,1%; allele frequency 7,83%) is the most common genotype, followed by the α_2IVS1:-5nt α/WT (genotype frequency 4,66 %; allele frequency 2,33%), and the $-\alpha^{20.5}$/WT (genotype frequency 2,11%; allele frequency 1,05%) genotypes; instead, in the beta globin gene, the βcd39/WT(genotype frequency 5,08%; allele frequency 2,54) is the most typical genotype, followed by the βIVS1:6/WT (genotype frequency 3,81%; allele frequency 2,75%) and the βIVS1:110/WT (genotype frequency 3,81%; allele frequency 1,90%) genotypes; and finally, in the delta globin gene, the δcd 27/WT (genotype frequency 6,35%; allele frequency 3,17%) is the most common genotype found, with the δcd142 C>A(Hb Fitzory)/WT (genotype frequency 0,42% %; allele frequency 0,21%) and the δIVS2:1/WT (genotype frequency 0,42%; allele frequency 0,21%) genotypes. These data show the importance of a careful assessment about haematological indices to carry out a correct diagnosis of genetic defects.

P17.48-M

Variations in ncRNA gene LOC284889 and MIF-794CATT repeats are associated with malaria susceptibility in Indian populations

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LOC284889 is an uncharacterized ncRNA gene on reverse strand to *MIF* mapped to 22q11.23. MIF, a lymphokine, regulates innate immune response by up-regulating the expression of *TLR4*, suppressing the p53 activity and has been shown to be involved in malaria pathogenesis. In this study, the possible effect of *MIF* variations on malaria susceptibility was investigated by re-sequencing the complete *MIF* gene along with 1 kb each of 5′ and 3′ region in 425 individuals from malaria endemic regions of the Orissa and Chhattisgarh states of India. The subjects comprised of 160 cases of severe malaria, 101 of mild malaria and 164 ethnically matched asymptomatic controls. Data were statistically compared between cases and controls for their possible association with *Plasmodium falciparum* malarial outcome. It is the first study, which shows that the allele *A* (rs34383331*T* > *A*) in ncRNA is significantly associated with increased risk to *P. falciparum* malaria [severe: OR = 2.08, p = 0.002 and mild: OR = 2.09, P = 0.005]. In addition, it has been observed that the higher *MIF-794CATT* repeats (>5) increases malaria risk (OR = 1.61, p = 0.01). Further, diplotype (*MIF-794CATT* and rs34383331*T* > *A*) *5 T* confers protection to severe malaria (OR = 0.55, p = 0.002) while *6A* (OR = 3.07, p = 0.001) increases malaria risk. These findings support the involvement of ncRNA in malarial pathogenesis and further emphasize the complex genetic regulation of malaria outcome. In addition, the study shows that the higher *MIF-794CATT* repeats (>5) is a risk factor for severe malaria. The study would help in identifying people who are at higher risk to malaria and adapt strategies for prevention and treatment.

P17.49-S

Epidemiology of Meckel-Gruber syndrome in Europe: a registry-based study

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Meckel-Gruber syndrome is a rare autosomal recessive lethal ciliopathy characterized by the triad of cystic renal dysplasia, occipital encephalocele and postaxial polydactyly. We present the largest population-based epidemiological study to date using data provided by the European Surveillance of Congenital Anomalies (EUROCAT) network of congenital anomaly registries. The study population consisted of 191 cases of Meckel-Gruber syndrome identified between January 1990 and December 2011 in 34 European registries. The mean prevalence of Meckel-Gruber syndrome was 2.6 per 100 000 births. The prevalence was stable, but regional differences were observed. There were 145 (75.9%) terminations of pregnancy after prenatal diagnosis, 13 (6.8%) fetal deaths, 33 (17.3%) live births and of these 11 (33%) neonatal deaths. In addition to cystic kidneys (97.7%), encephalocele (83.9%) and polydactyly (87.3%), frequent features include other central nervous system anomalies (68.8%), fibrotic/cystic changes of the liver (65.5% of cases with post mortem examination) and orofacial clefts (31.8%). Various other anomalies were present in 64 (37%) patients. As nowadays most patients are detected very early in pregnancy when liver or kidney changes may not yet be developed or may be difficult to assess, none of the anomalies should be considered obligatory for the diagnosis. Most cases (90.2%) are diagnosed prenatally at 14.3±2.6 (range 11-36) gestational weeks and pregnancies are mainly terminated, reducing the number of live births to one fifth of the total prevalence rate. Early diagnosis is important for timely counseling of affected couples regarding the option of pregnancy termination and prenatal genetic testing in future pregnancies.

P17.50-M

Genetic markers predicting menopausal age associate with diabetes and lipid traits in 11864 Finns

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Later age at menopause has been associated with decreased risk of cardiovascular diseases and increased life expectancy, but associations with diabetes are controversial and no genetic links have been reported. Additionally, smoking lowers and obesity raises menopausal age, which might disturb the seen associations. Stolk et al. reported of 17 genetic polymorphisms which associate with menopausal age (Nat Genet. 2012). We constructed a genetic score of menopausal age by summing the reported effect sizes (years/allele) of these polymorphisms in four Finnish cohorts (FINRISK1997, PredictCVD, Health2000 and Corogene) and studied its associations to common diseases and their risk factors. Linear, logistic and survival analysis corrected by age and geographical variables were performed in individual cohorts and combined in meta-analysis.
One-year increase in the genetic score (range -2.3 - 3.1 years) associated significantly with future diabetes in women (N=2831, hazard ratio=1.46, P=0.0083) but not in men (P=0.3). Interestingly, the genetic score associated nominally (P<0.05) with prevalent diabetes in men (N=5547, odds ratio=0.87, P=0.042) but no association was seen in women. In women nominal association of higher menopausal age score was seen also with higher triglyceride levels and lower HDL cholesterol. Controlling for BMI and smoking did not affect these associations.
Our results suggest that the genetic variants associated with the timing of menopause in women have different effects on metabolism in different genders. In women polymorphisms raising menopausal age raise also the risk of future diabetes 1.5-fold whereas in men the same variants have a slight protective effect on prevalent diabetes.

P17.51-S

Exome-wide association analysis of 4,522 individuals from the Oxford Biobank study reveals novel low frequency variants influencing human serum metabolite levels

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Small molecule metabolites are intermediates in disease pathways, and identification of genetic variants influencing metabolite concentrations could provide insight into disease pathogenesis. Common variant effects have been thoroughly scrutinised, but the impact of low frequency (LF) variants (minor allele frequency (MAF) <5%) remains largely unexplored.
Here we report an exome-wide association study of metabolite concentrations in 4,522 healthy individuals (age 29-53 years, 55% women) from the population-based Oxford Biobank study. Approximately 230,000 markers were genotyped using the Illumina HumanExome Beadchip and tested for association with 123 metabolites (80 lipoproteins, 15 lipids, 22 low molecular weight metabolites, and 6 ratios related to fatty acid saturation) quantified by nuclear magnetic resonance of serum samples. After log normalisation, inverse normalised residuals were generated adjusting for age, gender, and principal components. A linear mixed model was used assuming an additive genetic effect. In the single marker analyses, we identified 15 loci with genome-wide significant associations (p<5x10-8) for one or more metabolites. For two of the loci, the strongest signal involved a LF marker. We also performed gene-level tests: using SKAT for protein-altering variants (MAF<1%) we identified two novel genes (p<2x10-6) associated with alanine (PI16, p=4x10-7) and glycoprotein acetyls (C8A, p=2x10-6), both of them driven by single variants with large effects. An analysis focused on protein-truncating variants (using SEMGEM) revealed a significant association between COQ10A and glutamine (log10 (Bayes factor)=4.25). The identification of genetic variants influencing metabolite concentrations will allow us to explore the causal relationships between these metabolites, other metabolic risk factors and disease.

P17.53-S

A common functional polymorphism in miR-196a2 is associated with waist to hip ratio

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MicroRNAs (miRNAs) are small non-coding RNAs that function as crucial regulators in a broad range of biological processes. They have recently gained extensive attention as mediators of complex disorders and highlighted as promising diagnostic biomarkers for cardiometabolic diseases. Given the central role of miRNAs in gene expression, genetic polymorphisms in miRNA genes are expected to alter miRNA processing and function that may contribute to disease susceptibility. Here we retrieved 788 single nucleotide polymorphisms (SNPs) in all pre- and mature miRNA sequences and systematically investigated their association with 17 cardiometabolic traits. We used data of the largest meta-analyses of genome wide association studies (GWAS) on glycemic hemostasis indices, lipid traits, blood pressure, coronary artery disease, type 2 diabetes, and anthropometric measures including information up to 133,000 individuals. We found that a common variant in miR-196a2 (rs11614913:T>C) is significantly associated with waist to hip ratio (WHR) (p-value =3.4×10^{-5}). To explore whether this miRNA affect WHR, we examined the association of all miR-196a2 target genes with this phenotype and revealed *SFMBT1* to be significantly (p-value =2.8×10^{-6}) and *SMAD6* suggestively (p-value = 6.9×10^{-5}) associated with WHR. Additionally, a trans-expression quantitative trait loci (eQTLs) analysis in 762 subjects of the Rotterdam Study suggested an increased trend in the expression levels of *SFMBT1* and *SMAD6* in individuals carrying the risk allele. These findings may help improve our understanding of the regulatory role of miRNAs in fat distribution.

P17.54-M

A robust strategy for the calculation of Allele Frequencies from NGS data of pooled DNA samples

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Sequencing large number of individual genomes at high coverage, which is needed for population genetics studies, is still economically challenging despite substantial reduction in cost of NGS in recent years. An alternative approach is to sequence DNA from pools of individuals which has other potential benefits like: needing less DNA from each single individual and less time in sample preparation.
We have developed a robust strategy for the analysis of DNA sequencing data from pooled individuals and the calculation of allele frequency (AF) of identified sequence variations. We used CRISP [1] to call the variants, ANNOVAR to annotate them, and custom R-scripts to calculate AF. We applied this strategy in targeted re-sequencing (1.9Mb) of 600 (12 individuals * 50pools) Italian Multiple Sclerosis (MS) patients. For 129 variants, AFs have been independently validated by genotyping the single individuals with SNP-array. For each pool, the comparison of AFs in NGS pool data and those at the single individual level with SNP-array shows an excellent concordance between the two methods: mean correlation R^2=0.976 ± 0.004, and mean delta frequencies = (2.5 ± 0.2)*10^{-2}. Moreover, the comparison of AF with that of 1000 genomes (European individuals) for further 5980 SNPs showed an excellent concordance: mean difference of AF = (3.07±.05)*10^{-2}. Overall, we show that pooling the samples for population genetics and disease association studies is a robust and cost- and time-saving alternative to single sample NGS analysis.
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[1] doi:10.1093/bioinformatics/btq214

P17.55-S

Whole-genome sequencing of an Italian multiple sclerosis multiplex family identifies a novel functional variant in the GRAMD1B gene.

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BACKGROUND: While the role of common genetic variants is clearly established from recent studies on sporadic cases of multiple sclerosis (MS), the contribution of rare variants is unclear. AIMS: To identify rare genetic

variants contributing to MS susceptibility in an Italian multiplex family. DESIGN: SNP microarray genotyping and whole-genome sequencing (WGS) in 4 MS patients and 4 unaffected individuals belonging to an Italian multiplex family descending from a first cousin marriage were performed. RESULTS: We identified 437 variants with high functional impact, two of which under one of the two LOD peaks on chromosome 8p21.2 and 11q23.3. The first one is in the OR8G5 gene, an olfactory receptor gene under positive selection, while the second one falls within the GRAMD1B gene, causing an aminoacid substitution (S601P). This second variant is not present in dbSNP and in the 1,000 Genome project, and segregates within the family, being homozygote in 3 affected and heterozygote in the left MS patient. Sanger sequencing confirmed the segregation with the disease. GRAMD1B is a very conserved gene from yeast to human. It encodes a membrane protein, which is part of the GRAM containing domain family protein. In the mouse it is highly expressed in the CNS and in specific immune cell subtypes, like dendritic cells and neutrophils. CONCLUSIONS: The use uf WGS in an Italian MS multiplex family has been successful in identifying novel rare genetic variants. Further investigations are ongoing to explore the role of the variant on protein function and its involvement in MS.

P17.56-M
Statistical methods for the analysis of gene expression in single-family studies for genetic and complex disease

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Multiplex families are a powerful tool to investigate heritability of genetic components in complex disease. Heritability can be investigated using gene expression analysis with specific challenges. Previously developed approaches were focused on identifying significant genes across families but each family can present different genetic makeup producing heterogeneous gene expression. Moreover it is important to disentangle the strong genetic component of gene expression from the disease effect or other factors affecting gene expression, such as environmental exposures.
We developed a statistical method for the analysis of gene expression data from single-family studies to minimize the problem in interpreting these data. We applied the Ornstein-Uhlenbeck model previously used to describe evolutionary processes. The model has been transferred to a context in which the quantitative trait has different values in diseased and healthy individuals and it is defined by trees derived from family pedigrees to explicitly model the inheritance of gene expression, thus to identify those genes that are affected by the disease status. In order to validate the method, simulated datasets were generated starting from a family pedigree taking into accounts the genetic component and the disease status of every gene for every individual. We also used a real dataset from a family pedigree with 4 multiple sclerosis affected individuals. The results obtained on simulated datasets reports that the OU model provides increased sensitivity with respect to traditional gene expression analysis approaches. The analysis of the MS affected family also identified genes and pathways relevant to the disease.

P17.57-S
Simultaneous estimation of the locations and effects of multiple disease loci in case-control studies

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The genetic basis of complex diseases often involves multiple linked causative loci. Under such a disease etiology, assuming one disease locus in linkage disequilibrium mapping is likely to induce bias and lead to efficiency loss in disease locus estimation. An approach is needed for simultaneously localizing the positions of multiple functional loci. However, due to the increasing number of parameters accompanying disease loci, these estimates can be computationally infeasible. To circumvent this problem, we propose to estimate the main and gene-gene interaction effects and a nuisance parameter at the disease loci separately through a linear approximation. Estimates of the genetic effects are entered into a generalized estimating equation to estimate disease loci, and the procedure is conducted iteratively until convergence. The proposed method provides estimates and confidence intervals (CIs) for the disease loci, the genetic main effects, and the interaction effects between loci, with the CIs for the disease loci providing useful regions for further fine-mapping. We apply the proposed approach to a data example of case-control studies. Results of the simulations and data example suggest that the developed method performs well in terms of bias, variance, and coverage probability, regardless of the underlying number of disease loci.

P17.58-M
Evolutionary analysis identifies an MX2 haplotype associated with natural resistance to HIV-1 infection

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The protein product of the MX2 (myxovirus resistance 2) gene restricts HIV-1 and simian retroviruses. We demonstrate that MX2 evolved adaptively in mammals with distinct sites representing selection targets in rodents and primates; selection mainly involved residues in loop 4, previously shown to carry antiviral determinants. Modeling data indicated that positively selected sites form a continuous surface on loop 4, which folds into two antiparallel α-helices protruding from the stalk domain. Population genetic analyses demonstrated that natural selection operated on MX2 during the recent history of human populations: distinct selective events drove the frequency increase of two haplotypes in populations of Asian and European ancestry. The Asian haplotype carries a susceptibility allele for melanoma; the European haplotype is tagged by an intronic variant. Analyses performed on three independent European cohorts of HIV-1 exposed seronegative individuals with different geographic origin and distinct exposure route showed that the ancestral allele of the intronic variant protects from HIV-1 infection with a recessive effect (combined p value = 1.55x10-4). The same allele is associated with lower in vitro HIV-1 replication and increases MX2 expression levels in response to IFN-α. Data herein exploit evolutionary information to identify a novel host determinant of HIV-1 infection susceptibility.

P17.59-S
Improving power in family-based association of rare variants via wavelet-based test

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With the advances in next generation sequencing (NGS) technology, a huge amount of data on high dimensional genomic variant was generated and the association of rare variants with complex disease was analyzed for finding the etiology of disease. A variety of statistical approaches for the analysis of rare variants were developed in population-based designs. However, many common diseases such as cancer, diabetes, cardiovascular disease, immune disorders and psychiatric disorders are known to cluster in pedigrees. Recently, many researches have modified existing population-based methods for detecting the family-based association of rare variants such as family-based collapsing methods, family-based functional principal component analysis (FPCA) and extended FBAT method. Rare variants may provide contributions to detect disease gene, however, the existence of noisy signals reduced the power of association. In this study, we modified the wavelet-based test in family-based design to increase power by suppressing noise and therefore reducing false positives. Using simulations with several types of data structure, our proposed method generated a reasonable type I error rate and a comparable or better power (0.4~0.7). We demonstrated that our proposed method can increase power relative to other existing association tests (family-based smoothing FPCA, family-based collapsing methods and extended FBAT) in the presence of genetic heterogeneity. Finally, we illustrated our approach to analyze the association of rare variants using cardiovascular disease from the Framingham Heart Study.

P17.60-M
Whole exome sequencing: Follow-up of a rare non-synonymous variant in GRHL3 in a German family with nonsyndromic orofacial clefting

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations and has a complex multifactorial etiology. In the context of a large collaborative study, whole exome sequen-

cing (WES) was performed in two distantly related cousins of an extended nsCL/P family of German origin. After stringent filtering of variants shared between both individuals, one variant in the grainy-head like 3 (GRHL3) gene was identified as potentially causal. This variant, rs138381915, is located in exon 13 and the potential risk allele mediates an amino acid change (p.Arg490His) of the GRHL3 protein. Prediction programs (Polyphen, Mutation Taster) predict this alteration to be disease-causing. Sequencing datasets (ESP6500, dbSNP, 1000genomes) report the frequency of the minor allele at rs138381915 to be below 0.5 %. Literature search revealed GRHL3 as an interesting candidate gene for nsCL/P. Based on the hypothesis that rs138381915 might be the causal variant in the family, we first confirmed the variant in the two index probands by Sanger Sequencing. We then tested all other (29) family members for whom DNA was available for the presence of the putative risk allele. Of these (10 affected, 19 unaffected), the risk allele was observed in a heterozygous state in seven individuals, only two of them were affected. Five individuals carried the putative risk allele but were not affected. Eight affected individuals did not carry the putative risk allele. These data suggest that rs138381915 is unlikely to be a causal variant regarding the nsCL/P phenotype in this particular family.

P17.61-S
Novel mutations described in Saudi Arabia

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Jeddah is the second largest city in the Kingdom of Saudi Arabia with a population of over 5 million. The Genetic Medicine Department at King Abdul-Aziz University is the major referral center for genetic disorders in Jeddah and was established in 2005. Over this period 1842 patients and families with genetic disease were seen. Patients were assessed clinically and where indicated by genetic testing.They were categorized into the following types based on etiology: chromosomal, single gene disorders, multifactorial, mitochondrial and others. They were then further subdivided into categories. Most mutations identified were novel and different from the western literature. We will present the types of genetic diseases present and the novel mutations identified both locally and nationally and put forward recommendations for future studies including the current set up of a data bank will discussed.

P17.62-M
Contribution of SNPs (rs9939609 and rs8057044) and haplotypes of FTO gene to the genetic risk for obesity in children from Yucatan, Mexico

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The fat mass and obesity-associated (FTO) gene has been identified as a strong candidate for obesity-related phenotypes in several populations. Significant associations of the SNPs rs9939609 and rs8057044 with body mass index (BMI) and with the risk for obesity have been suggested in homozygotes AA. The central role of FTO might be through an effect on cerebrocortical insulin sensitivity. Each FTO risk allele increases BMI by 0.26 to 0.66 units (kg/m2), and the odds of being obese by ~1.3. In this study, we evaluated the association of the SNPs (rs9939609 and rs8057044) and haplotypes of FTO gene with the risk for obesity in children from Yucatan, Mexico; where child obesity is the first cause of morbidity. We included 155 obese children, and 189 non-obese healthy children under a case-control association study. Genotype and allele frequencies between cases and controls were compared using SNPstats software. Genotype and allele frequencies were distributed according to Hardy-Weinberg expectations ($p>0.05$) in cases and controls, except for rs9939609-FTO in controls ($p<0.05$). Significant differences were found for the heterozygous AT genotype of the SNP rs9939609-FTO ($p= 0.03$) between cases and controls, suggesting that the heterozygous AT genotype of rs9939609-FTO might be a genetic risk factor associated with child obesity in the population of Yucatan. No significant associations were found for the SNP rs8057044 nor for the haplotypes of FTO gene ($p>0.05$). However homozygotes for the A allele showed a higher mean BMI and higher waist circumference than TT or GG allele carriers.

P17.63-S
Molecular Analysis of *TYR, OCA2, TYRP1* , *SLC45A2* and *GPR143* Genes in 158 Patients with Oculocutaneous and Ocular Albinism

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Albinism is an heterogeneous group of inherited genetic diseases present at birth. This disorder can affect all ethnic backgrounds with an overall prevalence of approximately 1/17.000 people but prevalence of the different forms of Albinism varies considerably worldwide. It can be classified as Ocular Albinism (OA1) caused by mutations in the *GPR143* gene located on the X-chromosome, or as Oculocutaneous Albinism (OCA) an autosomal recessive inherited condition. OCA1-4 forms are recognized based on the expression of mutations in four different genes: *TYR*, *OCA2*, *TYRP1* and *SLC45A2*. A cohort of 158 OCA or OA1 subjects were recruited from the Medical Genetic Unit and Department of Pediatric Ophthalmology of Niguarda Ca' Granda Hospital of Milan (Italy) and characterized for *TYR*, *OCA2*, *TYRP1*, *SLC45A2* and *GPR143* gene defects, associated to the different phenotypes, in order to describe their frequencies variation in Italian population. We identified *TYR* mutations in 83 subjects (66,6% missense, 11,1% nonsense, 8,5% frameshift, 2,6% deletions in frame, 9,2% splicing mutations and 2% exons deletion), *OCA2* mutations in 34 subjects (70,2% missense, 5,2% frameshift, 15,8% splicing mutations and 8,8% exon deletions), *TYRP1* mutations in 4 patients (42,9% missense and 57,1% nonsense mutations), *SLC45A2* mutations in 12 patients (63,66% missense, 4,55% nonsense, 22,74% frameshift and 9.1% splicing mutations) and *GPR143* mutations in 7 subjects (14,3% missense, 14,3% nonsense, 42,8% frameshift, 14,3% splicing mutations and 14,3% exon deletions). Here we also report about the OCA1-4 and OA1 gene frequencies in our cohort of albino patients (mainly of Italian origin).

P17.64-M
Genetic landscape of populations along the Silk Road reveals a haplotype associated with hyposmia in Tajikistan's population

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Smell is a versatile mechanism for recognizing different odours and is mediated by olfactory receptors. While collecting phenotypes related to smell in six countries along the Silk Road, we found an increased rate of failure to discriminate odorants in individuals from Tajikistan respect to the other countries. Using haplotype-based association we linked this to a 15 kb region within olfactory receptor gene cluster on chromosome 6 (p-value 3.86e-05). This region is embedded in the largest intron of OR5V1 and is downstream OR11A1 and upstream OR12D3. We also analysed genetic variability in 1,114 unrelated samples either from the Silk Road and ten other worldwide populations at over 300,000 polymorphic sites and characterized population genetic structure of the Silk Road within a worldwide context with a resolution never obtained before. We identified genetic components peculiar to Central Asia and observed that Tajikistan behaves as an outlier population. Indeed Tajiks share a consistent number of unusually large stretches of homozygosity and have the lowest effective population size (Ne) among the studied populations, most likely as the result of past isolation and/or consanguinity. Altogether these novel findings clarify the complex genetic patterns of the Silk Road populations and suggest that the smell misperception phenotype observed in Tajikistan might be the result of a combination of genetic drift and relaxed selection at the olfactory receptors genes.

P17.65-S
Targeting a gene network of ADAMTS genes contributing to pediatric stroke

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Pediatric stroke (PS) is an important childhood disease and several genetic polymorphisms contributing to PS risk have been established in numerous candidate gene based association studies. Recently, we have reported results of the first family-based genome wide association study identifying a gene network of ADAMTS genes in the predisposition to PS. To investigate possible causative gene variants in the resulting linkage disequilibrium based candidate regions; we performed a next generation resequencing approach in 48 affected children and 48 unaffected siblings. The selected target regions of about 6.8Mb comprise 42 gene regions including ADAMTS2, ADAMTS12, ADAMTS13 and ADAMTS17. Custom target enrichment was performed using the NimbleGen SeqCap EZ Choice technology. The resulting DNA libraries were paired-end sequenced (100 cycles) on an Illumina HiScanSQ instrument yielding in 300 Gb sequence data in total and 87.1% bases with a QScore > 30. Sequence reads were mapped by using the BWA algorithm and analyzed by GATK yielding in 80% median target specificity and median target region coverage of 176x. Variant annotation was done by using SNPEff and Annovar software tools. A sibship disequilibrium test was applied on the 16,586 identified variants, 4060 of which were novel, to compare the

two sample groups. 32 significant (p<0.05) coding non-synonymous or UTR SNPs in 14 genes were identified and selected for validation using capillary sequencing and subsequent genotyping within the full cohort of 270 nuclear families. The resulting data may help to understand the genetic architecture of ADAMTS genes and their impact on PS.

P17.66-M
A genome-wide association study of Agreeableness suggests a novel association in the NAV2 gene in Korean women

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Data from genome-wide association (GWA) studies have been used to find the common variants of personality. In a previous study, we reported that neurotransmitters and the olfactory receptor 1A2 gene are associated with neuroticism in a cohort of young Korean women. However, many genetic variants that are highly associated with certain personality traits are still unknown. Here, we report on a meta-analysis of GWA data for personality in three cohorts samples (2045 individuals). All participants were of Korean ancestry. Personality traits were measured with the Revised Neuroticism-Extraversion-Openness Personality Inventory to assess five factors: Neuroticism, Extraversion, Agreeableness, Openness, and Conscientiousness. In either discovery stage, classical association analyses were performed under an additive model followed by meta-analysis using the weighted inverse variance method. We observed consistent direction of effect and significant association of the NAV2 gene and Agreeableness in either the discovery and combined stage (p=7.85×10-7, for meta-analysis). NAV2 gene involves in optic nerve development and sensory perception of smell and sound. We previously reported that the sensory system may play an important role in personality, and the present study leads to the same conclusion. The sensory system affects personality as a filter of the acceptance system, which may have an advantage to reconstruction.
This study was supported by a grant of the National Project for Personalized Genomic Medicine, Ministry for Health & Welfare, Republic of Korea (A111218).

P17.67-S
New genetic matching methods for handling population stratification in genome-wide association studies

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A usually confronted problem in association studies is the occurrence of population stratification. In this talk, we propose a decisive extension to the Cochran-Armitage Trend test in order explicitly take into account structures obtained from matchings and clusterings. We employ pairwise and groupwise optimal case-control matchings and present an agglomerative hierarchical clustering, both based on a genetic similarity score matrix. By simulations of genotype data under the null hypothesis we assess our framework, in order to affirm that it correctly controls for the type-1 error rate. By a power study we ascertain, that structured association testing using our framework displays reasonable power. We compare the results from our methods with those obtained from a logistic regression model with principal component covariates. We also highlight and discuss a possible false-positive association to Alzheimer's disease using the principal components approaches, which is neither reproduced by our new methods nor by the results of a most recent large meta analysis.

P17.68-M
The causal role of insulin-like growth factors and binding proteins in prostate cancer: a Mendelian randomization study

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Epidemiological studies reported positive associations of circulating IGF-I and IGF-II, and inverse associations of IGF binding protein 3 (IGFBP-3) with prostate cancer risk, whilst IGFBP-2 has been associated with low grade disease. However, systematic review findings have been inconsistent which may reflect confounding or reverse causality. We examined the causal role of IGF-I, IGF-II, IGFBP-2 and IGFBP-3 in prostate cancer using multiple genetic variants to construct allelic scores as instruments for measured circulating IGFs and IGFBPs, in a Mendelian randomization approach.
We investigated 1131 SNPs, previously reported to be associated with IGFs and IGFBPs, in ~700 population controls from the ProtecT study. We generated allelic scores that consisted of the most strongly -and exclusively- associated SNPs with each biomarker. Finally, we used the allelic scores and instrumental variable analysis to estimate the causal effect of IGFs and IGFBPs on prostate cancer in ~40,000 cases and controls from 21 studies included in the international PRACTICAL consortium.
IGF-I and IGF-II were positively associated with prostate cancer risk. The estimated causal odds ratios for the effect of a standard deviation (SD) increase in circulating IGF-I and IGF-II was 1.15 (95% CI 1.06, 1.26) and 1.14 (95% CI 1.02, 1.26), respectively. Notably, IGF-II increased risk for advanced and high grade disease. Conversely, IGFBP-2 and IGFBP-3 were not associated with prostate cancer susceptibility. In conclusion, this Mendelian randomization study provides additional support for a causal relationship between IGF-I and IGF-II and prostate cancer risk.

P17.69-S
Combining different sources of information to optimise genomic prediction of complex traits

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In the last decade there has been substantial progress in identifying genetic loci associated with complex phenotypes but limited progress in using genomic information to predict phenotypic performance. In this study, we assess the advantage of using previously published results to inform genomic predictors of complex traits. Our goal is to compare model performance with respect to trait architecture and latent population structure. We consider linear, additive models with different sparsity levels, either learned from the data using lasso or elastic nets, or determined a priori by considering markers and their corresponding effects from large meta-analysis association studies. We characterise the predictive signal captured by each model and explore whether prediction accuracy can be increased by combining these simpler predictors into a meta-model.
We evaluate predictive performance using height, body mass index and high density lipoproteins in two population cohorts, originating in Croatia and Scotland. We examine how to maximise prediction accuracy when the target individuals come from the same or a different population to the training samples. Our results demonstrate that between population prediction is possible using samples from the target population to perform model selection, subject to sample size and trait architecture. Furthermore, we show that a model combining the predictions from penalised regression with meta-analysis-based polygenic scores performs better than either model on its own. Our findings suggest that incorporating previous results into statistical models as well as exploiting the predictive signal from latent data structure can lead to improved predictions of complex traits.

P17.70-M
Technical issues of using Next Generation sequencing for rare-variant association

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To assess the role of rare variants in multifactorial diseases, next-generation sequencing in combination with targeted enrichment is used to provide genotypes. Subsequently, statistical methods such as SKAT are used to compare case and control data in various ways. For GWAS, the use of datasets from different sources for cases and controls has been adequately addressed. For NGS, the role of data quality is less clear. To test our ability to perform rare-variant association, we set up a pilot experiment, including 64 samples with juvenile myoclonic epilepsy, a multifactorial disorder, 64 with epileptic encephalopathy, a presumed monogenic disorder, and 64 healthy controls, in the same way as a larger experiment for a set of 348 genes. We analysed whether batch effects and other lab variables could affect the outcome of the association.
Between two series of library preparation about five months apart differences in coverage and complexity were visible, as well as differences in the number of detected variants, especially rare variants. Within a series of library preparation, different runs on the sequencing machine had different quality characteristics, but the differences in number of variants between runs were small. We tried various methods of correction, but no correction

removed batch effects beyond all doubts.
Based on our experiences, the use of control sequences from earlier experiments or even other labs cannot be recommended. It is likely to lead to spurious associations or lack of associations.

P17.71-S
A Script for Linkage Analysis of Rare variants

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For the past years genome-wide association analysis provided progress in detection of common genetics variants contributing to variation in many common disorders and quantitative traits. However, for many traits, genome-wide significant associations did not result in the establishment of the causal underlying variants. Now, whole-exome resequencing of large population samples and formation of databases of annotated functional variants may help to solve this problem. Causal functional variants with a large effect on the variation of traits are rare in populations, but can be aggregated in families. Analysis of samples from isolated populations makes possible to detect genetically informative families where rare variants co-segregate with diseases or traits. To facilitate a search for families with aggregation of the rare variants of interest from extended pedigrees or samples from isolated population and then to test its co-segregation with trait by the methods of linkage analysis we developed a software LARA (Linkage Analysis of Rare Allele). LARA combine commonly used package for linkage analysis MERLIN (Abecasis et al 2002) and PedCut software (Liu et al 2008) for the pedigree splitting into a set of sub-pedigrees. LARA searches for rare allele carriers, automatically generates and transfers the data, necessary for the analysis. LARA can help to test all functional variants located in the genomic regions already detected by linkage or associations methods. The software LARA is freely available at http://mga.bionet.nsc.ru/soft/index.html.

P17.72-M
Correcting for population substructure in rare variant - rare disease association studies

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Sophisticated techniques have been developed for genome wide association studies (GWAS) to correct for population substructure that may cause substantial inflation of test statistics and spurious associations. For rare variants in spatially structured populations the existing methods do not effectively control for stratification. We developed an approach to build up a control group that is most similar to the individuals in the case group considering all exomic variants especially also the rare ones. We show with simulations of real exome data that the power of association studies for rare variants can be optimized if case-control groups are similarity-matched. We also found that the power could be further improved when we increased the control:case group ratio by adding additional exome data.

P17.73-S
Functional linear model for regional association analysis of rare genetic variants in family-based samples

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Methods based on the linear regression models are widely used for genome-wide association analysis of family-based samples. To use these models for region-based analysis, a collapsing approach is commonly used. However its power decreases if effects of causal variants have opposite directions.
We developed a new method for the region-based association analysis of family-based samples, using techniques of functional data analysis. The individual discrete genotypes and effects of multiple variants in the analyzed region are considered as the continuous data, which can be described by stochastic functions constructed on the physical positions of the variants, using a finite set of basis functions. Thus, under the functional linear model, the genetic effects of the multiple variants are described by coefficients of the basis functions. The null hypothesis of zero values for the coefficients is tested by standard statistical tests. We introduced a covariance matrix defined through a relationship matrix in the trait inheritance model to take into account a genetic relationship between individuals. High power of our method is provided by the simultaneous consideration of genetic information on not only genotypes of multiple variants, but also their physical positions, and by taking into account related structure of the samples. Our method is implemented in the software package FFB-FLM available for free download (http://mga.bionet.nsc.ru/soft/FFB-FLM/).
This work is supported by RFBR grants 13-04-00272a, 14-04-00126a.

P17.74-M
Genome wide inbreeding estimation within Lebanese communities using SNP arrays

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Consanguineous marriages have been widely practiced, with variable rates, in several global communities depending on religion, culture, and geography. The populations of the Middle East are among those with the highest inbreeding level and frequency of inbred individuals. A genome wide analyses of 165 unrelated Lebanese has been performed either through the estimation of LOH (Loss of Heterozygosity) or through the FEstim algorithm depending on SNP frequencies. Relying on these genome-wide data that identify regions of homozygosity by descent (HBD), this study was able to estimate total inbreeding levels, remote consanguinity, and population admixture and structure. The inbreeding coefficient value was estimated to be 1.6% in offspring of unrelated parents (over 3 generations) and 8% in offspring of first cousins. In either case, the remote consanguinity (RC) value was approximately equal to 0.6% resulting from genetic drift or recurrent consanguineous unions. This RC value suggests that for any unrelated marriages in Lebanon, the mates could be related as third cousins or as second cousins once removed. Under the hypothesis that 25% of marriages occur between first cousins, the mean inbreeding (F) value of 2.2% found may explain the increased incidence of recessive disease within offspring. The LOH and FEstim genome wide approaches were applied to investigate the genomic similarity of Lebanese communities. Both approaches revealed a unique ancestral population of the four studied communities (Greek Orthodox, Maronite, Shiite and Sunni).

P17.75-S
Population genomics analysis in whole genome sequencing of 152 rhesus macaques

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Rhesus macaques (Macaca mulatta) are the most widely studied nonhuman primate model species in biomedicine, critical to various aspects of disease research. We applied next generation whole genome sequencing for 152 unrelated individuals (144 Indian-origin, 8 Chinese-origin) using either deep (30X) or low coverage (6X) strategies. Analysis using SNPTools identified 51.6 million SNPs. On average, Indian-origin individuals have >9.5 million variants, while Chinese animals have >12 million, representing much higher diversity than humans. Current effective population size (Ne) is estimated at 65,000 for Indian-origin animals, 79,000 for Chinese-origin. Both estimates are substantially higher than values for humans. Analyses also reveal dramatic demographic changes over time in details to more than 10 million years ago. Functional annotation in coding sequences found >250,000 missense variants and >4600 stop-codon-gained mutations. In addition, about 110,000 SNPs mapped to conserved ENCODE transcription factor binding motifs. We used position weight matrices from the JASPAR database to assess these SNPs and found >25,000 candidate variants that may significantly affect TF binding, and thus gene expression. We mapped rhesus SNPs to the 4% of the genome identified as conserved across 29 mammals, and found reduced SNP density and MAF, consistent with negative selection in those regions. We also applied a number of site frequency spectrum tests and found significant new evidence for both positive and negative selection in both coding and noncoding regions in the macaques. Analyses of LD and local recombination rates are in progress.

P17.76-M
Association of five confirmed risk gene polymorphisms with Rheumatoid Arthritis in the Algerian population

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Objectives: The aim of this study was to investigate the role of five con-

firmed gene polymorphisms (PTPN22rs2476601, STAT4rs7574865, IRF5rs2004640, TRAF1/C5rs10818488 and TNFAIP3rs6927172) in Rheumatoid Arthritis risk among the Algerian population.
Methods: The study sample comprised 110 patients with RA and 197 ethnically matched healthy control subjects. Each polymorphism was genotyped using a predesigned TaqMan® assay. Allele and genotype frequencies in patients and control subjects were compared by chi-square test and odds ratios with 95% confidence intervals CI.
Results: Statistically significant association of all studied polymorphisms with RA was detected. The strongest signal was obtained for PTPN22 (rs2476601) with an allelic P value = 10-11 (OR = 9.83, 95% CI [4.28 - 22.56]).
Conclusion: This case/controls study lead, for the first time in the Algerian population, to highlight the association of five risk genetic factors with RA. This contributes to the characterization of RA genetic component in a population still under genetic investigation.

P17.78-M
How does this Arab Genome differ from other genome?

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Whole genome from Caucasian, African, Chinese and Korean individuals have been studied and published to date. Here, we successfully generated, assembled and analyzed the first full genome of a 32 Saudi healthy volunteers from using NGS technology (5500xl Genetic Analyzer). Alignment of the Saudi genome with H19 references revealed nearly more than 1.7 million unique SNPs. However, SNPs comparison analysis with HapMap phase III populations showed the highest share was with ASW population (1,545,053) while the lowest share was noticed to be with JPT population (1,280,257). The SNPs counts and frequencies per chromosome were partitioned into heterozygous and homozygous categories ranging from 258,174 SNPs (chromosome 2) to 1,182 SNPs on Y chromosome. The SNP frequency was calculated by dividing the number of called SNPs by the length of the covered consensus sequence, omitting unknown regions from the reference genome. The heterozygous and homozygous SNPs frequency was calculated based on their share of the total number of SNPs. A de novo assembly of 9,011 contigs sequences was not represented in NCBI reference genome. This project is pointing to perform a whole genome/exome of 1000 Saudi individuals towards Establishment Saudi Genome Database for comprehensive view of genetics variant such as large structural rearrangement and SNPs. Conversely, the whole genome/exome of some of the common chronic diseases in the Saudi population is already started such as multiple sclerosis.

P17.79-S
Soluble CD40 ligand is regulated by membrane CD40 expression in platelet concentrates

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Introduction:
Soluble CD40 ligand (sCD40L) is a platelet-derived proinflammatory mediator that accumulates during platelet storage. Unfortunately, in some cases, high levels of sCD40L cause acute transfusion reactions (ATRs). We investigated 2 polymorphisms previously associated with high levels of plasma sCD40L in some diseases, in the aim to identify 'dangerous' platelet concentrates (PCs).
Material and methods:
Levels of sCD40L were measured by Luminex® in 142 PCs (EFS Auvergne-Loire). We performed a Tetra-primer ARMS-PCR to genotype CD40-rs1883832 [C>T] and CD40L-rs3092952 [A>G]. Differences of plasma sCD40L concentrations among groups of genotypes were compared by ANOVA followed by Bonferroni correction (significance when P<0.017).
Results:
Genotype frequencies didn't deviate from Hardy-Weinberg expectations.
The distribution of genotypes for CD40L-rs3092952 was 68, 43 and 30 for AA+A, AG and GG+G respectively. No difference was observed between the 3 genotypes in sCD40L levels.
The distribution of genotypes for CD40-rs1883832 was 68, 52 and 8 for CC, CT and TT respectively. sCD40L was higher in PCs derived from donors with TT genotype (TT vs CC: P<0.001; TT vs CT: P<0.001; CT vs CC: P=0.7).
Discussion:
The CD40-rs1883832 polymorphism has been reported to regulate CD40 expression. sCD40L may activate cells expressing CD40 receptor as in platelets. We hypothesize that those with TT genotype may express a high amount of CD40 on their surface and thus may capture sCD40L, becoming more activated, thus secreting more sCD40L.
Further studies are required in larger samples to confirm this result allowing the discard of PCs with high sCD40L amounts and consequently preventing ATRs.

P17.80-M
Inferring rare disease risk variants based on exact probabilities of sharing by multiple affected relatives

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Family based study designs are regaining popularity because large-scale sequencing can help to interrogate the relationship between disease and variants too rare in the population to be detected through any test of association in a conventional case-control study, but may nonetheless co-segregate with disease within families. When only a few affected subjects per family are sequenced, evidence that a rare variant may be causal can be quantified from the probability of sharing alleles by all affected relatives given it was seen in any one family member under the null hypothesis of complete absence of linkage and association. We present a general framework for calculating such sharing probabilities when two or more affected subjects per family are sequenced, and show how information from multiple families can be combined by calculating a p-value as the sum of the probabilities of sharing events as (or more) extreme. We also examine the impact of unknown relationships and propose methods to approximate sharing probabilities based on empirical estimates of kinship between family members obtained from genome-wide marker data. We apply this method to a study of 55 multiplex families with apparent non-syndromic forms of oral clefts from four distinct populations. Whole exome sequencing was performed by the Center for Inherited Disease Research (CIDR) on two or three affected members from each family. The rare single nucleotide variant rs149253049 in the gene ADAMTS9 was shared by affected relatives in three Indian families (p=2e-6), illustrating the power of this sharing approach.

P17.81-S
A Founder Effect for PPIB-associated recessive osteogenesis imperfecta in Acadian and Cajun Populations

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Mutations in PPIB, a gene that encodes cyclophilin B, a prolyl cis-trans isomerase, cause recessively inherited osteogenesis imperfecta (OI) that ranges in severity from moderately deforming to perinatal lethal. Among Acadians in New Brunswick and Cajuns in Louisiana, who represent geographically distinct populations derived from the same ancestral group in France, we identified 2 families from the Acadian group and 2 from Louisiana, both presumed to be from the Cajun group, with severe/lethal OI caused by a homozygosity for a previously unreported PPIB mutation (c.344-1G>T, IVS3-1G>T). All transcripts from this allele use a cryptic acceptor site 5nt into exon 4 that results in a frameshift and mRNA instability. Both Canadian families presented prenatally; the first family had features of a lethal skeletal dysplasia on ultrasound and on autopsy was thought to have perinatal lethal OI. The second family was thought to have a severe moderately deforming OI on ultrasound. The two families from Louisiana presented with clinical diagnoses of perinatal lethal OI. None had mutations in COL1A1 or COL1A2. There is no known consanguinity within these families or relationships between them.
Because of the probable founder effect for PPIB-associated recessive OI among Acadians and Cajuns, likely tracing back to early 17th century France, in pregnancies/children of Acadian/Cajun ancestry (and their antecedents) presenting with severe skeletal dysplasia, PPIB-associated OI should be considered. Targeted analysis based on ethnic background may help facilitate more timely diagnosis and subsequent genetic counselling.

P17.82-M
Genetic survival modeling with large-scale population cohorts

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Recent methodological development on linear mixed models has provided a common framework for heritability estimation, multi-locus association testing and genomic prediction of quantitative traits in population cohorts

with arbitrary relatedness structures. The possibility to use population cohorts rather than family structures opens up a multitude of new avenues also for genetic epidemiological research on time-to-event outcomes. However, connecting time-to-event outcomes to whole-genome sequencing data has so far not been computationally feasible with hitherto existing software. We introduce an R package ("GSM") for heritability estimation, multilocus association testing and genomic prediction of time-to-event outcomes that is scalable to sequencing data. The package implements a very flexible piece-wise constant hazard model that contains an individual-specific Gaussian random effect with an arbitrary covariance structure. Computationally, we transform the problem to a Poisson model, which we analyze by fitting interconnected Generalized Linear Models. The underlying computational algorithm is written in C++ to enable analyses with millions of genetic markers and events in thousands of individuals. We demonstrate the runtime efficiency of our implementation and give an example of heritability estimation and multi-locus association testing for cardiovascular disease related events. Our work extends the computational tractability of linear mixed models from quantitative traits to time-to-event outcomes and will prove useful, e.g., for combining information across individuals' genomes and their hospital records.

P17.83-S
Genome- wide association analysis of swallowing symptoms related to dysphagia in a healthy older adult cohort

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Background: Patients with swallowing difficulties (oro-pharyngeal dysphagia) caused by neurological damage (stroke, Parkinson's disease, ageing) show different recovery patterns which may be caused by genetic determinants.
Aim: To find an association between human genetic variations and swallowing impairments within the ageing cohort.
Materials and methods: We performed case-control genome wide association study (GWAS) of self-reported swallowing symptoms related to dysphagia. The analysis included 555 community dwelling, unrelated, older adults (mean years of age = 81.4; SD = 5.349) with known phenotype and genetic information consisting of 512 806 single nucleotide polymorphisms (SNP). Gene-based association analyses of these traits was also conducted. The genetic data underwent quality control procedures prior to the study. This included analysis of population architecture using Multidimensional Scaling of the genome wide genotype data.
Results: Analysed cohort showed European ancestry with no major population stratification. The results shown one genome wide significant SNP rs17601696 (P=4.83x10-8) from non-coding region of chromosome 10. Analyses of individual genes did not result in any genome-wide significant association**.**
Conclusion and future work: SNP rs17601696 may have an impact in swallowing impairment among elderly individuals. The results require replication in an independent cohort with appropriate phenotype/genotype data. Presented GWAS results will be replicated in the human model study using Transcranial Magnetic Stimulation (TMS). Identified genetic loci may play a role of potential markers to predict individual's outcome from swallowing impairments.

P17.85-S
Investigation of hellenic families with microscopic hematuria reveals the frequency of collagen IV mutations and evidence for activation of the unfolded protein response

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Familial hematuria(s) comprise a genetically heterogeneous group of conditions which include heritable glomerulopathies (Alport Syndrome (AS) and thin basement membrane nephropathy (TBMN)). AS is rare, caused by X-linked *COL4A5* or autosomal recessive *COL4A3/A4* mutations (ARAS), while TBMN is frequent. We sought to evaluate the prevalence of *COL4A3/A4* mutations among patients presenting with microscopic hematuria (MH), belonging to 91 Hellenic families. Also we studied 28 sporadic patients with MH and four patients with ARAS. We performed functional studies in cultured podocytes, focusing on the induction of the unfolded protein response (UPR) after overexpression of wild type or mutant *COL4* chains.
Among 91 families, heterozygous mutations were found in 11 (12,1%). Restricting the calculation to 68 families with ≥3 patients with MH, the positive finding is 11/68 (16,2%). Three heterozygous mutations were found in three of the 28 sporadic patients (10,7%). Altogether, among 52 heterozygous patients 17,3% reached end-stage kidney disease (ESKD). Of those aged >50 years, 26% reached ESKD, in keeping with previous findings that TBMN is not always benign. Functional studies showed that mutant *COL4A3/A4* chains expressed in podocytes are preferentially retained in the cells, compared to wild type chains. Mutant chains differentially triggered activation of the UPR pathway, as evidenced by activation of BiP, a sensitive ER stress marker.
TBMN may emerge as a more frequent cause of ESKD than AS. The ability of mutant chains to elicit the UPR pathway when overexpressed in podocytes may prove of functional significance and prognostic value.

P17.86-M
Variation in BTBD9 gene is associated with Tourette syndrome in the Polish population

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Tourette syndrome (TS) is a childhood-onset neuropsychiatric disorder manifested by tics. The anatomical location, number, frequency, complexity, and severity of tics change over time. The etiology of the disorder is unknown, though the predominant role of genetic factors has been established. Variants of the BTBD9 gene (rs4714156, rs9296249 and rs9357271) were reported to be associated with GTS in French Canadian and Chinese Han populations.
The study group comprised 162 TS patients, and the control group consisted of 180 healthy persons. The rs4714156, rs9296249 and rs9357271 variants of the BTBD9 gene were genotyped.
Analyzed SNPs indicated a strong linkage disequilibrium (D= 1, r2=0.938-1).). MAFs, genotype frequency, allelic, genotypic and haplotype association analysis within examined variants of the BTBD9 gene revealed no significant differences between controls and TS patients. However, there were significant associations between the BTBD9 gene variants and a clinical phenotype of TS. Minor alleles of all three SNPs were found significantly less frequently in patients with ADHD and were more frequent in patients with no comorbidities. There was a borderline statistical significance for minor alleles to be less frequent in patients with severe tics. All three SNPs were not found to be associated with the family history and the age of tic onset.
Our results indicate that the examined variants of the BTBD9 gene are not associated with the risk of developing GTS, but may be associated with comorbidity and tic severity in the Polish population.

P17.87-S
The investigations of susceptibility genes/variants related to type 2 diabetes in Turkish

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Rapidly increasing prevalence of diabetes in Turkey as worldwide has made diabetes as a public health problem which mostly uses the sources of health services on personal and social levels. As well as ethnic and geographical differences, contribution of multiple genes on the emergence of the disease makes type 2 diabetes (T2D) even more complicated to understand. It was noted that prominent genes in all of the studies are the genes coding proteins usually functional in pathways related to insulin release or activity. IRS1 and 2, KCNJ11, ABCC8, TCF7L2 and Adiponectin genes which are of prominent genes in literature have been scanned among type 2 diabetic individuals living in Konya region and risk allels were determined in our study. For this purpose, patients applied to Selcuk University, Faculty of Medicine, Endocrinology Department and diagnosed with T2D were included. Forty seven SNPs in six gene regions were genotyped among about 200 diabetic and 150 healthy individuals. Association analyses with disease and biochemical data and genotypes, Hardy-Weinberg analyses, genotype-phenotype relationship were evaluated statistically. The silent substitution R1273R and exon 16-3t/c of ABCC8 gene, SNP −11391Ġ→A in proximal promoter and SNP +276G→T in intron 2 of Adiponectin gene and intronic SNPs rs7903146 and rs12255372 in TCF7L2 gene were significantly associated with T2D (P<0,001). We also found an effect of the E23K variant in KCNJ11 gene on insulin secretion in our population (P<0.05). Consequently, our results suggest that ABCC8, Adiponectin, TCF7L2 and KCNJ11 genes are significant determinants of T2D development also in Turkish population.

P17.88-M
The T allele of rs7903146 in TCF7L2 is associated with type 2 diabetes in Iranian: a large population-based cohort study

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Introduction: The precise mechanisms underlying the development and progression of type 2 diabetes have not been elucidated yet; however, a combination of multiple genetic and/or environmental factors contributes to the pathogenesis of the disease. The two main pathophysiological mechanisms leading to type 2 diabetes are impaired insulin secretion and insulin resistance which have a significant genetic component. TCF7L2 is one of the genes involved in insulin secretion, and the TCF7L2 rs7903146 constitutes the best-established risk allele to be associated with diabetes. Therefore, this study was carried out to replicate the previous findings in Iranian population using samples from the Tehran lipid and glucose study (TLGS), a large population-based cohort study. Methods: This case-control study included 2173 affected patients and 2400 controls selecting among TLGS participants. The genetic variants on transcription factor 7-like 2 (TCF7L2) namely rs7903146 was genotyped genotyped using the Centaurus (Nanogen) platform in DeCODE genetics. Association of T-allele with type 2 diabetes was examined using plink software after age and sex adjustment. Results: In this study, the minor allele (T) of rs7903146 increased risk of type 2 diabetes 1.33 fold higher in case group compared to control group (OR:1.33; p= 1.8E-10). Conclusion: The findings revealed the association between the presence of T allele in rs7903146 and type 2 diabetes among Iranian population which confirmed previous result in other ethnicity. Keywords: Type 2 diabetes, TCF7L2, rs7903146, SNP, TLGS

P17.89-S

The first genetic study of Type 2 Diabetes in the Cypriot population

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Type 2 diabetes (T2D) mellitus is a chronic complex multifactorial disease of glucose metabolism. It is a serious worldwide public health problem which has reached epidemic proportions with an increasing prevalence and obvious substantial familial clustering. Therefore, T2D has been the subject of numerous medical-genetic studies aiming at the elucidation of the genetic mechanisms involved in the disease. Candidate gene studies, genome-wide association (GWA) scans and meta-analysis of these scans identified several genetic T2D susceptibility loci.
We initiated T2D genetic studies in the Cypriot population. We collected a representative number of diabetic and non-diabetic samples, extracted DNA and created the first Cypriot T2D DNA Bank. Twenty one already established in other population susceptibility loci were investigated in our population. Four of the 21 tested loci [*TCF7L2* rs7901695, *FTO* rs8050136, *HHEX/IDE* rs5015480 and *SLC30A8* rs13266634] that are among the strongest associated SNPs worldwide have been also associated with T2D susceptibility in our population. Furthermore, 4 statistically significant associations were detected between the following specific variables of the phenotype and the tested loci: 1) age at T2D onset with *THADA* rs7578597 and *TCF7L2* rs7901695, 2) body mass index with *FTO* rs8050136 and *KCNJ11* rs5219, 3) glycosylated haemoglobin levels of patients with *ADAMTS9* rs4607103 and 4) cardiovascular disease with *TCF7L2* rs7901695.
Our findings expanded the genetic assessment of T2D in a distinct population and reconfirmed 4 of the worldwide established loci. Our study population will be further studied for the identification of additional and maybe novel T2D susceptibility loci.
Funded by CRPF (ΥΓΕΙΑ/ΔΥΓΕΙΑ/0609(BIE)/03).

P17.90-M

The southern migration route: a supporting clue from aboriginal Vedda people of Sri Lanka

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Being located along the proposed southern migration route and the presence of earliest skeletal evidences of anatomically modern human (37,000 B.P.) with aboriginal Vedda population, the island of Sri Lanka could provide the knowledge of genetic variation of modern humans in South Asia. In order to reveal the genetic relationship of the Vedda people with the tribal groups along the southern coastal migration route, the present study compared the mitochondrial DNA (mtDNA) hypervariable segment 1 variations from 75 Vedda people from Sri Lanka with 33 world tribal groups from published data bases. The Vedda people signal out as an exceptional tribal group of South Asia having less than 30% of individuals sharing haplogroup M with 64% of haplogroups R30, U1 and U7. The latter two haplogroups were recognized as West Eurasian ancestry. In principal component analysis (PCA) some Vedda groups occupied separate positions while some were closely related to South Indian tribal groups. Most interestingly, by viewing PCA from the point of view of the Southeast Asian foragers, it is evident that their closely related groups are the Vedda people. This fascinating genetic footprint is suggestive of yet another piece of evidence of the dispersal of anatomically modern human out of Africa via the southern migration route. And also this mtDNA study highlights Sri Lanka's strategic location along the southern migration route, thereby providing a genetic gold mine, which will offer insight into the initial settlements and peopling of South Asia.

P17.91-S

RNA-Sequencing reveals differential gene expression between visceral and subcutaneous adipose tissue in Greek women undergoing abdominal surgery

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Adipose tissue is a major endocrine organ that contributes to whole body metabolic homeostasis. The role of body fat distribution in the development of obesity-related metabolic consequences, such as type 2 diabetes, hyperlipidemia and cardiovascular disease is of great importance. Fat depots from different areas of the body display distinct structural and functional properties and have specific roles in pathology.
We applied RNA sequencing (RNA-Seq) to quantify transcript levels in visceral and subcutaneous adipose tissue. RNA was extracted from 81 tissue samples (42 subcutaneous, 39 visceral) of women from the Greek population spanning the BMI range (sample collection is ongoing), who underwent bariatric surgery or surgical treatment for non-inflammatory disease. RNA-Seq was performed on the Illumina HiSeq 2000 platform with paired-end 49 bp sequencing. Reads were mapped using the GEM mapper with an average of 27.4 million reads per sample. We report here results on differential gene expression determined using DESeq. We have also performed genotyping of the above samples on the Illumina Omni 2.5 exome v1 chip and aim to report candidate regulatory variants through association of SNP genotype with mRNA levels for each tissue. Gene expression of the two types of tissues will be further tested with the levels of cardiometabolic biomarkers. We are also performing formaldehyde-assisted isolation of regulatory elements (FAIRE)-Seq on a restricted number of samples from both tissues to identify regions of open chromatin. Combining all the above information will contribute to our understanding of adipose tissue biology and as a result to obesity-related pathogenesis.

P17.92-M

Meta-analysis of Y chromosome haplogroups C, N and Q in Eurasian populations for the perspectives of proto-Bulgarian ancestry

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Bulgaria is situated on the presumed trajectory of the pioneer colonization of Europe. Since then it has been subjected to a series of demographic events with disputable impact on the contemporary Bulgarian gene pool. One of the most controversial issues of the Bulgarian past is the origin of the proto-Bulgarians, which were previously considered as a sparse Turkic population.
In order to delve into Bulgarian patrilineal origins we have performed a survey of Y-chromosome haplogroups followed by meta-analysis of haplogroups C, N and Q distinctive for Altaic populations.
The analysis was performed on a sample comprising 808 Bulgarian males using RFLP and DHPLC analysis. We have found that only 1.49 % of the contemporary gene pool belongs to haplogroups C, N and Q. Our results were used to upgrade and extend the distribution maps of these haplogroups and

to compare their frequency in 240 Eurasian (sub-) populations with more than 20 000 samples.
The comparison reveals a statistically significant difference in the distribution of the studied haplogroups between Bulgarians and Altaic populations as well as between Bulgarians and Eastern Slavic populations. Based on the novel historical studies which point to a substantial contribution of the proto-Bulgarians to the modern Bulgarian gene pool the obtained results suggest that there is no common genetic ancestry between proto-Bulgarians and present day Altaic populations as they reject the hypothesis of the Turkic origin of proto-Bulgarians.

P17.93-S
Forensic parameters and allele frequency distribution of 15 autosomic STR loci in a Mestizo population from the State of Yucatan, Mexico

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The State of Yucatan is a region with a high density of population with Mayan ascendancy at Southeastern, Mexico. Short tandem repeat (STR) polymorphisms are mainly used in forensic fields for paternity tests and personal identification. Since, there are no STR databases from Yucatan, Mexico, a sample of 200 mestizos was PCR-typed for fifteen STR loci with the Power Plex 16 Promega kit (D3S1358, THO1, D21S11, D18S51, Penta E, D13S317, D7S820, D16S539, CSF1PO, Penta D, VWA, D8S1179, TPOX and FGA). Genotype distribution by locus was in agreement with Hardy-Weinberg expectations for all fifteen STRs. The most highly polymorphic loci were Penta E and D18S51, showing 19 and 17 alleles, respectively. Heterozygosity index ranged from 61.5 for D3S1358 to 94.5 for Penta E. For each locus, allele frequencies were obtained, ranging from 0.003 to: 0.455 for D3S1358; 0.380 for THO1, 0.283 for D21S11, 0.203 for D18S51, 0.188 for penta E, 0.480 for D5S818, 0.243 for D13S317, 0.323 for D7S820, 0.328 for D16S539, 0.363 for CSF1PO, 0.278 for penta D, 0.390 for VWA, 0.345 for D8S1179, 0.468 for TPOX, 0.210 for FGA. The most discriminating loci were Penta E (PD = 0.981) and D18S51 (PD = 0.965). The combined power of exclusion was 0.9999999 in the studied population. Our results suggest that this system provide powerful discrimination and remarck the importance of the generation of local databases for STRs when these markers are being currently used in forensic casework.

P17.94-M
Retrospective analysis of live birth prevalence of children with Down syndrome in Denizli, Turkey

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Down syndrome (OMIM: #190685) is the most frequent chromosome abnormality among live births. Its prevalence increases with maternal age, and can be diagnosed by antenatal screening. The average incidence in the USA is 1/700-800 live births and 1-3/1000 births in the EU. We examined prevalence variations of DS in Denizli, Turkey, through a retrospective study. Sixteen years of survey data were retrieved from the two main state hospital registry records between 1994 and 2010, subject had diagnosis as ICD-90. We also obtained some demographical, marital and child bearing trends in Turkish population from Turkey Demographic and Health Survey and TurkStat. Additionally we search some data from EUROCAT for EU and CASP for USA. We identified 113 DS live births in Denizli for 16 years. The prevalence of DS was 9.07 per 10,000 live births before the year 2000 and 9.90 after 2000. The prevalence did not change significantly. The population in Turkey is still young; the fertility rate is high in women under 35 years old, in contrast to EU, and prenatal screening programs are extensively applied; for these reasons, the prevalence of DS has remained stable during these 16 years.

P17.95-S
Six novel loci associated with VEGF circulating levels identified by a meta-analysis of genome-wide association studies

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Vascular Endothelial Growth Factor (VEGF) is the most important pro-angiogenic factor, implicated in both physiological and pathological angiogenic processes. A previously published GWAS had identified four loci independently associated with VEGF levels, two on chromosome 6, one on chromosome 9 and one on chromosome 8 (Debette et al, 2011).
We sought to identify additional loci associated with circulating VEGF levels measured on ~13.000 individuals from six cohorts using genome wide association data imputed to the Phase1v3 release of the 1000 genomes. A GWAS of VEGF levels was performed in each cohort and the results were meta-analyzed using an effective sample size weighted meta-analysis approach.
Five chromosomal regions (5q14.3, 6p21.1, 8q23.1, 9p24.2, 10q21.3) containing SNPs associated at genome-wide significance with VEGF levels (p-value<5*10-8) were identified. Independence was assessed by conditional GWAS in a forward stepwise fashion, including in the association model the most significantly associated SNP at each step, and repeating this process until all SNPs independently associated with VEGF levels had been detected. Ten independent signals were identified including all four previously-reported loci as well as six novel loci.
In silico and de novo replication analysis will be carried out in ~2000 individuals from three additional independent cohorts, as well as functional validation using mRNA data and exploration of the association of these VEGF loci with various clinical endpoints.
Further, pathway analysis will be performed to look for biological processes most likely to be associated with the genes located in the identified loci and to help identify additional VEGF loci.

P18.01-S
Familial 17q12 duplication presented as SGA/IUGR and microcephaly during pregnancy: A counseling dilemma

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Recurrent genomic rearrangements of chromosome region 17q12, ranging from 0.3 to 2.1 Mb, have been described to be associated with different clinical phenotypes. Patients carrying a 17q12 duplication present with intellectual disability, developmental delay of various degree, epilepsy, schizophrenia, autisim, brain abnormalities, esophageal atresia and various renal and urinary tract abnormalities. In the normal population, duplication of 17q12 is quiet rare (<0.02%) and it penetrance estimate is around 21%. Hence, prenatal counseling poses a dilemma.
We present a baby boy with prenatal and postnatal microcephaly and IUGR/SGA. He was born to a healthy nonconsanguineous couple. Late amniocentesis (32 week) because of symmetric SGA/IUGR, microcephaly and echogenic intracardiac focus found 1.4 MB 17q12 duplication. Parents' CMA showed that it was inherited from his father. During counseling the father was found to have normal HC but he has dyslexia and some speech disturbances. Counseling in this case posed a dilemma since 17q12 duplication is known to have a variable phenotype and incomplete penetrance. The parents decided to continue with the pregnancy. The boy was born at term. Examination was normal except for microcephaly (HC< -2Sd).
Prenatal microcephaly has not been previously described in 17q12 duplication syndrome. The clinical significance of this prenatal finding adds to the counseling dilemma

P18.02-M

Evaluating a digital information resource for adolescents in genetic research - adolescent and parent perspectives on information requirements

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There is insufficient evidence regarding adolescent participants' information needs in genetic research. Previous information interventions have yielded little improvement in understanding and confidence in participation decisions, and have not specifically addressed the needs of adolescents. UK adolescents (aged 12-17) and parents with experience of participation in genetic research discussed their knowledge, attitudes, and information needs surrounding genetic research, in focus groups or interviews. A digital information resource (website) was developed following participants' suggestions, and subsequently evaluated for its feasibility and desirability by adolescents (n=21) and parents (n=5) with and without experience of genetic research. Thematic analysis was carried out on transcripts of focus groups and interviews, and on written feedback. Predominant attitudes to genetic research were that such research is prestigious and complex, and that participation in genetic research is a relatively simple proposition, until potential outcomes are explored. The evaluation of the resource suggests participants favour comprehensive information, presented using modern technology, which is accessible and manageable. Further, participants recommend that information is ‚customisable' to accommodate individual differences in requirements, concerning specific content and information quantity. Finally, the analysis suggests participant autonomy is an integral aspect of information provision for adolescents, and should be explicitly incorporated into information design. Digital technology can satisfy participants' preferences, though information resources should be designed with the specific needs of the adolescent population in mind. Questions remain regarding the ‚minimum essential information' in genetic research, but participants should be afforded as much choice as possible.

P18.03-S

Adolescent participation in genetic research - motivations and influences on adolescent and parent participation decisions

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Increasing numbers of young people participate in genetic research following changes in international regulations. There is a recognised need to support children and adolescents in making informed participation decisions, yet there is little evidence concerning how to do so. Moreover, adolescents are a distinct population from children and adults because of their cognitive development and social context. A thematic analysis of focus groups and interviews with UK adolescent patients (aged 12-17) who have previously participated in genetic research (n=7), and their parents (n=7), explored participants' experiences of decisions regarding research participation. The analysis suggests that participants prioritise subjective and interpersonal factors when making participation decisions, and that genetic aspects of research are subordinate influences on decisions. Adolescents cited primarily altruistic motivations, while parents referred to their children's illness and healthcare experiences as key to participation decisions. Further, the analysis demonstrates how motivations such as altruism and personal benefit are mediated by other aspects such as trust and personal histories, and highlights differences between adolescent and parental considerations regarding participation in genetic research. The potential influences of subjective or interpersonal influences on adolescent and parent decisions should be taken into account when inviting participation, in order to support autonomous participation decisions. Further, participant information should be tailored to each group's needs to reflect adolescent and parent priorities, and to ensure that participants are properly informed concerning genetic aspects of research which may not be viewed as a priority.

P18.04-M

Telethon Network of Genetic Biobanks: a key service for diagnosis and research on rare diseases

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Telethon Network of Genetic Biobanks (TNGB), composed of 10 members, was founded in 2008 to coordinate biobanks already supported by Telethon since 1993. Currently, TNGB stores 75,900 biospecimens from over 750 rare genetic diseases. Governed by a Coordinator and the Biobanks' Directors (Network Board), TNGB is also supported by both a Coordinator Emeritus and an Advisory Board which includes ethical-technical experts and a representative of Patients' Associations. Policies and activities are defined in the TNGB-Charter. TNGB has always focused on improving quality, visibility and catalogue access. Its interoperability is facilitated by an IT infrastructure, which greatly simplifies harmonisation and standardisation of all activities. The IT platform, managing samples' workflow, generates a centralised, continuously updated, online catalogue and also enables coordinated management and common rules for catalogue access based on a unique "Request Control Panel". Hitherto, over 300 scientific publications results from research conducted using TNGB-services. Through continued dissemination activities aimed at promoting TNGB-services, the interest for the Biobanks is also enormously increased among patients/families. Indeed, 9 agreements have been formalised between TNGB and Patients' Associations. TNGB is a member of EuroBioBank and some Biobanks are also part of Regional Nets. TNGB is a BBMRI-EU associated-member and is involved in BBMRI-IT node construction. Finally, TNGB is an associated partner of the RD-connect project. TNGB operates according to national and international regulations/recommendations and collaborates with qualified centres and regulatory bodies (Garante Privacy Authority) to review emerging ethical-legal and societal issues. General information and documents including the catalogue are available at www.biobanknetwork.org.

P18.05-S

Ethical, social and policy issues of biobanking for genomic research: a multidisciplinary and qualitative study

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This contribution presents an ongoing study of ethical, social and policy issues of biobanking in the context of the Lausanne Institutional Biobank (BIL), a large-scale hospital-based bioresource established in January 2013 at the CHUV Lausanne University Hospital to support prospective research in genomics. Specifically, the project aims to investigate:
- motivations of patients to accept or refuse to participate in the biobank;
- participants' and non-participants' concerns about issues of collective interest related to biobanking, such as: data protection, data sharing, exploitation for research, ownership and commercialization;
- participants and non-participants' perspectives about feedback of individual findings from research.
To explore these questions, we use a multidisciplinary approach where identification and analysis of key ethical, social and policy issues is informed by qualitative research. Data are collected through follow-up of broad consent using semi-structured interviews with biobank participants and non-participants, and focus groups with personnel in charge of recruitment. A second phase of the project will include a qualitative survey of opinions of the various stakeholders concerned by the future exploitation of the biobank, e.g.: lay citizens, patients associations, researchers, health professionals, policy-makers. Study outcomes will contribute to:
- the improvement and adaptation of informed consent requirements in the context of the biobank;
- the development of a framework for participative governance of the bio-

bank, involving various stakeholders concerned;
- the definition of criteria and procedures to disclose individual findings to biobank participants, in preparing for future research on data collected.

P18.06-M
Do we still need to follow the traditional model of face to face results disclosure for BRCA predictive testing? An examination of current practice in the Republic of Ireland (ROI)

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The traditional model of cancer predictive testing services is changing. Many genetic centres are now offering a choice to patients in how they receive their results instead of the typical face-to-face disclosure. Research has shown that it is feasible to change the current 2 visit model of initial consultation followed by results disclosure, without reducing patient satisfaction to a large degree (Sutphen et al, 2010). In view of this and the increasing demand on the ROI Breast Cancer (BRCA) predictive testing service, a 2 year retrospective study on patient preference in how to receive a BRCA predictive result was performed. The aim was to examine those who had been through the BRCA predictive process previously to study if an alternative to face-to-face result disclosure would have been an option they would have preferred. A questionnaire was used to assess this and results showed that 71.7% of respondents would have liked the option of obtaining their results by telephone or by letter. However, when asked about their actual experience of BRCA predictive results disclosure 40.6% did still prefer the face-to-face contact, while 44.9% would have preferred an alternative. Car parking and distance were the top two variables determining whether the surveyed showed a preference towards options or not, followed by sex and test result. This study shows that while the majority expressed a wish to have a choice, it is important not to underestimate the value of a face-to-face encounter. We are now reviewing our practice for BRCA predictive genetic counselling.

P18.07-S
Risk-stratified screening for cancer and response to personalised genetic information in the general population

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There is evidence that risk-stratified population screening based on multiple factors including a polygenic risk profile has the potential to be more efficient than age-stratified screening. Therefore, genetic information is likely to be used for cancer prevention strategies in the general population in the future. To understand issues of acceptability of such genome based screening in the public, we reviewed issues of genetic risk-communication, how disease risk is perceived and the behavioural response of people to genetic risk feedback. A systematic review was conducted based on inspection of 1948 abstracts and use of 25 studies. We found that though the general population has limited genetic literacy, they are interested in being informed of their genetic risk status. They are generally positive about using genetic information in disease prevention but not about provision of this information to employers and insurers. There is evidence of positive association between perceived risk and cancer screening behaviour such as uptake and mammography, particularly when risk is communicated by categorising into high, medium and low strata. Yet genetic risk feedback particularly that conveying small increases of risk has little or no effect on lifestyle changing behaviour. Also, personalised risk-communication is effective in improving knowledge and risk perception of the population. Personalised risk information is associated with only short term psychological distress including anxiety and fear but not with fatalism. Strategies of preventing cancer using personalised genetic information may potentially be acceptable to the general public. However, before implementation of risk-stratified screening, further empirical evidence is needed.

P18.08-M
Carrier screening for recessive disorders through exome sequencing

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Detection of carrier status for certain recessive Mendelian disorders is a well-accepted health-care practice in several countries since the 70s and aims at the prevention of frequent severe monogenic disorders. Exome sequencing in combination with the increasing knowledge of human pathogenic variation provide the possibility to perform a carrier screening for all the known recessive Mendelian disorders. Such a screening would allow for a much more informative genetic counseling and may alter the total prevalence of the known recessive disorders. In order to test this hypothesis we have used exome sequencing data from 104 individuals of European origin and have identified the total number of likely pathogenic variants in the >1600 recessive disorders for which the responsible gene is known. The mean value was 18.2 variants per individual. Consequently we have randomly paired these exomes in order to create 5356 fictive couples. 33.14% of these couples have at least one gene for which both members are heterozygous for a likely pathogenic variant. These preliminary results exhibit an upper estimate of at risk couples but more precise knowledge and definition of the pathogenic potential of each variant will render the carrier detection more accurate and make it a potent test for family planning.

P18.09-S
Call for a Standardized Genetics Clinical Laboratory Specialty Training Across the Globe: An Initiative for Clinical Molecular Genetics Training in Turkey

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Genetics discipline is composed of several clinical laboratory specialties, as defined by the American Board of Medical Genetics (ABMG): Clinical genetics, biochemical genetics, clinical cytogenetics, and clinical molecular genetics. Except for the clinical genetics, each specialty can be practiced by scientists with PhD and an appropriate training in human genetics. ABMG mandates a two-year, highly structured specialty training for the laboratory scientists before they become lab directors who can officially sign out patient reports. The Clinical Molecular Genetics specialty training program includes a preparation of a logbook documenting contribution to the reporting of 150 cases at different levels, i.e. performance and interpretation of certain number and variety of diagnostic test results, and sharing them with physicians and patients. In addition, rotations in Clinical Cytogenetics and Biochemical Genetics laboratories ensure cross-disciplinary exposure. Didactic lectures and hands-on laboratory training provide fellows competency in skills such as variant interpretation, risk estimation, clinical test development and validation, proficiency testing and regulatory aspects of running a certified clinical laboratory. The investigator, an ABMG board certified clinical molecular geneticist, aims to discuss the training outline that can be implemented in the rest of the world, especially in an era of rapidly advancing technologies that results in accumulation of variants in clinical laboratories with an unprecedented speed. In particular, the significance of a standardized variant assessment system across different laboratories (as outlined in Duzkale et al., Clinical Genetics 2013) and transferring variant data from clinical reports to public databases such as NCBI's ClinVar will be discussed.

P18.10-M
Clinical utility guidelines covering diagnostic next-generation sequencing (NGS)

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Clinical Utility Gene Cards (CUGCs) are disease-specific guidelines authored by international expert groups. They are dealing with the risks and benefits of the application of genetic tests in the clinical setting. Each document represents a balanced summary of the analytical and clinical validity, the clinical utility and cost-benefit issues. CUGCs offer quick guidance to all stakeholders, including clinicians, clinical geneticists, referrers, service providers and payers. Each CUGC is peer-reviewed and published by the European Journal of Human Genetics. CUGCs are also freely available on the websites of EuroGentest, the European Society of Human Genetics and Orphanet.
In order to adapt CUGCs to NGS approaches we have started to build up a NGS panel data collection. It contains data from NGS providers including panel name, tested genes, disease and genetic background. The overlap of tested genes between different providers can be determined by comparison and genes deemed essential by the providers can be easily identified, serving as the first step in the establishment of CUGCs for NGS-based genetic test applications in diagnostics. In a second step we have modified the disease-specific format of the CUGC guidelines and invited experts to put it to test. We here present the state of discussion.
A prototype of our data collection is available at the EuroGentest website: https://eurogentest.eshg.org/index.php?id=668. So far we have identified 28 laboratories having launched a total of 944 clinical NGS tests covering 2882 genes. We encourage NGS providers to contact us regarding their current services and to include them in the database.

P18.11-S
Dynamic Consent - A Patient Interface for 21st Century Research Networks

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Biomedical research increasingly relies on the application of novel technologies to allow data to be shared on an unprecedented scale. However, the procedures for ethical involvement of participants have not kept pace with these dramatic changes in research capability; mechanisms of informed consent remain static, paper-based, and organised around national boundaries and legal frameworks.
Dynamic consent (DC) is both a specific project and a wider concept that offers a new approach to consent, to meet the needs of twenty-first century research. It is a personalised, communication portal which allows interactions over time, enabling participants to engage in the donation of their tissue samples and personal information for research purposes, as much or as little as they choose.
The technical architecture of DC includes components that can securely encrypt sensitive data and allow participant consent choices to travel with their data and samples when shared with third parties. In addition to improving transparency and public trust, this system is of benefit to researchers by streamlining recruitment, and enabling efficient recontact of participants.
The interface facilitates two-way communication of information to stimulate a more engaged, informed and scientifically literate participant population where individuals can tailor and manage their own consent choices. To date, DC has mainly been developed in the context of biobanking, but it also has potential for use in other domains for a variety of purposes.
In this paper we present dynamic consent and show how it can be used as a tool for translational research and personalised medicine.

P18.12-M
Duty to recontact in clinical genetics: Asystematic review of the literature

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Introduction Many findings from NGS diagnostic techniques cannot be interpreted yet, but may provide medically relevant information in the future. However, guidelines on recontacting former patients if new actionable information arises are lacking. **Methods** As a first step in developing such guidelines, we conducted a systematic literature search on recontacting in clinical genetics in PubMed, Embase, Web of Science, and Google Scholar. Our search strategy identified 974 articles in all four databases. Fifty full-text articles in English language met our inclusion criteria, and were included in the review. **Results** Most literature is from the US (48%), followed by Canada (22%) and Europe (22%). In the literature recontacting is usually not regarded a legal obligation in clinical genetics. Most authors do consider recontacting to be desirable. Many articles argue that the responsibility for recontacting should be shared with the patient. We found no national or international guidelines, except for the 1999 ACMG policy statement on duty to recontact. Only four of the fifty articles described practical experiences with recontacting. These showed that patients were usually positive about the renewed contact. **Conclusion** Most authors consider recontacting to be desirable. The limited empirical evidence indicates that patients appreciate recontacting. Practical problems of implementing recontacting in clinical genetics are brought forward most often as argument contra imposing a duty to recontact. One of the challenges for the future will be to create ways to overcome these. Legal issues remain important. We therefore consider it important to develop guidelines on this topic for the NGS-era.

P18.13-S
The ethical dimensions and the tools for data sharing in genetics within evolving frameworks

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Data as well as biological sample international sharing is paramount in health research. While policy declarations from numerous research institutions and funders encourage such sharing a number of difficulties and needs are identified in practice to „make it happen". This movement in the context of the availability of large scale sequencing technologies for studying human genomic variation is confronted with legal and ethical aspects regarding privacy, confidentiality, clinically useful information and the duties attached. Issues related to identifiability, consent process and regulation of access challenge the existing framework. The evolving legal framework regarding exchanges of biological samples (no unified legal EU framework for research) and personal data protection (EU Directive in revision) is challenged. Examples from various consortia and projects are analysed to enlighten the different facets into play, from the P3G consortium (Public population projects in genomics and society), the international consortium on cancer genomics, European infrastructures such as BBMRI (Biobanking and Biomolecular Resources Research Infrastructure) or ESGI (European sequencing and genotyping) and other EU projects. The focus will be on Charts, Codes and tools to foster sharing, especially hSERN (human sample exchange regulation navigator) that gives information on theoretical and practical legal aspects for exchanging biological samples across borders and the BRIF initiative (Bioresource research impact factor) that aims at providing ways to recognise the efforts to make available quality bioresources and at measuring their use. Thus from policy willingness to incentives a whole culture of samples and data sharing is on its move, but not without difficulties.

P18.14-M
General practitioners and direct-to-consumer genomic tests: a survey in Emilia-Romagna region (Italy)

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Personal genomic tests (PGT) for disease risk assessment, based on genome-wide association study variants, have been offered directly-to-consumers (DTC) by several companies since 2007. Concerns regarding their potential adverse impact include, among others, lack of counselling, dubious test quality, unnecessary anxiety and medical interventions based on erroneous/misinterpreted results. To mitigate worries professional education on PGT-DTC has been advocated and the central gatekeeper role of family physicians has been highlighted.
Relatively few studies have been published on awareness, involvement and attitudes of healthcare providers on DTC marketing of PGT and, to the best of our knowledge, none in Italy.
A 2008 CDC survey showed that 42% of healthcare providers were aware of DTC-PGT, that 42% of them had at least one patient asking questions about having such a test and 15% had at least one patient who brought test results in the past year.
The preliminary results of a 2014 survey in the Emilia-Romagna Region (Italy), involving solely general practitioners, show that slightly more than 20% of the respondents are aware of DTC-PGT and that respectively 85% and 15 % of them feel unprepared or only partly prepared to answer questions about such tests. About 45 % of them have had at least one patient asking questions on purchasing or performed DTC-PGT during 2013. These data are coherent with the limited number of Italian companies directly marketing PGT and underscore the critical need to enhance primary physicians' information on genomics tests provided outside of the clinical setting.

P18.15-S
The research policy regarding disclosure of genetic research results: A historical perspective in Japan

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Currently, the return of genetic research results to research participants is a hot topic of debate throughout the world, yet no consensus policy has emerged. Regarding genetic research, Japan has government guidelines, entitled Ethical Guidelines for Human Genome/Gene Analysis Research, which have been set by three ministries in March 2001. These guidelines, together with the preceding document formulated by the government in 2000, Fundamental Principles of Research on the Human Genome, set the principle that a research participant has the right to be informed of his/her genetics information resulting from the research. This means that Japan has taken a stance emphasizing the right of research participants to receive their results since 2000. In the recent revision of the 2001 government guidelines, however, the stance of disclosure in principle was also retained, but the newly added stipulations resulted in researchers' discretion playing a significantly larger role, due to the strong influence of the Act on the Protection of Personal Information. In this study, we have identified two potential ethical issues, which need to consider in the revised guidelines. One is that they do not actually require researchers to offer opportunities for participants to express these wishes and opinions. The other is that one of the exemptions, which show the situation that researchers do not have to disclose, considers only the promotion of research activity, but not the interests of research participants. Based on these findings, we discuss their implications for international research community.

P18.16-M
Recent situation about rules of sharing, reuse and circulation of personal genome data in Japan

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Personal genome data is very important resource for biomedical researches. In very recent years, it is going to be used beyond previous research area and increasing in number with launce of a several large-scale genome cohorts. Although it has been used after acquisition of donor's informed consents and the review by the institutional ethics review boards, that are defined on the national guideline for the genome researches, ELSI (ethical, legal and social issues) are going to change rapidly in Japan. For example, discussions about broad consents and return of results issues are becoming more severe. Development of more secure technology for data-sharing and data-reuse are also desired as well as moral governance. Since Personal Information Protection Law will be revised in a several years in Japan, various experts f began to discuss how these new types of personal information should be protected. In the poster, these situations about the treatment of personal genome data will be reported.

P18.17-S
Genetic Counselling, Genetic Diagnostics, Genetic Prevention, Genetic Education in EB Centre Czech Republic in University Hospital in Brno

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Centre for patients with Epidermolysis bullosa congenita (EB) works at the University Hospital Brno since 2001, from 2012 as the highly specialized medical care centre. EB Centrum CZ is a member of the international network of EB centres and clinical experts. The Centre cooperates with DEBRA Czech Republic (member of the Czech Alliance for Rare Diseases) witch supports people with EB and their families and try for 10 years to engage people with EB to a full life. In EB Center CZ works a multidisciplinary team of health specialists which provides comprehensive care to all EB patients in CZ. The DNA analysis is performed for EB simplex (EBS) and dystrophica (EBD) (analysis of the genes for keratin 5 and 14, and collagen VII) . The mutation was found in 60% patients with EBS, in 67% patients with the dominant EBD and in all patients with the recessive EBD. In one patient was confirmed a rare form of EB caused by a mutation in the gene for plectin. Genetic counseling in our Centre was performed in more than 90 % families with the recessive form of EBD, in about 66 % families with the dominant form of EBD and in about 60 % families with EBS. EB patients from Slovakia, Russia and Ukraine are interested in consultation, genetic conselling and DNA analyse in EB Centre CZ. Activity of EB Centre CZ and DEBRA CZ are also focused on education and awereness for healthcare professionals, patients, their families and the public.

P18.18-M
Enhancing genetic counseling for Familial Alzheimer's Disease through improved phenotyping.

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Familial Alzheimer's disease (FAD), despite representing a rare condition, is attracting a growing interest. In clinical practice, we noticed that individuals with a family history of AD show considerable interest for the availability of genetic tests for early detection of the disease.
When informed about the availability of a genetic test for autosomal dominant mutations (PSEN1, PSEN2, APP genes) some individuals decide to be tested, while other refuse. Those who refuse to take the test are primarily motivated by lack of efficacious therapies. However, both those who decide to take the test and those who refuse it lament lack of clear information about the clinical development of the condition.
Indeed, we found out that, albeit rare, FAD cases are poorly phenotyped. In particular, we performed a systematic review of studies describing the phenotypic features of FAD cases sustained by PSEN2 mutations resulting in largely incomplete and low-quality data. Given the incomplete penetrance of some mutations, their variable phenotypic expressivity, and the lack of systematic and accurate phenotyping of FAD cases, the possibility of implementing genetic counseling procedures is strongly limited. This may also affect the capacity of individuals to react and cope with the communication of the test result should they turn out to be mutation carriers. Available guidelines do not clearly address this issue and thus may not provide sufficient guidance for clinicians and counselors. Based also on experience in other contexts, we propose criteria to improve FAD cases phenotyping aiming at enhancing the quality of genetic counseling activities.

P18.19-S
The efficacy of the teaching of the patients during genetic counseling session

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Studies evaluating the efficacy of the genetic counseling are few in number in Russia. In our study, we evaluated the efficacy of the genetic counseling for families with affected children or relatives by a number of parameters, such as change in the conversance of patients and the impact of genetic counseling on reproductive plans of the patients. The study was conducted in the outpatient department of the Research Centre of Medical Genetics (RCMG), the Russian Academy of Medical Sciences (RAMS), in 2007-2011. We interviewed 226 respondents aged 17 to 67 years. The survey was conducted before and after genetic counseling. Questionnaires have been analyzed using Statistica 10.0 software. We found that the conversance of the risk of an affected child birth after genetic counseling increased significantly (Wilcoxon test, $p < 0.05$). So did conversance of patients about the possibility of prenatal diagnostics (χ^2 - distribution, $p < 0.05$). However, when assessing the extent to which the patients increased their understanding of the causes of hereditary diseases (that was assessed by a series of test questions), we did not detect any changes in conversance of patients before and after genetic counseling. We concluded that these issues deserve a special attention when conducting genetic counseling. Also, we found no significant changes in reproductive plans of the patients after genetic counseling. Currently, there are many reasons that affect the reproductive plans of the family, in addition to an adequate understanding of the extent and meaning of genetic risk.

P18.20-M
Changes in the public's perception of clinical genetics in Cyprus: Reflections from the 20 years of experience of the Clinical Genetics Clinic (CGC)

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Having been established in 1994, the Clinical Genetics Clinic (CGC) is a pioneer in the field of clinical genetics in Cyprus. Over the course of the last 20 years, we have had first hand experience in how people's perception and acceptance of the offer of clinical genetics and genetic counselling has evolved. While in its infancy we observed frequently that patients experienced fear of being stigmatised, characteristic of small societies, if diagnosed with a genetic condition or if they pursued genetic counselling. Some patients also considered being referred to CGC as accepting to proceed with prenatal diagnosis. There was a lack of awareness that genetic counselling would give them the opportunity to discuss their options, receive psychosocial and educational support to make autonomous decisions. As a result, the CGC embarked on (and still continuous) organising public awareness activities, as well as educating patients/families and other health care professionals on common/rare genetic disorders and the process of clinical genetics and genetic counselling. In recent years, patient referrals increased substantially from wide range of specialists (paediatricians, OBGYN, neurologist, oncologist etc).There are more informed individuals/patients compared to before, who may inquire about or instigate referral to genetic counselling themselves as well. We grew in our team as well as services including the Cancer Genetics Clinic, established in 2006, to provide tailored genetic counselling to cancer patients. In this poster, we will elaborate on these points mentioned as well as others to reflect on our 20 years experience in offering clinical genetics.

P18.21-S
Employment and professional integration of genetic counsellors in France : a new collaboration in the healthcare sector

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The profession of genetic counsellors was founded in Europe in the 1980's in the United Kingdom. In France, it began in 2005 under the aegis of a law, following a report on the demographic situation in the health professions. Today, after seven years, we number 122 graduate genetic counsellors who can work in a variety of settings in multiple specialty areas of human gene-

tics. How are these new non-medical practitioners integrated into the multidisciplinary services of genetics? How are they recruited and with which status? What are the responsibilities entrusted to them? How are they perceived by geneticists working with them? We performed a literature survey to trace the history of the creation of this profession. To answer the underlined questions, we used socio-epidemiological studies through the elaboration of surveys regarding the education, the role and practice of genetic counselor. Studies were addressed to both genetic counselors and geneticists. Among the 122 graduate genetic counsellors, 94 are employed (77%), with an average monthly salary of 2,001.21 €/month. They are able to manage consultations only when no medical procedures are required. However, the responsibilities are dependent on the relationship established between the genetic counsellor and the medical geneticist. Overall, this profession has been quickly established in France and is the only one governed by a specific law at European level. Although this survey emphasizes inequalities in the practice of this new profession, including discrepancies regarding the administrative aspects, genetic counsellors are increasingly being integrated into all levels of healthcare service delivery.

P18.22-M
Challenges of web-based personal genomic data sharing

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In order to study the relationship between genes and diseases, the increasing availability and sharing of phenotypic and genotypic data has been advanced as an imperative within the scientific community. In parallel with data sharing practices by clinicians and researchers, recent initiatives have been observed in which individuals are sharing personal genomic data. The involvement of individuals in such initiatives is facilitated by the increased accessibility of personal genomic data, offered by private test providers along with availability of online networks. Personal webpages and on-line data sharing platforms such as *Free the Data*, *Consent to Research* and *Genomes Unzipped* are being utilized to host and share genotypes, electronic health records and/or family history uploaded by individuals. Although personal genomic data sharing initiatives vary in nature, the emphasis on the individuals' control on their data in order to benefit research and ultimately health care has seen as a key theme across these initiatives. In line with the growing practice of personal genomic data sharing, this paper aims to shed light on the potential challenges surrounding these initiatives. As in the course of these initiatives individuals are solicited to individually balance the risks and benefits of sharing their genomic data, their awareness of implications of personal genomic data sharing for themselves and their family members is a necessity. Furthermore, given the sensitivity of genomic data and the controversies around their de-identifiability, potential privacy risks and harms originating from unintended uses of data have to be taken into consideration.

P18.23-S
Opinion about reproductive decision-making among MMR mutation carriers of reproductive age

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The reproductive techniques such as prenatal diagnosis (PND) or preimplantation genetic diagnosis (PGD), although debated, are often legally forbidden in case of HNPCC syndrome, without considering mutation carriers' opinion about their reproductive options
We conducted a study in 43 individuals (probands and relatives) identified as Mismatch Repair mutation carriers of reproductive age (until 40 years for women, 50 years for men) exhaustively screened during the genetic oncology consultations in our institution. All of them received a closed questionnaire focusing on socio-clinical, possible causes of distress and reproductive preferences (natural conception, PND, PDG or adoption) whose answers were ranked from 0 to 4.
Complete data were available for 29 individuals (22 relatives, 7 probands:13 males, 39±5.6 years; 16 females, 31±6.8 years).
Main reasons of distress given in first were fear of transmitting predisposition for 17 individuals (9 men, 8 women) (58%) and fear of premature death for eight (4 men and 4 women, 27.5%). For 5 persons the fear of passing on deleterious gene took second place, so more than 22/29(75%) of this population regarded this problem as very serious.
PND / PGD and natural conception were equally reported (52% and 48%, respectively).
Among the subjects afraid enough by hereditary concern (22), only 13(59%) chose in first the new reproductive techniques and 9/22(41%) gave priority to natural conception
Neither reason of distress, nor gender were associated with reproductive choices
Conclusion: Although most subjects were afraid to transmit the predisposition, all of them did not give priority to reproductive techniques.

P18.24-M
Improved hereditary recurrent fevers diagnostics resulting from participation in the European molecular genetics quality network

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The hereditary recurrent fevers (HRF) are rare rheumatologic diseases characterized by a course of self resolving inflammatory episodes, inflicted by cells of the innate immune system, and mainly affecting connective tissue, skin and/or central nervous system. If untreated, some HRF patients may develop life threatening, secondary amyloidosis. HRF genetic diagnosis is increasingly requested for patients with recurrent inflammatory episodes of unknown origin. More than 10 HRF genes are listed in the Infevers database. An external quality assessment (EQA) program for the primary tested HRF syndromes (FMF, CAPS, TRAPS, MKD) has been provided by the EMQN since 2009. Fifty laboratories, mostly from European countries, participate in the scheme. Data demonstrating improved genetic diagnosis for HRF since 2009 will be presented; genotype error rates have dropped dramatically compared to a prior 3 year survey (2005-2008). Moreover, with low participation outside Europe, best practice guidelines for the genetic diagnosis of HRF have also been written by HRF genetic and clinical experts, addressing the scope of diagnosis and clinical significance of pathogenic, clinically debated, rare, novel or population specific variants. A simple interpretation chart concludes on the contribution of each variant type to the diagnosis of recessive or dominant HRF disease. Guidelines also addressed minimal details and indication for referral, technical quality assurance measures, variant description nomenclature and recommendation for further genetic testing. Our future goal is to expand the scheme to monogenic autoinflammatory diseases, and to implement EQA on new genetic diagnostic methods for HRF such as Next Generation Sequencing (NGS).

P18.25-S
Attitudes of genomic researchers, health professionals and students on returning incidental findings to whole genome research participants

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At the moment, there is no consent on how to handle incidental findings (IF) in human research. We are investigating the opinions of genomic researchers, health professionals and students on returning IF to research participants using a questionnaire available on line at the Welcome Trust that was kindly sent to us for translation to spanish and application in our country. The questions explore if the severity of the health problem incidentally found, the level of risk of getting the condition and the usefulness of the information affect whether the person think the result should be returned. Also, it asks the participants to first answer based on their own professional knowledge and second to answer imagining they are the research participants. The preliminary results show that respondents agree that information on preventable conditions should be returned to participants in genomics research; however, they were more likely to receive IF regarding a life-threatening non preventable condition than to return it to participants. More than ninety percent of subjects would return IF related to drug response or that could be relevant to their children. And 80% think someone should decide which types of IF to share. Also, 80% feel it is acceptable to have a ‚flexible consent' process where research participants could update and change what information they choose to receive, and almost 50% of respondents think results should be available for ever. As whole genome research advance in Mexico, studies to develop policies on how to handle IF become important.

P18.26-M
To know or not to know: Research participants want to know about incidental findings in WES-studies

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Introduction
In the last years the ethical and legal management of incidental findings in

massive parallel sequencing have been discussed. There is a lack of literature concerning research participant's perspective. The aim of this study was to investigate whether research participants want disclosure of IF's and what kind of IF's they want to know about.
Methods: 127 research participants in a study of gastrointestinal polyps were informed about Whole Exome Sequencing and the risk of IF's. They were asked to decide whether they A) wanted disclosure on IF's no matter whether the mutations were associated with a non-treatable or non-preventable condition, B) wanted disclosure on mutations associated with treatable or preventable conditions or C) wanted no disclosure at all.
Results: Participants who wanted disclosure of all IF's (A) accounted for the majority (n=78), 45 of the participants only wanted disclosure of mutations, which could lead to surveillance or treatment (B) and four participants did not want IF's to be disclosed at all (C).
Conclusion: The study showed that almost all research participants wanted disclosure of at least some types of IF's. The answers did not depend on age or sex. We suggest that well-defined IF's are to be disclosed in research projects and that the type of IF's (non-treatable and actionable) are categorized, discussed with the participant, and incorporated in the research consent form.

P18.27-S
Disclosure of genetic information to family members: does the French legal framework solve the dilemma?

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Genetic information is often considered as specific, among other biological information, because of its personal and family dimension. When a person is diagnosed with a serious genetic anomaly, the disclosure of this information can be relevant for other family members when prevention measures or treatment exist. The transmission of this information raises legal issues for professionals: how to preserve confidentiality and privacy of personal medical information? How to ensure the right to know of the relatives when the information to be disclosed can be of interest for their health? The French legislator tried, in 2004, to draw a balance between these principles by implementing the "genetic information procedure to family members. The lack of adoption of enforcement decrees made the law not applicable since a revision occurred in the new bioethics law (2011). This procedure tends to favor information of relatives by creating a primary legal obligation for the index subject to inform his family members. It also creates professional obligations notably in the ways this information has to be formalised and disclosed when the subject do not want to communicate it. The French legal system is almost complete) as many texts (legal and good practices) have enriched the procedure throughout 2013 In the light of these legal novelties we will make a comparative analysis to address -The equilibrium of the principles referred to in the law, -Their adequacy to the practices, -The remaining unclear points (responsibilities not to disclose, genetic information relating to minors)

P18.28-M
Gynecologic cancer is "sentinel cancer" for Lynch syndrome

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Lynch Syndrome (LS) arises by a germline mutation of MMR genes. Women with LS show a risk for endometrial cancer equal or greater than colorectal cancer. Specific surveillance and risk-reducing strategy could be proposed in LS patients. MSI and IHC MMR protein expression tests in tumor samples could represent an effective strategy for identifying LS in patients with GC (Gynaecological Cancers). Clinico-pathological features, MSI and Immunoistochemical (IHC) expression of MMR proteins were investigated on 78 tumour samples (62 endometrial, 7 ovarian and 9 cervical tumours) of 75 patients affected by GC. All patients referred to genetic Counseling Service of Varese Hospital from 2001 to 2013. Somatic test including IHC and MSI revealed absence of MMR protein expression in 44/64 and MSI in 36/61 GC. Fifteen GC showing MSI and loss of IHC MLH1 expression revealed MLH1 promoter hypermethylation. The mean age at GC diagnosis in LS patients was 46 years. Endometrial cavity was the prevalent site, and 28/38 GC tumors showed a pure endometriod histotype. Twenty nine out of 35 patients show GC as first manifestation of LS. MMR germline pathogenetic mutation was identified in 20 patients (10 patients were obligate carriers), the remaining five patients showed variants with unknown significance of MSH6 gene. In conclusion IHC, MSI and MLH1 methylation in CG patients under 50 years is an efficient strategy to identify LS. In addition clinico-pathological features including site, hystotype and presence of lymphocytes infiltration help to identify an additional subset of LS patients.

P18.29-S
New genomic technologies and medical genetics: How much time is needed? A preliminary study

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Clinical genetics services are time and labor intensive, since they include both counseling meetings as well as extensive patient related activities such as administration, summary letters, and the interpretation of new genomic technologies. With increasing pressure for cost effective medical care, information is needed to evaluate the time and efforts required for providing medical genetics services. An online survey was conducted among 151 professionals who practice medical genetics throughout the world (85.75% medical geneticists, 13.12% genetic counselors). The reported average amount of time required for counseling sessions for pediatric, oncogenetic, pregnancy with a malformed fetus and preamniocentesis counseling was significantly different: ~60,42,51,27 minutes respectively. The average time required to write summary letters varied from 30 to 41 minutes. The time required for literature searches was 31-60 minutes and for patient related activities, 42 minutes. The time for patient related bioinformatics search and for test interpretation was 57 minutes . CMA requires an average of 48 minutes for analysis and for genetic counseling each. Professionals with less than 10 years' experience needed more time than those with more than 10 years' experience. Time devoted to clinical work received the highest percentage followed by administration, research and teaching. This study emphasizes the complexity and time consuming demands of the practice of medical genetics in the era of advanced genomic testing; further consideration and assessment is required in order to determine how to adapt genetic services to the demands of cost effectiveness, without compromising the quality of patient care.

P18.30-M
Project Superhero: the kids are doing it for themselves

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Multiple endocrine neoplasia (MEN) disorders are autosomal dominantly inherited syndromes characterised by multi-glandular adenomas/carcinomas. AMEND is a patient group providing support and information resources to MEN patients. However; no information about MEN aimed specifically at children and young people currently exists. Inspired by 2011 research by Metcalfe et al(1), AMEND started 'Project Superhero!' which aims to 1) improve communication about the MEN conditions within affected families, 2) increase involvement and compliance by affected young people in their healthcare, and 3) provide families with a wider range of information about MEN to aid open discussion. A Family Focus Day (FFD) held in March 2013 involved 13 young people aged <18 [ages 8-18] from 6 families [2 MEN2a, 1 MEN2b, 2 MEN1, 1 control family]. Adults and young people completed self-assessments to test their knowledge about MEN. Adults' scores ranged from 3-5 (mean 4.3) for identifying five different glands; young people's scores ranged from 0-5 (mean 3.2). Knowledge levels (0 = no knowledge, 5 = complete knowledge): adults' scores ranged from 1-4 (mean 2.8) and young people's ranged from 1-4 (mean 2.3). Questions from the young people, included, 'Do I have cancer?' and 'Will this kill me?'. The findings were used to develop resources to answer their questions, which include MEN1 and MEN2 Medikidz™ comic books and 2 website animations. Evaluation of the resources is underway.
(1)'Parents' and Children's Communication About Genetic Risk: Qualitative Study Learning From Families' Experiences, *Metcalfe A, et al*, 2011, European Journal of Human Genetics'

P18.31-S
The Genome Clinic in Geneva: an example of a multidisciplinary task force for the clinical use of next generation sequencing

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The advances of next generation sequencing (NGS) technologies enable their application in clinical care. However, beside the clear benefits of NGS, such an implementation faces technical, ethical and financial challenges, in-

cluding data processing, storage and management, variant interpretation, genetic counseling (informed consent, management of variants of uncertain clinical significance, incidental findings), quality control as well as reimbursement questions. In order to optimally integrate the use of NGS into our clinical practice and to address these questions, we have created a multidisciplinary working group, the Genome Clinic task force.
This task force is composed of physicians and scientists, including clinical and molecular geneticists, bioinformaticians, bioethicists and a coordinator. During our weekly sessions, clinical cases of heterogeneous mendelian disorders that could potentially benefit from a NGS approach are presented, results and interpretation of analyzed cases are discussed, as well as issues related to bioethics, management and health policy.
During the pilot phase, we have validated 20 cases for whole exome sequencing followed by targeted bioinformatics analysis of selected genes. In addition, we have collaborated with the Swiss Federal Office of Public Health (SFOPH) in order to render NGS a reimbursable genetic test by the health insurance. We will present the results of resolved clinical cases as well as the outcomes of our interactions with the SFOPH.
In conclusion, this multidisciplinary task force has enabled us to deal with the multiple issues related to NGS in clinical practice and to ensure a high standard clinical service within this new and exciting field.

P18.32-M
From clinical suspect to molecular confirmation of Noonan syndrome; contribution of "best practice" genetic counselling

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Noonan syndrome (NS) is AD disorder, characterized by variable expressivity of clinical features such as: postnatal growth reduction, congenital heart disease, characteristic facial dysmorphisms and development delay. In ~75% of all NS cases, germline mutations involving RAS-MAPK signaling pathway genes (PTPN11, SOS1, RAF1, KRAS, NRAS, BRAF, SHOC2, MEK1, CBL) are causative.
We reported a case of 13-year-old girl [born at 36w by CS (BW 3250 g (~95°), BL 48 cm (~75°)] referred for genetic counseling due to growth retardation, facial dysmorphisms, development delay and learning disability. After birth she presented frequent vomiting, with failure to thrive and at 5 months of age underwent surgery for intestinal malrotation. Because of short stature, Growth Hormone (GH) therapy have been introduced at age of 3yrs up to 11yrs. Negative molecular testing for PTPN11 and SOS1 genes, normal female karyotype and aCGH analysis were observed.
Objective examination: H 138 cm, (<3°); W 33 kg, (<3°), no menarche, hypertelorism, eyelids ptosis with down slanting palpebral fissures, low-set and posteriorly rotated ears, high-arched palate, micrognathia, short and webbed neck, low hairline at the back of the neck, pectus excavatum, prominent scoliosis, joint hyperextensibility, bilateral pes planus and mitral valve prolapse disclosed by US.
Phenotype of our patient was suggestive to NS, thus further mutational screening has been requested. Missense mutation in exon 2 of KRAS gene (c.40G>A; p.Val14Ile) has been identified. Even though KRAS mutations are usually associated with NS severe phenotype with cardiac involvement (hypertrophic cardiomyopathy), this finding is not present in our patient.

P18.33-S
Orphanet UK: Ensuring quality of information

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Orphanet is the largest online resource for rare diseases and orphan drugs for all audiences. It provides free and direct online access to the most comprehensive classification for rare diseases, an "assistance to diagnosis" tool, the EUCERD's newsletter, thematic studies and reports and updated information about rare diseases and specialised services in around 40 countries. Orphanet UK (www.orphanet.co.uk) has been operational since 2005. To date it lists >300 expert centres, 125 laboratories, >300 patient organisations, >130 patient/mutation registries and >580 research projects and clinical trials.
Orphanet has established strong quality standards over the years considering the different situations of the countries part of the consortium. There are defined inclusion criteria for each activity.
Information is collected from official sources specific to each country. Once published data will be checked annually by post-validators, who are professionals working in the field of rare diseases with expert knowledge in the relevant activity. Orphanet UK has established several partnerships to post-validate its information. Rare Disease UK and Genetic Alliance validate patient organisations. ERNDIM validates EQA accredited metabolic laboratories in the UK. UK centres of expertise are validated by experts that are part of the EUCERD and research activities are validated by relevant patient organisations. Orphanet UK is also working to establish new partnerships to validate data about molecular and cytogenetic laboratories and data about clinical trials too. Every effort will be made to ensure that information is accurate, comprehensive and up to date.

P18.34-M
Attitudes of adult patients and parents of children with cystic fibrosis towards carrier screening for cystic fibrosis

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Cystic Fibrosis (CF) is a severe autosomal recessive condition with clinical symptoms such as chronic pulmonary disease and pancreatic insufficiency. CF affects approximately one in 2500-4000 Caucasians, while the carrier frequency is estimated at one out of 25 to 30. Carrier screening for CF has been available to individuals without family history of the disease since the early 1990s. However, very few screening programs have been implemented around the world to date. In order to assess social desirability of carrier screening for CF, it is important to study views and attitudes of key stakeholders, such as patients with CF and their family members.
The aim of this study was to assess views of adult patients and the parents of children with CF regarding carrier screening for CF. Participants were recruited from a register of patients at the University Hospital of Ghent. 134 questionnaires were distributed of which 112 were returned (response rate 83.5%). In overall, the attitudes towards carrier screening for CF were positive, with 80% of respondents thinking the procedure entails more advantages than disadvantages. Eighty-five percent of the respondents believe that the screening should be routinely offered to all couples planning a pregnancy, while 72.9% were of the opinion that the procedure should also be provided prenatally. Regarding future pregnancies, 46.1% would themselves choose for preimplantation genetic diagnosis, 43.6% would prefer to conceive naturally followed by a prenatal diagnosis. Others were ready to accept the risk of having an affected child, or opt for an adoption, 5% each.

P18.35-S
Predictive genetic testing in hereditary heart diseases: a single-center series of 304 subjects

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Hereditary heart diseases are typically characterized by autosomal dominant inheritance and delayed cardiac expression. Predictive genetic testing (PGT) is offered to asymptomatic relatives to allow targeted medical care with early therapeutics in order to reduce the risk of complications. Psychological issues related to PGT are complex and have been poorly studied. To evaluate our practices regarding PGT for hereditary heart diseases and study the behavior of relatives after the first information consultation, offering a waiting period before blood sampling. We retrospectively studied records from 304 consecutive relatives seen in our department and have requested PGT. Underlying diseases in the families were HCM (60%), DCM (17%), ARVC (15%), LQT (5%), Brugada syndrome (2%) and other (1%). There were 260 adults and 44 minors. At the time of the first consultation, the average age was 37 years, and 83 % of the relatives previously had a cardiac checkup. After multidisciplinary consultation, 22 relatives (8%) dropped out of procedure and 11 relatives (3%) performed blood sampling but did not come back to know their results. Blood sample was delayed for 70% of relatives and immediate for 30%. A total of 21 different genes were analyzed and most frequent ones were MYBPC3 (97), MYH7 (77), LMNA (37). A mutation was present in 36% of relatives and absent in 64%. We observed a high level of genetic uptake after initial consultation but a minority of relatives decided to stop or delay the procedure. These results suggest the benefit of a waiting period before blood sampling.

P18.36-M
Presymptomatic and predictive genetic testing in minors - a mini-review in preparation for new Danish best practice guidelines

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Aim: In preparation for the creating of Danish guidelines regarding predictive and presymptomatic genetic testing in minors, we have reviewed existing guidelines and policy papers as well as international conventions. Furthermore we have reviewed existing literature concerning the psychosocial impact of testing children.
Materials: Guidelines on the topic from four of the largest English-speaking genetic associations were reviewed. The Convention on the Rights of the Child and the Convention on Human Rights and Biomedicine were also considered.
Results: In the reviewed guidelines it is recommended to offer genetic testing if the test result will be of medical benefit to the child. In some guidelines emphasis is on *immediate* benefit. If there is no potential medical benefit it is recommended to defer genetic testing for adult-onset disorders until minors are able to decide for themselves. Some guidelines suggest that psychosocial factors in certain cases can justify genetic testing for adult-onset disorders. Requests for genetic testing for childhood-onset disorders can usually be met. Thorough genetic counseling and consideration of timing of the test is of importance in the decision-making process.
Discussion: There is a lack of knowledge about the psychosocial impact of testing children. The recommendations in the reviewed guidelines are based on the classic medical moral principles: Nonmaleficence, beneficence and respect for autonomy. The recommendations are in consistency with international conventions on the field, but statements within these conventions can be subject to interpretation. We call for increased awareness of the difficulties in defining "the best interest" of children.

P18.37-S
Presymptomatic testing in minors: Requests and pratices. Evaluation of pluridisciplinary consultations for the last 20 years

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Twenty years ago, the first presymptomatic tests (PST) began for Huntington's disease. Since then, PST have been extended to other diseases, in particular among minors. The practice of these tests is regulated by law within each European country and follows a number of principles, including respect for the «right to not know» and autonomy. In the case of children, performing such tests is complicated because all of these conditions cannot always be met. In the Department of Genetics in the Pitié-Salpêtrière Hospital, several protocols of PST are reported to the French Biomedicine Agency, in accordance with French law. We wanted to explore our practices regarding children. All patients under 18 years of age at the first consultation for a PST were included in this retrospective study. 175 children met the inclusion criteria and they were divided into 4 groups (cardiology, myology, neurology, oncology). Medical data but also access to the test or not, motivations, were collected. The average age of minors at the first consultation was 13 years. 69% of children have performed the test but this varies significantly ($p<0.05$) according to the pathology (46% in neurology and over 90 % in cardiology and oncology). 8.6% of children also said they did not want the test at the first consultation, underlying the importance of parental demand. The study by group of pathologies also notes that the reflexion time, the reasons given for doing the test vary according to the pathology and emphasize the importance of a differentiated care.

P18.38-M
Promotion of genetic services in the Slovenia-Italy cross-border region

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SIGN (Slovenian-Italian Genetic Network) is a project sponsored by the European Union with the aim to implement both the accessibility and the quality of genetics services in the Slovenia-Italy cross-border region. Eight Slovenian and Italian institutions have joined the project. One of the major objective of SIGN is to raise awareness in both the general public and medical specialists on the activities of the genetic services and on the new diagnostic options patients with rare diseases may be offered. For this purpose, training courses in medical genetics for paediatricians, gynaecologists and oncologists have been organized and several workshops for high school students have been conducted. All the project partners were actively involved in these meetings, allowing the reinforcement of the genetic network and the establishment of new scientific collaborations. In addition, a specific website, in both Italian and Slovenian, has been developed in order to help patients, doctors and students to better understand human and medical genetics (www.signgenetics.eu). The section dedicated to the general public contains information on human genetics, genetic diseases and their impact on the society described in a simple, non-technical way. The section for doctors and students offers a variety of educational tools, including fact sheets on several genetic diseases, examples of clinical cases useful to recognize and correctly diagnose specific diseases, video seminars of experts and clinical protocols. Another section is devoted to updating and promoting the results achieved within the project: it includes a description of the project partners and their clinical and research activities.

P18.39-S
The French Foundation for rare diseases: accelerating rare diseases research

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The French Foundation for rare diseases is an innovative cooperative framework dedicated to rare diseases research. Flagship of the second French National Rare Diseases Plan, co-founded by University Hospitals, Research organisations and Patients organisations, we act as a federative and strategic hub to accelerate scientific, clinical and social innovation by stimulating cross-sector cooperation to the benefit of patients affected by rare diseases. With our headquarters at the heart of the French Platform for Rare Diseases and seven regional coordinators, in direct contact with researchers all over the national territory, our priorities are driven by grounded needs and integrated into a national strategy with an international perspective. Our active support, spanning from basic to translational and clinical research, includes enhancing the access to high throughput technologies such as NGS, promoting international collaboration via dedicated partnerships, and accelerating the translation of research into clinical development through targeted links to orphan drug experts. Since rare diseases research is tightly linked to societal challenges, we are also actively supporting studies on social, economical, ethical impacts of rare diseases. We regularly initiate national working groups, open to international perspectives, to specifically target timely issues, including professional training needs, ethical and regulatory issues around data and bio-specimen collections, as well as the impact of new technologies in genetics, patients' consent to genetic testing and their paths through diagnosis and treatment. Through this unique range of actions, we aim to contribute to acquainted national public health and research policies and to the promotion of international multi-stakeholders cooperation.

P18.40-M
Rare Diseases week in Timisoara - a campaign with a good start

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„Volunteers for Rare Diseases" was created in „Save the Children" Timis in 2007 when students from the University of Medicine Timisoara, supported by teachers, wanted to work with people with special needs. The campaign launched on the International day of rare diseases was the opportunity to spread informations about this topic in community. An extension to a whole week dedicated to this was the next step.
Increase awarenes in general population, involving the local authorities, rasing the interes in this field for medical services employees and involving our students in volunteer work were main objectives.
In all the 5 years we used internet as a tool and mass-media campaign also. Volunteers made a street march in the dedicated day with flyiers distribution in downtown and placing posters. We organised round table at the local TVs with specialised medical staff. Each year we met the children in hospitals and settled lessons for parents.
With parents we discussed the common situations of various diseases, followed by monitorization of their skills yearly. „Rare Disease Day" has taken many forms over the years: symposiums, conferences, information campaign, march (more than 200 participants/year). The childrens with rare pathologies have in this way support for their integration into society. The

authorities realise the need for social protection with different ways to support this people. All the student involved can use informations about this pathology and early diagnose cases, encouraging prevention.

P18.41-S
Respecting autonomy while reacting to change: A review of current policy and empirical evidence on re-consent in longitudinal biomedical research

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Background: The need to balance the autonomy of participants and their consent to participate in biomedical research with the fast pace of scientific enquiry is becoming difficult as the number of projects grows and pressure increases at national and international levels to link project resources to facilitate access and data sharing.
Methods: We undertook a literature review from the perspective of longitudinal cohort studies and biobanks. We examined existing policy statements, academic literature on re-consent, and evidence from re-consent exercises.
Results: Guidance from policy bodies is vague, suggesting that re-consent should be sought if changes are made to the original protocol or if new research falls outside the scope of the original consent. Stakeholders' attitudes showed different approaches. Broad consent, with or without an oversight body is a popular alternative, while seeking re-consent for every study was also mentioned by a minority. Several alternatives along this continuum have been suggested by commentators and as a result of empirical studies. Actual and potential research participants want re-consent when a new use presents increased risks and is for a new unrelated condition, while commentators also suggest re-consent when the research is moving beyond existing use, such as for next-generation sequencing.
Conclusion: Practical difficulties, potential loss of participants and increased costs make re-consent for every new study unpractical and in some cases unfeasible. There is a lack of studies investigating actual participants and their positions on re-consent and research is needed to inform best practice guidelines for re-consent in longitudinal studies.

P18.42-M
Willingness to participate in the research of rare diseases: general public versus patients and their family members

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Background: Development of new approaches for prevention and diagnosis of rare diseases depends on willingness of people to participate in research. In this study we compared the research participation of general public represented by mothers of newborns with the proportion of patients with rare diseases and their relatives consenting to anonymous genetic research.
Material and Methods: General public participation was assessed from an intake of a pilot study for newborn screening (NBS) expansion involving 70,818 newborns from 58 Czech maternity hospitals. The willingness of patients and/or their relatives to donate samples for research was recorded from 318 consent forms obtained by 67 physicians during genetic counselling; only 240 forms were completed with regard to research.
Results: The intake of the NBS pilot study varied considerably by the maternity hospital ranging between 11% and 93% of mothers with a median intake of 75%. The willingness to donate samples for research differed substantially by the counselling clinical geneticist and varied between 0% and 100%; 82% of patients and their relatives consented to the research use of residual samples.
Conclusions: This study shows similar and quite high willingness of both the general Czech population and patients/relatives with rare genetic disorders to participate in research. The notable difference in recruitment between maternity hospitals as well as clinical geneticists points out the major factor also documented in literature for other conditions, which is the involvement of site or person offering the research participation.
This study was supported by projects LM2010004 and PRVOUK-P24/LF1/3

P18.43-S
50 years anniversary of Smith-Lemli-Opitz Syndrome

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50 years ago the Smith-Lemli-Opitz Syndrome (SLOS, RSH syndrome) was described for the first time in three male patients by David W. Smith, Luc Lemli and John Opitz. It was a clinical description showing microcephaly and hypogenitalism. 30 years later Tint and colleagues found the underlying defect in cholesterol biosynthesis (1994). The gene was identified by a group in Innsbruck (Fitzky et al., 1998), first mutations in SLOS patients have been detected by the same group and also by Wassif et al. (1998). Hence SLOS is a metabolic and malformation disorder caused by mutations in the DHCR7 gene. This gene encodes the α7 sterolreductase. Now more than 130 mutations are known and all reported patients are included in the DHCR7 database (http://databases.lovd.nl/shared/genes/DHCR7). Mutation spectra are different in European populations. Time since establishment of common founder mutations (c.964-1G>C, p.Trp151* and p.Thr93Met) is long enough (about 100 and 200 generations) to explain frequencies (1:100 for c.964-1-G>C) by genetic drift (Witsch-Baumgartner et al., 2007). There are modifiers of clinical severity of SLOS. Depending on SLOS patient´s maternal variants apoE 2, 3 or 4 the severity vary significantly (Witsch-Baumgartner et al., 2004). The fundamental problem of the disease is the lack of cholesterol during embryogenesis. Regarding therapies adding HMG-CoA reductase inhibitor (simvastatin) might ameliorate the severity (Jira et al., 2000; Haas et al., 2007). After 50 years the pathophysiology of SLOS is still not as clear due to multiple functions of cholesterol. To help patients it is still necessary to continue research on SLOS.

P18.44-M
Gonadal mosaicism in split-hand/foot malformation: Implications for genetic counselling

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Split-hand/foot malformation (SHFM) is a congenital limb defect affecting predominantly the central rays of the hands ± feet. SHFM is genetically and clinically heterogeneous with inter and intra familial variability in the clinical manifestations. Most cases are inherited in an autosomal dominant manner and a causative genetic alteration is detected in approximately 50% of patients affected. SHFM3 accounts for 20% of cases and is caused by tandem microduplications at 10q24. We report 2 cases of recurrent 4 limb ectrodactyly with phenotypically normal parents and 1 case of a female with SHFM3 and her difficult attempts at IVF due to an abnormally high number of affected embryos. Case 1: A brother and sister presented with isolated 4 limb ectrodactyly. Microarray analysis showed a *de novo* 10q24.32 microduplication, which was 434 Kb in size. Case 2: A mother with a child with 4 limb ectrodactyly was 16.5 weeks pregnant when abnormalities of the hands and feet were identified on fetal ultrasound. Microarray analysis confirmed the 10q24.32 microduplication, previously identified in the affected sibling. Case 3: The proband was a mother with SHFM3 and a microduplication at 10q24.32, approximately 540 Kb in size. Preimplantation genetic diagnosis of 16 embryos identified the microduplication in greater than 80% of the embryos. We speculate that SHFM3 may represent a subtype of ectrodactyly that exhibits a higher chance of gonadal mosaicism. Genetic counselling in *de novo* cases should reflect this observation. Further research of the complex inheritance of SHFM is essential to providing families with accurate counselling.

P18.45-S
Regulation of genetic information

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Background: Genetic information in Norway is regulated by The Norwegian Act of Biotechnology (2003). One of its purposes is to protect people against discrimination and stigmatization. According to the Act § 5-8 genetic information in terms of predictive, symptomatic or carrier status shall not be released to insurance companies or employers. **Purpose**: The purpose of this study was to gain new insight and broader knowledge regarding regulation of genetic information in Norway. The focus was how people from insurance companies, medical genetics, patient organizations and the Norwegian government experience the regulation of § 5-8. **Material and methods:** A qualitative method was used with in-depth interviews of seven participants. **Results:** The participants experienced the § 5-8 to be important. They stated that genetic information is private and can easily be linked to a person's identity. Genetic information can also easily be misused, misinterpreted and can result in healthy people thinking they have a diagnosis. Despite challenges associated with enforcing the paragraph, the participants believed that it has a major role in protecting people from discrimination and stigmatization. In terms of new technology and commercial forces they also thought the paragraph will be important. **Conclusion:** The paragraph 5-8 has a high significance for the participants. It will also be important for fu-

ture perspectives to prevent people from discrimination and stigmatization. In order to protect human rights, it is important to inform patients about the regulation.

P18.46-M
Chromosomal mosaicism in chorionic villi: implications for prenatal diagnosis and genetic counseling

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Chromosomal mosaicism in chorionic villi (CVS) can be either confined to the placenta (CPM) or generalized to the fetus (TFM). The probability of TFM depends mainly on timing and mechanism generating the mosaicism. Due to the variable distribution of the abnormal cell line, when a mosaic is detected in CVS a confirmatory amniocentesis should be performed to discriminate between CPM or TFM. We present a diagnostic experience on 52,673 CVS combining cytogenetic analysis of cytotrophoblast and mesenchyme and, in case of CVS mosaic, on amniocytes. A CV mosaicism was found in 1.81% of CVS. The stratification by category of chromosome abnormality indicate that mosaics involving 47,+mar or sex-chromosome aneuploidies have the highest risk of TFM (35.8% and 31.6%, respectively) while autosomal trisomies and 46,der karyotypes have a lower risk (6.9% and 5.2%, respectively). We will present the risk of TFM stratified by type of chromosome abnormalities distinguishing mosaic (MA) and nonmosaic abnormalities (NMA). Genetic counselling is challenging in case of CVS mosaicism and the need for data to calculate a risk of fetal involvement is vital to refine a personalized chromosome abnormality-based strategy of investigation to reduce the need for follow-up amniocentesis. While prenatal diagnosis is destined to become "molecular" with microarrays, NIPS and NGS, the large cytogenetic diagnostic experience on CVS presented in this study is helpful to evaluate limits and advantages of the new technologies that must be integrated in pretest and post-test counseling.

P18.47-S
Realising Genomics in Clinical Practice- Whole Exome Sequencing and the Patient Pathway

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Whole genome sequencing (WGS) and whole exome sequencing (WES) have been described as transformative technologies with potential to revolutionise patient care. As part of a bigger project addressing the ethical, legal, social (ELSI) and operational challenges likely to be raised by these technologies 'Realising Genomics in Clinical Practice', the PHG Foundation convened a workshop to explore the impact of these technologies on patient pathways. Key features such as the point and source of referral for sequencing will influence how WES/WGS will be translated into clinical practice. By comparing patient pathways currently in use in targeted approaches and whole exome sequencing, our aim was to illustrate the key operational differences and translational aspects that are likely to arise. In particular, we focused on three areas which seem to raise distinctive ELSI challenges: the nature and scope of consent processes and the degree to which they might need adaptation; technical aspects including the scope, construction and standardisation of data filters, and requirement for data sharing in order to validate and interpret findings; and the extent, timing and nature of disclosure of test results, including incidental or unsolicited information. We review our findings and evaluate the prerequisites for effective translation, including requirements for additional genetic counselling, and the educational and training needs of health care professionals, clinical scientists, patients and publics. These findings will be contextualised by presenting preliminary results from the entire Realising Genomics Project. Further information on the Realising Genomics project can be found at http://www.phgfoundation.org/pages/realising_genomics.htm

P18.48-M
Revisiting Hook: rates of fetal aneuploidy and other significant genetic aberrations in women presenting for prenatal care

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To date, health care providers have relied on modelled values to counsel pregnant women regarding their age-related risks for common aneuploidy syndromes, particularly in younger maternal age categories. The goal of this study was to provide a contemporary update using actual data from a single laboratory to derive specific age-related risks for common aneuploidies as well as significant non-aneuploidy fetal genetic aberrations.
QFQ-banded karyotypes from prenatal samples analyzed at a single biomedical from 1994-2012 were analyzed. Selected for study, were samples in which the only indication for karyotype was either maternal age ≥ 35 or maternal anxiety (patients <35 years) with exclusion of additional risk factors, such as maternal serum markers or ultrasound anomalies.
129,263 karyotypes from villi and AF were analyzed. As expected, autosomal aneuploidies and 47,XXY show a statistically significant positive association with maternal age while MX shows a borderline negative association only for CVS. Risk of non-aneuploid anomalies compromise a substantial share of chromosomal abnormalities in younger women. We present the rates of fetal aneuploidies and other significant chromosomal anomalies stratified by two periods of gestational age and, when applicable, by maternal age, which have been observed in real clinical practice. As standard prenatal screening strategies currently do not detect these non-aneuploidy DNA aberrations, the data presented in this study is critical for informed patient decision-making which should be a routine part of genetic counseling in the prenatal setting.

EMPAG EDUCATIONAL SESSIONS

EES1.1
Responding to guilt and shame in clinical consultations

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Genetic medicine is an area of health care that often deals with delivering profound and difficult information; it brings clinicians directly in contact with individuals and families who sometimes have to make very difficult and life-changing decisions. Genetic counselling involves the skill of giving information, advice and support to help guide patients through this process.
Adjusting to information and assimilating new knowledge can have significant implications for the life decisions patients make. It is now recognised as normal for the course of adjustment to involve a range of emotional responses including denial, sadness, anger and guilt before the stage of acceptance is reached. This process can take time, individuals can vary in presentation and sometimes they can get 'stuck' often resulting in unhelpful behavioural consequences and increasing distress. In order to be effective clinicians need to understand this process and be equipped to recognise and respond to emotional responses that may occur within routine consultations.
This workshop looks specifically at the nature of guilt and shame in the context of genetic medicine. Drawing upon a cognitive behavioural model of emotion we will consider how the difficult and corrosive emotions of guilt and shame can be understood as an attempt by the individual to assimilate information and actions into their pre-existing belief system.
Drawing on a combination of theory, reflection on clinical examples, and illustration by video, participants will be encouraged to consider how they can develop their clinical interview skills to identify and respond to expressions of guilt and shame in routine clinical settings.

EES2.1
Qualitative and quantitative methods in psychosocial research

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This presentation will provide an overview of qualitative and quantitative methods in psychosocial research in the genetics setting. In the first part of the session, we will cover three distinct types of qualitative research that have been used in psychosocial research in genetics: thematic analysis; discourse analysis; and deliberative approaches. Each approach is useful for particular kinds of research questions and problems. Case studies will be presented to illustrate the utility of each method, and the specific research questions it is able to address. Case studies will be drawn from the domains of genetic testing and counselling, popular representations of genetic risk, and research participation in biobanks.
The second part of the session will cover the key concepts and study types used in quantitative methodology, including survey studies, case control studies, cohort studies and randomised controlled trial, as well as examples of quantitative studies that may be carried out in the genetics setting to illustrate strengths and weakness of different types of designs. This will be followed by an introduction to the most commonly used validated instruments suitable for measurement of genetic counselling and testing outcomes. Finally the session will cover several health psychology theories that are particularly relevant to the genetic counselling and testing setting and may be used to provide the basis for formulation of study hypotheses and interpretation and analyses of findings.

EMPAG PLENARY LECTURES

EPL1.1
The impact of total gastrectomy upon e-cadherin carriers: experiences of eating

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Between 30%-50% of cases of Hereditary Diffuse Gastric Cancer (HDGC) are caused by mutations in the E-cadherin gene. CDH1 mutation carriers have an earlier than average age of disease onset, and greatly increased risks of developing stomach cancer. Individuals identified as at-risk, either because of their family history or as a result of DNA testing, need to make decisions about risk management, whether they will have risk-reducing surgery (total gastrectomy - RRG) or continue screening. This retrospective study interviewed 42 patients, 27 of whom had undergone RRG. In this paper we will reflect upon the impact of surgery on bodily integrity and look at people's experiences of living without a stomach. The paper will focus upon eating post surgery, and discuss the ways in which surgery impacts upon identity. We will demonstrate that following surgery, hunger and satiety are constructed as disembodied experiences or desires that need to be re-embodied. Finally, we will argue that the process of re-embodying these supposed „physiological" states raises a number of issues about the nature of hunger and satiety. These will be interrogated using an analytic framework in which internal states are understood as grounded within public criteria.

EPL1.2
Impact of rapid genetic counselling and testing on primary surgery and psychosocial well-being in newly diagnosed breast cancer patients: Findings from a randomized controlled trial

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Aims Female breast cancer patients carrying a BRCA1/2 mutation have an increased risk of contralateral breast cancer. We investigated the effect of rapid genetic counselling and testing (RGCT) on treatment decisions and psychosocial well-being. **Methods** Newly diagnosed breast cancer patients from 12 Dutch hospitals with at least 10% risk of a BRCA1/2 mutation were randomized to an intervention group (offer of RGCT) or a usual care control group (ratio 2:1). Study outcomes included uptake of direct bilateral mastectomy (BLM), cancer-specific distress, anxiety, depression, and health-related quality of life (HRQOL). Assessments took place at study entry, and at 6 and 12 months follow-up. **Results** Between November 2008 and December 2010, we recruited 265 women. Based on intention-to-treat analyses, no significant group differences were observed in percentage of patients opting for a direct BLM (14.6% (RGCT group) versus 9.2% (control group); OR 2.31; CI 0.92-5.81; p=0.08). Per-protocol analysis indicated that patients who received DNA test results before surgery (59/178 women in the RGCT group) opted for direct BLM significantly more often than patients who received usual care (22% versus 9.2%; OR 3.09, CI 1.15-8.31, p=0.03). No statistically significant differences were observed between groups over time on any of the psychosocial or HRQOL outcomes. **Conclusions** These results suggest that RGCT can be safely offered to newly diagnosed high-risk breast cancer patients. However, DNA test results need to be made routinely available pre-surgery in order to play a more significant role in surgical treatment decisions.

EPL1.3
Disclosure of psychosocial research results: a randomized study among GENEPSO-Ψ cohort participants

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Background: The disclosure of aggregate research results to long-term cohort participants is rarely done and no recommendation specifies the best way to achieve this ethical obligation. GENEPSO-Ψ cohort follows both *BRCA1/2* carriers and non-carriers to study their psychosocial and preventive behaviour. Our aim was to study the impact of different formats of dis-

closure document.
Methods: Among the 454 respondents to the 5- and/or 10-year GENEPSO-Ψ questionnaires, 76% wished to receive "*information about the survey results*". They were randomized in a 2x2 factorial design to study the impact of adding to the psychosocial research leaflet: 1) an up-to-date medical information sheet, 2) a photography of the research team with the names of the researchers.
Findings: Providing additional medical information did not change outcome measures. On average (possible range[0-10]) the document was considered satisfactory (6.8), interesting (7.0), useful (7.3), and understandable (8.8). The information provided on medical findings was regarded as insufficient by 35% of the sample but the document was regarded as more often increasing (28%) than decreasing (2%) trust in research; average trust in medical researchers was high (75.2, possible range[0-100]). The additional photography only increased the rate of people feeling the information about the research team was sufficient (90.1% vs 80.7%, p=0.041).
Discussion: Adding medical information to the leaflet presenting the psychosocial research results did not significantly change satisfaction and trust of cohort participants. The lack of huge recent advances regarding prevention among *BRCA1/2* carriers may explain their relative disappointment. Predictors of dissatisfaction will be further studied.

EPL1.4
Prevalence and detection of psychosocial problems in cancer genetic counseling

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Introduction: Although only a minority of individuals undergoing cancer genetic counseling experience high levels of distress, many experience a range of specific psychosocial problems related to genetic counseling. The aim of this study is to evaluate the prevalence of these problems, and to investigate which method for detecting psychosocial problems is most optimal during counseling. **Methods:** Individuals undergoing genetic counseling for cancer were invited to complete a questionnaire including the Psychosocial Aspects of Hereditary Cancer (PAHC) questionnaire, the Hospital Anxiety and Depression Scale (HADS) and the Distress Thermometer (DT) prior to, or immediately following, their counseling session. **Results:** The most frequently reported problems of the 137 participants were on the PAHC-domains 'living with cancer' (84%), 'family issues' (46%), 'hereditary predisposition'(45%), and 'child-related issues'(42%). Partial correlations between the PAHC, the HADS and DT were low. Previous contact with a psychosocial worker, and a previous cancer diagnosis were significantly associated with higher distress on the HADS, but explained little variance (9%). No variables were associated with the DT. Previous contact with a psychosocial worker, and having children were significantly associated with several PAHC domains, but explained a small percentage of the variance (2-14%).
Conclusion: The large majority of counselees experience specific problems related to cancer genetic counseling. No variables were identified as important predictors of distress or psychosocial problems. To detect experienced psychosocial problems, we recommend that all counselees complete a brief problem-oriented questionnaire like the PAHC, and not only a questionnaire measuring distress, as a routine part of cancer genetic counseling.

EPL1.5
Developing a group programme for BRCA1/2 mutation carriers who underwent prophylactic mastectomy

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Introduction - Prophylactic mastectomy in BRCA1/2 mutation carriers reduces the breast cancer risk significantly, but may have a profound impact on body image and self-esteem. In addition, some women report feelings of isolation and stigmatisation. Other issues to face are an increased risk for ovarian cancer, potential cancer risk for offspring, intimacy with the partner, communication with family and ongoing grief. **Methods -** As a result of our long-year studies on impact of prophylactic mastecomy a group intervention was developed with regard to the supportive-expressive needs of these women. With a maximum of 10 members, a closed 8-sessions group programme was started focussing consecutively on the following themes: 1) introduction 2) body image 3) social support and coping 4) social support and loss 5) partner relationship or dating 6) ovarian cancer risk 7) communication within the family 8) evaluation and future plans. **Results -** Seven women participated in the first group aged 26-52. The older women advised the younger and vice versa, for example with regard to mother-daughter communication issues. The group members reported high satisfaction, particularly they felt they were no longer isolated and they could share their experiences and learn from each other. One woman who already had mastectomy for previous breast cancer, felt she was different from the others.
Conclusion - A next group will focus on a more homogenous population with regard to the experience of having had cancer yet or not. After refining content and structure of the programme an intervention study will be established.

EPL2.1
Ok for us, not for them: Patients and genetic counsellors' experiences of NIPT and views on wider use

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In the UK non-invasive prenatal testing (NIPT) is now routinely offered for sex determination of pregnancies at risk of sex-linked conditions, and technology is progressing towards use for diagnosis of single gene disorders and detection of aneuploidy. We present the findings of a two-arm qualitative study investigating both genetic patients' and genetic counsellors'(GCs) experiences of NIPT for sex-linked conditions and their views on wider use of the technology. Forty (20 patients and 20 GCs) semi-structured interviews were completed and transcripts analysed using modified grounded theory methodology.
While both groups expressed that they felt positive about the use of NIPT for pregnancies at known genetic risk, they had concerns about broadening its use in the routine antenatal setting, particularly relating to the early timing in pregnancy and the apparent ease of using the technology. Shared concerns included the difficulties of ensuring informed consent, potential misuse of the technology for non-medical indications, and a diminished acceptance of disability in society. Patients articulated a strong sense of distinction from the general public, which included their use and perspectives of the technology, based on their lived experience, prior knowledge of a genetic condition and sense of 'genetic' responsibility. GCs discussed concerns about availability of necessary knowledge and time to appropriately offer NIPT in stretched routine services to provide the counselling and support they felt patients required. These findings highlight the importance of discussing and considering the issues surrounding wider implementation of NIPT including differences between those with prior genetic risk and the general population.

EPL2.2
Non-invasive prenatal testing (NIPT): opinions and interest among pregnant women in a country with relative low uptake of prenatal screening

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Objective: To study pregnant women's current uptake of prenatal screening for Down syndrome, and future interest in non-invasive prenatal testing (NIPT).
Methods: Online survey on the Dutch pregnancy Fair website, completed by n=389 pregnant women.
Results: Uptake of the combined test (33%) corresponded to the average uptake in the Netherlands. Most important reason to have this test was reassurance that the child is healthy (57%). Reasons to decline included: 'the results are just a chance' (49%), 'fear of miscarriage due to follow-up invasive diagnostics' (34%). 230 (59%) had heard of NIPT; 48% were interested in having NIPT, 24% were unsure, and 28% were uninterested. Safety (33%) and accuracy (30%) were considered most positive aspects, and 70% were willing to pay for it (average 150 euros). 41% expected that women would deliberate less before taking the test, 23% expected more women to feel obliged to take it. Testing for other diseases was considered positive, mostly because this could avoid suffering (75%). However, 38% expected that women cannot foresee the consequences of their choices, and acceptance of children with a handicap will reduce (35%). 42% found that women should be able to choose from a list of diseases, 25% preferred packages with different diseases to choose from, and 30% preferred a fixed list (no choice).
Conclusion: The results suggest that more women will have prenatal screening if NIPT is to replace the combined test. However, challenges for counselling are expected if NIPT is introduced, especially when widening the scope of testing.

EPL2.3
Received information and knowledge about Down syndrome among pregnant women and their partners coming for a first trimester combined (CUB) test? - Do they have the knowledge to make the decision

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Fetal diagnostic testing for chromosomal abnormalities such as Down syndrome (DS) is frequently used in Sweden. Prior to testing expecting parents do not routinely receive information about the conditions being screened for. The aims were to assess why expecting parents choose to undergo CUB-testing (combined ultrasound and biochemistry test), their perception of information, thoughts about invasive procedures, possible termination of pregnancy and knowledge about DS.
Method: From November 2010 to March 2011, 105 pregnant women and 104 partners answered a questionnaire after completing the CUB-test, at the Fetal Medicine Unit, Uppsala University Hospital.
Results: The most common reason for choosing a CUB-test is "to get a confirmation on a healthy baby" or "because you should do it". A majority had not been given information on what it means to live with a child with DS and many requested more information. Internet is the most common source for information about DS. A substantial proportion of pregnant women and partners have little knowledge of the medical, cognitive and social consequences of DS. Twenty-four percent had not yet decided about invasive testing if increased CUB-risk and almost half had not decided what to do about the pregnancy if DS was diagnosed.
Conclusions: A majority of expecting parents attending CUB-test had not received information about DS and requested more information. A substantial proportion of expecting parents have varying and in several aspects low levels of knowledge about DS and its consequences. Many had not yet decided what to do if DS was diagnosed.

EPL2.4
Diagnosis Down syndrome: a cross-cultural study of family experiences

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Purpose: Much has been written about family-provider interactions surrounding the diagnosis of Down syndrome (DS) and substantial resources have been devoted to helping health care providers feel more prepared to deliver the diagnosis of DS. However reports of parents receiving inaccurate information continue to appear in the literature.Moreover, anecdotal reports of parents feeling pushed to make unwanted choices, such as undergoing invasive testing or terminating a pregnancy following the diagnosis of DS, are becoming more common. In addition, limited attention has been devoted to understanding how family experiences vary from one country to another. Therefore, the purpose of this presentation is to compare family experiences in four countries (Ireland, Portugal, United Kingdom, USA) where differences currently exist in terms of options for prenatal testing and options for families following the diagnosis of DS. Method: 1185 parents of individuals with DS completed a survey which includes a variety of questions concerning family-provider interactions surrounding the diagnosis. In addition, interviews were conducted with a subset of parents. Results: Findings suggest that 50% of the parents were dissatisfied with family-provider interactions and most health care providers are not following the recommended guidelines regarding how to inform parents. Additionally, important cultural differences were noted. Conclusion: Findings from this study will help in efforts to improve parental satisfaction with family-provider interactions surrounding the diagnosis of DS. In addition, they will help ensure that cultural context is considered during the informing process.

EPL2.5
Dynamics of prenatal screening: blurring boundaries between normative frameworks

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Historically, distinct prenatal screening programmes are aimed at finding different types of conditions: 1) maternal/fetal diseases or markers requiring intervention or adapted care in order to secure healthy pregnancy outcomes for mother and child; 2) fetal disorders that the prospective parents may regard as a reason for abortion. Ethically, this is an important distinction. There is a widely shared consensus that for screening leading to no other possible interventions but abortion, prevention is a morally problematic category. This is why official documents define such screening in terms of what may be called the 'autonomy paradigm', where the aim is to help individual women/couples make autonomous reproductive choices. This is also reflected in counseling guidelines. Whereas some degree of professional directivity is morally acceptable (or even required) for the first type of prenatal screening, non-directivity is regarded as absolutely essential for the second. However, this distinction is increasingly under threat. It was already blurred with the introduction of 'dual purpose' ultrasound screening, but it will only be further undermined with the present dynamics of genetic technologies. Think eg. of NIPT for both serious fetal disorders and for gene expression profiles that are predictive of pregnancy complications. The expanding scope of prenatal screening will also provide more options for fetal therapy. This presentation will consist of a systematic exploration of the ethical challenges involved in this blurring of frameworks and conclude with ethics guidance recommendations for the future field of 'prenatal personalized medicine' (Bianchi).

EPL2.6
Stigma and reproduction: the place of stigma in reproductive decisions

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This paper reviews the impact of a genetic disorder on family life, reflecting its mode of inheritance, and then examines the social impact of a specific sex-linked disorder, hypohidrotic ectodermal dysplasia. The stigmatisation of HED affected males is as important in the accounts given by their womenfolk as the physical effects of the condition; this impacts on their feelings about transmission of the disorder to the next generation. Perspectives may also change over time, with grandmothers expressing more strongly their sense of guilt at having transmitted the condition, despite there being no question of moral culpability.
We then consider the broader impact of stigma on reproductive decisions. They can be impacted by stigma both (i) within families where the practical effects of a specific genetic disorder are well known, and (ii) in couples faced by decisions in pregnancy with no prior expectation of a fetus affected by a genetic disorder. In the former case, any decision made has implications for the self-esteem of affected family members. In the latter case, decisions about continuing or terminating the pregnancy may be affected by the parents' remembered past and/or imagined future responses to encountering an affected individual. In this way, parents' fantasies may shape their decisions.
The scope for parental fantasies to influence decisions is greater when technology amplifies the uncertainty attained in genetic investigations or ultrasound imaging. How will parents' fantasies of uncertainty play out in practice? What can genetics professionals do to promote respect for affected individuals?

EPL3.1
How do research participants perceive "uncertainty" in genomic sequencing?

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Introduction: The scope of uncertainty in genomic sequence information has no rival in health care delivery. We present data from adults participating in an NIH genome sequencing cohort study where perceptions of uncertainty are hypothesized to be key in predicting decisions to learn and act on genomic health information. Methods: We conducted six moderated focus groups with 39 randomly selected ClinSeq® participants, varying whether they had coronary heart disease and/or prior receipt of sequence results. We elicited perceptions of the uncertainties associated with genomic sequencing using writing prompts. Results: Participants perceived the uncertainty as a quality of the information. The majority of participants characterized uncertainty of sequencing information as "changing, fluid, developing, or ground breaking." These responses led to anticipation of more optimistic future outcomes. Fewer participants described uncertainty as "questionable, less accurate, limited, or poorly understood". These perceptions seemed to undermine participants' faith in the information, leading to feelings of disillusionment. Discussion: Our findings suggest that perceptions of uncertainty are related to epistemological beliefs and thus expectation of the information. Interventions to promote realistic expectations of genomic sequencing may mitigate adverse responses to uncertainty.

EPL3.2
Discussing clinical utility; The role of patients and their families

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These last years, many studies investigate the clinical utility of whole exome sequencing as a diagnostic method. Clinical utility is most commonly established on the basis of data that show the effectiveness or economic value of an innovation. Given that whole exome sequencing is a very new method, however, a multidimensional approach to clinical utility seems more appropriate, which also investigates its acceptability for patients .
This paper presents how patients and their families assess the utility of whole exome sequencing, and the diagnosis it produces. Our case-study is based on 20 in-depth interviews with patients and their families, who are involved as participants in a research project which aims to assess the clinical utility of whole exome sequencing as a diagnostic tool for children with hitherto unidentified developmental delay.
The case study provides insight into why these patients and their families want a diagnosis, and how they value the diagnosis that they eventually get. Their evaluations reveal not just the acceptability of WES as a diagnostic tool, but provide insight into how diagnoses are evaluated with respect to their daily caring practice and social lifeworld. We will argue that this provides important input to an assessment of the clinical utility of WES, which may inform how WES is to become part of clinical routine, and how it should be accompanied by counseling.

EPL3.3
Variants in Practice Study (VIP): High risk women's responses to receiving genetic test results for genomic variants associated with breast cancer risk

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Results from genome-wide association studies (GWAS) have identified common genomic variants that form an important component of the heritability of breast cancer risk. Data from Victorian high risk breast cancer families demonstrate that testing for these genetic factors identifies a significant aetiological group known as 'polygenic families' and provides clinically important information.
It is important to investigate lay and professional understandings of novel, complex genetic test information generated by SNP testing to identify the most effective ways for genetic health professionals to communicate this information to patients and the treating medical team.
Our qualitative study aimed to **assess patient and healthcare professionals' understandings of genomic variant data. This presentation focusses upon women's expereinces of receiving SNP results following their participation in a GWAS study: Variants In Practice study (VIP).**
Forty women attended an appointment at the familial cancer clinic, Peter Mac, Australia. Women had 1. previosuly been diagnosed with breast cancer 2. undergone BRCA1/2 mutation testing (no mutation found). Subsequently they were genotyped for 22 common genomic variants from which breast cancer risks were calculated.
Analysis of interview transcripts has revealed a number of preliminary themes, including study participation is motivated by feelings of altrusim and specifically responsibility for family members. Receiving SNP information was viewed very postively, particularly by those women who had previously undergone risk-reducing surgery, who felt their decision was validated by their polygenic result. In conclusion, this study suggests that SNP results are regarded as useful information for both the research participants and their families.

EPL3.4
To Disclose, or Not to Disclose? The Context Matters

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Progress in understanding childhood disease using next generation sequencing (NGS) portends vast improvements in the nature and quality of patient care. Ethical questions surrounding the disclosure of incidental findings (IF) persist, as NGS and other novel genomic technologies become the preferred tool for clinical research. Thus, the need for multidisciplinary discussions and disclosure practices on the return of results in paediatric research has never been more immediate. The aim of this study is to explore the views of investigators concerning the disclosure of IFs in the paediatric oncology context. Our findings reveal at least four contextual themes underlying the ethics of when and how young participants and their families could be made aware of these unexpected results during the course of their research participation: clinical significance of the result, respect for persons, scope of professional responsibilities and implications for the healthcare/research system. Moreover, this study illustrates heterogeneity of standards and approaches within the broader researcher community, and the need to recognize the multiplicity of contextual factors that characterize paediatric cancer genetic research, specifically. As NGS increasingly becomes a centerpiece for innovative genetic research in paediatric oncology, sober thought should be given to the possibility of discovering IF, and to proactive and anticipatory management of resultant data that conforms to bioethical norms. The authors intend to broaden the scope of ethical disclosure practices for paediatric participants, their families and the investigators who recruit them.

EPL3.5
Comparing the views of Australian parents, paediatricians and genetic health professionals about disclosure of genomic results

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Background: Genomic chromosomal microarray (CMA) testing for childhood investigations has increased diagnostic yields. However, CMAs also increase detection of incidental findings (IFs) and variants of unknown and uncertain clinical significance (VUS). Elucidating patient disclosure preferences may help clinicians anticipate the type of results about which patients want to be informed.
Methods: A questionnaire, using hypothetical scenarios, was designed to investigate and compare the perspectives of parents, paediatricians and genetic health professionals for result disclosure. Quantitative data were analysed using ANOVA and Kruskal Wallis tests. Open text data were analysed using content analysis.
Results: 147 parents, 159 paediatricians and 69 genetic health professionals participated and at least 89% of respondents in each category certainly or probably favoured disclosure of VUS as well as variants of certain clinical significance, with the lowest percentage being amongst parents, who were less sure of their disclosure preferences. There was consensus among respondent groups that knowledge of a variant of certain clinical significance would provide more practical and emotional utility compared to VUS. Parents demonstrated some different perspectives to health professionals; for example, they placed more emphasis on using knowledge of a VUS when considering future pregnancies (K.Wallis:$p<0.001$).
Conclusion: This study, together with a previous study investigating the opinions of a subset of these respondents for disclosure of IFs,(1) is the first Australian exploration of preferences for genomic result disclosure, with implications for clinical practice.
(1) Turbitt E, et al. Availability of treatment drives decisions of genetic health professionals about disclosure of incidental findings. EJHG 2014;doi:10.1038/ejhg.2014.11

EPL3.6
The experiences and views of health care professionals and researchers regarding the feedback of results in the context of next generation sequencing in oncology

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Next generation sequencing (NGS) allows the production of large volumes of sequence data (and potentially genetic results) and the ethical and practical issues regarding feedback of results become particularly pertinent to address. Should (any) results be given to research participants? If so, which results and who should provide them? Within two EU funded projects in oncology (CAGEKID and EUROTARGET), in order to gather researchers' and health care professionals' views and experiences on providing results we distributed a questionnaire to attendees of genetics meetings in Europe in 2013.
Of the 95 respondents, 88% work as researchers and/or clinicians in a field related to oncology and half (52%) use NGS in some aspect of their work; 56% of respondents state that they provide specific information about NGS to participants or patients before enrolling them in a study or using their samples for sequencing. The majority, 83% had never received requests from physicians or patients for access to NGS data to inform treatment decisions. Regarding feedback of results in a research setting, 54% or respondents think that results stemming from NGS studies should be provided to individual participants and 72% think that actionable incidental findings should be disclosed to participants. Finally, 53% of respondents think that specific measures and/or limitations should be implemented for the sharing of NGS data/results with colleagues in the scientific community. Such empirical data from stakeholders is a valuable contribution to the ongoing discussion of how to responsibly handle and feedback results to patients and research subjects.

EPL4.1
Parental influences on decision making in Duchenne/Becker clinical trials

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Introduction: Parents' decisions about enrolling children in Duchenne/Becker muscular dystrophy (DBMD) clinical trials (CTs) may be influenced by hopes and expectations, which when unrealistic may challenge informed consent. We explore influences on decision-making. Methods: The study team used a community-based participatory research approach. Parents of children in DBMD CTs were recruited for an online survey through Parent Project Muscular Dystrophy and clinics. Participants viewed a series of benefit and worry statements and rated each statement's influence on decision-making, and how much they expected and hoped/worried about each. Results: From the first 61 participants, CT decision-making was influenced by potential altruistic and individual benefits: learning generalizable information (97% slightly to strongly agree), CT resulting in a drug that works (82%), better future for other children (82%), improved quality-of-life for their child (82%), and parent doing everything to help their child (82%). CT worries most affecting decision-making were: child bothered by side effects (39%), eligibility for another trial (36%), and child not liking the trial (29%). Expectation and hope ratings were strongly correlated with decision-making influence ratings ($r=0.5$ to 0.8, $p<0.01$). Associations between expectations and altruistic decision-making influences yielded the highest correlation values; conversely, associations between hopes and individual-benefit influences yielded the highest values. Conclusions: Anticipated CT benefits influenced parents' decision-making more than worries. The relationships among hopes, expectations and decision-making influences for altruistic versus individual benefits will be further explored, to inform a new conceptualization of opportunities and challenges during informed consent that extends beyond knowledge-oriented concepts such as therapeutic misconception.

EPL4.2
The impact on children and parents of participation in clinical research trials for Morquio A syndrome and Sanfilippo A syndrome

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Clinical research trials of enzyme replacement therapy for Morquio A syndrome and Sanfilippo A syndrome are underway. This qualitative study explored the impact of participation on 7 children with Morquio A syndrome and parents (5/9 eligible families) and parents of children with Sanfilippo A syndrome (4/6 eligible families). Face-to-face semi-structured interviews were carried out, the interviews were transcribed and key themes identified through interpretative phenomenological analysis. The main questions explored were: motivations for taking part, impact on life, and views on the information received prior to the trial. Children described the key role their parents played in providing them with information and supporting them through medical procedures. They talked about what was "good" (e.g. making new friends) and "bad" (e.g. fear of needles) about taking part. Many parents felt there was no real choice as current management options are limited. Some saw the trial drug as treatment rather than potential treatment. Most felt that too much complex information was given during the consent process, but discussions with the clinical trial team facilitated understanding and provided emotional and practical support. Parents described negative impacts on employment and family life. Positive impacts include an improvement in their child's condition and a more optimistic outlook for the future. Overall, children and parents felt the advantages outweighed the disadvantages, and would recommend taking part in a similar trial to other children/families. These findings provide an insight into the impact trials have on children and parents' lives and identify potential improvements for future clinical trials.

EPL4.3
Why do parents request carrier testing in their healthy children? A comparison of genetic health professionals' and parents' views

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Many parents want to know the carrier status of their other children following the diagnosis of a child with a genetic condition. However, the reasons behind their desires for this information have not been fully investigated.
The aim of this study was to explore the reasons parents want carrier testing performed in their healthy children and health professionals' understanding of these reasons.
Semi-structured interviews were conducted with genetic counselors and clinical geneticists (n=17), and parents (n=25) of children with one of three genetic conditions (cystic fibrosis, haemophilia and Duchenne muscular dystrophy). Inductive content and thematic analyses were used to compare genetic health professionals' and parents' accounts of the reasons parents want to know the carrier status of their healthy children.
Genetic health professionals expressed views about parents' reasons for requesting, including that parents primarily want carrier testing to reduce their own anxiety and be reassured. Several professionals indicated that some parents 'just need to know', and others acknowledged that generally parents request testing with their child's best interests in mind. In contrast, parents stated they primarily wanted genetic testing in order to convey the information to their children. Parents felt that disclosing carrier status to their children would allow the children to make informed reproductive decisions or prepare themselves for having an affected child.
This mismatch in understanding between the genetic health professionals and parents of the reasons parents want carrier testing in their healthy children has implications for genetic counselling practice.

EPL5.1
What is the role of genetic counsellors? A systematic review of evidence

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In many countries, genetic counsellors are employed in specialist genetic centres. However, in Europe, the role and practice of genetic counsellors is at a critical stage of development: in 2014 genetic counsellors will have their first opportunity to formally register via the European Board of Medical Genetics. The Board states that the genetic counsellor must fulfil a range of roles, including providing information and facilitating psychosocial adjustment of the client. To examine the extent to which genetic counsellors fulfil the prescribed roles, we conducted a systematic review of the published relevant scientific evidence using the method described by the Centre for Reviews and Dissemination. We searched five relevant electronic databases (Medline, CINAHL, SocIndex, AMED and PsychInfo) using relevant search terms and handsearched four journals for research-based papers published in English between 1 January 2000 and 30 June 2013. Of 419 potential papers identified initially, only seven satisfied the inclusion criteria for the review and all studies were conducted outside Europe. The findings indicate that where genetic counsellors are utilised in specialist genetic settings, they undertake a significant workload associated with direct patient care and this appears to be acceptable to patients. Genetic counsellors manage cases related to a wide range of conditions, predominantly where the diagnosis has been clearly established. With the increasing burden on genetic counselling services, there is an argument for the increased use of genetic counsellors in countries where they are under-utilised. However, further research on the roles of genetic counsellors in Europe is required.

EPL5.2
Referral for breast cancer genetic counseling among Turkish and Moroccan patients in The Netherlands

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Introduction: Turkish and Moroccans are the largest minority groups in the Netherlands. Migrant breast cancer patients are underrepresented in family cancer clinics. The present study investigates the referral for genetic counseling and DNA testing among Turkish and Moroccan breast cancer patients.

Methods: Breast cancer patients were identified as Turkish or Moroccan using a name-based approach. Data were ascertained from medical registries of 6 participating hospitals in Amsterdam and Utrecht, The Netherlands. All patients had been diagnosed with a new breast cancer in the years 2007-2012. A control group included non-Turkish/Moroccan patients from the same hospitals.
Results: In total, 156 Turkish/Moroccan patients have been identified. Preliminary results show that no information about the cancer family history was found in 13 (8%) patient files, and insufficient information was found in 77 (49%) medical files (i.e. report of breast cancer family history only). Approximately 35% (n=55) of Moroccan and Turkish breast cancer patients fulfil criteria for breast cancer genetic counselling, of whom 40 (73%) were aged < 40 years at diagnosis. A total of 31 (56%) were actually referred for cancer genetic counseling and testing. These results will be compared to a group of non-Turkish/Moroccan breast cancer patients.
Conclusion: Our study shows that a large group of Turkish and Moroccan breast cancer patients is eligible for genetic counseling and testing due to a young age at diagnosis. Over half of the eligible patients are referred for genetic counselling. Attention should be paid to the completeness of the registration of cancer family history in the hospital records.

EPL5.3
Genetic counselling for Indigenous populations: an exploratory study from the perspective of Australian genetic health professionals

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It is well established that cultural factors impact on the provision of genetic health care in a range of populations. Indigenous populations are thought to have particularly low levels of access to genetic health services, and cultural issues may be a contributing factor. We present data from the first study of genetic health service provision to Indigenous Australians. This qualitative study aimed to identify elements of culturally-competent genetic health service provision for this population group. Twelve semi-structured interviews were conducted with genetic counsellors and clinical geneticists from around Australia who had experience delivering services to Indigenous Australians. Participants were asked to describe their experiences and comment on collective cultural needs they identified, as well as training and resources for health professionals working with Indigenous patients. Interviews were audio-recorded and transcribed with thematic analysis conducted on the data. The findings show that participants were reluctant to generalise the needs of Indigenous peoples. Some participants asserted that Indigenous peoples have needs that differ from the general population, while others felt that there were no collective cultural needs, instead advocating an individualized approach. However, being flexible and practical, taking time to build rapport, recognising different family structures and decision-making processes, as well as other socio-economic factors were all identified as important factors in participants' interactions with Indigenous patients. This research has implications for international policy, training and practice, in addressing the needs of global Indigenous populations in the field of genetic health.

EPL5.4
Attitudes toward consumer-targeted genetic testing in Japan

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Background: Development in genomics have radically changed our self-understanding, and "we have become biomedicalized" (Pálsson 2007). In US, consumers feel the need for tools to estimate the promises and claims of genetic testing services (Green et al, 2011). Ministry of Industry in Japan is preparing best practice guidelines for consumer-targeted genetic testing, but there is no legal regulation against genetic discrimination. However, few studies on public attitudes exist in Japan. Purpose: This paper shows Japanese citizens' attitudes toward consumer-targeted genetic testing and its regulation and address broad ethical, legal and social implications in East Asia. Methods: We employed a web-based questionnaire survey to investigate general perceptions in 2012. In total, 14,718 Japanese citizens completed (RR=37.0%). Results: 14% of respondents knew consumer-targeted genetic testing and just 3.1% had purchased before. 48.6% showed interests to use susceptibility testing on life-style related diseases, 35.9% for congenital disorders and 30.8% for PGx testing. On genetic testing for children, 27-33.3% of respondents agreed to share results with schoolteachers for "personalized education". They show fewer interests in talent identification testing. 56.2% wished to ban genetic discrimination by law. Discussions: Compared with past studies in South Korea and Taiwan, Japanese respondents showed fewer interests. We could explore the reasons why Japanese haven't been "biomedicalized" in spite of its innovative position in Asian countries. We'll compare these results with newly obtained data in 2014.

EPL5.5
Predictors of adverse psychological reactions to receipt of direct-to-consumer genome-wide profiling results

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The purpose of this study was to assess predictors of adverse psychological reactions among direct-to-consumer genomic test consumers. We analyzed data from the Scripps Genomic Health Initiative, which studied 2,037 individuals who underwent the Navigenics Health Compass, a commercially available test yielding personalized risk estimates for 28 complex diseases. The participants completed baseline and follow-up survey measures assessing demographics, personal and family health history, attitudes toward genetic testing, anxiety (STAI), test-related distress (IES), and reactions to receipt of results. One hundred thirty participants (6.4%) were defined as having an adverse psychological reaction based on changes in STAI and/or IES. These participants reported significant changes in emotions ($p<0.0005$) and the way they thought of themselves ($p=0.032$) after receipt of results, as well as differences in level of concern about their health ($p=0.024$) as compared to the rest of the study population. However, this group did not endorse a different profile of pre-test concerns at baseline, including concerns related to learning about personal disease risk or to not knowing how they would feel about their results ($p=0.785$, $p=0.902$, respectively). Further, neither were the number of conditions for which participants had elevated risks, nor the actual estimated risks disclosed significantly different in these participants ($p=0.696$, $p=0.123$, respectively). In this study population, while participants' self-report of feelings and concerns upon receipt of results align with their psychological measures, neither self-assessment of pre-test concerns nor genetic risk estimate information disclosed serve as predictors of distress upon receipt of results.

EPL5.6
"It is a very lonely path": Exploring experiences of establishing a genetic support group in Victoria, Australia

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The diagnosis of a genetic condition can be life changing. Genetic support groups have an important role in providing mutual or peer support, advocacy, and assisting in public and professional education for families affected by a genetic condition. People may be motivated to start a support group for a variety of reasons, but this can be difficult, and there has been little prior research conducted on the process. This research, which was part of the Master of Genetic Counselling program at the University of Melbourne, aimed to explore how members of the Genetic Support Network of Victoria (GSNV) experienced establishing or attempting to establish a genetic support group in Victoria. Seven semi-structured, in-depth interviews were conducted with nine participants. Using a narrative analytic approach, a number of concepts and themes were identified: participants found that setting up and running a support group could be lonely; they experienced being a support person for others as confronting; and felt they needed to acquire skills to help them establish their group effectively. Participants also needed mutual support and information from a genetic support group, and worked in partnership with health professionals and peak organisations to establish their group. These findings have implications for genetic counsellors who have skills in providing emotional support, training, and facilitating the running of, and access to, support groups. This research also suggests a role for the GSNV in providing practical assistance to those who wish to start a genetic support group in Victoria.

EPL6.1
Co-designing an Intervention to facilitate family communication about inherited genetic conditions (IGC).

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Introduction: Many parents experience difficulties in talking to their children about an IGC that affects their family. Parents often want to talk to their

children but are unsure about what to say, when to say it and are concerned about their child(ren)'s reactions. As a result, children may be given little or no information and they are often afraid of upsetting their parents by asking. The silence that occurs about the IGC can be detrimental to the long-term mental health and well-being of parents and children. Methods: To facilitate better family communication about the IGC, we designed a therapeutic intervention based on multi-family discussion groups (MFDG) used in systemic family therapy. To inform the intervention's development, a series of focus groups for parents, children, young people and genetic counsellors were held. Findings: MFDG were considered important for facilitating family communication. Families suggested MFDG should be held in welcoming environments that simultaneously facilitate involvement in group activities but also provide distraction from the emotionally challenging subject matter. MFDGs should be attended by families with similar IGC but not necessarily the same so that they could relate to and learn from each others' experiences. Parents and young people agreed that all family members should attend the MFDG although parents only, should attend the first session. Desired outcomes included: a happier home life, design of a communication tool kit for families use and the development of informal networks. Conclusion: The newly designed MFDG's effectiveness will be tested using a randomised controlled trial.

EPL6.2

A randomised controlled trial of a genetic counselling intervention to enhance family communication - the GIF study

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Background: Disclosure of genetic information within families to at-risk relatives is extremely important, but often problematic. A genetic counselling intervention, delivered post consultation, may result in increased access to genetic services by family members. This Australian randomised controlled trial aimed to assess the effectiveness of intense genetic counselling follow-up on numbers of at-risk relatives utilising genetics services.
Methods: Participants (probands) (n= 95) were recruited when attending a genetic service for diagnosis or genetic testing. The intervention group (n=45) received three telephone counselling interventions while the control group (n=50) received usual care.
After 18 months, clinical files were audited to look for differences in the percentage of at-risk relatives who contacted genetics services. Analyses were adjusted for clustering within families.
Results: Overall, 142/554 (25.6%) at risk relatives in the intervention arm contacted genetic services, compared with 112/536 (20.9%) in the control arm (adj OR 1.30 95%CI: 0.70-2.42). Subgroup analyses of genetic categories revealed substantial differences in contact percentages: cancers - 28% (intervention) vs 15%(control); cardiac - 32% vs 44%; CF carriers - 10% vs 13% and 'others' (including FraX, translocation carriers, SMA, Duchenne) - 39% vs 10%.
Conclusions: The GIF genetic counselling intervention, specifically designed to enhance family communication was found to:
• remain congruent with principles of genetic counselling practice
• be delivered successfully by different counsellors
• improve the ability of clients to communicate genetic information effectively
Findings have implications for health professionals who wish to assist clients in effectively communicating new genetic information to at-risk relatives.

EPL6.3

"What would you like to know?" Patients' attitudes towards communication of incidental findings emerging from new sequencing technologies

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In the near future, testing of single or few genes is expected to be replaced by whole exome/genome sequencing (WE/GS). which raises controversies on communication and management of incidental findings. However, few studies have analyzed the perspectives of those expected to be more affected by WE/GS: patients undergoing genetic testing (GT). A semi-structured interview was submitted to patients undergoing clinical GT with the aim of exploring their attitudes toward communication of genetic alterations other than those specifically searched for. Eighty-two patients, 22.8% females and 77.2% males, aged 18-83 years, were interviewed between June 2013 and mid-february 2014. Only a minority (36.6%) stated they had heard of WE/GS before. The majority of interviewed (58; 70.7%) stated they would like to be informed of any alteration; this answer was not influenced by previous knowledge, marital status, gender, education, and purpose of testing, while showed a significant correlation with having children: outof 48 with no children, 22 (75%) desired to know any alteration compared to 22 out of 32 (68%) having children (p=0.04). Although less aware (only 18% reported knowledge of WE/GS), younger people (18-35 years) were more likely to want to know any alteration (86% versus 70% and 60% in people aged 36-54 and 55-83, respectively). The main reason for choosing to be informed of any alterations was the willing to have a clear knowledge of own risks (89.7%), while helplessness and fear of an unpreventable disease were the main motivations for those willing to be informed only of preventable diseases.

EPL6.4

Genomic investigations: health care professional (HCP) and family experiences of managing incidental information in clinical practice

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Background
The ability to analyse the genetic code in ever greater detail means that the possibility of clinically relevant findings, that are unrelated to the original reason for a test, is increased. These Incidental Findings (IFs), especially when identified in children, may not bring health consequences for many years. This greater sensitivity of genetic testing poses challenges to the clinical encounter including consent to, and disclosure of results.
Study aims
1.Identify current practice, including consent and disclosure practices, surrounding IFs in a range of clinical settings in the UK
2.Investigate family and professional experiences and views about the ethical and practical issues raised by the discovery of IFs
3.Inform policy on the consent and disclosure practices of IFs in clinical practice
Methods
The findings from 23 clinic observations and 52 in-depth interviews were analysed thematically.
Findings
4 main findings will be presented:
1.The possibility of IFs is currently not discussed in any systematic way at the time of genetic testing
2.More results with uncertain clinical significance are being reported. HCPs communicate these to families in different ways
3.There is no clear consensus from HCPs and families on what and how incidental information should be disclosed
4.HCPs believe that current systems do not facilitate the follow up of IFs long term
Conclusion
Further debate is required to integrate genomic technologies into medicine whilst addressing the ethical challenges, particularly as genetics is mainstreamed.

EPL6.5

„Very often the answer's not black or white": Exploring communication in paediatric clinical genetic consultations

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Background: A large proportion of clinical geneticists' workload includes investigations for children with developmental delay where the underlying cause often remains unknown. New diagnostic technologies provide hope for a diagnosis for many of these children. However, results generated through use of these technologies increases the complexity of communication and uncertainty during genetic consultations. To date there has been limited research into the process of paediatric genetic consultations. This project investigates the process and experiences for clinicians and parents during these consultations.
Methods: This qualitative project investigated consultations across four Australian states. Theoretical framework: Symbolic Interactionism - meaning is derived, created and modified through social interactions. Data: audio-recorded consultations (n=32), parent pre-consultation surveys (n=32), and post-consultation interviews with parents (n=32) and clinicians (n=11). Detailed microanalysis (content, thematic and discourse) was completed

across data sets for enhanced understanding, triangulation and analytical rigour.
Results: Overall, the content of the consultations was similar although clinicians appeared to have different 'styles' of communicating and interacting with families. Parents largely understood the information and had realistic expectations regarding diagnoses. Where expectations were not met, parents were often disappointed. Clinicians described many professional challenges working in this area, both practical and emotional, especially the frustration of frequently being unable to answer parents' questions regarding the cause of the child's delay.
Conclusion: Detailed analysis of three complementary data sources provided rigorous and unique perspectives on impacts of new genetic technologies for clinicians and parents. Findings from this study will inform best practice in this area of medical communication.

EPL6.6
Communicating oncogenetic information: do gastroenterologists and surgeons discuss heredity with their patients and, if so, what and how?

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Background: Doctors need to investigate if there is an indication for DNA-testing and provide their patients with information. We aimed to gain insight in discussion of cancer genetic topics by gastroenterologists and surgeons as part of an intervention study, comprising a checklist for doctors, intending to optimize referral to genetic counselling of patients visiting the Gastro-Intestinal Oncology Centre Amsterdam. Insight into physicians' performance can improve referral and optimize patient understanding.
Methods: Following a pre-post design, both before and after introduction of the checklist, 40 consecutive, new patients completed a short questionnaire assessing the discussion of cancer genetic topics during the initial consultation. Additionally, after introduction of the checklist, initial consultations were audiotaped for a qualitative analysis of the discussion of cancer genetic topics. Data on family history and referral was collected from medical files.
Findings: Discussion of cancer in the family increased from 78% before, to 90% post-intervention (p=0.11). However, doctors infrequently asked about second-degree family members (pre: 50%; post: 65%; p=0.19) and age at which family members got cancer (pre: 57%; post: 70%; p=0.29). Qualitative analysis of the audiotapes indicated the use of multi-interpretable and vague questions.
Discussion: Contrary to expectations, cancer in patients' family members was discussed in most intake consultations. However, the suboptimal quality of the discussion hampers optimal referral for genetic counselling. Development of an alternative intervention might help better discussion of cancer genetic topics. Education for doctors is needed to improve knowledge and discussion of cancer genetic topics.

EPL7.1
Consent and confidentiality in clinical genetics: a qualitative study

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Background: Should healthcare professionals (HCPs) have a responsibility to ensure patients' relatives are aware of genetic risk? What kind of consent is required before genetic information is shared? And how might sharing information affect patient confidentiality? The UK has national guidelines regarding these matters. We explored HCPs' and patients' views.
Method: We conducted 13 HCP focus groups (n=60) and 31 patient interviews in the UK. Data were analysed thematically.
Findings: HCPs and patients felt genetic information should ideally be confidential to families, not individuals. Keeping individual confidentiality could constrain relatives' comprehension of risk and ability to make informed decisions about testing. But familial confidentiality and sharing information was difficult for HCPs to put into practice, due to limited time and practical resources and concerns about disrupting family dynamics. Sharing information across genetic services was also problematic, because of variations in how consent was documented and what it entailed, e.g. some genetic services gave patients an explicit choice of whether HCPs could share information with relatives, while others used an opt-out system where permission to share was assumed. Few patients remembered consenting and the options given.
Conclusion: Sharing information and familial confidentiality were viewed positively by most participants, but not consistently put into practice by HCPs. We therefore show that guidelines are not yet fully integrated into clinic. These issues will become more pertinent as increasing amounts of data about patients' relatives are produced though genome-wide testing. Ethical tensions warranting further research will be highlighted.

EPL7.2
Autonomy and emotions: Professional challenges in seeking consent to genetic testing

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Informed consent is the ethical and legal bedrock of clinical practice and research involving humans. This paper investigates consent in practice by exploring how professionals account for consent to microsatellite instability and immunohistochemical testing for features of Lynch Syndrome and biobanking for research purposes.
Twenty eight semi-structured interviews were undertaken with professionals responsible for seeking consent. Thematic mapping was carried out on transcribed data, which led to the identification of themes for more detailed analysis. Data were examined from a rhetorical discourse analysis perspective, which involved micro-examination of the discursive devices drawn on by participants in their talk.
Two themes encompass the communicative difficulties that were described: Autonomy (enabling choice) and handling emotional responses in interaction. Challenges pertaining to enabling choice involve different forms of opposition to respecting the autonomy of the person giving consent, both within and beyond the individuals involved in communication. Issues concerning handling emotional responses in interaction involve accounts of strong emotions both of those asked to consent and also of the professionals involved. These two themes were unevenly balanced between the settings with challenges of handling emotional responses almost entirely confined to bio-bank settings. This is likely to reflect the contexts of current and previous family experiences. In both settings, enabling autonomy becomes complex when the professional is not involved in a face-to-face interaction with the person asked to give consent. This work brings an alternative perspective to accomplishing consent, as an interactive process involving complex moral negotiations within relationships.

EPL7.3
Randomized controlled trial of a telephone-based peer support program for female carriers of a BRCA1 or BRCA2 mutation: Impact on psychological distress

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Objective: To assess the effectiveness of a telephone-based peer-delivered intervention, in reducing distress among female *BRCA1 or BRCA2* mutation carriers. The intervention consisted of trained peer volunteers contacting women multiple times over a four month period to provide informational, emotional and practical support.
Methods: 337 participants completed the baseline questionnaire and those reporting interest in talking to other mutation carriers were randomized to either usual care group (UCG) (n = 102) or intervention group (IG) (n = 105). Two follow-up questionnaires were completed: i) four months after randomization (Time 2, intervention end for IG) and ii) two months later (Time 3). Outcomes included breast cancer anxiety (primary outcome), unmet information needs, and cognitive appraisals about mutation testing.
Results: Over the study period, there was a greater decrease in breast cancer anxiety in the IG than UCG (p<0.01) and at Time 2, the IG's mean breast cancer anxiety scores were significantly lower than the UCG's. There was a greater reduction in unmet information needs in the IG than UCG (p<0.01), with unmet needs lower in the IG than UCG at Time 2 (p<0.01). There was a greater reduction in cognitive appraisals-stress in the IG than UCG (p<.01) with significantly lower scores found at Time 2 for the IG compared to UCG (p<.01). However cross-sectional differences were not found at Time 3 for any outcome measure.
Conclusion: The intervention is effective in reducing breast cancer related anxiety and unmet information needs in the short-term. Identifying strategies for prolonging intervention effects is warranted.

EPL8.1
Women's experiences following a prenatal diagnosis of fetal abnormality: The PeTALS project

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Introduction In Victoria, Australia, following prenatal diagnosis of a fetal abnormality, women and their partners are commonly offered a choice about whether to have an abortion or continue their pregnancy.
Objectives The PeTALS project aims to explore the psychosocial impact of prenatal diagnosis and identify professional and social supports utilised and needed at this time.
Methods This study is being conducted at 3 sites including two tertiary metropolitan hospitals and a private ultrasound clinic. A longitudinal case study approach is being used to collect questionnaire and qualitative data from women at three different time points – 6 weeks post definitive diagnosis of fetal abnormality, 6-9 months later, and 2 years post-diagnosis.
Findings 39 women were interviewed at the first time-point, regarding their experience of receiving a prenatal diagnosis (27 had various chromosomal aneuploidies, 6 had cardiac anomalies, 6 had other structural anomalies), and choosing to have an abortion (n=33), or continue their pregnancy (n=6). Women commonly experienced significant grief and overwhelming sadness; many described intense feelings of isolation from their partner, family and friends. Women who had an abortion described feeling negatively 'judged' and reported that their partners also experienced significant emotional impact.
Conclusions Women describe variable and sometimes inadequate levels of follow-up bereavement care and support. There is a need for increased support and counselling for couples following prenatal diagnosis. Providing prenatal testing and abortion in the absence of a full range of supportive options may be considered unethical; this is an important area for ongoing research.

EPL8.2
Experiences of young Huntington's disease carriers and their partners solliciting a prenatal and/or pre-implantation genetic diagnosis: a qualitative study

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Introduction: Huntington's disease (HD) is an autosomal dominant, incurable, late onset neurodegenerative disorder. There is an increasing demand for prenatal or pre-implantation diagnosis by couples in which one partner is a HD carrier and who want to avoid transmission of the disorder to their children. This exploratory qualitative study aims to gain insight into the decision-making process regarding reproductive options by young HD carriers and their partners, and into the way prenatal testing is being experienced. Materials and methods: Using thematic analysis, ten semi-structured interviews were conducted in HD carriers aged between 18 and 30 years, to document their attitude on and experience with the decision-making process that preceded their request of a prenatal or pre-implantation genetic diagnosis. Following topics were addressed: 1/ Which issues arose in the decision to have children?; 2/ Which issues arose in the decision to have prenatal testing (PD or PGD); 3/ How did you experience the actual procedure ?; 4/ How do you see the future with your children? Results: For young HD carriers not to choose for prenatal testing is not an option, nor is refraining from offspring. With regard to the emotional impact, major differences in the lived experience of PD and PGD are reported: the prospect of a termination of pregnancy causes great distress in women. Conclusions: With this exploratory study we gain understanding in the complex psychological process linked to procreative decision-making of young HD carriers and their partners that opt for a prenatal or pre-implantation diagnosis.

EPL8.3
Difficult decisions in prenatal diagnosis - patients' experiences of decision-making under uncertainty, and the implications for expanding the offer of prenatal testing.

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The practice of fetal anomaly detection has progressed tremendously from the first diagnoses of anencephaly by x-ray to modern 4-D imaging techniques and chromosomal microarrays. Although these new technologies will increase the range of abnormalities that can be detected prenatally, the amount of uncertain findings will also increase.The current practice of fetal anomaly detection has largely developed as the result of rapid transfer of research developments to practice with limited prior ethical reflection. To date, although "uncertainty" is often cited as a potential drawback to more detailed genetic testing in the prenatal setting, there has been little study of the impact of uncertainty on the decision-making process of patients. In order to gain insight into these processes and to better understand how to approach the ethical evaluation of new fetal anomaly detection technologies, a qualitative study was undertaken. In-depth interviews of 26 participants who had received an uncertain prognosis in their pregnancies were conducted. These interviews identified a number of key themes including managing information, values and decisional context, and trust. The findings raised a number of questions, regarding for example the usefulness of the notion of autonomy as a primary ethical principle in the prenatal context where parents must make decisions based on unclear and often changing information. These findings have implications for how new fetal anomaly detection technologies are evaluated and put into practice to ensure that the maximum benefit is achieved with the minimum harm.

EPL8.4
Offering a choice between 5 Mb and 0.5 Mb prenatal whole genome SNP array analysis: are pregnant couples able of making informed decisions?

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Background: We implemented whole genome SNP array instead of conventional karyotyping (CK) for prenatal diagnosis (PND). Array detects more clinically relevant anomalies and more genetic anomalies of yet unquantified risk for neurodevelopmental disorders, so-called susceptibility loci (SL). Using SNP array ensues the question whether pregnant couples want to know more and oversee the possible consequences. We explored whether pregnant couples at increased risk for aneuploidies were capable of informed decision-making when offered a choice between the clinical outcomes of a SNP array analyzed at 5 Mb resolution (comparable to CK) and a SNP array analyzed at 0.5 Mb resolution with or without SL. **Methods:** Consenting pregnant couples (N=143) received genetic counseling by phone and filled out the Measure of Informed choice (MIC) designed for this study. Choices based on sufficient knowledge and congruent with attitude were considered informed. **Results** *:* The MIC had sufficient internal consistency (Knowledge α=.74; Attitude α=.80). Median MIC knowledge score was 5, range 0-7. We considered a score ≥5 on the MIC knowledge scale as sufficient. Based on this criterion, 66% of the couples had sufficient knowledge and thus made an informed decision. However, 82% found it difficult to reproduce knowledge about SL. **Discussion:** Our results show that the majority of couples were capable of making an informed choice. However, the most complicated possible outcome of array, SL, was least understood. More research is necessary to assess what the psychological impact of SL is when couples have not well anticipated this possible outcome.

EPL8.5
SNP Array in prenatal diagnosis; first impressions on the psychological impact of receiving a susceptibility locus s a test result

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Background: Genomic SNP array as a first-tier prenatal cytogenetic test for all indications has been implemented in our laboratory. Array may also detect susceptibility loci (SL) for neurodevelopmental disorders, with an unquantifiable risk for the fetus. SL may psychologically challenge the couple in their decision whether or not to continue their pregnancy. Since we implemented SNP array in September 2010, our policy was to disclose the presence of SL. We conducted this study to explore the psychological impact of receiving a SL. **Methods** In our pretest counseling was no emphasis on the possibility of receiving a SL. Twelve pregnant couples whose fetus was affected with a SL were asked for participation postnatally. Two couples and six men refused participation. Consenting participants (*N*=13, four men and nine women) were interviewed by phone. The interview was structured with a fixed set of questions. **Results** Receiving their SL was shocking to 10 out of 13 participants. Postnatally, all participants considered their child healthy without concern about the SL. They stressed the importance of a pre and posttest counseling and their wish for a choice regarding SL disclosure. Eleven participants indicated they wished to learn about their SL again. **Conclusions** Although our group is small, it is one of the first studies providing a preliminary insight into how couples experienced receiving a prenatal SL. Further studies have to be carried out in order to investigate the (dis)advantages of reporting SL in a prenatal setting, from medical as well as from psychological point of view.

EPL8.6

Professional views about prenatal aCGH-testing

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Background: Array-comparative-genomic-hybridization (aCGH) in pregnancy allows a higher detection rate and shorter turnaround time compared to karyotyping. However, the test can also reveal findings that are (a) not related to the reason for which the test was done (b) relevant only much later in life, and (c) uncertain. Little empirical data exist on health care professionals' (HCPs') views about these issues.
Aims: To explore the views of UK HCPs about: the type of information that should be sought and disclosed; timing of disclosure (i.e. during-pregnancy/after-birth/when the information is medically-actionable); and who should decide about these issues.
Methods: Q-methodology, which combines qualitative and quantitative approaches, was used to explore the views of 45 HCPs (Genetic-health-professionals, lab-scientists, fetal-medicine-experts).
Results: Preliminary data suggest that: HCPs either prioritise parental-choices, or their own judgements about clinical relevance to determine disclosure.
Most HCPs support aCGH testing in pregnancies for further investigations of abnormalities found on scans, but would not currently wish to offer it otherwise.
Where aCGH uncovers adult onset predispositions, many argue that these should be disclosed because (a) they might be relevant to one or other parent, and (b) they are not confident that systems can be set-up to delay disclosure to nearer the time of clinical relevance.
Conclusion: HCPs support the introduction of prenatal aCGH into clinical practice in certain circumstances, but are concerned that consent procedures, education of professionals outside of genetics and national-guidelines are not yet in-place to address all the ethical issues.

EPL9.1

Predictive testing for Huntington Disease: Lessons learned from 24 years' experience

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Predictive testing for Huntington disease commenced in Sydney, Australia, in 1990, at one genetics service. The same counsellor (a social worker) has coordinated and provided counselling for predictive testing at this service for 24 years. Over this time 554 people have been seen through the predictive testing process, with additional provision of social work assistance to the 19% of mutation carriers who have become symptomatic. This long term perspective has provided some valuable insights into dealing with the challenges of the process and impact of predictive testing. This paper presents our recommended procedures, based on lessons learned, for various aspects of the process including pre-result assessments, timing of test clinicians' knowledge of result, testing of minors, testing of siblings, protocol flexibility and the therapeutic relationship versus duty of care, and follow up. Utilisation of these procedures, together with expert counselling skills, is likely to achieve the aim of maximising client support and autonomy while minimising harm arising from the outcome of predictive testing for Huntington disease and similar late-onset neurogenetic conditions.

EPL9.2

Patient views on the delivery of predictive test counselling services for Huntington's Disease

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Huntington's disease (HD) is a progressive neurodegenerative disorder characterised by involuntary movements, cognitive impairment and psychological symptoms and is inherited in an autosomal dominant manner. Predictive testing by direct mutation analysis for individuals at risk of HD has been available since 1993. Internationally agreed guidelines for predictive testing have been published and recently updated but variability exists in *how* testing is delivered between centres. Whilst a substantive literature exists on the impact of predictive testing, few studies have looked at how the predictive test counselling process is received by tested individuals. This evaluation study sought the views of individuals who had a predictive test for HD at our centre over a 5 year period (2007-2012). 44 of 100 eligible individuals completed and returned a questionnaire designed for the purpose of the study. Descriptive statistics were used to present the quantitative data and a thematic analysis was conduced on the free text comments. Overall, participants were positive about their experience and valued getting information and support as well as building a good relationship with their genetic counsellor. However, 14/44 participants found the testing process too long and for 7/44 participants the journey time to the hospital took > 2 hours. Proposals for improving the service included a more tailored approach that took greater account of prior experience. In addition participants welcomed the inclusion of information resources such as video clips highlighting a range of testing experiences, and also advocated more focus on post test follow up and support.

EPL9.3

Quality issues in genetic counselling practice for presymptomatic testing: a European Delphi study

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Genetic counselling for presymptomatic testing is complex, bringing both ethical and practical questions. There are protocols for counselling but a scarcity of literature regarding quality assessment of such counselling practice. Generic quality assessment tools for genetic services are not specific to presymptomatic testing. Our aims were to identify aspects of effective counselling practice in presymptomatic testing for neurodegenerative disorders as a basis for developing a quality assessment tool. We used the Delphi method to ascertain the views of relevant European experts in genetic counselling practice. Ethical approval was obtained and panelists were anonymous to other contributors. Questionnaires were sent by electronic means to a list of 45 experts, who each contributed to 1-3 rounds (Medical Doctors, Geneticists, Genetic Counsellors, Genetic Nurses, and others). In the first round, we provided a list of relevant indicators of quality of practice from a literature review. Experts were requested to evaluate topics in four domains: a) professional standards; b) service standards; c) consultand's perspective; d) protocol standards. We then removed items receiving less than 65% approval and added new issues suggested by experts. The second round was performed for the refinement of issues and the last round was aimed at achieving final consensus on high standard indicators of quality, for inclusion in the assessment tool. The most relevant indicators were related to (1) consultand-centred practice and (2) advanced counselling and interpersonal skills of professionals. High standard indicators are being used to develop a new tool for quality assessment of presymptomatic testing counselling practice.

EPL9.4

Experiences and implications of young women undergoing predictive BRCA testing under the age of 30

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Background: This qualitative study focuses on experiences of a sample of young female BRCA carriers who had predictive genetic testing before the age of 30 and explores their motivations for testing and implications of receiving a positive (bad news) result.
Methods: Following appropriate informed consent procedures participants were recruited through the Cancer Genetics Service for Wales. Semi structured interviews were conducted face-to-face with seven participants. Interviews were transcribed in full and analysed using thematic analysis.
Results: The motives for testing and perceived advantages described by participants were similar to those identified in previous studies with older participants, such as increased awareness and knowledge, and feeling more in control. However some of the perceived disadvantages described by participants were specific to young women. These included feeling pressured to make important life decisions earlier than they would have liked, for example decisions about when/if to have children, and about risk reducing surgery. Participants also reported feeling abandoned or forgotten because of the loss of ongoing clinical contact or feeling 'stuck waiting' for screening to begin. None of the participants however, felt that these disadvantages were sufficient reason to regret having had the test at a young age.
Conclusions: Findings in this small study suggest that having BRCA predictive testing can have positive outcomes for young women. However they should be encouraged during pre test counselling to explore the decisions and choices they may be faced with in the event of a bad news test results and may benefit from ongoing support/follow up.

EPL9.5
The experiences of BRCA1/2 mutation positive women in Northern Norway

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This qualitative study explores how women who have been diagnosed with a mutation in BRCA1 or BRCA2 experience living with risk of cancer development, as well as their need for psychosocial and informative follow-up. The study also investigates whether learning and coping seminars (LCS) contribute to a greater sense of coping. Focus group interviews were performed with female BRCA1 and BRCA2 mutation carriers when they attended LCS. The interviews were performed immediately before and after the LCS. 17 women participated in two focus groups in two different LCS seminars (10+7). The following themes were discussed: Their personal reactions after being identified as mutation carriers, experiences with the risk management program, decision making and experiences regarding risk reducing surgery. In addition, the participants expectations and experience with participating at the LCS were discussed. They were also encouraged to expound upon any other issues important to them. The data were analyzed using content analysis as described by Knodel.
Preliminary results indicate that the participants had experienced random and different information regarding risk reducing surgery. They regarded the LCS as a valuable source for information and psychosocial support, and suggested that future LCS should be available for female BRCA1/2 mutation carriers shortly after receiving their unfavourable test results. Other themes as feelings of loneliness and fear of cancer development were also identified in this study.

EPL9.6
Genetic test declining and high personal colorectal cancer risk perception in DNA mismatch repair gene mutation families

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Purpose: About half of people from mutation-carrying families do not undergo genetic counselling and/or testing to identify their mutation status and risk of colorectal cancer (CRC). We studied perceived CRC risk and qualitative analysis of reasons for declining in this group.
Patients and Methods
We studied 26 participants (mean age 43.1 years, 14 women) in the Australasian Colorectal Cancer Family Registry who were relatives of mismatch repair gene mutation carriers; who had not been diagnosed with any cancer at the time of recruitment and who had declined an invitation to attend genetic counselling and/or testing at the time of interview. Bounded estimates of perceived CRC risk over the next 10 years, understanding of genetic testing and CRC risk, reasons for declining testing and self-reported colonoscopy screening were elicited during a face-to-face semi-structured interview.
Results: A sub-group of decliners (31%) unconditionally rejected genetic testing compared to conditional decliners who would consider genetic testing in the future. Mean perceived 10-year risk of CRC was 54% [95% CI 37, 71] in unconditional decliners, compared with the mean perceived 10-year risk of CRC of 20% [95% CI 5,36] in people who conditionally decline genetic testing. This difference remained after adjusting for potential confounding factors (age, gender and reported screening colonoscopy).
Conclusions: The unconditional decliner group perceive themselves to be at 3.26 times higher risk than conditional decliners. Novel interventions in general practice clinics may improve genetic testing uptake and/or appropriate colonoscopy screening for this high-risk and under-serviced group.

EMPAG POSTERS

EP01-S
Counselling issues arising from microarray in prenatal testing

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Microarrays have replaced conventional karyotyping for the detection of chromosomal imbalance for the large majority of cases in the prenatal setting. Our clinic began to offer microarray in 2010. The test was initially restricted to women found to carry a fetus with a structural abnormality on ultrasound, in accordance with studies that suggested an increase in detection rate of a chromosomal imbalance not detected by karyotype. More recent studies have examined offering microarray to all women undergoing invasive procedures for reasons such as advanced maternal age, increased risk on screening and parental anxiety. In our clinic, a large proportion of our clients are referred for increased risk counselling post screening. Within this group, many women undergoing invasive testing to exclude chromosome abnormalities have opted to have microarray with the view of greater detection of syndromes that have a significant clinical impact. Our counselling includes the risk of detecting a CNV of unknown or uncertain significance. Case examples will illustrate the complexity of information, coping with uncertainty, counselling issues and implications for practice.

EP02-M
Factors affecting PGD decision making in Israeli BRCA1/2 mutation carriers

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Aim: In 2006, the Israeli Ministry of Health issued directives to expand PGD to encompass hereditary breast-cancer syndrome. This study aimed to decipher the factors affecting PGD decisions among Israeli BRCA1/2 mutation carriers or a spouse of a carrier.
Methods: We used a qualitative-phenomenological approach. Participants were 19 Jewish Israeli women, who requested PGD for BRCA1/2 mutation detection for their future embryos, at the Sheba Medical Center. All women were either carriers (n=14) or spouses of a male carrier (n=5), of one of the three Ashkenazi founder mutations in BRCA1/2. Of carriers, six were diagnosed with breast cancer. All women are married, and all but three had at least one child. We invited the women to narrate what motivated their choice to opt for PGD. Thematic analysis was used to unveil experiences and preferences related to the issue.
Results: Three significant factors were found to be associated with PGD: Prior infertility treatment (n=12); The existence of frozen embryos after fertility preservation treatment, done before chemotherapy (n=5); and Family history of ovarian and/or early-onset breast-cancer (n=10). Although most women underwent PGD (n=16), the majority (n=12) ended by declining the selection process, primarily for clinical, bureaucratic and financial difficulties.
Discussion: This study details factors associated with the choice to undergo PGD, in order to prevent the passing on of the `bad` BRCA1/2 mutation. Although, PGD for BRCA1/2 mutation is allowed in Israel, it is not funded by the Israeli healthcare insurance.
This study was funded by the Israel Cancer Association grant.

EP03-S
Prenatal genetics service: the service users' perspective

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Prenatal Genetics is about to become yet more complex with the advent of array comparative genome hybridisation with some centers already having implemented this approach. Various research studies looking into the process of the prenatal genetic diagnosis interaction highlight that clinicians' and service users' can have different goals, purposes and values regarding prenatal testing. Informed patient decision making requires that information provision is responsive to patient interests. Clinical audit is one method to elucidate the needs of these service users. An advocate in the UK for this approach of information provision, support and care that is responsive to the current needs of service users is the national charity Antenatal Results and Choices (ARC), which provides non-directive support to expectant and bereaved parents throughout and after the antenatal testing process. ARC provides guidance on the principles of good practice when supporting parents through a diagnosis of fetal abnormality. The South West Thames Regional Genetics service is responsible for a population of 2.2 million people and undertakes approximately 400 prenatal appointments annually. To assess the ongoing needs of our prenatal service users, a clinical audit was conducted through the use of a questionnaire which was sent to 150 families. The responses were then corroborated with data from the clinical genetic notes. The collated results were annonymised for the purpose of analysis. This retrospective clinical audit will present areas of good practice already in existence and others which may provide opportunities to improve patient experience with the advent of emerging new genetic technologies.

EP04-M
An easy test but a hard decision: non-invasive prenatal testing and emerging ethical issues

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Non-invasive prenatal testing (NIPT) using cell-free fetal DNA in maternal serum can be used to test for specific genetic disorders. The aim of this study, part of the RAPID project, was to explore ethical issues around NIPT for single gene disorders. We used a qualitative cross-sectional design and recruited carriers of four autosomal recessive conditions: cystic fibrosis, thalassaemia, spinal muscular atrophy and sickle cell disease. Data were collected via focus groups or telephone interview and analysed thematically.
Parents were overwhelmingly in favour of NIPT: those with deceased children were especially keen to reduce the chance of fetal loss, while many valued knowledge of fetal status in the first trimester. Obtaining written consent was considered important to emphasise the potential impact of the results on the pregnancy and to avoid possible routinisation of the test, as parents reflected that although the test was easy, subsequent decisions could be hard. Parents felt that giving the mother time to discuss the test with her partner was required before blood was taken. Where fathers declined carrier testing, participants felt that mothers should be able to request a test, but the father should be aware the result might convey potentially unwanted information about his carrier status if the fetus was affected. While many of the ethical issues echo those for invasive testing, due to the ease of non-invasive testing, it is important that it does not become routine and that informed decision-making by parents is supported by professionals.

EP05-S
The phenotype and genotype of Bartter syndrome in Maltese patients

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We describe the clinical phenotype and genotype in a Maltese cohort with Bartter's syndrome.
Children with a genetic diagnosis of Bartter's syndrome until December 2013 were included. Gender, gestation, birth weight, occipitofrontal circumference (OFC), age at presentation,age and clinical phenotype at diagnosis, were documented.
Three female and one male were identified with age range of 2-15 years. All pregnancies were complicated by polyhydramnios and prematurity. Median gestational age was 33 weeks. Birth weight ranged from 1.5kgs-2.1kgs. All OFC measurements were above P90. Two children were diagnosed clinically at birth, one child at 2 years and one at 6½ years. One child has severe spastic diplegia from complications of prematurity and one child has delayed speech. All have a small triangular face, small chin and body weight along a low percentile. Two children have been diagnosed with nephrogenic Diabetes Insipidus. All have borderline low-normal potassium levels, normal eGFR and parathyroid hormone, and bilateral nephrocalcinosis. Molecular genetic analysis in the coding region of the KCNJ1 gene revealed a homozygous c.277T>G mutation in all patients; all parents are heterozygous for the same variation. A random sample of 100 Maltese and a 100 Belgian DNA samples, revealed the heterozygous state in 1 Maltese sample and none in the Belgian samples.
To our knowledge the c.277T>G mutation is reported once, in a compound heterozygote Italian patient. It would appear that the c.277T>G variation is a Maltese mutation since it presents in the homozygous state in all patients. Some phenotypic features are shared by all patients.

EP06-M
Attitudes of pregnant women and male partners towards non-invasive prenatal testing (NIPT) and widening the scope of prenatal screening

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Non-invasive prenatal testing (NIPT) and its potential to test for multiple disorders has received much attention. This study explores women's and men's attitudes towards NIPT, and their views on widening the scope of prenatal testing in a country with a low uptake of prenatal screening (the Netherlands). Five focus groups with low-risk pregnant women (n=28), three focus groups with men (n=19) and 13 interviews with high- and low-risk pregnant women were conducted. Participants felt that current prenatal screening has great disadvantages such as uncertain results and risk of miscarriage from follow-up diagnostics. Characteristics of NIPT (accurate, safe and early testing) could therefore diminish these disadvantages of prenatal screening and help lower the barrier for participation. This suggests that NIPT might allow couples to decide about prenatal testing based mostly on their will to test or not, rather than largely based on fear of miscarriage risk or the uncertainty of results. The lower barrier for participation was also seen as a downside that could lead to uncritical use or pressure to test. Widening the scope of prenatal testing was seen as beneficial for severe disorders, although it was perceived difficult to determine where to draw the line. Participants argued that there should be a limit to the scope of NIPT, avoiding testing for minor abnormalities. The findings suggest that NIPT could enable more conscious decision-making for prenatal screening. However, to ensure voluntary participation, especially when testing for multiple disorders, safeguards on the basis of informed decision-making will be of utmost importance.

EP07-S
Case Ilustrations of the utilization and uptake of NIPT-counselling and management issues

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Non invasive prenatal testing (NIPT) for aneuploidy using cell free DNA in maternal circulation has had a dramatic impact on prenatal screening and diagnosis. In Melbourne, Australia, NIPT is offered by several different service providers with out-of-pocket costs ranging from $500 - $1,200. Testing is largely performed in the USA and turn around times vary from 10 days to 3 weeks. Some centres provide clients with genetic counselling. At present, clients attending our public hospital may access NIPT as a first tier screening option or after first or second trimester screening. We have observed that many women who receive an increased risk result for Trisomy 21 will utilize NIPT before committing to an invasive test such as amniocentesis. Personal experiences that appear to influence this decision include IVF pregnancies, history of infertility, advanced maternal age, religious/spiritual beliefs and a desire to further clarify their risk. Counselling for this group of women requires significant time to explore the various pathways and possible outcomes, and the limitations and benefits of each.
Case examples will be used to illustrate our experiences with clients taking up NIPT, including management of false positive results, inappropriate use of NIPT and failure to achieve results. Decision making processes and counselling issues arising from NIPT will be explored.

EP08-M
Monitoring of congenital anomalies in the population of the Republic of Moldova

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In the Republic of Moldova, the monitoring of the CA using since 1991, and from 2009 we are working to incorporate our registry in the EUROCAT. Were studied the prevalence and sharing of CA on the basis of genetic monitoring for the period from 2008 to 2012. The overall prevalence of the CA for the 5-year period amounted to 18.92 per 1000 newborns. The maximum frequency of the CA was noted in 2008 - 20.3 per 1000 births, the lowest in 2009 - 18.36 to 1000 newborns. Prenatal screening of pregnant women using non-invasive and invasive diagnostic techniques has allowed to reduce the average frequency of the birth of children with CA for 7.2% to 17.56 per 1000 newborns. In the structure of the CA by prevalence leading place is occupied by CA of the musculoskeletal system (22.5 ± 2.58%), multiple malformations (22.02 ± 2.98%) and the CA of the circulatory system (19.12 ± 4.48%). The prevalence of individual nosological forms of CA (esophageal atresia, cleft lip/palate, omphalocele, Down syndrome) are consistent with those of the international register of EUROCAT. Results of questionnaire of 158 women given birth to children with CA showed that 85.6% of women do not take folic acid in the first trimester of pregnancy. 74.5% of the mothers drank alcohol during pregnancy. Influence of the teratogenic factors noted at 22.2% of cases.Monitoring data allow to plan and carry out preventive measures to reduce the birth rate of the children with CA in Moldova.

EP09-S
The impact of the perceived severity of genetic conditions on the attitudes towards genetic testing and termination of pregnancy

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Introduction: It is known that different factors (e.g. education, religion, severity of genetic conditions) may influence decisions regarding genetic testing and/or termination of pregnancy.
Objective: The aim of the present study was to investigate the extent to which the perceived severity of various conditions moderates the decision to opt for genetic testing and/or termination of pregnancy.
Methods: The study was conducted on adult couples from the general population in Romania. Participants were asked to complete questionnaires assessing the perceived severity of 30 conditions, the attitudes towards genetic testing and the attitudes towards termination of pregnancy.
Results of the study are being analyzed and presented in detail.
Discussion & Conclusions: This study was aimed at integrating these three concepts in order to have a better understanding of the factors associated with the decision to opt for genetic testing or terminate a pregnancy, in the general population. Implications of the impact of perceived severity of genetic conditions on the decision to opt for genetic testing and termination of pregnancy are being discussed.

EP10-M
Development of Myriad Individualized Medicine Units

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Since its foundation 20 years ago, Myriad genetics has become a leading molecular diagnostic company dedicated to making a difference in patient's lives. Through the discovery and commercialization of transformative tests to asses a person's risk of developing disease, guide treatment decisions and assess risk of disease progression and recurrence. These test services achieve top-quality assessment enabling top-quality information for the patient based on test results. Myriad laboratory operates under the highest standards of lean efficiency allowing result reporting much faster than average. In its aim to improve patient's quality of live and deliver top-quality information to the patients, Myriad Spain has developed a plan to create Individualized Medicine Units (IMUs). These multidisciplinary units located into the health system offer professional cancer counseling services. Different professionals including nurses, geneticists, genetic counselors, oncologists and psychologists will provide genetic testing education to patients and medical specialists. Thus, IMUs will become single organization structures which will permit a degree of coherence in providing genetic tests to counseled individuals. With the creation of IMUs access to an integral service with added value will benefit individuals affected of cancer as well as individuals at risk to develop it. IMUs are patient centered structures which intend to implement equal opportunity, help specialists to interpret genetic test results as well as to get genetic guided medical and preventive options closer to patients.

EP11-S
The disclosure of direct to consumer genetic testing: how to regulate them?

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Direct to the consumer genetic test (DTC GT) service is an indisputable and increasing phenomenon which portray internet society. It lets hypothesize that common people search for more health information and more sense of responsibility on their health behaviors. Indeed, proponents of DTC GT argue that making consumers able to calculate the relative risk of developing certain diseases may result in improved compliance with health-screening practices and more healthful lifestyle choices. The advertisements have a tendency to highlight these benefits and minimize any possible limitations. Moreover, consumers purchase these tests without the obligatory involvement of the health care provider, leaving free interpretation and use of genetic data. Despite these aspects, no concrete evidence about the attitudes towards, knowledge and use of DTC GT tests by members of the general population exist. Findings of previous researches indicate a low level of awareness of direct-to-consumer genetic testing impact, because of the hypothetical nature of many studies, use of not representative samples of the population and too little evidence from users. It is necessary to provide a theoretical framework of DTC tests use and to collect systematic data on

cognitive and behavioral DTC GT effects in the general population, in order to achieve progress in the policy arena, regulatory oversight, insights for consumers to make aware decisions and reduce the potential for misinterpreting genetic test results.

EP12-M
From NICE guidance to clinical practice: the challenge of setting up a service for Familial Hypercholesterolaemia

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In 2008 NICE published guidelines for the identification and management of Familial Hypercholesterolaemia (FH). A key priority was the identification of people with FH using DNA cascade testing.
This led to a scoping exercise in how to implement this service in the south central region. This included who, where, when & what kind of service. More importantly who would pay? The Wessex FH Cascade Testing is about to be launched in April 2014, commissioned by 12 Clinical Commissioning Groups (CCGs) within the new NHS structure with pump priming by the South Central Cardiovascular Network (SCCVN) and support from the British Heart Foundation (BHF) This is a narrative on how this was achieved, including the challenges of commissioning a new service and the opportunities afforded and how this has impacted thus far on the Genetic Service as a whole.
In March 2013, the Cardiovascular Disease Outcomes Strategy (CVDOS) specifically highlighted the need to improve the detection and management of people with Familial Hypercholesterolaemia (FH).
Action 5: The NHS Commissioning Board will take the lead, working with the Chief Coroner as appropriate; to improve the processes for identifying inherited cardiac conditions. The National Clinical Director for Heart Disease will work with all relevant stakeholders to develop and spread good practice in relation to FH and sudden cardiac death.

EP13-S
Developing genetic counselling in Portugal: education, practice and the growing of the profession

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As high-throughput genomic technologies became widespread and genetic healthcare workload expands, there is a call for education and training professionals to translate this changing landscape into appropriate care as well as for the health education of general population. In that sense, genetic counsellors are fully skilled professionals that could play an important role on the provision of safe and ethically adequate practice.
The first Portuguese genetic counsellors have completed their formal training in 2012. The Portuguese course in genetic counselling is accredited by the European Board on Medical Genetics (EBMG), in accordance with the proposed standards for education and core competences for professional practice, and in line with the harmonizing efforts of professional's education across different countries and healthcare settings. Moreover, a national association of genetic counselling professionals have been created. With this report we aim to provide evidence on the development of genetic counselling in Portugal, its educational program and current challenges on the establishment of the profession.
A growing body of research focusing diverse settings of the genetic counselling provision has been undertaken recently in Portugal. This includes the professionals' training needs for effective practice, quality issues of counselling, or the set of constraints affecting service delivery. Emergent challenges to continue the development of genetic counselling in Portugal are the recognition of the genetic counsellor profession, the harmonization of practice at national level, and the development of a clinical and counselling supervision network.

EP14-M
Reaction of maternity generations to human genetics in Japan

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Background:Demand for prenatal genetic testing has been increasing in general citizens, despite that they have less literacy on human genetics. This study aimed to educate human genetics to maternal generations and evaluate the reactions to the education and their understandings. Method: We recruited 10 mothers of pre-school children who accepted the program. We designed a lecture program, followed by focus group interviews (=FGI), which were conducted twice. After having an event on genetics with children, further planning and operation were carried out together with the mothers, then FGI were conducted again. Main theme of this program was to understand and accept a concept, "*everybody is different therefore everybody is valuable*". The transcript data were qualitatively analyzed. The study protocol was approved by the ethical committee of the affiliated institution. Results: After series of genetic education, participants naturally accepted "*everybody is different*". Accordingly, some of them changed their attitude to have more confidence to themselves, and some have more tolerance to the children. Most importantly, participants start thinking that learning genetics is useful for children to stop bullying and discrimination, and want nearby friends to learn about it.Discussion: Through genetic education, negative images of heredity that originally participants possessed were drastically disappeared. Therefore, it is important to educate human genetics and to give messages to recognize human diversity simultaneously.

EP15-S
And if I announce a genetic rare disease,are there particular recommendations? That must I know?

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For some people, the disease announce is a shock. This announcement is going to change the course of life of the patient and its perception of the future. We know that is not only the subject which is going to receive the impact of the announcement but a whole family system. Numerous measures implemented have encouraged the improving how the disease is announced to the patient. Indeed, in our presentation, we shall see in what an announcement of genetic rare disease is a particular announcement. We speak about: the representation of the disease rare genetics, the extreme rarity of these diseases, the absence of „name" for many, the notion of orphan disease, the consequences of the dysmorphology, the difficulty giving of the information. It's essential that the doctors who announce the genetic rare diseases know what is has the work psychically for a patient, a family confronted with the genetic rare disease. It will be there a facilitator for the announcement and the care of the families.

EP16-M
Informing best practice in presymptomatic genetic testing for Huntington disease

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Introduction: International guidelines for offering pre-symptomatic genetic testing for Huntington disease (HD) first developed in 1994 have been recently reviewed (MacLeod et al, 2012). In light of these recent recommendations, the experience and views of clients who had undergone testing at an Australian HD genetics service operating since 1991 were explored. Method: Semi-structured telephone interviews with 14 participants were transcribed, de-identified and coded for thematic analysis using NVivo data management software. Results: Six main themes were identified: (1) motivations for testing; (2) access to services; (3) information and client-centred care; (4) perspectives on the guidelines; (5) support person role and impact; and (6) the afterwards. Motivations for testing were similar to previous studies including reducing uncertainty, family planning and to inform risk status of existing children. Participants felt they were seen promptly by the service, that they were provided with sufficient information about testing, their care was client-centred and their needs were met at the time of testing. Some participants wanted testing immediately, but understood the rationale in the guidelines for delaying testing until the second counselling session. Involvement of a support person varied, and those who involved their support person only at the results-giving session described a greater emotional impact of the result on the support person. Some individuals reported a significant long-term psychological impact after receiving a negative test result. Conclusion: Recommendations for practice include the involvement of support person/s from the initial session, and to provide long-term follow-up to all individuals undergoing pre-symptomatic testing, regardless of result.

EP17-S
Myotonic dystrophy type 1 families: anticipation as a decision making behavior for presymptomatic DNA testing of asymptomic children

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Myotonic dystrophy (dystrophia myotonica, DM) is the most frequently inherited neuromuscular disease of adult life. The classical DM, known as

Steinert disease or DM type1, is a multisystem disorder with an autosomal dominant inheritance associated with the presence of abnormal expansion of CTG trinucleotide repeats in DMPK gene on chromosome 19q13.3. Anticipation is a well known phenomenon in DM1. The greater size of the expansion of CTG repeats is associated with earlier onset and more severe symptoms in the following successful generations. This study presents different decision making choices of parents for presymptomatic -DNA testing of their asymptomatic children in DM1 families. This decisions are defined by the following medical and psychological aspects: genetically verified DM1 diagnosis in one of the parents, family history data for a relative with DM1 symptoms, making a choice about the asymptomatic sibling after receiving an information for affected fetus in second pregnancy of DM1 patient.

EP18-M
Ten years' experience of pre-symptomatic genetic testing for late onset neurodegenerative diseases

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Introduction: Pre-Symptomatic genetic Testing (PST) for late onset monogenic neurodegenerative diseases, such as Familial Amyloid Polyneuropathy (FAP), Machado-Joseph Disease (MJD) and Huntington's Disease (HD), is available to at-risk healthy individuals. The aims of this study were: 1) to analyze the motivations for a PST; 2) to access the present clinical situation of the subjects; 3) to inquire about the individual treatment and preventive options; 4) to evaluate the reproductive options; 5) to analyze feelings of regret after PST.
Methods: This retrospective study consisted of telephone interviews of 168 carriers who underwent PST at our Medical Genetics Unit between 2000 and 2013 (145 FAP, 11 MJD, 12 DH).
Results: Most subjects failed to formulate, retrospectively, a motivation for PST, either than mentioning a positive family history (46%). From all patients that tested positive for FAP, 45% present peripheral neuropathy, 19% have undergone liver transplantation and 10% are medicated with *Tafamidis*. 11/23 patients with MJD and HD have psychiatric illness. From 43 pregnancies were reported - 6 patients underwent prenatal genetic testing and 5 couples pre implantation genetic diagnosis. 24 subjects mentioned having decided not to have children because of the test result. 18 subjects presented feelings of regret after performing PST.
Conclusion:
The complexity of PST calls for discussions about testing that are tailored to the testing context and the individual's needs and preferences. Hence, it might be useful to access the long-term psychosocial consequences of PST, as well as to ensure that adequate health and reproductive care are accessible.

EP19-S
Educational-support groups for daughters of BRCA mutation carriers: a valuable addition to patient care

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Introduction: The social workers of the department of Medical Genetics of the University Medical Centre Utrecht twice a year organize educational-support groups for young woman at risk for hereditary breast and ovarian cancer (either yet untested daughters of BRCA1/2 mutation carriers or mutation carriers)
We started these group sessions in 2008 because we noticed:
- parents with a BRCA mutation worrying about their daughters <25 years
- young womens' need to be in contact with peers
Although there is no medical benefit of knowing one's mutation status before the age of 25, the risk and uncertainty of (not) knowing are usually hard to deal with.
Methods: The group meetings are separated two-hour psycho-educative support sessions with 5-8 participants. Themes include:
-Timing of the DNA testing
-Family influence on decision making
-Talking about BRCA carriership and breast cancer within your family, with friends, at work or school
Results: We have conducted 11 group sessions. Over 60% of the participants joined more than once. Participants experienced positive effects such as an increase in knowledge and nuance. Their feelings are recognized by their peers and thereby contributing to normalization and sense of release. The participants and their reactions endorsed us to continue these meetings.
Conclusion: The purpose of the educational-support groups is that participants feel recognized in their particular situation and can share their experiences with peers. This helps them in learning how to deal with the (possible) genetic predisposition and being able to make their own decisions thoughtfully.

EP20-M
Living with uncertainty: the experiences of young healthy Italian women who have undergone genetic testing for hereditary breast and ovarian cancer

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Introduction. Testing of the BRCA1 and BRCA2 genes can identify inherited mutations that predispose to breast and ovarian cancer, and thus provides those women with the opportunity to engage in risk-reducing behaviors and programs. The information derived from the test, however, also exposes women to the responsibility of making difficult decisions and raises important new issues that may affect how they manage their lives.
Aims and methods. The aim of this qualitative study was to explore, through in-depth interviews, the experiences and behaviors of 22 young Italian women (11 mutation-positive, 11 mutation-negative, mean age: 35.21) who participated in BRCA testing. All the women had a known BRCA mutation in the family but no personal cancer history. Interview data were collected and analyzed in accordance with the grounded theory approach.
Results and conclusions. The following psychosocial themes were found to be affected by the results of BRCA testing: childbearing intentions, future projects, family support, feelings towards children and partners, risk perception, attitudes towards risk management strategies. Test results appeared to have a definite but generally not severe impact on the participants' lives although, in some cases, negative emotions were denied in words but expressed through behaviors. The results also showed that the main predictor of negative feelings was a previous experience of cancer in the family. These findings may help clinicians better understand women's experiences of BRCA testing and thus develop adequate, culturally and ethically sensitive interventions in the areas of effective communication, support and care.

EP21-S
The initiator and timing of referral to breast cancer genetic counselling; an exploration of everyday person-centered practice

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Objective: The referral process for genetic counselling in breast cancer patients may be compromised by patient-related factors, like patient's age, referral initiative or cancer history. This study aimed to characterize this referral process in daily clinical practice.
Methods: During genetic counselling a checklist was filled in for each consecutive counselee affected with breast cancer assessing educational level, the initiator for referral and the ethnic background as reported by the counselee. Chi-square tests were used to assess associations between patient-related factors and initiator of referral and timing of genetic counselling.
Results: Included were 96 consecutive breast cancer patients referred to cancer genetic counselling: 52% of them were referred on their own initiative versus 48% on their doctor's initiative. There was no significant relationship between initiator of referral and time elapsed since diagnosis, age at time of diagnosis, number of first-degree female relatives and number of first degree relatives affected by any cancer.
Discussion: Patients' interest in genetic testing is not clearly related with time elapsed since diagnosis. Family history seems to play a role in the timing for referral.
Conclusion: One out of two breast cancer patients plays an active role in the referral for genetic counselling. However, we did not establish a relationship between initiative for referral and time since diagnosis.

EP22-M
The impact of objective versus subjective risk on emotional distress in breast cancer women

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Objective: The aims of this study were to assess the impact of objective versus subjective risk on emotional distress and to investigate the extent to which cognitions mediate the impact of risk perception on emotional distress in a sample of breast cancer women. **Method:** In a retrospective quasi-experiment, a convenience sample of 53 breast cancer women (mean

age 51.92 years, SD=10.33) completed questionnaires assessing subjective and objective risk emotional distress and cognitions. **Results:** Subjective risk and objective risk are positively correlated (p<.01). Both subjective risk and objective risk cannot to predict emotional distress. No association was found between subjective or objective risk and cognitions. Alternatively, cognitions are an important predictor of emotional distress. Moreover, cognitions were found to mediate the relationship between risk estimation and the emotional distress. **Conclusions:** Results suggest that risk estimates (both subjective and objective) have a limited capacity to predict emotional distress in breast cancer women. The impact of risk estimates is mediated by cognitions which are an important predictor of emotional distress. Collective findings in this area will provide a better understanding of the mechanisms responsible for emotional distress in breast cancer women and will suggest potential psycho-social intervention

EP23-S
Predictive TP53 testing in adolescence and young adults: a case series

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The growing awareness of the contribution of genetics in cancer has resulted in increasing numbers of young people (16-27) at risk of Li Fraumeni syndrome (LFS) presenting to the Peter MacCallum Familial Cancer Centre for predictive testing. The unique needs of young people in combination with the complex and unpredictable nature of cancers associated with LFS has led to a considered method of genetic counselling practice being employed when supporting these young people through predictive testing. The methodology adopted by genetic counsellors at the Peter MacCallum Familial Cancer Centre incorporates many of the recommendations made in a locally developed model for the provision of genetic counselling to young people. This yet to be published model, was developed by a group of Melbourne based genetic health experts and adolescent health experts in response to the growing number of young people seeking advice from Familial Cancer Centres. It aims to counter the apprehension had by many genetic health professionals in working with young people by providing practical evidence based strategies.[1] The strategies employed aim to engage and empower the young person throughout the process and include mandating direct contact with the consultand prior to the consultation. Ensuring one on one time is had for a period of the consultation to allow for unrestricted discussion and overt discussion with the young person about the level of involvement of their parents. Ten consultations with young people requesting LFS predictive testing utilising this methodology will be reviewed and discussed. [1]Duncan RE, Young MA. *J.Pers.Med*. 2013.10(6),589-600

EP24-M
Attitudes of women at high risk of ovarian cancer to population-based risk prediction and stratification

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Ovarian cancer is the fifth most common cancer amongst women in the UK, and accounts for more deaths than all the other gynaecological cancers combined. As early stage symptoms are few and non-specific, ovarian cancer is often diagnosed at an advanced stage and survival rates remain low. There is an opportunity to improve outcomes through advances in risk stratification, early detection and diagnosis. A population-based ovarian cancer risk prediction and stratification program incorporating genetic testing is being developed. A previous focus group study with individuals from the general population identified a need and support for the proposed program. This qualitative interview study was undertaken to explore attitudes of women at high risk of ovarian cancer (strong family history or BRCA1/2 mutation carriers). Eight women participated in one-on-one semi-structured interviews to explore their experiences of learning about ovarian cancer risks and attitudes towards the proposed program. Overall women had very positive attitudes towards the introduction of a population-based risk prediction and stratification program for ovarian cancer. Women felt that it would be of interest to other women in the general population and would raise awareness of the disease. Women drew on their own experiences of genetic testing, screening, risk-reducing surgery and interactions with health professionals to offer suggestions for the successful implementation of the program. Suggestions included providing counselling before and after receiving risk estimates, different formats for presenting risk estimates and providing information regarding ovarian cancer symptoms, effectiveness of screening, preventive options and lifestyle factors to reduce cancer risks.

EP25-S
Genetic counselling for inherited cardiac conditions in Wales: A service evaluation

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The management of Inherited Cardiac Conditions (ICCs) is one of the most rapidly evolving areas in cardiology. The last decade has seen major changes in the diagnosis and management of ICCs. These were previously thought to be rare isolated disorders; however, it is now recognised that collectively they are common and are associated with the tragedy of sudden arrhythmic death syndrome.

A service evaluation was designed to investigate service users and Genetic Counsellors' experiences of genetic counselling at the time of a funding freeze for genetic testing for ICC's in Wales. Information was sought about service user's reactions and responses to information about genetic testing restrictions, psychosocial issues, and service improvements. A qualitative study which used in depth semi structured interviews with three service users and two genetic counsellors; a theme orientated discourse analysis was used to extract meaning from the data.

The service users' accounts supported existing theory that effective communication in genetic counselling is partially dependent on believing that the GC is interested in service users' personal circumstances. In this sample the unavailability of genetic testing contributed to feelings of uncertainty and concern for at risk family members.

As a result of this study, clinicians may wish to consider re-contacting service users who agreed to have a DNA sample stored during a freeze in NHS funding for ICC mutation screen testing. Service users with DNA banked for a prolonged period may require additional information or support to cope with the implications for their family.

EP26-M
Does and should breast cancer genetic counselling include lifestyle advice?

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Introduction: To optimally inform counselees about their and their relatives' risks, information about lifestyle risk factors, e.g. physical activity and alcohol consumption, might be discussed in breast cancer genetic counselling. This study explored lifestyle discussion in consultations during breast cancer genetic counselling. *Method:* First and follow-up consultations with 192 consecutive counselees for breast cancer genetic counselling were videotaped and coded for discussion of lifestyle topics (e.g. physical activity, diet, alcohol, smoking). Counselees completed online questionnaires before the first and after the final consultation. *Results:* With 52 (27%) counselees lifestyle was discussed, either in the first or final consultation, or both. Counselees mostly raised the topic (60%). Counsellors provided information about lifestyle risk factors to 19% and lifestyle advice to 6% of the counselees. Discussion of lifestyle was not associated with counselees' characteristics or causal attributions. Post-counselling, more affected counselees considered lifestyle as a factor contributing to their breast cancer (29%) compared to pre-counselling (15%; p=.003). *Conclusions:* Information and advice about lifestyle risk factors is infrequently provided, both with breast cancer unaffected and affected counselees and with those who did and did not consider their lifestyle as a cause of their breast cancer. A debate is recommended on how to incorporate lifestyle information in breast cancer genetic counselling. Modifiable lifestyle factors could be discussed more frequently to optimally inform counselees about possible ways to reduce their risk. Counsellors should be educated about effects of lifestyle and studies are needed on how to best integrate lifestyle information in breast cancer genetic counselling.

EP27-S
What ethical and legal challenges are associated with implementing risk-stratified screening for common cancers?

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The prospect of using multiple common genetic variants for population-

wide genotyping and personalised screening for common cancers raises some significant ethical, legal, social and organisational challenges. Whilst risk-prediction modelling performed as part of the Collaborative Oncological Gene-Environment Study (COGS) suggests that genotyping can effectively target screening and treatment, questions remain about the optimal age for sampling, analysis and reporting. Ethical guidance relating to the genetic testing of children generally focuses on highly penetrant variants which are strongly predictive of future ill health. In contrast, the multiple common variants identified through the COGS project are less predictive, and much more work needs to be done to assess the utility of this genetic knowledge particularly in children, when combined with lifestyle factors over a lifetime.
With these caveats in mind, the ethical, legal and social challenges associated with two alternative models for managing genotypic information in a personalised screening programme are presented: a targeted database in which data and samples are accessed solely for genotyping for risk prediction for a single type of cancer; and a generic database in which samples and data are retained indefinitely for multiple conditions. These ethical, legal and social challenges are discussed in terms of the potential implications for the ethical principles of autonomy, beneficence, and justice. These also inform relevant recommendations from the COGS project which illustrate some of the translational challenges of moving from modelling into practice.

EP28-M
Impact of genetic counselling in women undergoing invasive prenatal diagnosis for advanced maternal age

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A cross-sectional survey using a self-administered questionnaire was performed with the aim of exploring the impact of genetic counselling in women undergoing invasive prenatal procedures because of advanced maternal age.Out of 637 questionnaires mailed to women who underwent prenatal diagnosis 1 -6 weeks before, 221 (34.69%) were returned; of those, 52.9% belonged to women counselled through an individual session, while 47.1% women had attended a group session.The majority of women (85.5%) defined the information received during counselling as clear (with 15.4% considering it as „extremely clear", regardless of the type of counselling received) and helpful (overall 99.1%).Twenty-two (10%) stated their expectations about CVS/LA had changed after counselling.Out of 192, 140 (72.9%) reported satisfaction about the counselling process regardless of the type of counselling received. Although more women (52.6%) stated they preferred, or would have preferred, individual counselling, the highest satisfaction rates were reported by women attending group sessions.

EP29-S
Cancer genetic counseling in Iceland

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Cancer genetic counseling was formally set up at Landspitali - The National University Hospital of Iceland in 2007. Clinical genetics use the services of the Genetical Committee of the University of Iceland the Icelandic and the Cancer Registry to make accurate electronic pedigrees and risk assessment. Biobanks allow for up to 60-70 years old samples to be tested. Icelandic founder *BRCA* mutations are the 5193G>A in *BRCA1* and 999del5 in *BRCA2*. The latter has been traced back several hundred years. The prevalence of the *BRCA2* mutation is 0.6-1% in a population of 320.000 and the estimated number of carriers is 12-1500 at screening age; 25-70 years. We have identified 40 *BRCA2* and three *BRCA1* founder mutation families. A counselee not found in the pedigree database, does usually not belong to a *BRCA* family. A little over 750 individuals have been tested for the founder mutations identifying 200 carriers of the *BRCA2* mutation, 15 of the *BRCA1* mutation and 2 with a new *BRCA1* mutation. The number, age of onset and types of cancers vary considerable in the *BRCA2* families. The 45 pedigrees, with 38-1250 individuals each, showed that 38 female carriers had been diagnosed once with breast cancer, two with bilateral breast cancers, two had been diagnosed with three or more breast cancers and 11 with ovarian cancer. Seven males have had prostate cancer and eight breast cancer. Other cancers were pancreatic, kidney, colon and thyroid. For prevention, 15 females and one male have had mastectomy and 7 had oophorectomy.

EP30-M
Is communication with relatives discussed in the final consultation for breast cancer genetic counselling?

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Background Counselees in cancer genetic counselling are responsible for discussing possibilities for DNA-testing, risk estimations and surveillance advice with their relatives. We describe whether communication with relatives is discussed in final genetic counselling consultations, whether counsellors provide advice and whether counselees share their intention. *Methods* Consecutive new female counselees who were the first of their family to seek breast cancer genetic counselling were included from 2008 to 2010. We report on counselees whom received an indication for DNA-testing for themselves or an affected relative and/or a follow-up consultation. Final consultations were videotaped (n=152). Questions, information and advice about family communication were coded. Counselees' reactions to the counsellor's advice were scored on the level of agreement. Furthermore, counselees' intentions were transcribed. *Findings* Communication with relatives was discussed in 73.0% of the final consultations. In 33.6% the counsellor provided advice about family communication and a large majority (n=47, 92%) of the counselees responded with agreement. Almost half of the counselees (46.3%) expressed an intention about discussing risk information and most explicitly stated which relative they would inform (42.7%). Few counselees expressed when (9.2%), how (5.9%) or where (1.3%) they intended to discuss information with relatives. *Discussion* Almost half of the counselees expressed an intention to share risk information with one or more relatives. However, most counselees were vague about how and when they intended to do this. As risk information may be poorly transferred within families, counsellors could explore counselees' intentions and discuss how best to discuss risk information with relatives.

EP31-S
Genetic counselling in post-genomic era

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With the surge of genetic tests and technologies, genetic counsellors are faced with the challenge of translating emerging scientific knowledge into practical information for patients, clinicians and public health policy makers. The new tests and technologies also are associated with new psychosocial and ethical considerations. New guidelines are needed for each new discovery of the genomic impact on phenotype, pathology and disease while "old" syndromes and "old" pathology, continue to require attention. In the new post-Human Genome Project era, genetic counsellors will be an integral part of translating genomic discoveries into beneficial impact on human disease, health care, and medical benefits. The needs for genetic counselling should be designed into genomic research at the onset. Genetic counsellors need to handle old while rapidly assimilating new information and the principal challenge is to be up to date and updated. [World J Med Genet 2013 May 27; 3(2)]

EP32-M
Attitude toward genetic testing of children for common disease risk in Japan

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[Purpose] The purpose of this study was to assess the attitude of Japanese general public toward genetic testing of children for common diseases, and to clarify factors related to the attitude by nationwide opinion surveys conducted in 2009 and 2013. **[Methods]** In the survey in 2009, 4,000 people (age, 20-69) were selected from the Japanese general population by a stratified two-phase sampling method. In 2013, 2000 people were selected in the same way. They were queried about the following topics in a mail survey: attitudes toward genetic testing for disease susceptibilities of children for common diseases, interest in medical genomic studies, level of genomic literacy and awareness of the benefits and risks of medical genomic studies. The factors related to the attitude were examined using logistic regression analysis. **[Results]** The response rate was 52.5% (2,009/3827) in 2009 and 60.3% (1161/1925) in 2013. The genetic testing for disease susceptibilities of children was favored by 58.8% people in 2009 and 58.4% in 2013. The interest in medical genomic studies did not so change between in 2009 and 2013; belief of "scientific development has more advantage than disadvantage" was a little increased. Interest in medical genomic studies, belief in science and awareness of risks were significantly related to favorable attitudes

in each survey. It was suggested that interest, belief and awareness of risks were the factors related to the outcome of positive attitude change toward genetic testing for children. We will also show the attitude toward informed assent to children in genetic research.

EP33-S
Opinions Of Hearing Parents About The Causes Of Hearing Loss In Their Deaf Children Compared With Gjb2 (Cx26) Genetic Testing Results In Three National Republics Of Russia

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Hereditary hearing impairment (HI) caused by GJB2 mutations is frequent sensory disorder. The results of research on molecular basis of HI are widely used in practice as various genetic test systems. However, primary subjective opinions of parents about causes of HI in their children should be taken in to account at the interpretation of genetic testing results especially in regions where genetic testing has not yet been widely used in public health. We conducted the first sociological research based on surveys of hearing parents of deaf children in three national Republics of Russia: Sakha (n=101), Tuva (n=61), and Bashkortostan (n=21) for analysis of subjective opinions of parents about causes of HI in their children followed by comparison with results of genetic testing of GJB2 gene. Most of respondents (73.8%-86.1%) chose answer "non-hereditary" for question about presumptive causes of HI of children and their opinions are more likely based on the absence of deaf relatives. Subjective opinions of parents are inconsistent with genetic testing results, despite different contributions of GJB2 mutations (16%-71%) in studied regions, and in many cases the announcement of testing results may have severe psycho-emotional influence on parents.

Study was supported by RFBR (#12-04-00342_a, #12-04-98520_r_vostok_a, #12-04-97004_r_povolzhye_a, #14-04-01741_A), SBRAS Integration project #92 «Ethnogeny of indigenous peoples in Siberia and North Asia: comparative, historical, ethno-social and genomic analysis», the Sakha Republic President grant for Young Researchers for 2014 (RP#80), RAS Program «Fundamental Sciences for Medicine» (#30 for 2013-2015), and «Scientific and Educational Foundation for Young Scientists of Republic of Sakha».

EP34-M
State anxiety as an affecting factor to the perception of the information during the genetic counseling and the reproductive decision making process

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This paper will show the results, part of longitudinal study /more than 5 years/, of the factors that influence the perception of the information during the GC and the reproductive decision making process. Till now we explored the effect of: type of diagnostic procedures, advanced maternal age, family reproductive history. This study focuses on the pathologic rates of the SA. SA is inducted and is a result of the concrete situation, not a typical personal trait. Purpose: Analyzing the anxiety rate during the GC and the reproductive decision making process. Task: Examination of the SA rate with State-Trait Anxiety Inventory (STAI). The object of the study are 100 women, who were pointed for GC because of biochemical screening results and risk pregnancy. Hypothesis: The need of making reproductive choice after the GC can increase the rates of SA. Results: In most of the cases of women without higher rates of trait anxiety pathologic rates of the anxiety during the process of GC are observed. Conclusions: Through the GC process the SA is increased and this affects the subjective perception of the information and the reproductive decision making process. This suggests creating an algorithm for good practices for psychological support in the process of genetic counseling.

EP35-S
Content analysis of informed consent for whole-genome-sequencing offered by direct-to-consumer genetic testing companies

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Due to rapid development and a decrease in the price of high throughput sequencing technologies, whole genome sequencing (WGS) has become increasingly available in the research and clinical settings and is also being offered commercially by direct-to-consumer (DTC) companies. This offer amplifies the already identified concerns regarding informed consent for both WGS and the DTC offer of genetic tests. The aim of this project is to study the websites of companies advertising WGS DTC to analyze whether they address the recommended elements outlined by Ayuso and colleagues (2013) regarding consent for WGS in the clinic, which include, among others, pre-test counselling, description of the test process, expected benefits, possible risks, voluntary nature of participation, confidentiality and privacy, informing relatives or not, the storage and future use of samples, management of incidental findings and specific informed consent. Preliminary analysis reveals that the consent information on the websites of Illumina and Gentle, at least mention the majority of these items. Furthermore, both companies require consumers to obtain the test and/or results through a physician. However, involving a physician, and addressing these aspects on their website do not ensure an adequate informed consent process. Indeed, challenges of informed consent include, among others, the potential overload and complexity of the information regarding the process and the potential results, the (limited) ability of individuals to completely understand the information given, and biased understanding. This empirical study contributes valuable information toward the ongoing debate on the responsible offer of WGS in the commercial realm.

EP36-M
Return of whole-genome sequencing results in paediatric Research: a Statement of the P3G international Paediatric Platform

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Background: Whole-genome sequencing (WGS) is expected to have a significant impact on the field of human genetics in the near future. The sheer volume of information generated by WGS raises the question of whether or not to return such results. This dilemma is exacerbated in the context of paediatric research as particular issues are raised: the rights of parents to access their child's genetic information; the best interests of the child; the right (not) to know; the utility of information; consent, and counseling.
Objective: The aim of this research was to develop guidance on how to address the return of WGS results in paediatric research. We worked with the P^{3}G International Paediatric Plateform to build a common tool.
Methods: To formulate the Statement, we: (a) reviewed the legal and ethical norms applicable to the European and Canadian research communities and the relevant literature to identify both existing and emerging guidance; (b) conducted a qualitative study of stakeholder groups; (c) developed recommendations; and (d) validated the recommendations through consultations with a large number of stakeholders (e.g. genetic researchers, community-based organizations).
Results: We propose a Statement to address the issues of when WGS results: i) should be returned; ii) should not be returned (and identify exceptional circumstances); and iii) described the procedures for the communication of results.
Conclusion: It is anticipated that the Statement will not only provide a template for paediatric research using WGS, but also guide researchers and ethics committees.

EP37-S
Disclosure of genetic information within the family: evaluation of the model letter accompanying a new French decree

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In France, the recent publication of the decree of June 20th 2013 (n° 2013-527) modifies professional practices in medical genetics. It suggests a protocol regarding the transmission of information to relatives after a genetic diagnosis of a serious condition with the possibility of preventative or care measures. If a mutation carrier refuses to directly inform other members of

the family, one option is to call on the services of a genetic professional for the transmission of this information. This decree includes a specific model letter that can be sent to relatives by genetic professionals to invite them to make an appointment at a genetics center to access genetic counselling.
As part of a group comprising a geneticist and six genetic counsellors, we aimed to study the impact of this letter, both in terms of patient understanding and feelings. Evaluation was based on a brief interview after reading the model letter. Two groups were recruited either from "classical" genetics consultations or from "the general population".
We present here the results of 148 questionnaires. Overall, this type of letter appears to be an acceptable procedure for the majority and participant understanding was satisfactory, nevertheless two paragraphs were frequently read twice to be understood. The relevance of a third paragraph was frequently questioned. The main feeling is concern, while anxiety appears to occur with lower frequency. In light of these results, we discuss the need to formulate a new model letter accommodating as many people as possible to standardize practices.

EP38-M
Experiences of being a carrier and mother of a child with hemophilia

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We have conducted a qualitative study to explore women's experiences of being a carrier of the X-linked disorder hemophilia. We collected data through in-depth interviews with 16 women who were carriers and mothers of a child with hemophilia. In analysing these narratives, we adopted an interpretative approach to highlight the personal experience of being a carrier of an X-linked disorder, how meaning is negotiated, communicated and lived out within a wider social context. Preliminary analysis suggests that carriers experience feelings of worry and guilt after having a son with hemophilia. A striking feature in our study is that those who already knew they were a carrier, and thought this knowledge would prepare them, still experienced the event of having a child with the diagnosis distressing. Those who were diagnosed as carriers after having a son with hemophilia seemed to go through a different trajectory, focussing primarily on the child and not so much on their own carrier status. Although open communication about hemophilia and inheritance within the family makes coping with the disease easier, it might not hinder distressing feelings. More nuanced knowledge into experiences and challenges of mothers of children with X-linked disorders could prepare genetic counsellors, clinical geneticists and other professionals to understand their situation and facilitate an empowering approach.

EP39-S
The parenthood and the child rare disease: there is specificities?

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About 3 million people are concerned by in France and even if the media and the French Foundation of Rare Disease are proactive in discussing these diseases, their increased number and specifications are difficult to keep up with.
A preexisting child presenting with a rare genetic disease will also be a source of psychological disturbances. An overview of the current literature showed that many dimensions are involved in parents with children diagnosed with rare genetic disease: social, financial, familial, professional, educational, medical and psychological. However this analysis was done on a little number of studies and very little is detailed on the specificity of the rare genetic disease. Also these elements are usually studied to individual levels and not necessarily at the family level. And, if a child is diagnosed with a rare genetic disease, the whole family will be concerned, will suffer and will need to adapt to this new situation. It seems that parents are showing signs of psychic weakness but no prediction can be made on the impact this will have on the family system nor on the factors of protection of weakness.
But are there specificities in the rare genetic diseases?

EP40-M
The impact of carrier identification on children's wellbeing from parents' and children's perspectives

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The identification of sickle cell disorder carriers is routinely reported to parents following newborn screening in England. Although parents express intentions to provide carrier results to children in late childhood, little is known about when carrier status is provided or how children adapt to knowledge of their status. This is despite advice from European guidance that testing be deferred to avoid negative psychosocial detriment in childhood. This study explored the impact of disclosure of sickle cell carrier results on children's wellbeing via separate semi-structured interviews with 8 mothers and their children (M = 9.50 years, range = 6.92 - 14.56 years). Thematic analysis explored themes within and between each parent-child pair. Parents provided partial carrier information to children at around age 7; some parents acknowledged children's residual anxiety yet others did not report adverse effects following disclosure. By contrast, children reported significant anxiety, nightmares, withdrawal, distraction in school and feared negative responses from peers, which they had not discussed with parents or others. Parents and children reported the desire to have more developmentally appropriate information to improve understanding. Poor parental communication and lack of understanding of a carrier status caused children's maladaptive adaptation to results. Greater emphasis should be placed on aiding discussion between parents and children following carrier status disclosure acknowledging how difficult it can be for children to have these discussions, the availability of resources for families and further research regarding the long-term impact of carrier status on children's wellbeing.

EP41-S
Impact of Hemophilia on the Psychological Health of Hemophilia Patients

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Purpose: Psychological factors have a significant impact on quality of life for patients with chronic diseases such as haemophilia. The aim of this study was to evaluate psychological aspects of haemophilia. Sf-36 is one of the most beneficial instruments for assessment of life quality in haemophilic patients. Methodology: The study included 60 patients with A and B severe haemophilia, with the age between 16-40 years old, during 2012-2013. The results of SF-36 questionnare were examined on the whole group, on age group respectively 16-25 years old and 26-40 years old. The health condition declared by the haemophilic patients was correlated with Beck depression parameter. Results: Evaluation on the whole group showed the lowest scores on the domains D4=45,2+/_19,4 of the pain, D1=35,1+/36,6 physical performed functionality and vitality D7=44,2+/38,1. There were statistically significant differences between the two groups on D1 and D4 ($p<.0.1$). We observed significant differences in social relationships, mental health and the general state of health between the two groups ($p<.05$). The correlation between health score and Beck depression parameter is not significant. Conclusions: Knowledge of the psychological characteristics of haemophilia patients are useful in developing a plan for social integration and finding a healthy balance.

EP42-M
Creation and started by a therapeutic patient education for gaucher's disease patients: the french experience

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Therapeutic patient education (TPE) is an essential part of chronic disease care, enabling to achieve autonomy in health-related decisions, a better quality of life and better health outcomes. In order to elaborate an educational program adapted to patients, a working group* was made up including physicians, nurses, psychologists and the patient's association.
The first step was an evaluation of the patient's needs based on patients and professionals' interviews. This investigation brought to light a plurality of representations and real-life experience of the disease as well as numerous repercussions on the working life, the social life, the balance of the couple and the family.
Several preferential themes have been identified to design the educational program which contains:
-A personalized consultation in order to identify the patient needs (educational diagnosis) and to organize his educational training
-Three educational group sessions of approximately 2 hours. The choice of sessions for a given patient will be negotiated by the physician and the patient together. They concern the following themes:
a) Better understanding of Gaucher's disease and its consequences (definition, symptoms, natural evolution, follow-up and complications)
b) Living with the disease in everyday life (real-life experience, fatigue and pain management, identification of available resources)
c) Better appropriation of his (her) treatment (criteria of choice of the specific treatment, additional treatments, oral treatment and drip administration)

-A follow-up consultation, support and evaluation of patient's progress
The program is based on interactive educational methods meeting quality criteria of adult's training and it is accompanied by varied educational tools.

EP43-S
Psychological support of BRCA1/2 carriers: audit of patient satisfaction with clinical psychology appointments, issues discussed and the offer of psychological support for women considering risk reducing mastectomies

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BRCA1 and BRCA2 carriers who live in the south east of England are offered an appointment in a one-stop BRCA clinic at Guy's hospital following identification of a gene mutation. Carriers are offered a choice of appointments with specialists in genetics, breast surgery, gynaecology, oncology and psychology. In addition regular support groups and patient education days are provided. Dedicated psychological support is provided by a clinical psychologist. We will discuss the types of interventions offered and the range of issues addressed.
In addition we will present data from a satisfaction survey carried out with patients who opted to see the Clinical Psychologist in the BRCA follow up clinics between January and March 2014. This survey found that 92% of patients felt completely listened to and understood, 85% felt completely able to talk about the things they needed to and 85% felt that the appointment helped them feel they could move forward a great deal or to some extent with their issues.
UK guidelines recommend that all women considering risk-reducing mastectomy are offered psychological support. The local protocol is that all women seen in the BRCA multidisciplinary clinic are offered an appointment with the Clinical Psychologist. An eighteen month audit of the offer of these appointments (by the psychologist) to the women is currently underway. Preliminary data show that 46% (12) of the total number of women eligible for an appointment (26) were offered an appointment. Possible reasons for this discrepancy and tentative recommendations for practice will be discussed.

EP44-M
Using Patient Reported Outcome Measures in clinical genetics services: Pilot studies in six UK clinical genetics centres.

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Background: Patient Reported Outcome Measures (PROMs), short self-completion questionnaires capturing aspects of health or health-related quality of life, have recently gained prominence in healthcare evaluation worldwide. Historically, the quality of UK clinical genetics services has been evaluated using process measures e.g. patient waiting times, numbers of patients seen. Whilst current approaches to quality are important, little attention has been paid to patient outcomes because these have been difficult both to specify and to measure. This paper reports on pilot studies using a new clinical genetics-specific PROM, the Genetic Counselling Outcome Scale (GCOS-24) in six UK clinical genetics centres.
Methods: Patients were asked to complete GCOS-24 before and after clinic attendance. Satisfaction data were also collected in some centres. Data were analysed using analysis of variance and bivariate correlation. Centres provided feedback on feasibility of PROMs data collection and lessons learned.
Results: Five centres demonstrated statistically significant improvement in patients' GCOS-24 scores following clinic attendance with usable samples sizes of 42, 45, 54, 55 and 74 patients respectively ($p<0.001$). One centre had insufficient matched pre-clinic and post-clinic questionnaires to enable a useful analysis. GCOS-24 improvement scores correlated significantly with patient satisfaction.
Conclusions: Findings demonstrated that participating clinical genetics centres can deliver significant measurable patient benefits and that GCOS-24 has potential to be a useful supplement to existing methods of evaluating quality of routine clinical genetics services. Useful next steps could include developing methods for optimising patient response rates and for integrating PROMs information into continuous quality improvement cycles in clinical care.

EP45-S
Fitting the Pieces Together - Adoption Issues in Genetic Counselling

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Adoption is a unique situation which can present a number of challenges within a genetic counselling session. Primarily, adoption may limit the communication of information between at-risk relatives, with difficulties in notifying either a child who was adopted out at birth, or the biological family of an adopted individual, of genetic risk information. However, many other clinical and psychosocial issues can also be raised in these cases which likewise need to be addressed.
In Queensland, Australia, the adoption process is regulated by a single State Government organisation. The current *Adoption Act 2009* legislation came into effect in February 2010 and aims to balance the privacy of those individuals who do not want to be contacted with people's right to access information about their birth parents or a child who was adopted. For new couples wishing to express interest in adopting a child, multiple assessments are required to determine their suitability as parents, including close examination of their medical history.
Three cases will be presented which highlight some of the various challenges that adoption can present within a cancer genetic counselling context. The outcome of each case will be discussed, including the specific clinical and psychosocial issues that were raised. These include the assessment of eligibility for genetic testing, provision of accurate risk management advice, facilitating communication of genetic risk, addressing psychosocial issues and the potential discrimination an individual with a genetic condition may face if they are wishing to become an adoptive parent.

EP46-M
„BRCA isn't all about boobs"...or is it? Lessons from a BRCA support group

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Within the Plymouth region of the Peninsula Clinical Genetics Service we identified 71 women who had tested positive for a BRCA1 or BRCA2 mutation. Letters were sent to these, inviting them to attend a support group. Twenty-seven women responded with twenty-five attending the group. Numbers were almost precisely equal between BRCA1 and BRCA2, with age ranging from early twenties to over 65. Most attendees had known about the gene change in their family for 4+ years. Motivation for attending focused on sharing experiences and meeting other women in a similar position. Reporting of beneficial aspects of the group tallied with this, with women particularly appreciating the chance to see the outcome of surgery. While distressing stories were shared, all participants reported feeling positive about the event. It was noted that, with appropriate facilitation, groups achieved an effective balance; sharing personal stories while also appreciating individual circumstances. Professional concerns about causing distress to those earlier in their journey were not realised, as participants took an active role in reassuring and supporting one another. Some women listed "helping others" as their main motivation for attending. Discussion at the end of the session helped provide a plan for future groups. It was identified that group support should be two-fold; combining continuity through consistent small groups, as well as the opportunity to divide according to stage of treatment / topic of interest. It is notable that, while women with cancer felt well supported, those undergoing prophylactic mastectomy felt that they lacked a clear pathway.

EP47-S
Differential binding of CREB, USF, and c-Myc to the Calreticulin Human Specific -220C may be linked with the evolution of higher brain functions in human

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We have previously reported a human-specific nucleotide in the promoter sequence of the calreticulin (CALR) gene at position -220C, which is the site of action of valproic acid. Reversion of this nucleotide to the ancestral type, -220A, co-occurs with severe deficit in higher brain cognitive functions. This mutation has since been reported in the 1000 genomes database at an approximate frequency of 0.0009 in humans (rs138452745). In the current study, we compare the pattern of protein binding between -220C and -220A using electrophoretic mobility shift assay (EMSA) by oligonucleotide probes representing 24 base pairs encompassing -220C>A. Antibodies reactive against transcription factors CREB, USF, and c-Myc were used to identify the specific proteins involved in complexes with DNA. Significant increase was observed in the overall protein complexes binding to the -220C allele vs. -220A. The transcription factors, CREB, USF, and c-Myc, were differentially bound to -220C, represented by supershifts. We propose that differential binding of CREB, USF, and c-Myc to CALR nucleotide -220C may be linked with the evolution of higher brain functions in human.

EP48-M
Association between COMT Val158Met genotype and personality traits in healthy female subjects

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Dopaminergic genes are associated with a broad range of behaviors. The enzyme catechol-O-methyltransferase (COMT) is involved in extrasynaptic dopamine degradation in prefrontal cortical areas. The relatively common methionine (Met) for valine (Val) substitution at codon 158 of the COMT gene (Val158Met) results in an enzyme with 3-4 times lower rates of catabolism, increasing dopamine levels in prefrontal cortical regions. Among the characteristics of dopamine is important to mention the brain reward systems, infant attachment and adult human personality, particularly higher scores in Val carriers were reported for the Extraversion scale of the NEO Five-Factor Inventory and the Novelty Seeking and Persistence scales from Cloninger's-TCI questionnaires. The aim of the study was to compare the COMT Val158Met functional genetic variant with personality traits, assessed using self-reported Big Five Questionnaire (BFQ-2) and Temperament and Character Inventory-Revised (TCI-R). We tested a possible interaction among these variables on a cohort of 154 healthy female students, recruited at the "G. d'Annunzio" University, Chieti. A significant correlation has been observed between Val carriers and the BFQ-2 Dynamism subscale ($r=-.17$; $p<.05$). Moreover we observed significant correlations among Val carriers and two TCI-R's temperament traits: Reward Dependence ($r = -.16$; $p < .05$) and Persistence ($r = -.16$; $p < .05$). We found a significant correlation ($r = -.22$; $p<.01$) between the Val carriers and Cooperativeness, a factor of the character side of the personality, measured through the TCI-R.

EP49-S
Psychological aspects of living with EDS.

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EDS patients suffer from a loss of control (body, time, environment, emotions). Inevitably this results in a loss of identity. Four elements stand out: the danger of living a restricted life, the risk of social isolation, the feeling of uselessness and the problem of being discredited. Especially the latter is very hard to endure. Patients are caught in a typical paradox: successful coping, often at the expense of superhuman efforts, makes other people doubt about the gravity of the illness. Yet, they have no other choice but to do their very best. There is always this pending and lurking question: "Are you really trying enough?" The underlying assumption is that effort always yields positive outcomes. If someone does not get any better he is being held responsible. It is this social response that makes a disease stigmatizing and tiring, not the disease in itself. We take a social psychological stance: not a person's character is determining his behavior, it is the context. As a consequence: we should reconsider our notion of empowerment. The traditional medical belief that 'rational' communication of information and compliance in following treatment regimens form the basis for patient empowerment is in line with a social discourse that propagates mastery and responsibility. It overlooks some important elements of illness experience. By listening to and talking with our patients we, practitioners, by definition, legitimize their experiences. This is where authority meets vulnerability. Only then are patients capable of moving forward.

EP50-M
Psychosocial outcomes and uptake of testing in non-pregnant and pregnant women offered carrier screening for fragile X syndrome (FXS)

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Population carrier screening for FXS identifies women at increased risk of having an affected child, and provides information about their own health risk. Debate surrounds educational and counselling complexities inherent in such screening. We assessed aspects of decision-making, in non-pregnant and pregnant women offered FXS carrier screening. Women were approached through general practice, obstetric or ultrasound clinics, received written information and telephone pre-test counselling with consent. At home, women decided about testing, and completed a questionnaire (Q1), returned with their buccal sample if tested. Premutation (PM) or grey zone (GZ) results were discussed by telephone and women offered genetic counselling; test-negative results were mailed. 1237 women (702 non-pregnant and 535 pregnant) initially consented; 71% and 59% were tested, respectively: PM (0.4%); GZ (2.0%). 85% had good knowledge (≥7/10 correct). 77% non-pregnant and 67% pregnant women had positive attitudes. Pregnant women were less depressed and less stressed than non-pregnant women on Depression Anxiety Stress Scale; no differences between tested and non-tested. Only a few women had scores outside of the normal range. Decisional conflict (DC) scores were subtly different between groups. Most significant differences were seen in the DC uncertainty subscale: non-tested less certain than tested women ($p< 0.0001$); pregnant less certain than non-pregnant women ($p< 0.0001$). The majority of women offered screening had good understanding with minimal psychosocial impact, possibly related to pre-test counselling embedded within this study, an important element for consideration in screening programs. Overall, women supported availability of being offered screening, although testing before pregnancy is preferred.

EP51-S
Post-mortem genetic testing requested by a family member: challenges for genetic counseling

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We report a family where post-mortem genetic testing identified a predisposition to melanoma and pancreatic cancer. The index case referred to genetic counseling presented a cancer of unclear origin (pancreatic/ovarian) at age 58. A sister had died of pancreatic cancer at 59, the mother had died of melanoma at 48 and an aunt was diagnosed with melanoma at 32 and breast cancer at 52 and died of a head and neck cancer at 60. Analysis of *BRCA* 1 and 2 genes was normal. Analysis of *CDKN2A* gene was discussed but declined by the patient for different reasons among which were the lack of efficacy of surveillance measures and clear guidelines. The patient gave consent for DNA storage for further analysis and research. After her death, one sister requested genetic testing of *CDKN2A* through a Canadian genetic team. With the consent of the patient's husband, the medical authority in Switzerland and the ethical committee in Canada, analysis of *CDKN2A* was performed in Canada and a pathogenic mutation was identified. Subsequently, the patient's adult children who live in Switzerland requested genetic counseling and opted for testing. Post-mortem genetic testing is commonly performed in the setting of forensic autopsy (for example following sudden cardiac death) but it presents some specific challenges when requested by a family member and performed on a stored DNA sample. This case raised legal and ethical questions about consent, practical questions regarding reimbursement of the test and awareness of psychological aspects of genetic counseling for offspring.

EP52-M
Reciprocity among genetics professionals in Europe - A UK:Dutch experience.

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Recent literature has highlighted that collaborations among genetics professionals in different countries can help to facilitate genetic counselling practice development. Reciprocity is one manner of achieving this. Reciprocity has been identified by the Transnational Alliance for Genetic Counselling (TAVC) as a useful entity in allowing for progression of the international genetic counselling community as it allows for reflection on current methods of genetic counselling in one's own and other countries. Furthermore, the December 2013 issue of the Journal of Genetic Counselling was dedicated as a special issue concerning a global perspective of genetic counselling, in particular highlighting the value and drawbacks of student exchange experiences as a component of study programs. Currently this seems to be the most common method of gaining such experience. Here, I present the experience of a UK trained and registered genetic counsellor visiting a Netherlands based genetics service and I reflect on the benefits and drawbacks for staff from genetics centres engaging in these opportunities. This is the first report of this kind and it is hoped that it may help to facilitate future reciprocity among these two countries. Furthermore, a Dutch perspective of a UK based service is also provided.

PUBLISHED ABSTRACTS

J01.01
Cytogenetic analysis of first trimester missed abortions

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BACKGROUND: Approximately 15% of all clinically recognized pregnancies are spontaneously aborted and ~60-70% of these are attributable to detectable chromosome abnormalities . Cytogenetic analysis is an important component in the assessment of human malformation in early failed pregnancies. **METHODS:** A total of 32 patients with missed abortion between 7-11 weeks gestation underwent cytogenetic analysis of chorionic villi, using R-banding cytogenetic techniques. **RESULTS:** Among 32 examined cases, 14 (43,75%) were with abnormal karyotype and rest of 18 (56,25%) were normal. Of the 14 cases with an abnormal karyotype, there were 3 structural abnormalities and 11 numerical aberrations. When analyzed by maternal age, the rate of abnormality for first-trimester losses was 38,88% in women younger than 35 years, and 42,85% in those 35 years or older. **CONCLUSIONS:** A total of 43,75% of the cases with missed abortion had an abnormal karyotype and the percentage was a little higher at advanced maternal age. Karyotyping of spontaneous losses in the first trimester beginning with the patient's second loss provides clinically important etiologic information and decreases the number of evaluations necessary for recurrent pregnancy loss.

J01.02
An analysis of the cells of trophectoderm with the use of aCGH within the scope of PGS, pregnancy rate and the effect of maternal age

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Chromosomal abnormalities are a major cause (in 70 %) of miscarriages in the early stage of pregnancy and in the failure of an embryo implantation. Many aberrations are of maternal origin. The number of aberrations increases with the age of the woman.
530 embryos were analysed from 299 patients with the use of aCGH within the scope of PGS, from that 256 embryos from 158 patients with the heartbeat of fetus were used for the calculation of pregnancy rate. The results of IVF cycles of 247 patients withouth PGS was used as a control group. The group of women was further divided into the group A) women younger than 35 years and group B) women over the age of 35. 34,6 % of embryos with aberrations was found in the group A, and 48,1 % of embryos with aberrations was found in the group B. Total pregnancy rate in both groups with PGS was 63 %. The pregnancy rate was 25,8 % in the group of women older than 35 years without PGS. The percentage of pregnancy rate increased to 68 % in group B. This represents an increase of 42,2 % for IVF success in the group B.
The increase of aberrations is obvious after the age of 35, here are the chromosomal aberrations a clear cause of the failure of IVF. It was confirmed that the exclusion of embryos with chromosomal aberrations can eliminate the effect of the age of woman on the success of IVF.

J01.03
Array CGH in prenatal diagnosis

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Following confirmation of array cgh as first tier diagnostic test for individuals with intellectual/ developmental delay, multiple congenital anomalies and autism spectrum disorders there has been a lot of discussions regarding using this test for prenatal diagnosis. In different countries there has been different approach to this case. Considering the possibility on finding CNVs of unknown significance leading to counseling dilemmas the general trend has been to use this technique in cases of ambiguity identified in routine chromosomal karyotype or where a normal karyotype is reported in a sonographic report of congenital anomalies. In the period of 24 months starting December 2011 till December 2013 we performed array CGH for 32 amniotic or chorionic villi samples. From these 32 samples, 3were inconclusive and of the 29, 5 showed a significant CNV. 3 of the 5 had problems in their sonography and 2 were being studied for de novo markers. It appears that array CGH Is an instrumentAl tool in clarifying complicated karyotypes and a significant test for fetuses with abnormal sonography findings.

J01.04
The AZFc region: crossroad between male infertility and recurrent pregnancy loss in women

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Introduction: Male factor infertility comprises approximately 30-50% of all infertilities. A great proportion of these patients have spermatogenesis failure with a genetic cause. After Klinefelter syndrome, Y chromosomal microdeletions (YCM) in AZF regions are the most frequent cause of male infertility. There are evidences that male factors can potentially affect fertilization, human embryo development and viability and placental proliferation. According to the recent studies, there is a potential association between Y chromosome microdeletion and recurrent pregnancy loss (RPL). Screening for Y chromosomal microdeletion in AZF region in men with non-obstructive infertility and spouses of women with RPL was the focus of this study.
Material and Methods: This study was carried out with a total of 65 male samples which included 35 non-obstructive infertile men, 15 males from couples with RPL and 15 fertile males as a control. Genomic DNA was extracted from blood lymphocytes. Screening of AZF deletion was performed by multiplex polymerase-chain reaction using 19 sequence tagged sites (STS) sets of primers.
Results: In 35 men with non-obstructive infertility only 1 subject was detected to have Y chromosome microdeletions in SY254 and SY157 and SY255. Results showed no Y chromosome microdeletion in 15 males of couples with RPL and their controls.
Conclusion: Amongst the various regions of AZF, often only microdeletion in the AZFc region could end up with fertility, thus mutation screening at the AZFc region is strongly recommended in males whose spouses have recurrent pregnancy loss after ruling out other RPL causes.

J01.05
Chromosomal abnormalities and Y chromosome microdeletion amongAzosperima/Oligospermia Saudi Patients

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A prospective cytogenetic and molecular study was conducted on 123 Saudi men with azoospermia to estimate the prevalence of common genetic abnormalities associated with male infertility. Routine cytogenetic studies were performed to screen for chromosomal abnormalities whereas multiplex PCR methods were employed to screen for submicroscopic microdeletions of the AZFa, b and c regions located on the long arm of the Y-chromosome. Chromosomal abnormalities were detected in 21 patients (17%). Among the 21 patients with cytogenetic abnormalities, 15 (71%) had the Klinefelter's syndrome 47,XXY karyotype, 4 patients showed different large deletions of the long arm of the Y-chromosome with or without 45,X cell line mosaicism, one patient showed low 47,XXY/46,XY mosaicism and one patient had a chromosomal translocation between chromosomes 2 and 5. The molecular studies revealed submicroscopic microdeletions of the Y-chromosome in 3 (3%) of the 102 patients with a normal male karyotype. These Y-chromosome microdeletions involved regions AZFc in all three cases and AZFb in two cases.
Our studies revealed that the prevalence of chromosomal abnormalities in azoospermic Saudi patients is similar to that of major international studies. However, the prevalence of submicroscopic Y-chromosome microdeletions in patients with a normal karyotype was noticeably lower than what have been reported internationally but consistent with a few studies performed in Arab countries including Saudi Arabia. Our findings therefore strengthen the assumption that other genetic and/or non-genetic factors may play a major role in male infertility in Arab patients.

J01.06
The Relationship Between The Index and the Blood Disease Thalassemia and a Sample Report

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Background and Aims: Thalassemia is a severe hemoglobin disease, recognizing by CBC and hemoglobin electrophoresis primarily. At first, CBC and hemoglobin electrophoresis was performed for the index case recognized as α-Thalassemia but finally experimental studies clearly demonstrated that it was different type of thalassemia. In the present study, we report a case of β-thalassemia mutations which is called -101 C>T relative to the transcription start site of β-globine gene, and is a β+-Thalassemia case, but it has been observed with different indices.
Methods: The patient was admitted based on hematologic indices as

α-Thalassemia, and diagnostic tests for α-Thalassemia, including GAP PCR, ARMS-PCR for α-Thalassemia were performed and all examinations were normal. For increased confidence, sequencing for patient's β-globins' gene was performed. We also observe more cases with the same index.
Results: Our findings, present the incidence of mentioned mutation in β-globin gene in β+ patient, in contrast to the hematologic indices for β-Thalassemia heterozygote. Moreover, this case has no resemblance to other β+-Thalassemia cases and it can be mistaken as α-Thalassemia. (For example: The - 88 C> A mutation relative to the transcription start site, which several of our indices present beta-Thalassemia).
Conclusion: As mentioned above, our outcomes are extremely similar to the results of CBC and hemoglobin electrophoresis of α-Thalassemia, but the patient was β-Thalassemia. Such conditions as cited above are hazardous and end to affected neonates if the other partner is β-Thalassemia patient. In this condition, Sequencing of β-globins for one of the couples could be helpful.

J01.07
Comprehensive carrier screening for severe recessive pediatric disorders in Chinese population by next-generation sequencing

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The global birth prevalence of single gene disorders is as high as 16.9%,among which recessive disorders account for 43.7%. Of 7719 disorders with suspected Mendelian inheritance, more than 1000 are recessive with an established molecular basis. Preconception genetic testing and counseling has led to significant declines in the incidence of Tay-Sachs disease and other several severe recessive diseases. And extension of preconception screening to more severe disease genes has also been attempted in some developed countries.
Here, we report a preconception carrier screen for 658 severe recessive childhood diseases in Chinese population. All the exons and their flanking sequences (±30bp) of 546 target genes were enriched by hybrid capture, sequenced by next-generation sequencing (NGS). At a resultant 160x average target coverage, 93% of nucleotides had at least 20x coverage, and mutation detection/genotyping had ~95% sensitivity and ~100% specificity for substitution, insertion/deletion, splicing, and gross deletion mutations and single-nucleotide polymorphisms.
In 199 DNA samples (91 males and 106 females), the average genomic carrier burden for severe pediatric recessive mutations was 1.47 and ranged from 0 to 6. The distribution of mutations among sequenced samples appeared random. Among the 199 individuals,118(59 couples) had once or more times adverse pregnancy outcomes such as miscarriage. The carrier burden of these individuals was 1.89, which is higher than general population.
Preconception carrier screening by NGS is feasible to the general population, especially the individuals with adverse pregnancy outcomes. And it may be an economical way to reduce the incidence of severe recessive pediatric disorders.

J01.08
Non-invasive prenatal diagnosis of fetal sex using multiplex-PCR

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The uncovering of cell free fetal DNA (cffDNA) in maternal serum of pregnant women (1997) has provided an approach for applying of cffDNA as a non-invasive method for prenatal diagnosis (PND). The determination of fetal gender is the first step of the PND. It is especially important in fetuses at risk of sex-linked disorders and in conditions associated with ambiguous development of the external genitalia.
Peripheral blood sample (10 ml) was obtained from 100 pregnant women that referred to the diagnostic laboratory for routine pregnancy tests during 6th to 10th weeks of gestational age. Cell-free fetal DNA was extracted from the maternal serum. The cffDNA was not enriched. The conserve sequences of SRY and GAPDH genes as a marker for fetal gender and for internal control respectively were amplified by Multiplex-PCR using specific primers for their reigns. We considered; amplification of just internal control indicates female fetal gender and amplification of internal control and SRY conserve sequence indicates male fetal gender. Latterly, all the collected results were compared with the real gender of the newborns.
Early determination of fetal gender provides the opportunity of deciding and employing early treatment designed for fetuses at risk. However, this study aims to validate a simple, reliable and applicable method for non-invasive prenatal diagnosis of fetal gender. In this presentation we will demonstrate our data regarding fetal gender. Furthermore, we will present sensitivity and specificity of this study.

J01.09
„Filling the gap" : the prenatal phenotype of two cases of rare microdeletion syndromes

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We describe two cases of prenatal detection of unexpected interstitial deletion syndromes that were recognized by CGH-Array after evidence of ultrasound fetal anomalies and normal karyotype
Case 1: A 43 years old woman in her fourth pregnancy referred for genetic counseling because of cystic hygroma with normal fetal biometry measurements at the first trimester. Level II ultrasonography: micrognathia, low-set ears, lower limb abnormalities. CGH-Array was performed revealing a deletion syndrome of 10,6 Mb on chromosome 6 (locus 6q11q14.1). After termination the fetal pathology showed frontal hypertricosis, small nose, long filtrum, macrostomia, thin lips, micrognathia, and low-set ears with abnormal lobes.
Case 2: A 41 year old woman in her third pregnancy. During the second trimester the pregnancy was complicated by intrauterine growth restriction and unilateral fetal club foot. A microdeletion of 496 kb at 17q21.21 was detected by CGH- Array. This mutation has been reported in only a limited number of families and with a variable phenotype. After termination the physical examination of the fetus demonstrated high forehead, hypertelorism, bulbous nose, thin lips, pointed chin, low-set simplified ears, camptodactyly, tales equinovarus, and reduced musculature in the lower limbs.
Both deletions were shown to be de novo. Understanding the pathogenesis of fetal anomalies and correlations between the pre- and post-natal phenotype is difficult and limited because of a lack of prenatal data from cases reported after birth. The clinical spectrum of microdeletion syndromes could be expanded greatly if more prenatal cases would be described and published in the open literature.

J01.10
Chromosomal abnormalities in couples with reproductive failures and/or those included in ART programs

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Chromosomal abnormalities (CA) are among the major genetic causes of reproductive disorders. A**im:** to investigate the prevalence and profile of CA among couples with deferent type of reproductive failures, as well as to assess the value of karyotyping in the routine work-up of couples, referred for ART. **Material and methods:** The study included a total of 947 individuals with reproductive failure. They were divided into the following groups: (1) 273 couples with two or more spontaneous abortions; (2) 193 women and 208 men from couples with infertility +/- one or more unsuccessful IVF procedure. All patients were analyzed cytogenetically for detection of major chromosomal abnormalities.
Results: Chromosomal abnormalities were found in 26 (2.74%) of all investigated individuals, 10 (2.08%) males and 16 (3.43%) females. The determined prevalence of CA was - 4.76% among couples with recurrent abortions (4 times more frequently in women than in men) and 3.24% in individuals (more frequently men) with infertility. The most common type of CA in group (1) (92% of these cases) are balanced structural rearrangements, carried mainly (83%) by female; in group (2) – CA of sex chromosome (62% of these cases), more frequently (62%) in male. **Conclusion:** The results of current study demonstrate the significance of chromosomal pathology in etiology of reproductive failure and emphasise the need for thorough genetic work-up in couples referred for infertility. Karyotype analysis should be an integral part of diagnostic work up in couples with reproductive problems especially those undergoing assisted reproductive procedures.

J01.11
Study of chromosomal abnormalities involved in couples with reproductive failure

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BACKGROUND Infertility is a medical problem affecting a significant proportion of the population, up to 15.0% of couples of reproductive age. Genetic pathology is an important part of the general human pathology due to the increasing number of genetic diseases. The purpose of this study was to establishing a correlation between the presence of structural and numerical chromosomal abnormalities in one of the partners and their reproductive development.
METHODS During the period from August 2007 to December 2011, 2195 patients with reproduction problems, who had received in our hospital, were investigated for this retrospective study, and the frequency of chromosomal abnormalities was calculated. The control group consists of 83 fertile couples who had one or more children in history, was investigated by karyotype.
RESULTS Of the 2195 patients investigated by classical cytogenetic techniques 91.12% had normal karyotype and 8.88% had chromosomal abnormalities, the most common chromosomal changes are polymorphisms. Numerical chromosomal abnormalities were detected in the proportion of 0.65% in infertile men and 0.62% in infertile women in the study group.
CONCLUSIONS: Recently a possible association between infertility and chromosomal abnormalities have been reported with a significant statistically association. Our study shows that there is not association between chromosomal abnormalities and infertility problems,but this study needs to be confirmed with further investigations on a larger control group to establish the role of chromosomal aberrations in the etiology of infertility.

J01.12
FISH assessment of chromosomal aneuploidies in infertile males

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Background. Reproductive failure is one of the most important issues for the population at the age of procreation and approximately 15% of the couple encounters reproductive difficulties. In the last years it was taken in consideration the hypothesis that not only somatic chromosomal anomalies but also germ cells chromosomal aberrations could lead to reproductive failure.
Aim: In this study we used multicolor FISH probes for chromosome 13, 18, 21, X and Y to evaluate the aneuploidy incidence in sperm cells from infertile males.
Methods: The study lot included 35 males with infertility and oligoasthenoteratozoospermia (OAT) and 20 males with normal fertility and normal semen characteristics for which the conventional cytogenetic investigation using peripheral blood revealed a normal karyotype. The fluorescent signals for these chromosomes, were analysed at least 10000 cells/patient.
Results: The overall chromosome disomy and nulisomy in OAT group was higher than the one identified in the control group. By comparing the incidence of the disomy in the OAT group, the highest incidence was the sex chromosome disomy, followed by the disomy of chromosomes 13, 21 (equal values) and then 18. The nulisomy incidence in the OAT group was higher for sex chromosomes, followed by the nulisomy of autosomes 13, then 21 and 18.
Conclusion: During these days, for patients with OAT, intra cytoplasmic sperm injection (ICSI) is frequently used, and it is important to inform the patients that the use of spermatozoa for fertilization could be associated with an increased risk of aneuploidies in embryos.

J01.13
Cleidocranial Syndrome with Premature Ovarian Failure

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Our patient family had twenty members that five of them (three brothers, One sister and father) suffered from oligodontia , enamel hypoplasia , dental decay, Mild proportionate short stature , frontal bossing , Hx of recurrent upper respiratory infections , sinusitis, bilateral clavicle bone hypolplasia , pectus carinatum , short & tapering fingers , lumbar lordosis and thorasic scoliosis.
My proband was a 33 years old female with premature ovarian failure and chromosomal abnormality in peripheral blood (%40 mosaicism of deletion of 6p2 and %4 45 XO in fifty metaphase) , other family members had not any fertility problem and chromosomal abnormality.
We gussed Our diagnosis in this case is Cleidocranial Dysostosis and ocurrance chromosomal abnormality and this syndrome probability resulted frome location of this gene (CBFA1) in short arm of chromosome 6 and varibilty of mosaicism 45 XO phenomenon in ovarian tissue.

J01.14
Mutations in cyp21ohb gene of women with reproductive dysfunction

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The interest in non-classical forms of congenital adrenal hyperplasia (CAH) and its impact on human reproduction was increased. The vast majority of all cases of CAH (95%) was due to deficiency of the enzyme of 21-hydroxylase. Defect of this enzyme is able to lead a miscarriage.
In aim to determ the ratio of the number of gene replica (b) and pseudogene (a) on exon 3 in CYP21OHB was conducted molecular genetic testing at 46 women, among them 26 women with infertility and 20 women- control group. The presence or absence of the mutation in the test region was determined by PCR using a specific primer.
In result of analyses was determined that equal ratio of the number of gene replica (b) and pseudogene (a) on exon 3 (b=a) was detected in 20 patients, representing 76,9% as compared with a group of fertile women (95%). In the group of women with the disorder generative function there are in 6 patients noted an altered replica of the gene «b» and pseudogene «a» on exon 3 (b<a) towards the control (23.1% and 5% respectively).
The present changes in the ratio of the number of replica gene and pseudogene were due to deletion a gene (b) and in this case, probably, it is a mutation which leads to development of reproductive disorders in the homozygous condition.

J01.15
Genetic counselling for pregnant woman with CPT II deficiency - a case report

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Carnitine palmitoyltransferase II (CPT II) deficiency is a autosomal recessive disorder of long-chain fatty-acid oxidation. The three clinical presentations are: lethal neonatal form, severe infantile hepatocardiomuscular form, and myopathic form. While the former two are severe multisystemic diseases characterized by liver failure with hypoketotic hypoglycemia, cardiomyopathy, seizures, and early death, the latter is characterized by exercise-induced muscle pain and weakness.
The lady aged 27 was referred to our hospital at 18 weeks of gestation. Her first child was dead at 9 months old triggered by infection. Postmortem revealed that the boy had CPT II deficiency. Hence she and her husband had genetic test for CPT II and both of them were diagnosed as carrier. At the genetic counselling at previous hospital, they decided not to have prenatal test in the future. We talked about that again and confirmed their will.
At 39 weeks of gestation, she delivered a boy, 2650g, Apgar score 9(1)/9(5). We monitored the boy in Growing Care Unit to avoid low glucose level which could cause catabolism, and the cord blood was sent for genetic test. The genetic test showed the boy is carrier as well as his mother.
If the mutations have been identified in an affected family member, molecular genetic testing of at-risk relatives can reduce morbidity and mortality through early diagnosis and treatment. In this case, the patient did not choose to take prenatal test, however, it would be worth to know if the fetus is affected to prepare neonatal care.

J01.16
Reduced fertility and sperm DNA fragmentation

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Conventional semen analysis is widely used to assess man's reproductive potential, giving no information about sperm genome. Sperm DNA integrity assessment is not a routine diagnostic, requiring specific markers that may indicate that sperm DNA fragmentation assay is needed. The aim of this study was to investigate possible markers of sperm DNA damage. Semen analysis was performed for 75 men and 8 sperm donors. According to WHO criteria all patients were divided into groups: normozoospermic, teratozoospermic and asthenozoospermic. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay used to assess sperm DNA fragmentation. Sperm DNA fragmentation rates in normozoospermic group (0,47±0,17) and control group (0,21±0,04) were equal (p=0,17). Sperm DNA fragmentation rate in teratozoospermic group (0,62±0,1) was

significantly higher compared to control (p=0,02). No difference was found between DNA fragmentation rate in group with reduced sperm motility (p=0,30). Sperm DNA fragmentation had no effect on sperm motility. Patients were also divided into groups on the basis of predominant sperm head morphology: amorphous (n=27), bulb (n=20), vacuolated (n=6) and others (n=22). The level of sperm DNA fragmentation (1,31±0,30) among patients with vacuolated sperm head as a predominant morphological form was higher compared to control (p=0,02). No significant differences were found between sperm DNA fragmentation level among patients with amorphous or bulb sperm heads and control group. In case of teratozoospermia sperm DNA fragmentation assessment should be recommended. Sperm head vacuoles may be a possible marker of DNA damage. Supported by Carl Zeiss, RF President's scholarship and RFBR.

J01.17
Association of MTHFD and RFC1 polymorphisms with the risk of Down syndrome in Romanian population

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Introduction Down syndrome (DS or trisomy 21) is a genetic disease resulting from the presence of an extra copy of chromosome 21. Although advanced maternal age represents the major risk factor for DS, most of DS children are born from women less than 30 years of age and is seems that different mechanisms are responsible for chromosome 21 nondisjunction in young women compared to older ones, each of them potentially affected by an impaired folate/Hcy metabolism. The objective of this study was to evaluate the correlation between gene polymorphisms MTHFD G1958A and RFC1 G80A and the risk of Down syndrome. **Materials and methods** Our study included 26 women that gave birth to DS babies and 46 control mothers of healthy children. Genomic DNA was isolated from whole blood, using peqGOLD blood DNA mini kit (ATP Biotech) following the manufacturer's instructions. The MTHFD G1958A and RFC1 G80A mutations were investigated by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. **Results** The combined RFC-1 80(GA or AA)/ MTHFD1958GG genotypes compared with the reference RFC-180GG/ 1958GG genotype was associated with increased DS risk (OR 0.18 [0.02-1.41] P= 0. 08), but not statistically significant. **Conclusions** This is the first study in a cohort of Romanian mothers of DS children in comparison with control mothers, analyzing RFC1 G80A and MTHFD G1958A polymorphisms as a maternal risk factor for meiotic nondisjunction of chromosomes 21, causing DS.

J01.18
Genetic deletion of Pelota in mice leads to embryonic lethality at an early post-implantation stage

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Pelota (*Pelo*) is ubiquitously expressed, and its genetic deletion in mice leads to embryonic lethality at an early post-implantation stage. In our study, we conditionally deleted *Pelo* after the establishment of embryonic stem cells (ESCs) and showed that PELO depletion did not markedly affect the self-renewal of ESCs or their capacity to form teratomas. However, *Pelo*-null ESCs differentiation into extraembryonic endoderm (ExEn) was severely compromised during embryoid body (EB) formation studies. Failure of *Pelo*-deficient ESCs to differentiate into ExEn was accompanied by the retained expression of pluripotency-related genes and alterations in expression of components of the bone morphogenetic protein (BMP) signaling pathway. Further experiments have also revealed that attenuated activity of BMP signaling is responsible for the impaired development of ExEn in *Pelo*-deficient cells. Collectively, our results convincingly show that PELO plays an important role in the differentiation of ESCs, into ExEn through activation of BMP signaling. Hence, the observed early embryonic lethality in conventional *Pelo*-deficient embryos could be attributed to the defects in ExEn formation during early embryonic development.

J01.19
Gene variation of TLR4 in patients with Endometriosis

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Endometriosis, defined as the presence of endometrial tissue outside of the uterus, is an estrogen-dependent chronic inflammatory condition associated with degrees of pelvic pain and infertility. Toll-like receptors play a key role in immune response, by regulating inflammatory reactions and activating adaptive immune response to eliminate infectious pathogens and cancer debris. Polymorphisms in TLR4 have been shown to be associated with increased susceptibility to diseases such as inflammation and cancer. Ectodomain of TLR4 protein consists of 21 leucine-rich repeats (LRRs) that are crucial for its dimerization and signaling. The aim of this study was to determine gene variations of TLR4 in patients with endometriosis.
Sixty three blood samples were recruited from endometriosis patients referring to Royan Institute along 2012-13, who have been confirmed by laparoscopic surgery. Ethical approval forms were obtained prior to the samples collection. The control group was consisted of fertile women who had no history of inflammatory disease or using any related drugs.
DNA was extracted by kit, special primers were designed, the LRR coding region was amplified by PCR and products were analyzed by sequencing. among 63 patient, 6 of them had a heterozygote single nucleotide polymorphism (SNP) (rs120475167) G/A which changes aa E to K, and 4 patients had heterozygote SNP (rs120475108) G/A (aa R to K), and 2 patients had heterozygote SNP (rs120475032) G/A(aa D to N).
According to our finding we suggest that the three aforementioned new SNPs in TLR4 gene can be associated with endometriosis.
Key words: Endometriosis, gene variation, polymorphism, TLR4.

J01.20
The association between endothelial nitric oxide synthase gene Glu298Asp polymorphism and spontaneous pregnancy loss

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Aim: The endothelial nitric oxide synthase gene (eNOS) polymorphisms have been associated with reduced vascular NO production or increased level of homocysteine and related to pregnancy losses. In the current case control study it was aimed to find out the possible role of eNOS E298D polymorphism in spontaneous pregnancy loss. Material and Methods: Twenty-three spontaneously aborted fetal materials, 22 mothers who had these abortions and 86 healthy control cases were enrolled in the current results. The genomic DNAs were isolated from aborted materials and peripheric blood samples and genotyping for target polymorphic allele was done by real time PCR technique. Results: Current results showed that eNOS (Glu298Asp) polymorphisms were significantly associated with spontaneous abortion (p=0.011). Conclusion: The present study identified the strong association between eNOS gene polymorphisms and spontaneous pregnancy loss risk.

J01.21
18p partial trisomy with fetal ascites inherited from a mother with familial 18p deletion syndrome

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The deletion 18p syndrome is one of the most common chromosome abnormalities characterized by dysmorphic features, growth and mental retardation with a poorer verbal performance. Until now, no reliable phenotype map for the characteristic clinical findings such as mental retardation, post-natal growth retardation and typical facial features has been established yet.
Molecular karyotyping holds the promise of improving genotype-phenotype correlations for frequent chromosome conditions such as the 18p-syndrome. Until now, there have been 9 reported families with transmission of del (18p) from a mother to a child (including our study).
We describe a female baby, 46,XX,der(18)t(18;20)(p11.32;p11.2) with fetal ascites, transmission of deletion 18p from a mother and her affected families with partial monosomy 18p of different sizes owing to unbalanced translocations.

J01.22
Structure of prenatally-identified polyploidies - a retrospective study

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Objective: The objective of this study was to identify the frequencies and types of prenatally-identified polyploidies through amniocentesis or chorionic villus sampling (CVS), and to compare them with polyploidy identified in terminated pregnancies. Materials and Methods: Fetal karyotypes were investigated from cell cultures and banded with GTG. Amniocytes were also screened with FISH and QF-PCR was used in the CVS cases. Results: We report here prenatal diagnostics through amniocentesis for 3 triploid fetuses.

Classical anomalies were observed during ultrasound scans: severe oligoamnios, intrauterine and placental growth retard with morphologic modifications. Each case presented with biochemical risk (trisomy 21 or trisomy 18). Fetal karyotypes post-amniocenteses showed 69,XXX (66.66%), and 69,XXY (33.33%). These represent 0.27% of the amniocentesis investigations. There were 75 CVS investigations performed, and one case (1.33%) of 69,XXY triploidy was identified through QF-PCR and subsequently confirmed by karyotype. Cytogenetic investigations were also performed in 172 cases of terminated pregnancies, and 17 instances of polyploidy were discovered (9.88%). Eleven of these were triploidy, and the most common karyotypes were 69,XXY (63.63%), followed by 69,XXX (27.27%) and 69,XYY (9%). Six cases of tetraploidies were discovered: 92,XXYY (50%), 92,XXXX (33.33%), 92,XXYY/46,XY (16,66%). The maternal age was between 22-32 years old in cases with triploidy as compared to 28-43 years old in cases with tetraploidy. Conclusions**:** Polyploidies are relatively frequent in terminated pregnancies, but can also be present the first or second semester of pregnancy in live fetuses. Obtaining fetal karyotypes in all cases which present severe fetal abnormalities can elucidate the etiology.

J01.23
First Trimester Combined Screening in a General Pregnancy Population in Voronezh Region Russia

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Early diagnosis of chromosomal abnormalities shall be secured by means of mass prenatal screening of pregnant women in the first trimester. The frequency of chromosome trisomy 21 (Down syndrome) constitutes 1:680 in Voronezh Region.
A general screening population of pregnant women presenting in their first trimester underwent combined screening for fetal trisomy 21 and 18 using serum markers and nuchal-translucency (NT), nasal bone (NB) measurement.
Pregnancy outcome was determined by invasive testing or phenotypic evaluation of the newborn.
During the 2012-2013 in Voronezh Region we examined 36 566 expectant mothers (86.5% out of women registered in first trimester).
As a result of prenatal screening, 451 women were selected to high-risk group of chromosomal anomalies (CA) (1.2% of all examined at a cut-off level at 1: 100). The PAPP-A; free ß-HCG was carried out on Delphia. The calculation of risk was evaluated with the FMF software. These women are recommended to pass the invasive diagnostics. We carried out 284 invasive procedures for karyotyping of fetal cells. In 97 cases (34.2%) CA was diagnosed by cytogenetic methods, including FiSH. Trisomy 21 was the most common among the detected fetal CA (47 cases). Trisomy 18 was detected in 13 cases; trisomy 13 - 9; triploidy syndrome - 10; Turner syndrome - 8 cases. Other CA was detected in 10 fetuses.
The expected number of trisomy 21 in the studied cohort is 54 cases. Thus the «Prenatal Diagnostics» program allows effectively diagnosing 87% trisomy 21 and preventing the birth of children with CA.

J01.24
Prenatal diagnosis of unbalanced translocation 11,18 - pitfalls of FISH rapid prenatal diagnosis

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FISH screening in uncultured amniocytes, is a standard procedure for the rapid detection of chromosomal aneuploidy in prenatal diagnosis, in order to detect the most common aneuploidies, involving chromosomes 13, 18, 21, X and Y. It is easy to perform, don't need cell culture, the risk for misdiagnosis is low (~0.4%) and, principally, allow results in less than 24 hours.
We report the case of a young pregnant woman with an abnormal ultrasound scan, revealing a foetus with multiple anomalies, including cardiopathy, short femur and clenched hands. It was the first pregnancy of this young, health, non-consanguineous couple with a normal family history.
Amniocentesis was performed and FISH aneuploidy technique, in uncultured amniocytes, was applied, with normal results. Cytogenetic analysis of amniotic fluid revealed an unbalanced translocation involving chromosome 11 and 18, with deletion of 11q24-qter and trisomy of 18q11.2-qter, confirmed by FISH.
The foetus had several anomalies consistent with trisomy 18 phenotype.
The FISH rapid prenatal aneuploidy test is, taken in account its possibilities and limitations, a powerful tool for the clinician in the care of pregnant women. It's limitations, it cannot detect cytogenetic abnormalities such as mosaics, translocations or rare aneuploidies, do not allow this technic to be used as an independent, stand-alone technology and must be performed in conjunction with standard cytogenetic testing for clinical diagnosis.
So, it seems likely that, for the foreseeable future, banded chromosomes will remain an indispensable tool in the genetic diagnostic laboratory.

J01.25
Analysis of association between the number of CGG repeats in FMR1 gene with IVF failure

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Infertility is a common clinical problem. It affects 13% to 15% of couples worldwide. In Vitro Fertilization is a common medical treatment which fails in some cases. IVF is not successful for several reasons. FMR1 Gene (Xq27.3) with CGG repetitive sequence -through their impact on autoimmunity - is one of the genetic factors influencing IVF failures. In normal subjects, the number of iterations is 5 to 54 and in relation to normal ovarian function the number of CGG repeats in the FMR1 gene are 26 to 34. The occurrence of these three repetitive sequences is three genotypes as normal, homozygous, and heterozygous. Heterozygous genotype has 2 modes: with the occurrence one of two alleles greater than 34 sub-genotypes creates het-norm/high and less than 26 sub-genotypes creates het-norm/low. In this study, correlation studies are of case - control and long PCR method. Samples are peripheral blood of 50 women with a history of at least 3 failed IVF, karyotype and normal MTHFR gene and 50 matched controlled group. Total Lab Quant software was used to determine the number of iterations and SPSS 17 software was used in data analysis. Analysis done using frequency tables and coefficients determined by measuring the number of iterations shown by p-value equal to 0.002 indicates a significant relationship between het-norm/low sub genotype in FMR1 gene and IVF failure.

J01.26
Analysis of association between the number of CGG repeats in *FMR1* gene with recurrent abortion in Iranian patients

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Most frequent anxieties in pregnant women is recurrent abortion with prevalence of 1 per 300 pregnancies which is occasionally caused through autoimmunity disorder. Recurrent abortion has been observed in pregnant women with autoimmune antibody such as Anti-phospholipids and Anti-TPO. The number of CGG repeats in *FMR1* gene correlates to autoimmunity. Increasing in *FMR1* repeat number is correlated to fragile X syndrome. Normal range is considered 5-54 repeats. The Normal function of ovarian is correlated to (26-34) CGG repeats in 5'UTR-*FMR1*. Accordingly two distinct types of ovarian sub genotypes are *Het-norm/low*, *Het-norm/high* with fewer 26 and more than 34 repeats respectively. Autoimmunity has been reported to be associated to *Het-norm/low* sub genotype. This study was designed to evaluate the relationship between the number of CGG repeats with recurrent abortion in Iranian patients. In this investigation, 50 women with normal karyotype and normal MTHFR genotype (with at least two kids) were compared to 50 women with 3 or more recurrent abortions for the number of *FMR1*-CGG repeats. Long Range PCR was performed to amplify the 5'UTR of *FMR1* gene and results were analyzed using the TOTAL LAB software. In conclusion our finding shows that 14 normal persons and 10 patients with recurrent abortion have high correlation with *Het-norm/low* sub genotype. So there was a statistically significant difference in the number of CGG repeats of women without abortion compared with women with 3 or more recurrent abortion (P-value <0.05). This is the first report to show the correlation of *FMR1*subgenotypes and recurrent abortion.

J01.27
Hereditary thrombophilia and pregnancy loss

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Pregnancy loss and adverse pregnancy outcomes (APO) result from a complex interaction of environmental and inherited factors. Although the etiology of more than half of such events remains unclear, gene mutations leading to a hypercoagulable state, such as factor V Leiden, factor II prothrombin, methylenetetrahydrofolate reductase (MTHFR) mutation, are often found associated with pregnancy loss.

Polymorphisms in genes for factor II prothrombin (20210G→A), factor V Laiden (1691 G→A) and MTHFR (677C→T), were determined in 121 women with history of pregnancy losses and in 51 women with healthy offspring and no history of APO (control group).
Mutations in investigated genes were found in 25.6% women of study group. Analyses showed that inherited thrombophilias were found in women with 2nd and/or 3rd (50%), rather than those with 1st trimester recurrent pregnancy loss. Women with a history of 3rd pregnancy loss showed the highest prevalence of examined thrombophilic genotypes. Group of women with the history pregnancy loss, as well as fetal chromosomal abnormalities and congenital malformations, also showed a significantly higher presence of thrombophilic genotypes than the control group. Mutation 677C→T in MTHFR gene was the most frequently found polymorphism among the examinees form the study group. Odds ratio analyses showed that women with thrombopilic genotypes have 5 – 10 times greater risk for APO.
Established association of inherited thrombophilias, especially 677C→T mutation in MTHFR gene, to late pregnancy loss, and fetal malformations and chromosomal abnormalities implies that these mutations might be significant risk factors for a broad spectrum of APO.

J01.28

The importance of appropriate genetic counseling and informed consent before beginning IVF procedures: two case reports

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In the Hospital of Lithuanian university of health sciences were counseled two couples which in the past used unsuccessfuly intensive infertility treatment regimen. The first couple was unable to conceive for 7 years: they applied two unsuccessful IVF procedures, however gave birth to a healthy boy. The second couple applied two IVF procedures and did not conceive for four years.
After the karyotype testing a chromosome translocation was identified: in the case of the first couple to a female, in the case of second couple to a male. The karyotypes of the first couple were the following: 45 XY, der(13;14)(q10;q10)of a male and 46XX of a female. The karyotypes of the second couple were respectively 46XY and 45 XX , der(13;14)(q10;q10)
The possibility of miscarriage and of anomaly were explained and further recomendations to couples were provided. After the counseling families are better able to decide on further family planning and prenatal diagnostics, if necessary. These cases present the importance of quality of health care services for the adjustment of the diagnosis, and the need of genetic counseling and appropriate genetic testing before ART because the main requirements for the informed consent include rationality, sufficient and clear information, free will and the form of consent conforming to the legal acts

J01.29

Prenatal genetic tests of pregnant women who were exposed to psychoactive drugs

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Backround: The effect of psychoactive substances on fetal developement has recently become a great concern. There's not enough data regarding this subject.
Methods: A retrospective study of cases from year 2009-2010 included 20 cases of pregnant women who were exposed to psychoactive drugs during their pregnancy. A control group of 30 cases of pregnant women who were not exposed to any psychoactive substances was randomly selected. Out of these cases the data of the use of drugs during pregnancy, prenatal utrasound evaluation (nuchal translucency thickness, morpho-pathologic alterations of organs), biochemical prenatal tests (markers indicating the risk of Down's syndrome and spina bifida) was collected.
Results and conclusions: No pathologic fetal ultrasound findings were observed in the control group of 30 pregnant women who didn't use antipsychotic drugs during pregnancy. Pathological fetal ultrasound findings (minor) were observed in 3 out of 20 pregnant women treated with psychoactive substances. The strongest associacion was observed between neuroleptics and ultrasound changes, $p<0.01$. Pathological fetal ultrasound findings were observed in 3 out of 13 pregnant women exposed to neuroleptics during pregnancy. The relationship between exposure to antidepressants and pathological ultrasound findings was statistically significant, $p<0.038$. Pathological fetal ultrasound findings were observed in 2 out of 8 cases of antidepressant treatment during pregnancy. The relationship between exposure to psychoactive substances during pregnancy and biochemical markers indicating the risk of Down's and Edward's syndromes and spina bifida was statistically insignificant. No increased risk of spina bifida was observed in any group of patients.

J01.30

Polycystic Ovary Syndrome and MTHFR C677T polymorphism in mexican women

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Introduction: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in reproductive age patients, high homocysteine level has been reported in PCOS, the MTHFR-C677T gene polymorphism have been associated with hyperhomocysteinemia and decrease of folic acid. **Objective:** To determine MTHFR C677T polymorphism frequency in Mexican women with polycystic ovary syndrome. Material and methods: We included 64 patients with PCOS diagnosis according to Rotterdam criteria and 101 Mexican mestizos as reference group (M). The genotyping was performed by PCR/RFLP technique. The M group presented Hardy-Weinberg equilibrium. **Results:** In the PCOS group (n=64) the MTHFR C677T genotypic frequencies %(n) were distributed as follows: CC 33 % (21), CT 48 %(31) y TT 19 %(12). The allelic frequency of the C allele was 57 %(73) and T was 43 %(55). The genotypic frequency in the reference group (n=101) were CC 31 %(31), CT 50 %(51), TT 19% (19) and allelic frequency for C allele 56 %(113) and for T allele 44 % (89). The allelic frequency comparison between both groups were no statistically different ($p>0.05$). Conclusion: In PCOS group the MTHFR-C677T genotypic frequency was 19 % and the allelic frequency for T allele was 46%. The genotypic and allelic distribution between both groups (group-PCOS vs group-M) was similar. This study showed no association between MTHFR C677T polymorphism and PCOS.

J01.31

Multilocus methylation defects at imprinted genes in miscarriages from women with recurrent and single pregnancy loss

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Genomic imprinting is an epigenetic phenomenon, which is involved in regulation of embryonic development and placental function. Previously we have reported that the multilocus methylation defects (MLMD) at imprinted genes may be among molecular processes, which are responsible for dysfunction of imprinted loci in pathology of early embryonic development. We hypothesize that MLMD at imprinted genes may cause karyotypically normal miscarriage, particularly among women experiencing recurrent miscarriage.Differential methylation of 51 imprinted genes were examined in first-trimester spontaneous abortions (SA) from women who have recurrent (group RPL, from 9 (data of GoldenGate Methylation Cancer Panel) to 105 (Methylation-specific PCR (MSP) SA) and single (group SPL, from 6 (data of GoldenGate Methylation Cancer Panel) to 114 (MSP) SA) pregnancy loss. Sixty induced abortions were investigated as a control group. All spontaneous and induced abortions had the normal karyotype.
Our results provide evidence that MLMD at imprinted genes in the group RPL was more frequent than that in SPL ($15x10^{-2}$ and $5.2x10^{-2}$, respectively, $p<0.01$) with predominance of somatic epimutations ($10x10^{-2}$ and $3.9x10^{-2}$ respectively, $p<0.01$) and multiple hypomethylation ($9x10^{-2}$ and $4.4x10^{-2}$ respectively, $p<0.01$). Frequency of MLMD in both groups at maternal loci was higher than that at paternal one ($12x10^{-2}$ and $6.4x10^{-2}$, respectively, $p<0.05$ for RPL; $3.6x10^{-2}$ and $1.4x10^{-2}$, respectively, $p<0.01$ for SPL). Therefore, the RPL is characterized by multilocus somatic hypomethylation of imprinted genes and MLMD at imprinted genes on maternal loci that associated with abnormal maintenance of maternal imprinting in somatic cells.

J01.32

Genetic association of phase II detoxification genes with recurrent pregnancy losses among Moldavian women

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Background:
Recurrent pregnancy loss (RPL) is a multifactor and distressing disease. In this study, we aimed to investigate the relationship between the polymorphism of GSTM1, GSTT1, GSTP1 and the pregnancy loss.

Methods: A case-control study of 100 women with RPL and 100 healthy women was conducted. Have been investigated DNA of 100 healthy children aged up to 17 years for comparative analysis of GST polymorphisms in Moldova with other countries. PCR, PCR/RLFP methods have been used for polymorphisms of GSTM1, GSTT1 and GSTP1 genotyped.
Results: We found that 57% of the cases with RPL and 47% of the controls had the GSTM1 null genotype (OR= 0.72, 95%CI = 0.49-1.07). On the other hand, 28% of the cases and 35% of the controls had the GSTT1 null genotype (OR = 0.72; 95%CI = 0.47-1.10). The frequency of GSTP1 gene of the cases and control is the following: GSTP1 Ile/Ile - 49% and 51%; GSTP1 Ile/Val - 42% and 43%; GSTP1 Val/Val - 9% and 6%. No significant difference have been established (p>0.05) The comparative analysis performed in the healthy population of the RM and other states have found that the frequency of GSTM1 null genotype in Moldova was similar to that in Ukraine, Italy , Russia, Egypt and Brazil (p>0.05), whilst the frequency of GSTT1 null genotype and GSTP1 polymorphism was significantly different to that countries (p<0.05).
Conclusion: The polymorphism of GSTM1, GSTT1, GSTP1 cannot be associated with the risk of recurrent pregnancy loss of Moldovan women.

J01.33
Alpha globin gene mutations in Kurdistan and Kermanshah provinces, Iran

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Alpha thalassemia is one of the most common hemoglobin disorders in the world and its severe clinical form (e.g. H disease) could be transfusion dependent. In this study we genotyped cases with history of blood transfusion or suspicious to H disease who referred to blood transfusion centers and or hospitals in Kermanshah and Kurdistan provinces. Alpha thalassemia was diagnosed on the basis of hematologic index and hemoglobin electrophoresis of patient (before blood transfusion) or patient's parents. DNA was extracted using salting out method and alpha globin mutations were investigated using multiplex Gap-PCR for common deletions and direct sequencing for point mutations. One hundred and ten thalassemia patients were recruited and screened using hematologic index and hemoglobin electrophoresis. 10 patients with diagnosis of alpha thalassemia were tested. --Med mutation was most common allele that was found in 5 chromosome (25%), followed by -α3.7 in 4 (20%), -α20.5 in 3 (15%), polyA1 in 3 (15%), -αIVSI (-5nt) in 3 (15%) and -αcd59 in 2 (10%). Deletional H disease (-α/--) was diagnosed in 4 cases (40%) and non-deletional H disease (--/αTα) in 4 cases (40%). Two cases (20%) showed αTα/αTα genotype. Two patients were blood-transfusion dependent, from which the first one received regular monthly blood showed --Med/αcd59genoyype. The second cases with -α20.5/α3.7α genotype received blood occasionally. This survey indicated diversity of alpha globin mutations and also different clinical manifestation of the H-disease.

J01.34
Holt-Oram syndrome as result of prenatal exposure of valproic acid

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Holt-Oram syndrome (HOS) is caused by TBX5 gene mutation and it is estimated to affect 1 of 100 000 individuals. This condition is inherited in an autosomal dominant pattern, but most cases result from new mutations in the gene. HOS is characterized by skeletal abnormalities of the hands and arms (upper limbs) and heart problems. The diagnosis of HOS can be established clinically. Clinical signs include upper limbs malformations, anomalous heart structure (eg. atrioseptal and ventriculoseptal defects or Fallot tetrada) and cardiac conduction disease, which can cause bradycardia or tachycardia. HOS can be confirmed through TBX5 gene mutation molecular genetic testing - DNA sequence analysis or array comparative genomic hybridization (array CGH). A case report of Holt-Oram syndrome in 6 month girl is represented from Kaunas hospital of LUHS, when mother has been taking a valproic acid 500mg/day during pregnancy for epilepsy treatment. The antiepileptic drug valproic acid has teratogenic side effect and is a potent inducer of neural tube defects and other abnormalities, such as cardiac, skeletal and limb deffects. When valproic acid cannot be avoidened in pregnancy, the lowest possible effective dose should be prescribed. Also it is recommended to use folic acid to 5 mg/day. A usage of folic acid should be started before pregnancy, so family planning and prenatal consultation is recommended for women with epilepsy.

J01.35
Stage-dependent expression of HomeoboxA10 gene in human endometrium

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HOXA10 gene belongs to Homeobox gene family and encodes DNA-binding transcription factor that regulates variety of downstream genes. HOXA10 gene has a well-characterized role in uterine organogenesis during embryonic development and functional endometrial differentiation and growth in adults. It is actively involved in cell proliferation through the regulation of hundreds of genes.
To evaluate the expression profile of HOXA10 gene during menstrual cycle, endometrial tissues were collected from 21 healthy fertile women undergoing laparoscopy for tubal ligation surgery, in menstruation, proliferative and secretory phases. For this respect ethical approval and informed patient consent was gained for the use of tissue sample. Total RNA was extracted from tissues using TRIzol reagent and cDNA was subsequently synthesized. Quantitative expression analysis was performed using the real-time PCR system.
Results showed stage-dependent manner of HOXA10 gene expression in endometrium tissues as notably increase of expression level in the secretory phase in comparison to the menstruation and proliferative phases.
This finding suggests that HOXA10 gene may have an important role in regulating endometrial cells proliferation and development in menstrual cycle and it is important for establishing conditions necessary for implantation. Up-regulation of HOXA10 in secretory phase during the window of implantation may be one of the potential molecular mechanisms of fertility .This gene can be noted as one of the candidate genes that its aberrant expression may contribute to the etiology of infertility and gynecological disorders.

J01.36
No alteration in PRLR gene in Iranian women with idiopathic hyperprolactinemia

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Hyperprolactinemia is seen in a group of infertile women .These women are treated for this problem because high amounts of prolactin can affect fertility by changing LH and FSH quantity.The increase in prolactin levels could be due to a disorder in the prolactin receptor, so investigating the geneome of this receptor which is located on chromosome 5 might be useful in finding the reason for this problem.
In this study, the infertile women referred to Royan infertility institute, diagnosed with idiopathic hyperprolactinemia whose pituitary MRI results were normal, were compared with the control group who were fertile women, for any changes in their prolactin receptor gene. The techniques used in this study were PCR-SSCP and Sequencing .the DNA was extracted from the blood samples by salting out method and then amplified by the polymerase chain reaction .The purified PCR products, were sequenced by the sanger sequencing technique in order to confirm the SSCP result.No change in any of the eleven exons of the prolactin receptor gene was detected neither in control group nor in the patients group, consequently it can be concluded that idiopathic hyperprolactinemia in the affected infertile women, is not in association with any change in the genome of the prolactin receptor.To our knowledge this is the first study on this gene in this group of patients.

J01.37
The role of ID gene family in transformation of endometrial lining of uterus during menstrual cycle

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The inhibitor of DNA binding family members (ID1,ID2,ID3,ID4) inhibit activity of basic helix-loop-helix transcription factor and have an important role in cell growth ,differentiation and angiogenesis.
Due to the high proliferation and angiogenesis in the endometrial lining of the uterus during the menstrual cycle, it seems that this gene family may be involved in the occurrence characteristics of this tissue.
This study focused on expression of ID gene family by real-time PCR in 21 healthy fertile women undergoing tubal ligation surgery, between 20-45 years old to investigate the possible role of this gene family in endometrial tissue changes during three phases (menstrual ,proliferative , secretory) of menstrual cycle. For this respect ethical approval and informed patient consent was gained for the use of tissue sample.
Data revealed high levels of mRNA expression for ID1, ID2, ID3 and ID4 in proliferative phase. Also, all members of this gene family showed more significant expression levels during the secretory phase than the proliferative phase of the menstrual cycle.
These results suggest for the first time that ID gene family has a dynamic role in the proliferation and angiogenesis of endometrial cells. We propose that changes in expression pattern of this family can be reviewed in gynecologic disorder and infertility that related to embryo implantation.

J01.38
Polymorphisms of *Toll Like Receptor* 2, 3 and 4 in patients that do and do not enter labour spontaneously at term

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To assess the association of polymorphisms of *Toll Like Receptors (TLR's)2,3 and 4* with the delay in onset of labour at term pregnancies, patients delivering at >37 weeks and without preeclampsia, IUGR or a history of preterm delivery were prospectively evaluated. *TLR*2 Arg753Gln, *TLR3* (c.1377C/T) and *TLR4*Asp299Gly and Thr399Ile polymorphisms were genotyped by using PCR-RFLP. Patients labouring spontaneously before the 41st week were compared with those who did not labour spontaneously until this week in terms of baseline characteristics, *TLR* 2, 3 and 4 polymorphisms. The same comparisons were also performed by using 40th week cut-off. Chi-square test, two sample T test or Man-Whitney U test were used for comparisons as appropriate. 79 patients delivering after 37 weeks were evaluated. All had CC genotype for *TLR*2 Arg753Gln and *TLR*4 Thr399Ile. There were no significant differences for *TLR*4Asp299Gly GA and *TLR*3 (c.1377C/T) polymorphisms between patients spontaneously entering or not entering labour until the 41st week; the same was true when the 40th week cut-off was used. Delay in onset of labour at term pregnant patients does not seem to be affected by presence of *TLR* 2, *TLR* 3 or 4 polymorphisms. Further studies are needed.

J01.39
Prenatal diagnosis of AIPL1 related Leber Congenital Amaurosis (LCA) in the Iranian population: application of novel informative markers rs11658369 and rs8066853

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Leber congenital amaurosis (LCA) shows clinically and genetically heterogeneous disorder which is caused by a large number of mutations in at least 17 different genes. In the present study the application of two genetic markers including rs11658369 and rs8066853 markers located in AIPL1 genetic region were genotyped and their application were investigated in prenatal diagnosis of the disease in the Iranian population. The markers were genotyped using newly designed specific primers by Tetra-primer ARMS PCR in 150 unrelated healthy individuals in the Iranian population. Analysis of the genotyping data using GenPop program, indicated the presence of informative haplotypes (>5%) with strong linkage disequilibrium for the markers and the AIPL1 gene. The efficiency of these haplotypes as a suitable tool in prenatal diagnosis of LCA was evaluated in two Iranian families with one affected child with an already diagnosed W278X mutation in the AIPL1 gene, through an APEX microarry screening and sequencing analysis. The transmission of the normal and affected alleles form parents to their affected child and the fetus was confirmed by using the haplotypes obtained by genotyping of rs11658369 and rs8066853 markers. Interestingly, in line with the mutation results, the genotyping data also confirmed the transmission of normal alleles to fetuses. The families now were given birth to children with normal vision as confirmed by ophthalmic diagnosis. The data suggested that rs11658369 and rs8066853 could be suggested as novel informative markers for linkage and prenatal diagnosis of AIPL1 associated LCA in the Iranian population.

J01.40
Analysis of pedigrees of families with reproductive losses

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Given the important role of genetic factors in the genesis of reproductive losses in families with miscarriage, we analyzed taking into account the reproductive disorders in families. When analyzing families accounted reproductive losses and infertility among relatives of the 1st, 2nd, 3rd and 4th degree of consanguinity. Analysis was conducted among 226 families with one miscarriage, 277 families with two miscarriages, 117 families with three miscarriages in history. The comparison group consisted of 201 families in history, which marked the birth of a healthy baby. Conducted under the EBM calculations showed that the presence of relatives in the pedigrees of families of women with reproductive losses is IP = 2,47 (CI- 95% 1,62:3,75) . Among the male relatives of the families of miscarriage, this indicator was IP = 2,18 (CI- 95% 1,27:3,59). In general, the odds ratio for relatives with reproductive disorders, excluding sex pedigrees in groups with reproductive losses and in the comparison group was IP = 2,79 (CI- 95% 1,95:3,98). Thus, in families with reproductive losses 2.5 times more relatives with reproductive disorders, compared with the families of the comparison group. This indicates a hereditary component, leading to reproductive losses. Availability unspecified hereditary factors, both by women and by men contributes to further research to find genetic markers defining gender reproductive disorders.

J01.41
Methylenetetrahydrofolate reductase C677T and A1298C polymorphism is not associated with oligozoospermic and azoospermic infertile male patients in the Turkish population

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Folate pathway plays significant role in the cell phsiology by participating in the DNA repair, methylation and genomic stability. Optimal function of the pathway is essential for high metabolic activity of the testicles. Methylenetetrahydrofolate reductase (MTHFR), the key enzyme of folate metabolic pathway, was reported to be five times more active in the testicles compared to other organs in a study with adult mice. This is the first study researching the association of MTHFR 677C>T (rs1801133) and 1298A>C (rs1801131) polymorphisms with infertility of the idiopathic nonobstuctive azoospermic and oligozoospermic patients. Study population included nonobstuctive 75 azoospermic and 62 oligozoospermic nonconsangiuonous infertile patients referred to Department of Medical Genetics of Trakya University between 01.03.2012-01.06.2013 due to the infertility who had been diagnosed based on the clinical examination and spermiograms (World Healh Organisation standarts, 2010) .All patients had normal karyotype without Y microdeletion. Melting curve analysis with labeled probes and primers designed by the manufacturer's (NLM Diagnostics, Italy) and Real Time Polymerase Chain Reaction method (Qiagen, Rotor Gene) have been used. There was no statistically significant association of MTHFR C677T and A1298C polymorphisms and infertility in the study population (p>0.05). The differences in the dietary folic acid intake between populations, even between the different regions of the same geographical area may cause the different results. The similarity of our results with other studies on Caucasian population can be explained by the patients included in this study live in the Trakya region of Turkey and they were being exposed to similar enviromental factors.

J01.42
The association study of MSH5 C85T and MLH3 C2531T polymorphisms in infertile men with non-obstructive azoospermia

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Introduction: Genetic factors cause about 10% of male infertility. However, the etiology of the majority of male infertility cases including non-obstructive azoospermia remains idiopathic. Defects in DNA repair during spermatogenesis are thought to underlie some types of testicular failure. Evidence is accumulating the mismatch repair proteins MSH5 and MLH3 play a crucial role in spermatogenesis. In the present study, we investigated the association between MLH3 C2531T and MSH5 C85T polymorphisms and developing non-obstructive azoospermia.
Methods: In a case-control study, peripheral blood samples were obtained from 110 non-obstructive azoospermia patients and 102 proven fertile men. DNA was extracted by using salting out method. Y chromosome

microdeletions were studied using Multiplex PCR. MLH3 C2531T variants were analyzed using the tetra-amplification refractory mutation system-PCR (4P-ARMS-PCR) method in the patients and controls. MSH5 C85T variants were determined by PCR-RFLP assay in the studied groups. SNPSTAT program was used for the detection of allelic and genotype frequencies and the association between non- obstructive azoospermia and the mentioned polymorphisms.
Results: 14 patients (12.7%) showed Y chromosome microdeletions and therefore were excluded from our study. No association was detected between MLH3 C2531T and MSH5 C85T polymorphisms with developing non-obstructive azoospermia in Iranian patients.
Conclusions: Our results suggest that these polymorphisms may not be considered as a genetic risk factor for non-obstructive azoospermia at least in Iranian population. Variability of the results in other populations may be explained due to differences in the ethnic backgrounds.

J01.43
Effect of oxidative stress on *KDM5D* expression in mature mouse testis

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Male factors are important causes of infertility. Two major causative factors of male infertility are oxidative stress (OS) and genetic factors. OS damages the sperm plasma membrane, the genome integrity and expression profile of genes involved in spermatogenesis. *KDM5D* or *SMCY* is one of these genes which its alteration is associated with male infertility.
In this study the expression profile of *KDM5D* gene was evaluated in testis tissues of infertile after OS induction.
Oxidative stress in adult mice testis was induced by injection of the 1:10 concentration of tertiary-butyl hydroperoxide (TBHP). Adult male Balb/c mice were randomly selected. Case group included treated mice by TBHP for 2 weeks and control group treated only by injection of dH_2O. Induced ROS levels in testes tissue samples of all mice measured by flow-cytometry. Consequently the expression of *KDM5D* gene was quantitatively measured in samples of both groups by real-time PCR.
According to Flow-cytometry results, an increase of oxidative stress in ROS treated mice in comparison to control group was observed. Moreover, the expression level of *KDM5D* gene was lower in TBHP treated mice than that of in control mice.
Oxidative stress can have detrimental effects on testicular tissue and alters the expression of some genes which are involved in spermatogenesis.

J01.44
The case of prenatal diagnosis Pallister-Killian syndrome in Sverdlovsk region (Russian Federation)

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Pallister-Killian syndrome or Tetrasomy 12p is an uncommon aneuploidy, which may present in the prenatal period with an ultrasonographically detected fetal abnormality.
A 30-year-old woman was referred to our center for evaluation of a frontal edema in her fetus. The pregnancy was uncomplicated and there was no significant medical history of the family. The initial screening scan was performed at 13 weeks gestation and an increased nuchal translucency (3, 8 mm) was obtained. At 14 weeks gestation, the expert ultrasound examination demonstrated increased nuchal translucency (4,1mm) and heart defect (tricuspid regurgitation). The couple was counseled concerning the options of invasive tests for karyotyping of fetus.
The parents accepted to perform a placental biopsy at 15 weeks gestation.
The cytogenetic analysis has shown that all cells of placental villus had a supernumerary, metacentric marker chromosome.
Prenatal molecular assay for detecting aneuploidies and microdeletion syndromes (Prenatal BoBs™), that was obtained in parallel with cytogenetic analysis has defined a gain of chromosomal copy number in group autosomal probes (region 12p13).
Using the method fluorescence in situ hybridization (FISH) we have confirmed that the extra chromosome was derived from chromosome 12.
The parental karyotypes were subsequently checked and were both normal.
On the basis of this, given the poor prognosis for Pallister- Killian syndrome and after counseling, the couple elected to terminate the pregnancy at 17 weeks gestation.

J01.45
Association FBLN5 gene polymorphism with pelvic organ prolapse.

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Pelvic organ prolapse - multifactorial disorder characterized by a loss of pelvic floor support leading to the herniation of the uterus into or through the vagina. One of the most important genes encoding proteins of elastic fiber matrix assembly and function is *FBLN5.* We used tagged SNP approach to increase the genetic coverage of the *FBLN5* gene (Haploview 4.2 software). The genotyping of all eleven selected SNPs was performed using a PCR-CTPP (polymerase chain reaction with confronting two-pair primers) and real-time PCR. The study sample set included patients (n=210) diagnosed with stage III-IV prolapse based on the Pelvic Organ Prolapse Quantification (POP-Q) examination, and controls (n=292) were women without prolapse and no prior history of prolapse surgery. Multiple logistic regression analysis adjusted for age, body mass index and vaginal parity was applied to evaluate the associations between *FBLN5* SNPs and POP in the entire set and in the strata with/without perineal trauma and fetal macrosomia. The top association signal was found for SNP rs2018736 (protective effect for the minor allele A; recessive model) in the entire set: P =0.0026, OR=0.42, 95% CI:0.24-0.75; in the strata with perineal trauma: P =0.0018, OR=0.27, 95% CI:0.11-0.64; and in the strata with fetal macrosomia: P=0.013, OR=0.14, 95% CI:0.03-0.71. The results of the haplotype analyses were consistent with the single SNP analysis. The results are clinically important providing a rationale for fibulin-5-targeted therapy in women combining genetic and clinical determinants of higher risk for POP.

J01.46
Preimplantation genetic diagnosis (PGD) for beta thalassemia and birth of a healthy boy after 4 times of therapeutic abortion

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A couple who was referred to us for prenatal diagnosis (PND) for beta thalassemia had experienced 4 consecutive pregnancies with diagnosis of thalassemia major. After four times of therapeutic abortions, the couple decided to have healthy child using preimplantation genetic diagnosis (PGD).
Ovulation induction and ovum fertilization were performed in Erfan Hospital, Tehran, Iran. In the 3rd day, a single blastomere from each embryo (in total 8) was removed and given to us. For molecular PGD, a multiplex nested PCR, using several STRs markers linked to HBB gene and also amplifying part of the β-globin gene, was done. Out of 8 tested cells, 3 blastomers were thalassemia minor, 2 homozygous normal and one thalassemia major. Also two embryos did not give us conclusive result on being affected or normal due to allele dropout (ADO).
The family decided to transfer 3 embryos. Three weeks after embryo transfer, pregnancy test was positive and a single pregnancy was continued up to the 11th week gestation. The family agreed on fetal testing. CVS was followed. Genetic testing for beta thalassemia and chromosomal aneuplidies showed a healthy boy who was carrier of thalassemia minor. A healthy boy was born on the Feb 4, 2014 by cesarean section. Similar test on placental sample confirmed our findings.

J01.47
Genomic variability study: CNVs analysis in women with premature ovarian failure (POF)

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Premature ovarian failure (POF) is defined by amenorrhea of at least 4 to 6 month duration, occurring before 40 yr of age, with two FHS levels in the postmenopausal range. Genetic basis for POF are FMR1 gene premutation and chromosomes anomalies detected by karyotype but its etiology remains unknown in more than 80% of cases. Copy Number Variation (CNVs) form an important class of human genetics variants. Array Comparative Genomic Hybridization (Array-CGH) analysis ia able to detect submicroscopic chromosomal rearrangements with a higher genomic resolution.
In this study we selected 32 women affected by POF, with normal FMR1 premutation and normal karyotype. This cohort have been analysed by Array-CGH 8x60K Agilent platform with a resolution of 100Kb, to identify an association between CNVs and POF. We observed 23 CNVs in 12 patients (37.5%): 3CNVs on the X chromosome and 20 on autosomal chromosomes; 14 duplications and 9 deletions; the rearrangements size were between

122Kb and 91.26Mb. In conclusion our data, accordingly with already published data, confirm the utility to use array-CGH to detect CNVs genome-wide for POF diagnosis and to identify new possibly pathogenetic genes.

J01.48
Expression of HIF and VEGF genes and pregnancy loss

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15% of human pregnancies are known to end in spontaneous abortion before 12 weeks of gestation. A disbalance of cytokines and growth factors can affect negatively early stages of human embryogenesis. The aim of the study is to determine the expression level of the growth factors genes in miscarriage patients.
Study was performed on the RNA samples from two groups of women. The first group included of women with a missed abortion or spontaneous abortion. The control group included women with normal pregnancy. The samples of RNA were extracted from decidua and chorion. The level of the gene expression was assessed using the two-step reverse transcription probe-dependent fluorescent real-time polymerase chain reaction.
Hypoxia inducible factor (HIF) is the primary molecular sensor responded to oxygen tension changes. HIF as transcription factor regulated many cellular processes, for examples angiogenesis, invasion, cell survival. HIF in hypoxia condition provides a potent stimulus for VEGF synthesis and is essential for development of maternal and placental vasculation in early human pregnancy. In the case of miscarriage the level of HIF-1 gene expression was reduced in the chorion. Analysis of gene expression vascular endothelial growth factor in physiological pregnancy showed that mRNA levels of this gene was significantly lower in decidua compared to the chorionic tissue ($P = 0.032$). In case of miscarriage the level of VEGF gene expression in chorionic tissue was not different from decidual tissue and significantly lower compared with the level of gene expression in the control.

J01.49
Paternal and maternal origin of Primary ovarian insufficiency (POF/POI) caused by FMR1 gene premutation - using Repeat Primed PCR (RP-PCR) method

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Patients and methods: Early ovarian depletion criteria were consistent with international protocols: secondary amenorrhea, ovarian failure up to the age of 40 years, levels of FSH≥ 40 IU/L, and low estrogen levels. We investigated the CGG trinukleotide repeats in the FMR1 promoter region by hybridization with radiolabeled DNA probes. The premutation CGG repeat number was 55-200, and the gray zone was between 45-54 repeats. **Results:** In a 38-year-old woman suffering from premature ovarian exhaustion we found deviations on both alleles of FMR1 gene during the Repeat Primed PCR (RP-PCR) tests. On one allele the CGG repeat number was 76. On the other allele the CGG repeat number was located in the so-called gray zone (CGG 52). Given that on both alleles deviations were found, we examined the family. We found a 29/52 CGG repeat number in her mother, and a 76 CGG repeat number in her father. The menopause of the mother occurred at the age of 46, the neurological examination of the father for the Fragile X Associated Tremor Ataxia Syndrome is in progress. In the brother of the female patient, a CGG repeat number 29 was detected. **Conclusions:** The genetic examination of the premature ovarian failure is very important for the patient and her family also, because the genetic results have serious influence on the reproductive possibilities and family planning of the premutation carriers.

J01.50
Maternal variant gene RHD and clinical effects during pregnancy and at birth

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During her second pregnancy, a 35 year old patient known with RH:1 status surprisingly has an anti-RH1 allo-immunization at 24 weeks of pregnancy. Prenatal non-invasive RHD genotyping on fetal maternal plasma was performed using real-time PCR (TAQMAN technology) studying three regions of the RHD gene (exons 4, 5 and 10) and shows amplification of the 3 exons. The maternal DNA sequencing allows to identify the type IVa RHD variant characterized by lesions of exons 2, 3 and 7 : RH1 corresponding antigen exposed the patient to the risk of anti-RH1 immunization. The titer of the anti-RH1 increases dramatically in late pregnancy (maximum title 1024), however, without sonographic fetal anemia. At birth, the child is RH:1 and has a minor anemia 120 g / l but a positive direct antiglobulin test (due to maternal anti-RH1 antibodies on the surface of his erythrocytes). But at the 12th day, the child presents a deep hemolytic anemia at 52 g / l; requiring 4 CGR transfusions (12th day, 13th day and 30th day). The sequencing of the RHD gene of the child at birth can highlight the two copies of the RHD gene: an allele with the standard RHD gene inherited from the father, and the other with RHD variant (type 1 DIV) identical to mother's. This case shows the possible clinical consequences of anti-RH1 alloimmunization in a mother carrying a variant of the RHD gene.

J01.51
Diagnostic algorithm for differential diagnosis in high-risk pregnancies identified by prenatal ultrasound screening: two case reports

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Hereby, we present 2 case reports of pregnancies with an initially high risk of congenital anomalies, detected by 1st and 2nd trimester prenatal ultrasound screening (US), that was significantly reduced by a complex follow up approach. Case 1: a 34-year-old woman (12th week-of-gestation: w.g.) was referred due to increased nuchal translucency (NT: 4.5 mm) and suspected hygroma colli cysticum detected by 1st trimester US. Following CVS we performed examinations comprising standard karyotyping, QF-PCR, arrayCGH, MLPA of subtelomeric regions and SMN1 gene, sequencing of DHCR7 gene and TORCH serology with normal outcomes. US performed in the 16th w.g. proved regression of NT and absence of other congenital anomalies, except for hyperechogenic bowel (CFTR negative). Fetal ECHO and 3D US were normal. In Case 2 a 30-year-old woman (22nd w.g.) was examined due the absence of nasal bone and renal pelvic dilatation on 2nd trimester US. Following AMC and cordocentesis we utilized identical diagnostic algorithm (except MLPA) and furthermore sequencing of FMR1 and FGFR3 genes with negative results. Both families were reassured and thus opted for continuation of their pregnancies, which resulted in the in term delivery of apparently healthy children (male and female). Our complex diagnostic algorithm allowed exclusion of the most of severe prenatal affections that were suspected by US screening and substantially decreased their risk. These case reports substantiate the diagnostic utility of our algorithm and underscore the importance of multidisciplinary approach to high-risk pregnancies. Supported by NT13770-4, FNM 00064203 and OPPK CZ.2.16/3.1.00/24022.

J01.52
The utility of conventional cytogenetics and aCGH analysis in the diagnosis of the products of conception

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During November 2012-January 2014, 42 samples were analyzed by conventional cytogenetic methods and 5 cases by aCGH technique (Array Comparative Genomic Hybridization). The analyses were performed for early miscarriages or for induced pregnancy termination due to severe fetal ultrasound abnormalities. The samples evaluated were from products of conception between 7 and 23 weeks of pregnancy, in patients aged between 24 and 41 years.
Depending on the biological sample received, either, chorionic villi, epithelial tissue, amniotic fluid or fetal cord blood was used for analysis. The karyotyping was successfully achieved in 40 cases and the aCGH analysis for all 5 cases.
Of the 40 cases analyzed by conventional cytogenetics, 23 (57.5%) had abnormal karyotype and in 17 cases (42.5%) no structural or numerical chromosomal abnormalities were identified.
Among the abnormal cases we identified: 13 homogeneous autosomal trisomies, 3 mosaic abnormalities, 2 unbalanced structural chromosomal abnormalities, 1 trisomy and unbalanced structural chromosomal aberration, one triploidy, one monosomy X, 2 double autosomal trisomies, one clonal chromosomal instability.
ArrayCGH analysis identified trisomies of chromosomes 21 and 22. In one case genomic aberrations were identified, including genes involved in embryonic development. For two cases with high gestational age the abnorma-

lities identified were correlated with the severe changes in ultrasound for which the decision of termination was taken, namely cardiac malformation in one case and sacral tumor formation for the second.
The results of our study sustain the importance of cytogenetics analysis for miscarriages, and aCGH may bring important information for pregnancies with ultrasound anomalies.

J01.53
Qf-pcr in Prenatal Diagnosis: preliminary evaluation of advantages and pittfalls

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Quantitative fluorescence PCR (QF-PCR) is widely used for the rapid prenatal diagnosis of common aneuploidies by the analysis of short tandem repeats (STR). The amount of each allele is quantified by calculating the ratio of the peak height or area. In our laboratory for standard risk pregnacies (screening by combined test or maternal age) we replace the Short Time Culture (STC) chromosomal analysis of chorionic villi (CV) by (QF-PCR) while we hold STC+LTC for different indications (parental chromosomal abnormalities, uncommon aneuploidy in previous pregnancy, ultrasonographic abnormalities, included NT>3,5). In all QF-PCR positive and when structural or mosaic anomalies are suspected we perform STC too. We also support the karyotype analysis of amniotic fluid (AF) by QF-PCR in > 19th weeks pregnancies. 5% of the 417 CV and 10% of the 95 AF gave an aneuploid result, consistent with the classical karyotype of both STC and LTC. In case of poor sample QF-PCR allowed to resolve maternal contamination dubts (14 cases). One case of vanishing twin, 9 mosaic karyotype cases, zygosity of 6 twins, a dubt Array-cgh on Y chromosome ring duplication also were supported by the QF-PCR results. We further analyzed 13 parental Periferal Blood (PB), to investigate Primer Binding Site Polymorphism (PSP) vs Sub Microscopic Duplications (SMD) or Somatic Microsatellite Mutation (SMM) for D13S1492, D18S386, D21S1442, DXS1187, DXS981, DXS2390, DXYS267, DXYS218, TAF9/TAF9L, BRAF/BRAFP1)and others microsatellites.

J01.54
QF-PCR: our experience in prenatal diagnosis and recurrent miscarriages

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Our center is responsible for invasive prenatal diagnosis from the 90s, every year we process by conventional cytogenetic analysis about 1200 samples between amniotic fluid (AF) and chorionic villi (CVS), and about 70 samples from recurrent miscarriages. In 2010 we introduced the QF-PCR analysis for the most common aneuploidies (chromosome 21, 18, 13, X and Y) as support to cytogenetics in :
1. AF samples: when there is a need to for a rapid response in cases of advanced maternal age, clinical suspicion of fetal anomaly or in amniocentesis performed late (more than 20 weeks)
2. CVS samples: in all cases, in conjunction with cytogenetic analysis of cytotrophoblast by direct preparation (STC) or to exclude maternal contamination in chorionic villus DNA used in molecular studies. Over the last three years, we extended QF-PCR analysis to recurrent miscarriages because traditional cytogenetic testing is labour-intensive and has a significant failure rate, especially when the sample quality is poor. In this case we investigate a greater number of chromosomes: 13, 15, 16, 18, 21, 22, X and Y, whose aneuploidies are the major cases of miscarriage.
In this work we report a summary of our experience, using CE-IVD QF-PCR systems CY5- labelled. In prenatal diagnosis QF-PCR system proved to be an effective and specific support to cytogenetic analysis, and a useful and reliable tool to diagnose aneuploidies in spontaneous miscarriages, reaching a pathology's diagnosis in 45% of cases and we're considering to replace cytogenetic analysis with this molecular system.

J01.55
The correlation between sperm DNA fragmentation and recurrent abortion in Iranian population

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Background: as previous studies have mentioned that sperm DNA quality may be related to unexplained recurrent abortion, this association study was designed to evaluate the degree of sperm DNA fragmentation using the SCSA (Sperm Chromatin Structure Assay) on sperm from couples with unexplained recurrent abortion compared to sperm from men of the general population without evidences of infertility, as control group. **Method:** After collection of ejaculated sperm from 60 couples with recurrent abortion and 30 couples with proven fertility, semen specimen were freezed by placing aliquots immediately under neat freezing conditions using liquid nitrogen. After thawing at approximately 37°C, sperm cells were stained with fluorescent intercalating dye, Acridine orange, after exposure to acidic conditions. Flow cytometry analysis was used to separate ssDNA as fragmented DNA from dsDNA as integrated DNA reported as DFI (DNA Fragmentation Index). Current references indicate that DFI more than 27% correlates with poor fertility potential. **Results:** In this study significant relationship between DFI over 27% of couples with recurrent pregnancy loss (43.3%) compared to normal group (13.3%) was observed (p<0.05). **Discussion:** The SCSA technique is considered to be the most time and cost effective, precise and repeatable technique for sperm DNA fragmentation assay. Due to the noticeable rate of abortion cases in iranian population, sperm DNA fragmentation analysis should be considered as a contributing factor in this incidence, as a screening test. Therefore treatment of DNA fragmentation may be important in reducing abortions.

J01.56
The Y chromosome gr/gr subdeletion is not associated with recurrent pregnancy loss

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Recurrent pregnancy loss (RPL) is defined as the miscarriage of two or more consecutive pregnancies in the first or early second trimester of gestation. RPL may be associated with several factors including endocrine, anatomical, psychological, infectious, thrombotic, genetic, or immunological causes. Approximately 50% of cases remain unexplained, Thus, treatments are only marginally successful. The majority of the testing for RPL assesses the woman, however, male factor is rarely discussed and has been poorly evaluated in RPL. The Y chromosome partial microdeletions including gr/gr microdeletion have been associated as a risk factor for infertility. The aim of the present study was to investigate whether Y chromosome gr/gr microdeletions was associated with RPL in a Iranian population.
Methods: The subjects were 88 male partners of couples where the female partner had experienced t or more RPLs. Fifty proven fertile males from the general population were also analysed as a control group. DNA extracted from peripheral blood was tested for Y chromosome gr/gr microdeletion using a multiplex PCR.
Results: gr/gr deletion was detected in 7 (8.64%) patients and 2(4.16%) fertile controls. However, the difference was not statistically significant (p>0.05).
Conclusion: gr/gr microdeletion in the Y chromosome does not appear to be important in the aetiology of RPL. However, ethnic variations may result in different consequences in other populations.

J01.57
An apparently balanced Robertsonian translocation rob(13;14) associated with a small chromosome marker identified in prenatal period

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We present a de novo balanced Robertsonian translocation (13;14) identified in prenatal period. The women, aged 32 years, asked the prenatal diagnostic because she had a previous pregnancy with 21 trisomy. The chorionic villous sampling made at age of 12 weeks of amenorrhea was followed by prenatal analysis by FISH technique, using Aneuvysion® kit and confirmed a 46,XX chromosomal formula. Cell culture indicated an apparently balanced Robertsonian translocation rob (13;14) and a small chromosome marker. The chromosomal analysis in both parents was normal. We repeated the prenatal analysis after amniocentesis at age of 17 weeks of amenorrhea. The G banding confirmed the presence of both anomalies: an apparently balanced Robertsonian translocation rob (13;14) and a small chromosome marker. The C banding indicated a possible derivative chromosome der (13;14) with two centromeres. To identify precisely the chromosomal anomalies we asked the help of Prof. Thomas Liehr, and the FISH analysis, made at University of Jena, with probes for centromeres of chromosomes 13/21 and 14/22, and probes for all acrocentric p-arms confirmed that one break appeared in the short arm of chromosome 13 and the second one in the centromere of chromosome 14. The latter leads to alphoid DNA derived from chromosome

14 being present on both derivative chromosomes. Thus, fetus has the chromosomal formula: 46,XX,t(13;14)(p11.2;p11.1)dn. The immunohistochemistry attested one active centromere on the chromosome marker and two active centromeres on the large derivative chromosome. We gave to parents the genetic counseling and they decided to keep the baby, which is normal.

J01.58
Premature chromatid separation in a woman with unexplained recurrent pregnancy loss; A case report

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The current case reports show an increased frequency of lymphocyte cells with premature chromatid separation (PCS) involving all methaphase chromosomes in blood cultures of a woman with RPL. Heparinised peripheral blood cell culture was made for the current couple with RPL. We analysed metaphase spreads from a couple with RPL using a rigorous GTG banded protocol to score the affected chromosomes. No structural and/or numerical chromosome abnormalities were detected in the current couple but multiple PCS was detected in mitotic spreads from 38-year-old mother. These findings from a proband case provide evidence for a possible association of PCS and RPL.

J01.59
Sex chromosome rearrangements and implications for the phenotype

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Sex chromosome structural abnormalities are frequently found in individuals with reproductive failure or disorders of sexual development. X-chromosome rearrangements are identified in patients with Turner' syndrome, usually in a mosaic form. The most frequent structural abnormality in these cases is the X isochromosome for long arms, although a ring X-chromosome or other variants can also be found. Additionally, other X-rearrangements are detected in patients referred for reproductive failure, infertility or even in woman with no clinical indication, including X translocations, partial deletions or duplications. In balanced X-autosome translocations, breakpoint position and replication behavior may influence phenotypic outcome. Rearrangements on the Y chromosome include: X-Y translocations, Y-autosome translocations, isochromosomes, rings, partial deletions and inversions. Dicentric Y isochromosomes for the short arm or ring Y chromosomes are the most frequent abnormal Y chromosomes found in infertile patients and in Turner syndrome, in mosaic with 45,X cells. In all these cases, it is important to well characterize the derivative chromosomes in order to establish a good genotype-phenotype correlation, to provide information about the role of different X/Y chromosome regions or loci in the clinical manifestations of patients.
For this purpose, we have collected patients with different sex chromosome rearrangements detected with karyotyping: X-autosome translocations (3); X isochromosome (3), X deletions (4); ring X (1); X inversion (1); Y-autosome translocations (4); Y isochromosome (1). The rearranged chromosomes have been analyzed using FISH, MLPA or aCGH when appropriate. After a detailed clinical assessment, genotype/phenotype correlations have been performed.

J01.60
Management of sex differentiation disorders at University Hospital Hassan II Fes

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Sex differentiation disorders represent all the abnormalities in development of the gonads, the genital tracts, and the external genitalia. Disorders of sexual differentiation are due to genetic defects or endocrine imbalance. Sex-determining genes (SRY gene) dictate the gonadal sex whereas the fetal testicular hormones determine the somatic sex during sex differenciation. Abnormal sexual development causes unconformity between gender identity and gender role.
The aim of this study was to evaluate the frequency, the genital anatomy appearance, the diagnostic and the surgical management of disorders of sex development (DSD) discovered during the neonatal period and the enfance
Between September 2009 and Marsh 2013, 30 patients with abnormal sexual development were identified in our unit. First-line testing included biology measurement and imaging. A surgical management was offered for some patients. Sexual dimorphic with genital ambiguity was the first reason of consultation. One patient had male breast development. All clinical evaluation suggested genital ambiguity.
This presentation points out the need of an accurate diagnosis of sexual differentiation disorder during the neonatal period. The intervention of a multidisciplinary team is essential as well for assignment of sex as for therapeutic guidelines.
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J01.61
Polymorphic locus 820AG of IGF-2 gene as a possible marker of fetal development disorders

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Insulin-like growth factor-2 (IGF-2) is a mitogen, growth and differentiation modulator for many cell types. It is mainly expressed during the prenatal development, and its activity strongly depends on the genomic imprinting. Genomic imprinting in the chorionic tissues of spontaneously eliminated human embryos has been studied on the model of 820AG (Apa1) of the IGF-2 gene locus. Methods. Isolation and purification of DNA and RNA, PCR-RFLP, RT. Results. Molecular and genetic analysis was performed of the polymorphic locus 820-AG IGF2 in 107 samples of DNA extracted from the chorionic tissues of spontaneously eliminated human embryos within 5-10 weeks of gestation. The loss of imprinting of the IGF2 gene was analyzed in 41 samples of the chorionic villi cells in human. In 90% of cases, the loss of imprinting was detected. Presence of AG genotype of SNP 820AG of IGF2 gene was shown to cause more than a 7-fold increase in the risk of embryo elimination (OR = 7.72, CI 95% 3.31-18.04). Conclusion. The loss of genomic imprinting of the IGF2 gene may be an important cause of the miscarriages in human.

J01.62
Decreased expression level and chromatin incorporation of histone acetyltransferase CDY1 in testicular biopsies of infertile men with non- obstructive azoospermia

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Background:
Many cases of male infertility associated with a severe impairment of spermatogenesis. During the last stage of spermatogenesis (spermiogenesis), haploid spermatids endures complex changes to differentiate into spermatozoa, this process includes chromatin modifications mediated by different histone modifying enzymes. Chromodomain Y (CDY) proteins encoded by the CDY family of genes, are characterized by two functional motifs, a chromodomain and a histone acetyltransferese catalytic domain. A testis specific CDY protein, named CDY1, binds to methylated histone regions through its chromodomain, and then causes hyperacetylation of genes involved in sperm chromatin condensation. This study aimed to investigate the relative mRNA expression and chromatin incorporation of chromodomain Y1 (CDY1) protein in the testis tissues of infertile men.
Material & Method:
Local ethical approval was gained for this study and informed consent was given by patients. Testicular biopsies were collected from 31 infertile men referred to Royan Institute and underwent testicular sperm extraction (TESE). These samples distributed into 4 groups: obstructive azoospermia (positive control), severe oligoasthenoteratozoospermia, non- obstructive azoospermia and sertoli cell only syndrome.
Using qRT-PCR and nucleosome-ELISA methods, the mRNA levels and chromatin incorporation of CDY1 was evaluated in the tissue samples.
Result(s):
Our data significantly showed lower expression and chromatin incorporation of CDY1 in all 3 sample groups with spermatogenesis defect in comparison to positive control.
Conclusion(s):
This data demonstrated that the defective epigenetic role of the histone acetyltranferase CDY1 may be associated with male infertility.

J01.63
Conventional CGH vs FISH and karyotyping in detection of chromosomal abnormalities in first-trimester spontaneous abortion

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Approximately 10-15% of clinically recognized pregnancies terminate with spontaneous abortions. Half of them are associated with chromosome abnormalities (CA). Cytogenetic analysis of chorionic villi has limitations such as high rate of culture failure, maternal cell contamination and poor chromosome morphology. FISH method with target probes doesn't allow to receive full information about fetal genome. CGH is the only DNA-based screening method that can detect chromosomal imbalances in a single experiment. In this study 60 abortion specimens were analyzed by G-banding, FISH and CGH (Table). Overall, CA were detected in 25 specimens. Karyotyping was unsuccessful in 31 samples, while CGH and FISH analyses were successful in all cases. G-banding analysis showed normal karyotype in 21 cases and detected abnormalities in 8 cases (32% of CA). FISH using probes targeting chromosomes 13, 18, 21, X and Y detected CA in 15 samples (60% of CA), but in two of these cases wasn't able to find double trisomies which were revealed by CGH. CGH detected CA in 21 samples (84% of CA) but missed triploidy in 4cases. Aneuploidies detected only by CGH were all confirmed by FISH with corresponding probes. CGH showed the highest detection rate of CA in comparison with karyotyping and FISH. CGH is a suitable technique for the detection of CA in spontaneous abortion with the exception of polyploidy that can be detected by FISH.

Comparison of karyotyping, FISH and conventional CGH analysis (n=60)

№	Karyotype results	FISH results	CGH results
1-9	46,XX	XX	Normal
10-21	46,XY	XY	Normal
22	48,XY,+2,+13	XY,+13	+2,+13
23	46,XX,der(13;D),+13	XX,+13	+13
24	47,XX,+15	XX	+15
25-26	47,XY,+16	XY	+16
27	47,XX,+18	XX,+18	+18
28	45,X	Monosomy X	Monosomy X
29	46,X,+18	Monosomy X,+18	Monosomy X,+18
30-35	Unsuccessful	XX	Normal
36-42	Unsuccessful	XY	Normal
43	Unsuccessful	XX	+2
44	Unsuccessful	XY	+7,+8
45	Unsuccessful	XX	+15
46-47	Unsuccessful	XY	+15
48	Unsuccessful	XX,+21	+15,+21
49	Unsuccessful	XY	+16
50	Unsuccessful	XY,+18	+18
51	Unsuccessful	XY,+21	+21
52-53	Unsuccessful	XX,+21	+21
54	Unsuccessful	XX	+22
55	Unsuccessful	Monosomy X	Monosomy X
56-59	Unsuccessful	Triploidy,XXY	Normal
60	Unsuccessful	Triploidy,XXX	Normal

J01.64

Is apolipoprotien E polymorphisms associated with spontaneous pregnancy loss

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Objective: Apolipoprotein E has three major isoforms (Apo E epsilon2, epsilon3 and epsilon4) and has important functions in nerve development and repair. To evaluate the association of Apo E polymorphisms and spontaneous abortion we studied the genotypes of spontaneously aborted fetuses, their mothers and control cases. Methods: In the current case control study, three different groups of aborted materials, mothers and healthy control were compared. Target gene of Apo E2/E3/E4 alleles were analysed by real time PCR. Results: The E2 and E4 alleles showed high prevalence in aborted materials comparing both with their mothers and healthy control group (p=<0.0001). The E4 allele was higher in fetuses comparing with their mothers (p=<0.0001). Conclusion: Apo E2 and E4 alleles seem to be contributing to the thrombophilic risk factors as a seconder parameter to spontaneous abortions.

J01.65

Parental idendification of aborted materials for chimerism and other molecular etiological parameters by microsatellite STR profilling

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Aim:Structural and/or numerical chromosomal abnormalities are responsible for 50-60% of the first trimester miscarriages, 20-25% for seconder and 5-10% for the third trimester miscarriages.In the current study it was aimed to use the microsatellite STR markers for the parental identifying of aborted materials.***Materials and Methods:*** Sixteen aborted materials and their parents were included in the current study.Thin skin biopsies from aborted materials and peripheral blood-EDTA samples from parents were used for total genomik DNA isolation. All samples were identified by using AmpF*ℓ*STR(®) Identifiler (Applied Biosystems) PCR Amplification Kit that provides15 different STR markers and compared.***Results:***One maternal originated trisomic (trisomy 13) aborted material and one is maternal and the other is paternal originated triploidic aborted materials were identified. One aborted material was showed maternal heterodisomic profile in the current results. ***Conclusion:***These results suggest that it is possible to identify the disomy, chimeric profiles and parental originated molecular cause of abortion by Identifiler Kit that provides specific microsatellite STR markers.

J01.66

Beta globin gene mutations in Kurdistan provinces, Iran

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Introduction: More than 95% of beta-thalassemia mutations are point mutations in the beta-globin gene in global population. This study was performed to determine beta globin gene mutation in blood transfusion dependent patients with in Kurd ethnicity in Kurdistan province in west of Iran.
Materials and Methods: Transfusion dependent patients were enrolled into the study. Diagnosis of beta thalassemia was confirmed using patients' parents' hematologic index and Hb electrophoresis. DNA was extracted from 5 ml of patient's blood using standard salting out method. Common mutations in Kurdish population were investigated by ARMS-PCR method. Unknown cases were investigated by direct sequencing of beta globin gene. Genotype frequency and allele frequency were calculated.
Results: Sixty eight transfusion dependent beta thalassemia patients (35 male and 34 female) with mean age of 16±6.89 years old were entered in the study. IVSII-1 had most common allele frequency in 37 (27.21%) chromosomes following by Fr8-9 in 22 (16.18%), IVSI-1 in 13 (9.56%), C36/37(-T) in 11 (8.09%) and IVSI-110 in 7 (3.67%).
Conclusion: IVSII-1 and Fr8-9 were the most common mutations in beta globin gene in Iranian Kurd thalassemia patients, which is in alignment with previous studies among Kurdish population.

J01.67

Results of prenatal tests in pregnancies after assisted reproductive technologies

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In vitro fertilisation (IVF) and other assisted reproductive technologies (ART) are effective treatments for infertility and are widely provided in developed countries. However recent scientific publications suggest that there is an elevated risk of major structural malformations, imprinting defects and such syndromes as Prader-Willy, Angelman, Wiedeman-Beckwith. The aim of the study was to analyse if ART are associated with increased risk of genotoxicy for chromosomal nondisjunction in meiosis. Study included analysis of 50 families that prenatally were diagnosed chromosomal anomalies (Down, Turner, Klinefelter, Edwards, Patau syndrome). And control group included 70 families with healthy children. The results of present study showed, that in chromosomal anomalies group three cases of children with chromosomal anomalies were conceived after ART. In control group all healthy children were conceived naturally (p 35 years old) and the occurrence of trisomy (p<0.05). The number of observed pathological cases is not so big to make exact conclusions, but results of present study supports hypothesis, that ART are associated with greater risk of chromosomal anomalies in conceived children.

J01.68

Associations of prenatally detected choroid plexus cysts with biochemical risk for congenital disorders

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Background: The choroid plexus cysts are one of the fetus ultrasonography

findings which concerns parents about their child's health. Usually cysts are found in an estimated number 1 % all performed ultrasonographies. The aim of the study to evaluate the risk of Down, Edwards syndromes and neural tube defect when choroid plexus cyst is found.
Materials and Methods: There were calculated risks of Down, Edward's syndromes and neural tube defect (NTD) by using second trimester biomarkers (alpha fetoprotein, human choriongonadotropin, free nonconjugated estriol) for patients with choroid plexus cysts. A control group was selected randomly with calculated risks and without any abnormal ultrasonography findings. These risks were compared between these two groups.
Results: 12 pregnancies with diagnosed CPC were included in this study during the year 2012. By comparing calculated risks for chromosomal abnormalities and NTD between test and control groups, we found out that there are no statistical significant difference.
Conclusion: There is no statistically significant data that choroid plexus cysts increases the risk of Down, Edwards syndromes and neural tube defect.

J01.69
Frequency of miscarriages twins

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Conducted a study to determine the frequency of twins in the structure of abortion among 968 families with miscarriage. Families with one miscarriage were 40.74 %, with 2 miscarriages 41.82 %, with 3 or more miscarriages 14.4%. Frequency of miscarriages twins was 0.88 %, which corresponds to the population frequency of 0.82 % of the Kaluga region. Comparative analysis showed that the differences are not significant at OR = 1,08 (CI- 95% 0,40:2,97). Thus, the recorded rate of reproductive loss miscarriage consisting of twins does not exceed the prevalence of twin births. This allows us to assert that the twin has no effect on the incidence of reproductive losses in the early stages of pregnancy and is comparable with the frequencies of registrable live births.

J01.70
Molecular management of 46,XX testicular DSD: About four North African cases

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The XX male syndrome (OMIM 400045) now termed Testicular DSD (Disorder of Sexual Differentiation) is a rare genetic condition (1:20,000 to 25,000 male newborns) characterized by a spectrum of clinical presentations, ranging from ambiguous to normal male genitalia. In males without genital ambiguity, the diagnosis is often made during investigation of infertility or delayed puberty with a frequency of 0,9% among azoospermic males.
Here, we report four observations of 46,XX men detected in our genetic counselling during genetic evaluation of infertility with azoospermia, hypergonadotrophic hypogonadism and testicular hypotrophy. Chromosomal abnormality revealed by two karyotypes in different laboratories was refined by molecular investigation of SRY gene using FISH (n=2) and PCR amplification (n=2) as well as AZF loci by multiplex PCR protocol.
The prevalence of XX male syndrome among our azoospermic men serie is 0,97% (4/412). Molecular analyses demonstrated the presence of Yp SRY gene in the four patients with absence of Yq AZF loci.
Review of literature shows that SRY positive Testicular DSD is the common variety (85%) and that PCR amplification of SRY is more appropriate, than FISH, to detect a small amount of Y chromosome translocated on to the X chromosome and to detect the Y-chromosome material in mosaic forms: XX-SRY positive/XX-SRY negative. Moreover, SRY-negative cases (15%) should undergo further molecular testing to explore the presence of SOX9, SOX3 or Sox3 promoter microduplication or microdeletion, or cryptic mosaicisme for Y chromosome. Recently, RSPO1 gene seems to be implicated in Testicular DSD associated with hyperkeratosis.

J01.71
Fluorescence in situ hybridization (FISH) on interphase nuclei in the uncultured chorionic villi samples from spontaneous abortions

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Background: Approximately 15% of all clinically recognized pregnancies end in spontaneous miscarriage. The most frequent cause of spontaneous miscarriage is fetal chromosome abnormalities such as autosomal trisomy, monosomy X and polyploidy. Molecular cytogenetic technique has been introduced in the genetic analysis of miscarriages in addition to the conventional karyotyping and provides new insights into this field. The present study displays frequency and spectrum of chromosomal abnormalities in embryos derived from spontaneous abortions in the western regions of Ukraine.
Methods: The study population consisted of 100 embryo tissues from women with the final diagnosis spontaneous abortions. Cytogenetic analysis was performed in the uncultured chorionic villi samples (CVS), using interphase FISH: CEP 13/21, CEP14/22, CEP22, CEP15, CEP 16, CEP17, CEP 18, CEP Y, CEP X, BACs RP11-2P5, RP11-89H21,RP11-973L24.
Results: Among the abortions the gonosomal constitution of XX prevailed (n = 58), followed by XY (n=42). Chromosomal abnormalities were found in 39 cases (39%): autosomal trisomies in 19 cases (49%), gonosomal monosomy X in 14 cases (36%), polyploidy in 6 cases (15%) (5 cases 69, XXN and 1 case 92, XXYY). Autosomal trisomies involved chromosome 14 (one case), 15 (4 cases) 16 (8 cases), 18 (3 cases), 21 (2 cases) and 22 (one case).
Conclusions: The present study demonstrates the value of FISH on CVS as an adjunct for understanding the etiology of SAs for cases in which karyotype is not available with conventional methods of cytogenetic studies. Autosomal trisomies was predominant followed by a gonosomal trisomy and polyploidy.

J01.72
A rare case of prenatal diagnosis of partial trisomy 14q co-existing 9p deletion from familiar translocation, with Arnold-Chiari malformation

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We report a case involving a familiar translocation between chromosomes 9 and 14 resulting in an unbalanced chromosome complement, with partial monosomy 9p (9p23->pter) and trisomy 14q (14q32->qter),in prenatal diagnosis.
A 40-year-old woman, after a miscarriage, underwent amniocentesis at the 17th week of gestation because fetal ultrasound revealed spina bifida and malformations. The dosage of the alpha fetoprotein in the amniotic fluid is >360000 UI/ml
RHG banded chromosome analysis on cultured amniocytes showed additional chromosomal material on short arm of chromosome 9.
Father's karyotype was normal. Mother's karyotype showed an apparently balanced reciprocal translocation between chromosomes 9 and 14. Fluorescence in situ hybridization (FISH) using WCP and subtelomeric probe confirmed the suspect.
Mother's karyotype is 46,XX,t(9;14)(p23;q32)
Fetal karyotype is 46,XY, der(9),t(9;14)(p23;q32)mat, which results in a monosomy of part of the short arm of chromosome 9 with a concomitant trisomy of the distal portion of the long arm of chromosome 14.
Fetal autopsy revealed a fetus with: lumbosacral myelomeningocele, mycrognathia, nose insellato, clubfoot, hypertelorism, ears low-set, diaphragmatic hernia, hydrocephaly, cerebral and posterior cranial fossa malformations compatible with Arnold-Chiari II syndrome.
Cytogenetic analysis is extended to the whole family and her brother, who presents fertility defects, shows the same traslocation.
There are the features clinical of chromosome 9p deletion syndrome, and neural tube defects due at a rare partial trisomy 14q. The peculiarity of the case is to find an Arnold-Chiari malformation associated with chromosomes aberration.

J01.73
Identification of the novel mutation c.618 C > A in HSD17B3 gene in four additional Tunisian patients with 46, XY DSD and molecular confirmation of a specific founder haplotype in the Tunisian population

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HSD17B3 isoenzyme is present almost exclusively in the testes and converts delta 4 androstenedione to testosterone. Mutations in the HSD17B3 gene cause HSD17B3 deficiency and result in 46, XY Disorders of Sex Development (46, XY DSD). This study aimed to search for mutations in HSD17B3 gene in six Tunisian patients with 46, XY DSD by DNA sequencing.
Polymerase chain reaction (PCR) amplification and subsequent sequencing of all the coding exons of HSD17B3 gene were performed on genomic DNA from all the patients and some available families members and revealed the presence of the novel nonsense mutation in the exon 9 (c.618 C > A) leading to the substitution p.C206X. the mutation was present in a homozygous state in two patients and in heterozygous state in four patients. The mutation p.C206X abolished a HhaI site, this propriety was used to confirm the mutation's presence in the patients and families members and its absence in 50 controls. The mutation p.C206X was found in six patients who belonged

to different families raising the possibility of a common founder. Genotyping using microsatellite flanking the HSD17B3 gene was performed and haplotype study showed that the c.618 C > A mutation occurred in a specific founder haplotype in the Tunisian population. The identification of this founder mutation has important implications towards genetic counseling in relatives of these families and the antenatal diagnosis. Our results showed also that HSD17B3 deficiency could not be a rare etiology of 46, XY DSD in the Tunisian population.

J01.74
Prenatal diagnosis of trisomy 5 mosaicism

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We report a case of prenatal diagnosis of a fetus with trisomy 5 mosaicism, a rare cytogenetic anomaly. These cases were very rare but pose a definite problem in prenatal cytogenetic diagnosis
A 37-year-old woman, after two miscarriages, underwent amniocentesis at 18 weeks of gestation because of advanced maternal age. Cytogenetic analysis revealed 26 clones with normal female karyotype and 2 clones from two culture vessels with trisomy 5.
The fetus karyotype was a mosaic: 47,XX,+5[2] / 46,XX[26].
A diagnosis of trisomy 5 mosaicism in amniocytes indicates an increased risk for fetal abnormalities, a confirmatory placental sampling may be helpful, whereas a fetal blood sampling have a very limited value.
Trisomy 5 mosaicism may be another example of tissue-limited mosaicism; then the fetal blood sampling could be falsely reassuring.
No further invasive testing was performed until 21 weeks gestation and level II ultrasound examination showed a fetus with intrauterine growth retardation (2°c), tetralogy of Fallot and hypertelorism. The rest of the fetal anatomy was within normal limits.
The couple interrupted the pregnancy.
The autopsy showed hypertelorism, saddle-backed nose. Exploration of the thoracic viscera showed heart disease complicated by characters of the tetralogy of Fallot. No further malformations were found in abdominal viscera. At the opening of the skull there was brain colliquation.

J01.75
Reduced gene expression and chromatin incorporation of histone demethylase JMJD1A in testicular biopsies of infertile men

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Background:
Spermatogenesis is a unique process in male reproductive system, which requires precise epigenetic regulation of gene expression. JMJD1A is a critical epigenetic modifier element that highly expresses in male germ cells and activates expression of genes by demethylation of H3K9me2/me1 modification. This study aimed to evaluate the expression and chromatin incorporation of JMJD1A protein in testicular biopsies of infertile men.
Material &Method:
Ethical approval and informed patient consent was gained for the use of tissue samples. Testicular biopsies were collected from 31 infertile men referred to Royan Institute and underwent testicular sperm extraction procedure. These samples were classified into the following four subgroups: obstructive azoospermia (as positive control, n=8), severe oligoasthenoteratozoospermia (n=7), complete maturation arrest (n=8), and sertoli cell only syndrome (n=8). The expression pattern and chromatin incorporation of JMJD1A in testicular biopsies were measured by quantitative real-time PCR and chromatin-ELISA techniques, respectively.
Result(s):
Our finding revealed that the expression and chromatin incorporation of JMJD1A were significantly decreased in severe oligoasthenoteratozoospermia, complete maturation arrest, and sertoli cell only syndrome groups in comparison to obstructive azoospermia patients.
Conclusion(s):
This study indicates that JMJD1A deficiency in testis tissues can result in defective spermatogenesis in human infertile males.

J01.76
Sperm head morphology as a predictive mark of DNA fragmentation

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In case of teratozoospermia the most appropriate spermatozoa from those with abnormal morphology have to be chosen. It seems very important to know if there is any relationship between sperm morphology and its genome quality. The objective of this study was to investigate whether specific sperm head abnormalities predict sperm DNA fragmentation. Semen analysis was performed for 84 patients attempting IVF clinic for spermogramm and 8 sperm donors according to WHO criteria. Sperm head morphology was assessed using strict Kruger's criteria. The following sperm head forms were analyzed: normal, big, small, bulb, amorphous, round, double, with vacuoles. The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay used to assess sperm DNA fragmentation. Sperm DNA fragmentation rate was significantly higher in patients than in control (0,64±0,08 vs. 0,21±0,04; p<0,05). The correlation between sperm DNA fragmentation and vacuolated sperm heads was found (r=0,32; p=0,005). The sperm DNA fragmentation was not associated with the frequency of the spermatozoa with other head abnormalities. Vacuolated sperm head should be treated as a marker of sperm DNA integrity abnormalities and might be considered a useful trait for more efficient selection of appropriate sperm used in fertilization. Supported by Carl Zeiss, RF President's scholarship and RFBR

J01.77
Chromosomal pattern in 256 Indian children presented with congenital malformation and mental retardation

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Chromosomal abnormalities are reported in 1 of 150 live born and 50% of the spontaneous abortion. Chromosomal aberrations cause major alterations at molecular level resulting in rearrangement and/or deletion of nucleotides, which pose serious impact on clinical manifestation. All such aberrations are not well characterized into syndromes; however, cause malformation and mental retardation. We describe chromosomal pattern in 256 children presented with congenital malformation, growth and mental retardation. Peripheral blood samples were processed for conventional G-banding analysis. Numerical and structural abnormalities are presented in the table below. A total of 106 children (41%) were detected predominantly with Down (65%) and rarely with Edward (3%) and Patau syndrome (1%). Among sex chromosomal abnormalities, Turner syndrome (12%) was higher than Klinefelter syndrome (1%). Down syndrome and Turner syndrome were of both classic and variant pattern. One child had both trisomy 21 and XXY pattern presenting Down syndrome and Klinefelter syndrome together, which might reflect a complex growth pattern at pubertal age. Structural abnormality was detected in 18% where autosomes were involved in 14% children. Phenotypic expression of these cases was not always straightforward for diagnosis of a typical syndrome and thus, a phenotype-karyotype correlation was essential for understanding clinical management. The detail data on 256 children highlights the necessity of routine karyotyping for clinical understanding, and also for genetic counseling of parents for introducing a preventive module.

Sex		Chromosomal aberrations detected in 106 of 256 children								
		Numerical						Structural		
		Autosomal			Sex-chromosomal			Autosomal		Sex-chromosomal
	N	DS	ES	PS	KS	TS	X/XY		DGS	
M	81	40	2	1	1^		1	10	1	1
F	69	29	1	0		13	0	4	0	3
Total	150	69	3	1	1^	13	1	14	1	4

^included in DS; M male, F female, N normal, DS Down syndrome, ES Edward syndrome, PS Patau syndrome, KS Klinefelter syndrome, TS Turner syndrome, DGS De George syndrome

J01.78
Association of H3K9ac and H3K9me2 modifications with impaired spermatogenesis

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During post-meiotic stages of spermatogenesis, histones of sperm chromatin are replaced by transition proteins (TNPs) and protamines (PRMs). Expression of TNPs and PRMs rise at final stages of spermatogenesis to compact chromatin for normal function of sperm. Any failure of their expression is associated with impaired spermatogenesis. Epigenetic factors such as histone actyle transferases and histone demethylases are involved in regulation of these genes. Therefore evaluation of relative modifications

such as H3K9ac and H3K9me2 in regulatory regions of mentioned genes in testicular biopsies of infertile men can represent better insight into molecular mechanisms of infertility.
In this study based on spermogram and pathological features of infertile men referred to Royan institute, testes tissue samples were collected from four groups including sever oligoasthenoteratozoospermia, complete maturation arrest, sertoli cell only syndrome, and hypospermatogenesis group as positive control. Expression of TNPs and PRMs were evaluated by qRT-PCR. Also, chromatin immunopercipitation coupled with real time-PCR was performed to evaluate the incorporation of H3K9ac and H3K9me2 into regulatory regions of mentioned genes. Consent was obtained from patients according local ethical approval.
Results showed significant decrease in expression of TNP and PRM genes in all groups compared to positive control. These findings also confirmed by ChIP data revealed decreased incorporation of H3K9ac (activating mark) and increased incorporation of H3K9me2 (repression mark) into regulatory regions of above genes in all groups vs. positive control.
The finding implies significant association of histone modifications with altered expression of sperm chromatin condensing genes and impairment of spermatogenesis in male infertility.

J01.79
A Prenatal Ring chromosome 13 with CIR and abnormal genitalia

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Ring chromosomes are unusual chromosomal alterations that occur in 1/50,000 human fetuses, although they have been found from nearly all human chromosomes. Mostly, they are consequence of two breakpoints in both arms, followed by fusion of the proximal ends generating a ring with loss of the distal genetic material and resulting in clinical features mimikking terminal deletion syndromes. Here we report a "de novo" prenatal male case with a ring chromosome 13 [r(13)], which was diagnosed after an amniocentesis performed because a suspicious of ambiguous genitalia and CIR (Intrauterine Growth Retardation). There was no family history of chromosomal anomalies, and the pregnancy evolution was normal with a "non-invasive prenatal DNA test on maternal blood" performed on the 13th gestational week, informed as a normal male. In the 19th gestational week an amniocentesis was performed because a suspicious of ambiguous genitalia and CIR and although the QF-PCR was informed as a normal male, the karyotype showed a r(13) and the array-CGH showed a terminal 13q deletion (chr13:104,727,326-112,193,513) involving 48 genes, including the EFNB2 gene. Deletion of the EFNB2 gene (OMIM:600527) has been already reported to be implicated in genital malformations and growth retardation, which helped in the prenatal counseling of the couple, who decided to interrupt the pregnancy.

J01.80
The necessity of specific genetic marker based molecular study of the AZFa region in infertile men

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Introduction: The relation between microdeletions in the Y chromosome and developing male infertility has been studied in several populations. The majority of published studies were designed according to the European Academy of Andrology (EAA) and European Molecular Genetics Quality Network (EMQN) guidelines for the better detection of AZF (Azoospermic factor) microdeletions. However, there are several reports indicating that using the EAA and EMQN suggested sequence-tagged site (STS) markers will result in false positive and false negative results at least in detection of AZFa microdeletions. The aim of the present study was to evaluate the accuracy of recommended STS markers for detection of AZFa microdeletion in Iranian patients with non-obstructive azoospermia.
Methods: A total of 100 Iranian non-obstructive azoospermic infertile men and 100 proven fertile men were selected for the molecular study of Y chromosome microdeletions in the AZFa region according to the EAA and EMQN guideline using sY84 and sY86 STS markers. In addition, the presence of sY176 and sY182 STS markers was investigated using multiplex polymerase chain reaction (M-PCR).
Results: Using sY84 and sY86 primers, we found only one patient who had AZFa microdeletion. However, with the use of sY176 and sY182 markers three new patients were detected with AZFa microdeletion.
Conclusion: It seems that the primers and STS markers recommended by the EAA and EMQN guidelines may not apply to all populations and it is recommended to design a population based STS panel at least for the study of AZFa region for better detection of microdeletions.

J01.81
An NGS-based test for the identification of individuals carrying recessive genetic mutations

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We developed qCarrier, an NGS-based approach targeting +4000 known mutations in over 200 genes causing recessive diseases, for testing couples undergoing assisted reproductive treatments in order to reduce the odds of passing a recessive or X-linked disorder to the offspring.
In contrast to SNP-genotyping platforms, NGS technologies are not limited to detecting previously known or certain types of mutations (i.e. point mutations or small indels), and can efficiently detect a wider range of disease-causing mutations. The qCarrier is based in sequence capture, followed by high-throughput sequencing Bioinformatic analysis is keystone in the process, as it combines algorithms optimized for the identification and annotation of different types of mutations (point mutations, indels, copy-number and balanced rearrangements).
For analytical validation of the test, we obtained DNA from 57 unrelated individuals: 39 patients and 18 previously genotyped controls. The validation set was composed of 49 different known mutations, including 29 SNVs, 13 indels and 25 CNVs causing diseases such as cystic fibrosis, phenylketonuria, spinal muscular atrophy, hypothyroidism, thalassemia, or Duchenne muscular dystrophy. All but one (48/49) different mutations were correctly scored in the blinded study and only one deletion-type mutation remained undetected. This information allowed us to finely tune the algorithm to reach maximum sensitivity. All single nucleotide changes were validated and no known recessive mutations were called in the control samples.
After initial deployment in the clinical setting we assessed the presence of disease-causing recessive mutations in 52,1% of the analyzed samples (11/21).

J01.82
Association between Cytochrome P450 2C19 (CYP2C19) gene polymorphism and the risk of endometriosis in Iranian population

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The aim of current study was to investigate the association of rs11592737 (A/G) polymorphism of CYP2C19 gene with the risk of endometriosis in Iranian women. 100 patients with endometriosis and 100 controls with no laparoscopic evidence of endometriosis were included in this study. Samples were analyzed for rs11592737 (A/G) single nucleotide polymorphism (SNP) in CYP2C19 gene using Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR).Our data revealed a significant difference in the distribution of rs11592737 genotypes between endometriosis patients and controls (P=0.002). Despite GG genotype, AG Genotype was more frequent in patients than the control samples (P<0.001, OR=3.14, 95% CI 1.66-5.96). Significantly, those cases with A allele showed an increased risk of endometriosis compared to the control group (P=0.02, OR=1.63, 95%CI1.08-2.44). No significant difference in the allele frequency has been seen in the different stages of endometriosis (P value=0.59). The results of this study suggest that rs11592737 (A/G) SNP of CYP2C19 may be associated with a higher risk of endometriosis among Iranian population.

J01.83
Prevalence and distribution of somatic genomic variations in placental tissues from anembryonic pregnancies

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High incidence of somatic mutations is a hallmark of abnormal embryogenesis. Recent data indicate the significant impact of copy number variations (CNV) into etiology of early pregnancy loss. However mechanisms of their origin are still poorly investigated. We aimed to estimate the somatic CNV incidence in placental tissues - cytotrophoblast (CT) and (EM) from 6 anembryonic pregnancies (AP) with normal karyotype using SurePrint G3 Human CGH+SNP 4×180K Microarray Kit (Agilent Technologies). Altogether 84 CNVs were found. Twenty-four rearrangements (21 polymorphic and 3 unique, which are absent from the Database of Genomic Variants) were de-

tected in both tissues indicating their meiotic or early mitotic origin before the germ layers divergence. On the other side, 34 (including 2 unique) and 26 (including 16 unique) variations appeared to be tissue-specific for EM and CT, respectively, originating from mitotic errors after the tissues divergence. So, at least 60 (71%) CNVs appeared de novo in somatic cells and 18 (30%) of them were unique. Sixteen CNVs from 18 were deletions. Eight of these variations were larger than 1 Mb and all of them were confined to the CT (del4p12, del4q13.1-q13.2 - 2 cases, del7q21.11, del10q21.3 - 2 cases, del17q21.33-q22 - 2 cases). The most interesting potentially pathogenic affected genes were GABRG1, GABRA2, GABRA4, GABRB1 (GABA family is responsible for implantation and endometrial decidualization, dysregulation of which is associated with AP), EPHA5 (may help to organize developing body plan), and CTNNA3 (controls trophoblast invasion). This study was supported by Russian Foundation for Basic Research, grant 14-04-32047.

J01.84
Detection of MED12 exon 2 gene mutations in Iranian women with Leiomyomas

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Introduction: Uterine leiomyomas are non-cancerous tumors arising from the smooth-muscle layer of the uterus that may grow as a single tumor or in clusters. Despite their benign nature, fibroids can cause a variety of health problems including, abnormal menstrual heavy bleeding, pelvic pressure and pain, pregnancy complications and reproductive problems. It has been suggested that mutations clustered in exon 2 of the MED12 gene, located on Xq13.1, are responsible for a majority of uterine leiomyomas. According to the role of ethnicity in developing fibroids, the aim of the present study was to investigate the frequency of MED12 exon 2 mutations in uterine leiomyomas of Iranian patients.
Methods: Genomic DNA was extracted from 50 fresh uterine leiomyomas tissue samples by a phenol-chloroform method. PCR-SSCP was used to detect mutations in the MED12 exon 2 and the flanking intronic regions. Fragments with altered banding patterns were sequenced on an ABI 3730XL automated DNA sequencer.
Results: Five type of MED12 gene heterozygous mutations were detected in 24 (48%) of the leiomyomas samples including, 12 (24%) missense mutations, 5 (10%) in-frame deletions, 4 (8%) single nucleotide variants affecting splicing,1 (2%) deletions/insertion-deletions spanning the intron 1-exon 2 boundary resulting in exon skipping, and 2 (4%) frame shift deletions. No mutation was detected in normal myometrial tissue.
Conclusion: Our study confirms the role of MED12 mutations in the pathogenesis of uterine leiomyomas, regardless of ethnicity. Therefore the gene could be an appropriate therapeutic target for uterine leiomyomas.

J01.85
Epigenetic analysis of regulatory region of CYP19A1 in granulosa cells of patients with polycystic ovarian syndrome

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Polycystic ovarian syndrome (PCOS) is a complex genetic endocrine disorder among the women of reproductive age. Hyper-androgenemia is one of the main clinical features of PCOS. Aromatase, the key enzyme for estrogen biosynthesis, is encoded by CYP19A1 which is comprised of an unusually large regulatory region including 10 tissue-specific promoters. In human cells CYP19A1 is expressed in gonads via promoter PII.
The aim of this study is to evaluate the acetylation and methylation levels of lysine 9 of histone 3 (H3K9ac and H3K9me2), in PII promoter region of CYP19A1 in granulosa cells of PCOS patients referred to Royan Institute for in vitro fertilization (IVF).
Six women under ovulation induction treatments for IVF consisting of three PCOS patients and three controls (women without ovulation problems; non-PCOS) groups were selected. For this respect, ethical approval form was filled before any experiment. We sequenced PII of CYP19A1 to confirm no genetic variations. After ovary puncture, follicular fluid was collected and granulosa cells were extracted via Sil-Select Plus gradient. Specific histone modifications were quantified via chromatin Immunoprecipitation (ChIP) coupled with real-time PCR.
Our results clearly demonstrate that in PCOS patients, incorporation of H3K9ac in PII of CYP19A1 is significantly higher than non-PCOS patients, whereas, the level of H3K9me2 in PCOS patients is decreased compared with the control group ($p<0.05$).
For the first time our experiments suggest that epigenetic alterations via histone modification in PII promoter of CYP19A1 can play a critical role in the development of PCOS.

J01.86
Epimutation of RB1 gene promoter is accompanied by hypermethylation of repeated genome sequences in human miscarriages with aneuploidy

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High level of chromosomal mosaicism is observed during global epigenetic reprogramming in early human embryogenesis. Previously, we have shown a high incidence of epimutations at RB1 gene (16.4 x 10-2) in placental tissues of aneuploid embryos with mosaicism. Moreover, hypermethylation of RB1 promoter was associated with smaller size of aneuploid clone. It was suggested that epimutations of RB1 are signs of global disturbance of epigenetic landscape in aneuploid placenta. To attend this question we analyzed level of retrotransposon LINE-1 promoter DNA methylation in the extraembryonic mesoderm and the cytotrophoblast cells of embryos with complete and mosaic aneuploid karyotype with (n = 14) and without (n =20) RB1 promoter epimutation, euploid miscarriages (n = 17) and induced abortions with normal karyotype (n = 19). There were no differences of LINE-1 methylation between extraembryonic mesoderm and cytotrophoblast cells in all studied groups. DNA methylation index of LINE-1 was significantly higher in group of mosaic aneuploid embryos with RB1 epimutation (58%) in comparison with complete and mosaic aneuploid embryos without RB1 epimutation (53%), euploid miscarriages (42%), and induced abortions with normal karyotype (52.5%) ($p<0.01$). Thus, for the first time we have shown that epimutation of RB1 gene promoter is accompanied by hypermethylation of repeated genome sequences in first trimester miscarriages with aneuploidy. This study was supported by Russian Foundation for Basic Research, grant 14-04-01003.

J01.87
The evaluation of adiponectin and its receptors (AdipoR1 and AdipoR2) genes expression in rat polycystic ovary syndrome models

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Background: Strong association between hypoadiponectinaemia and the occurrence of poly cystic ovary syndrome (PCOS) suggests a pathogenic role for adiponectin. We aimed to evaluate the expression of the adiponectin and its receptors (AdipoR1 and AdipoR2) genes in immature and mature PCOS rat model that were exposed prenatally to androgen excess.
Methods: Four pregnant Wistar rats in the experimental group were treated by subcutaneous injection of 5 mg free testosterone on day 20 of pregnancy while the controls (n=4) received only 500 ml of solvent. Female pups (14 cases and 18 controls) of each mother were randomly divided into 3 groups and sacrificed at 3 stages of life: day 0 (new born, n=10), day10 (10-day-old, n=10), and day 75-85(adult, n=12). RNAs were extracted from ovarian tissues and relative expression levels for adiponectin and its receptors genes were measured using TaqMan Real-Time PCR.
Results: The expression levels of investigated genes were not significantly elevated in newborns (Adiponectin: 1.119, AdipoR1: 1.594, AdipoR2:1.112 fold), while a significant decrease was detected in 10-day-old rats (Adiponectin 0.292, AdipoR1 0.26, and AdipoR2 0.161 fold ($p\leq0.05$)). We also observed a marginally significant increase in adiponectin gene expression at puberty (2.682 fold, $p=0.08$).
Conclusion: The results of this study showed that the expression of adiponectin and its receptor genes is changed in prenatally androgenized rats. These changes may alter the normal expression of steroidogenesis regulatory genes and consequently impair the normal development of ovaries and follicles.

J01.88
The Role of Early Development in Intra-individual Genetic Variation of Normal Human Fetuses

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Age-related events is one of origins of midlife copy number variations (CNVs) between tissues in non-genetic diseases; however mosaicism is prevalent in preimplantation stage and misscarriages. We aim exploring fetal mosaicism and its origins in apparently normal fetuses aborted due to maternal indications.
DNAs from 22 tissues of each fetus were studied by array Comparative Genomic Hybridization (array CGH) using 195000 probe slides in simple loops separately designed for each of two studied fetuses. Reciprocal CNVs as high confidence CNVs validated by qPCR. Functional analysis was performed by Gene Ontology (GO).
About 60 CNVs was observed in each fetus. The frequency of reciprocal CNVs varied from 2 to 18. Analysis of CNVs by array CGH and qPCR showed that changes were not mostly integer multiples. Some of CNVs were shared between both fetuses, some were found in the same tissues and some in different tissues. GO showed that altered genes are mostly involved in embryonic development. Tissues clustering according to CNVs revealed those from the same embryonic origin in some cases are close together in a cluster; however, there were large disagreements with clustering of embryonic layers derivatives.
According to distribution pattern of frequent CNVs their origin should be early development, some preimplantation and some postimplantatation. CNVs with low frequency seem to be occurred in later stages. Each organ inherits CNVs with a unique pattern regarding extensive cell mixing/migration in embryonic development. Shared CNVs between fetuses are mostly known hotspots; those occurred in same tissues might have functional role.

J01.89
Prenatal detection of fetal aneuploidy on the Ion Torrent Proton Platform

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Noninvasive prenatal testing (NIPT) for fetal aneuploidy detection via massively parallel sequencing has been successfully implemented in a number of high throughput clinical laboratories. Automation and parallelization of the complex workflow has reduced turnaround time and labor while maintaining high sensitivity and specificity. As these developments have improved workflow issues, the availability of new sequencing platforms on the market has introduced additional flexibility in implementation. Platform flexibility should encourage competitive pricing, foster innovation and ultimately, improve patient satisfaction.
We examined the performance of the MaterniT21™ assay using the Ion Torrent™ Proton Sequencer (Life Technologies™, San Diego California). One hundred and fifty-four patient samples, including sixteen from women carrying a known trisomy 21 fetus, as determined by fetal karyotyping, were analyzed. Libraries were prepared and sequenced according to manufacturer's recommendations. Sequenced reads were aligned, filtered for quality and normalized for GC bias. Robust statistics were then applied to identify positive samples with a z-score greater than 3.
Fetal aneuploidy status was correctly determined for 154/154 pregnant females, including 16 carrying a T21 fetus. Though the current Proton workflow requires more labor than is optimal for a production environment, significant improvements in that respect are anticipated in the launch of the Ion Chef template preparation system. Sequencing time was brief at < 3 hours and data analysis consistent with standard platforms. In summary, the performance of the MaterniT21™ assay on the Ion Torrent Proton platform in this limited study suggests the possibility of its suitability for implementation in a clinical environment.

J01.90
DNA methylation and demethylation patterns in human spermatogenesis

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We studied the distribution of 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) in human spermatogenic cells from testicular biopsy and ejaculate samples. Among analyzed dividing cells several types were detected: mitotic diploid and polyploid spermatogonia and meiotic spermatocytes at the pachytene, diplotene and diakinese stages. Chromosomes were identified by QFH/AcD staining. Cytosine modifications were detected by indirect immunofluorescence.
The distribution of 5mC in mitotic chromosomes from spermatogonia was band-specific: R-bands and pericentromeric heterochromatin of chromosomes 1, 9 and 16 were enriched in 5mC. Pachytene chromosomes showed less obvious 5mC-banding, with the most intensive DNA methylation in the peritelomeric regions. Diplotene and diakinese cells demonstrated high, but almost homogeneous DNA methylation with increased intensity of signal in chiasmas and heterochromatic regions. Mature spermatozoa contained 5mC.
5hmC was completely absent in mitotic and meiotic chromosomes of spermatogenic cells. Hydroxymethylation was identified in 8.8% of post-meiotic (haploid) spermatid nuclei and in up to 13.89% of spermatozoa, suggesting that they are actively demethylated.
Thus, mitotic chromosomes from spermatogonia and meiotic chromosomes from spermatocytes demonstrate band-specific methylation patters, but lack 5hmC. The presence of 5hmC in some post-meiotic spermatids and spermatozoa suggests that genome of these cells undergoes active DNA demethylation.
Supported by RFBR, Administration of St.Petersburg, OPTEC grant and stipend from RF President.

J01.91
Elucidation the chromosomal aberration impact on ovarian reserve: A retrospective clinical report

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Constitutional chromosome abnormalities are among the major contributors to the genetic causes of reproductive disorders. Despite all of worldwide efforts have been made so far, prognosis for mosaic X chromosome aberration below 30% of aneuploidy has yet to be established. The purpose of this study was to assess the quantity and quiddity of chromosomal aberrations that may negatively affect ovarian health causing premature ovarian failure (POF) and diminished ovarian reserve (DOR). In this purpose, retrospective observational study of clinical features and biological parameters was performed. A total of 531 individuals who were referred to our ward from 2007 to 2014 because of amenorrhea and poor responders to gonadotropins were selected. High resolution chromosome analysis by GTG banding was carried out on peripheral blood lymphocytes cultures. Supplementary tests also were performed when required. Of the 531 cases who were assessed for chromosomal defects, 52 showed abnormal karyotype. 22 cases were found to have cell lines with different level of X chromosome variation. Seven cases who were sex reverse sex determining region Y (SRY) negative, five cases with abnormal X chromosomes, three cases with structurally abnormal autosomes and four individuals carrying X-autosome translocation were diagnosed. The overall prevalence of chromosomal abnormalities was 9.8% which 2.1% of it belongs to normal variable chromosome features. The frequencies of chromosomal alterations were 5% and 1.7% in POF and DOR females, respectively. The results confirm previous observations and emphasis on the critical role of chromosome abnormalities as one of the possible etiologies for ovarian follicular attrition.

J01.92
Unique case of fertility in SRY-positive female

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We report a unique case of fertility in 29-years-old female with cryptic Y chromosome mosaicism. At the birth the patient presented female genitalia with clitoral hypertrophy, which was corrected surgically. Moderate virilization of female genitalia and high level of 17-OHP allowed to diagnose of congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency. DNA analysis found heterozygous CYP21B V281L mutation. From newborn to present time the patient is under the supervision and treatment by an endocrinologist. Menstrual cycle appeared at the age of 13 years after the hormonal stimulation. At the age of 23 years she married and after 4 months of unprotected sexual intercourse pregnancy appeared naturally. Pregnancy ended in a birth of a healthy boy by caesarean section at 38 months. Subsequently, the patient was observed by a gynecologist because of ovarian cysts, and surgical laparoscopy with biopsy was performed. Histolopatology of right gonad showed disgerminoma. On this occasion, the patient was aimed at cytogenetic and molecular-genetic examinations. Chromosome analysis of cultured lymphocytes showed mosaic 46,XX[47]/45,X[2]/46,XY[1]

karyotype. FISH analysis with CEPX and CEPY probes on peripheral blood lymphocytes and buccal cells confirmed complex sex chromosome mosaicism. Following cell clones were found in lymphocytes and buccal cells, respectively: 45,X (5% and 0%), 46,XX(94% and 75%), and 46,XY (1% and 25%). QF-PCR analysis, performed for chromosomes 13, 18, 21, X and Y, and multiplex PCR for 18 Yq STSs confirmed minor Y chromosome mosaicism with a presence of SRY, ZFY and AMELY loci, and not detected a chimerism and Y-microdeletions.

J02.01

Angiotensin-converting enzyme (ACE) I/D and alpha-adducin (ADD1) G460W gene polymorphisms in Turkish patients with tinnitus

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Objective: Tinnitus is the perception of experience of sound in the head or ears in the absence of external source. A number of factor influences tinnitus like hearing loss, age, noise exposure and hypertension. Angiotensin-converting enzyme (ACE) insertion/deletion (I/D) and alpha-adducin (ADD1) G460W polymorphisms have been associated to hypertension previously and this polymorphisms may be related to tinnitus. Therefore we aimed to investigate the relationship between tinnitus and angiotensin-converting enzyme (ACE) I/D and alpha-adducin (ADD1) G460W gene polymorphisms Methods: The patient group was composed of 89 individuals and the control group was composed of 104 individuals. ACE I/D polymorphism was carried out using polymerase chain reaction (PCR) method and ADD1 G460W gene polymorphism was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Results: ACE I/D polymorphism did not show any difference between the patient group and the controls. There was a significant difference in genotype ($p<0.01$) and allele frequencies ($p=0.0212$) of ADD1 G460W gene polymorphism between patients group with tinnitus and controls. The odds ratio for the (GW) genotype was 2.56, 95% CI=(1.39-4.71) ($p<0.01$). Conclusion: Our results demonstrate for the first time an association between ADD1 G460W gene polymorphism and susceptibility to tinnitus. ADD1 G460W polymorphism may play an important role in the pathophysiology of tinnitus.

J02.02

A novel mutation of SGK1 gene in central serous chorioretinopathy

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Purpose: Central serous chorioretinopathy(CSCR) is a mystery characterized by leakage of fluid under the retina that has a propensity to accumulate under the central macula. SGK1 which has an important role in many epithelial ion transport system may have a role in retinal pigment epithelial pump function whose disturbance is one of the main mechanism in development of CSCR. The aim of this study is to investigate whether SGK1gene variants are associated with CSCR
Materials and methods: We enrolled patients who were diagnosed with chronicCSCR (n=32) and unrelated individuals as a control group (n=32). For DNA extraction and PCR amplification followed standard methodologies. SGK1 gene was sequenced using BigDye® Terminator v3.1chemistry.
Results: We identified a novel mutation M32V (2/32) in the patient group (6, 25 % and AF.0,031). rs1057293 (p:0,68) is located in the encoder region of the SGK 1 gene but not associated with CSCR. An intronic rs1743966 (p.028) is also, not associated. We have also identified 3 more intronic mutations; 143951C>A(1/32; AF: 0,015), 147117A>T(1/32; AF: 0,015) and 145725 del TTAC(1/32; AF: 0,015).
Discussion: M32V is located on the region of 1-60 amino acids which is necessary for localization to the mitochondria. This mutation is probably important for the energy metabolism and plays an important role in the cellular response to hyperosmotic stress and other stress stimuli. Both rs1057293(p.0,68) and rs1743966(p.0,27) are not associated with CSCR. Probably these 7 snp are promising but it's difficult to conclude the association between these intronic mutations; 143951C>A, 147117A>T, 145725del TTAC- and CSCR.

J02.03

The occurrence of the rs61749246, the c.*2G>T of the FZD4 gene, in Slovak patients with retinopathy of prematurity

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Retinopathy of prematurity (ROP) is a complex disease affecting the development of retinal vasculature in premature infants. The International classification of ROP divides the development of the disorder into 5 stages. The environmental factors can influence the development of ROP, so the appropriate care may decrease its incidence. However, a genetic predisposition to ROP is suggested. Recently, mutations in genes FZD4, LRP5, TSPAN12, and NDP, were ide identified in 3 to 12% of the cases with ROP. Nowadays, our cohort consists of 11 premature newborns with different stages of ROP (stage 1 -3) treated at the Clinic of Neonatology in Martin. We performed the sequence analysis of the coding exons of the three genes - FZD4, TSPAN12 and NPD an identified rs61749246 (c.*2G>T of the FZD4 gene) in 3 patients with ROP. Two were heterozygous in stage 1, and one was homozygous in stage 2 (T allele frequency 0.18). The control group (n=50) of premature newborns without ROP contains two heterozygotes for rs61749246 (T allele frequency 0.02). The rs61749246 (MAF = 0.008 in dSNP) is the polymorphisms of the second G after the TAA stop codon of the FZD4 gene. It has been shown, that there are preferred nucleotides around the stop codon necessary for efficient translational termination. We suggest that this SNP is involved in the pathogenesis of ROP, probably based on gene expression changes during the eye development. We will enlarge our cohort focusing on higher stages of ROP and develop in-vitro translation assay for this SNP.

J02.04

Case report of a patient with sensorineural hearing loss due to compound heterozygosity of 35delG and G200R mutations in gene GJB2

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Hearing loss is the most common birth defect and the most prevalent sensorineural disorder and affects about one in 1,000 neonates. More than half of prelingual deafness cases are due to genetic factors. About 70% of all hereditary hearing loss cases are classified as nonsyndromic and recessive. Mutations in the genes GJB2 and GJB6, that are located in the locus DFNB1 and encode gap junction protein connexim 26 and connexin 30, are the main cause of nonsyndromic sensorineural autosomal recessive hearing loss. DFNB1 has digenic patterns of inheritance. The 35delG mutation in gene GJB2 is the most common mutation in DFNB1 in many European populations. We describe the case of 18 months-old boy attended genetic counsellor due to profound sensorineural hearing loss. Bidirectional sequencing analysis of gene GJB2 showed two mutations: c.35delG and p.Gly200Arg. The condition of heterozygous carrier of G200R mutation was found also in mother, father found to be a heterozygous cariier of 35delG mutation. Patient with compound heterozygosity of 35delG and G200R mutations in gene GJB2 that we found has never been described before. Precise genetic diagnosis is crucial for genetic counselling.

J02.05

Detection of mutations in selected regions of genes GJB6, MT-RNR1 and MT-TS1 in Slovak population associated with non-syndromic deafness

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Hearing loss represents frequent heritable disease that can be transmitted by all known types of inheritance. More than 50 genes are responsible for deafness. The gene GJB6 has high sequence and function similarity to the gene GJB2, which causes 50% of autosomal recessive non-syndromic deafness. Possibility of a bialelic heritability of these genes requires a detection of the presence of mutations in the gene GJB6. Detection of mutations that cause non-syndromic hearing loss in mitochondrial genome offers valuable information about their prevalence in Slovak deaf patients. Due to uniparental inheritance, information about frequency and occurence of these mutations is valuable for professional genetic counseling. In present study we

analyzed the coding sequence of the gene GJB6 and selected regions of genes MT-RNR1 and MT-TS1 of mitochondrial genome of 321 Slovak patients with non-syndromic hearing loss. In this group, not more than one mutation in the gene GJB2 was detected per person. In the gene GJB6 were detected three types of polymorphisms and two different frameshift mutations in heterozygote state. Also a 309 kb deletion in this gene was detected in one patient. Presence of the 309 kb deletion in the gene GJB6 was detected in Slovak population for the first time. In the gene MT-RNR1 was detected one type of pathogenic mutation (A1555G) and four types of potential pathogenic mutations. No pathogenic mutations in the gene MT-TS1 were detected.

J02.06
Genetic background of hearing loss among group polish CI patients

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Hearing loss (HI) is a significant medical problem in Poland and worldwide. The cause of hearing loss can be genetic or environmental. Currently the background of genetic hearing impairment is an area of intensive research conducted by many groups. Recently worldwide intensive studies are conducted to clarify the genetic basis of hearing loss. To date, more than 60 non-syndromic deafness genes and more than 1000 deafness-causing mutations have been described. The most common variants responsible for an isolated HI with recessive type of inheritance are mutations in the GJB2 gene (in particular the deletion of guanine at position 35 (35delG)) and therefore the search for genetic basis of hearing loss for diagnostic purposes usually includes only analysis of GJB2 gene, whereas mutations in each of the remaining genes associated with the process of hearing which can also cause hearing loss are not investigated.
According to the preliminary functional analysis, pathologic changes (caused by mutations of GJB2 and GJB6 genes) did not comprise spiral ganglion cells, which are crucial for the results of cochlear implantation.
The aim of our study was to estimate the prevalence of genetically related HI among patients with cochlear implants (CI). We have analyzed 1218 patients diagnosed with congenital hearing loss who received CI. Search for mutations was performed by various molecular methods. Our results shows that genetic defects of various genes are the most common reason of HI among patients with cochlear implants.

J02.07
KCNQ4 mutation spectrum in Slovak patients with non-syndromic hearing loss

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Non-syndromic deafness is one of the most common sensory impairment in humans. Most forms of non-syndromic deafness are associated with permanent hearing loss caused by damage to structures in the inner ear. The severity of hearing loss varies, can change over time and can occur at any age. The cause of non-syndromic deafness is complex, with more than hundred genes so far identified; however, some of these genes have not been fully characterized. Different mutations in the same gene can be associated with different types of hearing loss All this aspects contribute to the complexity of the disease and markedly hamper DNA diagnostics.
In this study we focused on gene KCNQ4, whose mutations lead to DFNA2, a subtype of autosomal dominant non-syndromic deafness that is characterized by progressive sensor-neural hearing loss across all frequencies. The KCNQ4 gene encodes protein called potassium voltage-gated channel KQT-like protein 4, which is part of a protein family that forms channels to transport positively charged potassium atoms between neighboring cells.
In our work we analyzed 324 NSHL patients without mutations in Gjb2 gene, which is most prevalent disease gene. Up to now we found one new deletion in gene KCNQ4 in exon 10 that causes frame shift, some pathological mutations and also several frequent polymorphisms.

J02.08
Mutational analysis of MIR184 in Iranian Keratoconus patients

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Keratoconus is a noninflammatory corneal thinning disorder and the major cause of cornea transplantations in the Western countries. Despite intensive biochemical and genetic investigations, its underlying cause(s) remains poorly understood. It has been suggested that mutations in *VSX1* and *SOD1* may contribute to disease presentation in some cases. Linkage analysis in an affected Irish pedigree ultimately in 2011 led to identification of a mutation in the seed region of *MIR184* (+57C>T) as the putative cause of keratoconus in the pedigree. In a subsequent screening of 790 patients of European or Indian descent, two novel causative mutations (+3A>G, +8C>A) in *MIR184* were identified. Notably, mutations in *MIR184* have recently been reported in two pedigrees, each affected with ocular diseases that affect the cornea. MiRNA 184 is the most abundant miRNA of the cornea. Here, *MIR184* was screened in 47 unrelated Iranians affected with keratoconus by direct Sanger sequencing. Only one variant allele (+39G>T; rs41280052) was observed in one patient. The same variation has previously been observed in control and keratoconus affected individuals at similar frequencies, suggesting that it is not a cause of keratoconus. Although the sample size was small, it is evident that mutations in *MIR184* are not a common cause of keratoconus among Iranian patients. They were not observed among the 94 chromosomes of the patients screened.

J02.09
Comprehensive analysis of keratoconus genetic factors

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Keratoconus (KTCN) is a thinning and anterior protrusion of the cornea resulting in altered refractive powers, and loss of visual acuity. KTCN is a multifactorial disorder in which both environmental and genetic factors are involved. Among the environmental factors, frequent eye rubbing and contact lenses wearing are mentioned. Family form of the disease and the coexistence with other genetic disorders indicate genetic factor involvement. Genetic studies have led to the identification of several loci on different chromosomes, linked to KTCN. However, only few reports indicated causative genes in these loci. Such examples are DOCK9, and MIR184. For most of the remaining KTCN loci, single genes were analyzed. Additionally, majority of previous reports concentrated on sequences variants in exons only. This leaves a gap in the studies of KTCN genetics.
The aim of this project was to analyze the DNA sequence information available in the databases of SNVs located within known KTCN loci. Previous studies in KTCN focused on protein-coding sequences. We extended the analyzes to include miRNA genes located in analyzed loci to investigate another genetic factor toward assessment of KTCN complexity. Additionally, sequencing of mitochondrial genome in KTCN patients from Polish population was performed and the data was incorporated into genetic analysis.
The KTCN development does not depend on a single change in the gene, but on the accumulation of numerous sequence variants. The complexity of KTCN etiology causes the need to find appropriate approach to investigate this disease.
Support: National Science Centre, Poland, grant no. 2011/03/N/NZ5/01470

J02.10
Megalocornea should be suspected in cases with hypotonia, mental retardation and macrocephaly: neuhauser syndrome an easily missed diagnosis

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Neuhauser syndrome, also known as Megalocornea-mental retardation syndrome (MMR), is a very rare autosomal recessive disorder. It can be diagnosed via clinical features: megalocornea, developmental delay, hypotonia and some dysmorphological signs. Genetic etiology is still unknown. About 40 cases have been reported in the literature. Here we report a further MMR case. Proband is a 13 month-old girl. Because of macrocephaly, motor and mental retardation, hypotonia and facial dysmorphic features, she was referred to pediatric genetics subdivision for genetic counseling. She was the fifth child of nonconsanguineous parents from small village and born at 35 week of gestation. In the newborn period she was hospitalized for prematurity. At 9 and 11 month of age, she was rehospitalized because of feeding problems and bronchopneumonia. On admission, her weight was 6,6kg(<3centile), height 76cm(25-50centile) and head circumference 46cm(97centile). Macrocephaly, broad forehead, hypertelorism, megalocornea, strabismus, low set ears, short columella and long philtrum were present. Corneal diameters were higher than 13,5mm bilaterally and intraocular pressures were normal. Laboratory tests revealed hypogammaglobulinemia. Echocardiography showed secundum ASD (4mm). On MRI, bilateral cerebral atrophy and thin corpus callosum were observed. Karyotype and subtelomeric FISH were normal. This case is one of the few MMR cases having immunedeficiency.

J02.11

The role of the vitamin D receptor signaling pathway in the development of axial myopia in children

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The aim of this study was to investigate an association of the VDR (vitamin D receptor) A-3731G gene polymorphism with the occurrence of axial myopia in children. We examined 53 girls and 24 boys aged 4-17 yr from Russian population: 19(38 eyes) with high myopia, 44(88 eyes) with medium myopia, and 14(28 eyes) with emmetropia. The gene polymorphism was identified with PCR-RFLP. We have found a higher proportion of carriers of VDR A(-3731)allele in the medium myopia group compared to the emmetropia group (32% and 7%, respectively, OR=4,45, 95%CI 1,13-17,53, p=0,016). It is known that the variation of the A(-3731) allele frequency is significant between various populations, being the highest one in Africans (about 74%) and the lowest one in Caucasians (about 19%). The A(-3731) allele is an ancestral while the allele G(-3731) is mutant. Null hypothesis for explaining the mutant allele frequency raise in Caucasians is that this is a result of a random selection, for example, genetic drift during the period of human migration out of Africa. Alternative hypothesis is that the difference in the polymorphism variation is not random, being the result of natural selection. The latter may be true if the G(-3731) allele gives some benefits for survival outside Africa. We hypothesize that the vitamin D receptor is a key regulator of the eye growth, and G(-3731) allele gives more benefits in preventing against myopic eye growth. The increase in G(-3731) allele rates in non-African populations might be explained by changing of ultraviolet radiation intensity.

J02.12

Ophthalmologic status of patients with Waardenburg syndrome

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Waardenburg syndrome (WS) is a genetically heterogeneous syndrome characterized by pigmentary abnormalities and congenital sensorineural hearing loss. WS has been classified into 4 main phenotypes: WS1 (OMIM193500) with dystopia canthorum, WS2 (OMIM193510) without dystopia canthorum, WS3 (OMIM148820) with dystopia canthorum and upper limb abnormalities, WS4 (Waardenburg-Shah syndrome, OMIM277580), with the additional feature of Hirschsprung disease. WS1 and WS3 are both caused by mutation in the PAX3 gene. The overall incidence is 1/42,000 to 1/50,000 people. Here we report on 12 patients with WS (24 eyes, 4 boys and 8 girls aged from 4 mn to 15 yr). The period of follow-up supervision of the patients lasted from 6 mn to 15 yr. 7 patients had mothers with WS, the rest had parents without WS symptoms. Routine basic ophthalmic, pediatric and audiological observation was performed for all patients. All of them suffered from congenital sensorineural deafness. Ophthalmic status of all patients included dystopia canthorum, epicanthus, heterochromia irides. 5 patients (10 eyes) suffered from congenital ptosis, converging strabismus with surgical correction. 10 children (20 eyes) had refraction anomaly as hypermetropia and astigmatism corrected with spectacles. 2 patients (4 eyes) had refraction anomaly with nystagmus. All patients (24 eyes) had long lacrimal ducts that imitated symptoms of congenital dacryocystitis in early childhood and required to probe lacrimal ducts repeatedly. 10 patients had skin hypopigmentation. Early ophthalmic and audiological checkup and treatment were the necessary conditions for function optimization and social adaptation

J02.13

A novel variation in 3'UTR of SLC26A4 gene in an Iranian family with Pendred Syndrome

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Background: Mutations in SLC26A4 gene that encodes pendrin is the second common cause of autosomal recessive deafness.Given that no genetic changes that lead to Pendred Syndrome (PS) in the 3'UTR region of SLC26A4 has been reported yet, here, we introduce the first data about a 3'UTR variation of SLC26A4 gene that has been segregated to PS.
Methods: Selecting pedigrees have been done with considering some clinical criteria and homozygosity mapping with flanking STR markers following conventional sequencing were performed the effect of the novel identified alteration on RNA folding was assessed using the mfold program.
Results: According to homozygosity mapping and confirmed clinical evidences such as goiter, vestibular aqueduct enlargement and positive perchlorate discharge test, we report a novel alteration in the 3'UTR of SLC26A4 gene led to PS. In addition, bioinformatics analysis showed that the introduced altered allele destabilized RNA structures; although, the Dot matrix analysis of wild-type and mutant form of selected sequence has not been showed any difference based on minimum free energy (ΔG= -21.5).
Conclusion: Our data suggest that identified variation might alter mRNA folding and it is conceivable that this new alteration can cause PS in the family under study.

J02.14

New polyurethane drug delivery system used for bevacizumab - an alternative to classic treatment of retinopathy of prematurity

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The retinopathy of prematurity (ROP), a multifactorial disease which is quite similar to familial exudative vitreoretinopathy, affects preterm infants with low birth weight and/or gestational age. The molecular identities of the genes which are involved in ROP remain uncertain, although the evidence for a genetic component of this disease is strong. Bevacizumab, known as Avastin, is an angiogenesis inhibitor who belongs to the class of monoclonal antibodies and it is considered as a possible treatment for patients diagnosed with cancers because it binds selectively to VEGF; some researchers have noticed impressive results in the field of severe ROP by testing it as an alternative remedy to the diode laser photocoagulation (the treatment of choice). The obtaining and characterization of eye drops based on polyurethane microparticles used as a drug delivery system for bevacizumab were the main aims of this research. The microparticles were synthesized using a polyaddition process coupled with a spontaneous emulsification. The microparticles' solutions were characterized by pH, size, and stability measurements; their irritation potential was evaluated on the mice skin by non-invasive techniques (tewametry, mexametry, and corneometry). A drug release study was performed by monitoring the degradation of these polyurethane microparticles for three weeks in two different media: simulated body fluid (SBF) and phosphate buffered saline (PBS); the influence of ultrasounds on the degradation of polyurethane microparticles was also evaluated. The results suggests the obtaining of a non-irritating material, with good stability, which presents a very slow degradation, beneficial for drugs which require low release rates.

J02.15

A report on a 4q25 deletion upstream of PITX2 in a child with unilateral Rieger syndrome

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Rieger syndrome (RS) is a rare autosomal dominant disorder characterized by specific ocular, dental and umbilical anomalies. Mutations in *PITX2* and *FOXC1* genes explain about 50% of RS. Deletions of coding exons and chromosomal translocations and more recently a deletion of an upstream regulatory region of *PITX2* have been described. Our case is a girl aged 13 months who was referred in our Center for Rare Genetic Ophthalmologic Diseases (CARGO) because of unilateral eye anomalies and umbilical malformations, suggesting RS. The examination revealed posterior embryotoxon, irido-corneal adhesions and pupillary anomaly on the right eye. She had redundant periumbilical skin as well as anal anteposition. Sanger sequencing of *PITX2* and *FOXC1* was normal. CGHarray analysis revealed a de novo 4q25 deletion upstream of *PITX2*. The deletion involved the upstream non coding region of *PITX2*, similar to two previously reported RS patients (Volkmann et al. 2011, Reis et al. 2012). The conserved non coding elements upstream the gene were showed to be involved in regulation of pitx2/PITX2 expression in a zebrafish model (Volkmann et al. 2011). Disease-causing mutations outside the coding regions have been reported in other diseases, such as deletions of the downstream regulatory region of *PAX6* in aniridia (Lauderdale et al. 2000). The identification of this additional case highlights the importance of the loss of the upstream regulatory region in this disease and the importance of screening for this type of deletion in RS patients optimizing genetic counseling for this family.

J02.16

A novel mutation of the USH2C (GPR98) gene in an Iranian family with Usher syndrome Type II

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Objective: Usher syndrome (USH) is an autosomal recessive disorder illustrated with retinitis pigmentosa (RP) and sensorineural hearing loss with or without variable vestibular dysfunction. USH is genetically and clinically heterogeneous. At least fifteen loci and eleven genes identified in USH. Usher syndrome is divided into three subtype: type I (USH1); type II (USH2); and type III (USH3). USH2 is the most common form of Usher syndrome, responsible for moderate to severe hearing deficits, retinitis pigmentosa started around or after puberty associated with normal vestibular responses. Three loci associated with USH2 have been reported: USH2A (*USH2A*), USH2C (*GPR98*), and USH2D (*WHRN*). Defects in *GPR98* are the cause of Usher syndrome type 2C (USH2C). Here, we report on a consanguineous Iranian family with two affected individuals for whom we identified a novel mutation in *GPR98* (*VLGRI*) gene. **Methods:** After performing homozygosity mapping using microsatellite (STR) markers,we approached whole exome sequencing (WES) to detect the disease-causing mutation of homozygous regions. To confirm the identified homozygote variant in *GPR98*, sanger sequencing has performed in all family members. Consequently we sequenced 100 normal control to ensure detected variant would not be a polymorphism. **Results:** We identified a new mutation in *GPR98* segregating with USH2C in this family. The missense mutation c.10019T>G leading to p.Val3340Gly. **Conclusion:**This mutation is the second one, which has been reported for USH2C in Iranian population by our group. To the best of our knowledge, this is the first report of a genetically confirmed case of USH2C using WES in Iran.

J02.17

The analysis of the GJA3 gene in patients with hereditary congenital cataract from Bashkortostan Republic (Russia)

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Introduction: Cataracts are one of the leading causes of blindness in humans, and mutations in the connexin 46 (GJA3) and the connexin 50 (GJA8) genes cause congenital cataract. Different mutations in these genes lead to the development of distinct cataract phenotypes. The aim of the study was to analyze the GJA3 gene in patients from Bashkortostan Republic affected with congenital cataract.
Objective: DNA samples of 40 unrelated patients with isolated form of hereditary congenital cataract from Bashkortostan Republic were analyzed.
Methodology: The analysis was performed by direct sequencing of coding regions of the GJA3 gene.
Results: Three different nucleotide alterations were detected. In one patient of Tatar ethnic origin with zonular form of cataract the deletion c.del1126_1139 was detected; one patient of Tatar ethnic origin with zonular cataract and microcornea carried the missense mutation c. 398 G>A (p.Arg133Gln). Both alterations were found in the heterozygous state, hadn't been described in literature earlier, and presumably are functionally significant mutations. In one patient new nucleotide substitution c.231C>T (Phe77Phe) in the heterozygous state was found.
Conclusion: Thus, three previously undescribed structural changes in the gene GJA3 were detected in hereditary congenital cataract patients from Bashkortostan Republic. To determine their functional significance further investigation are required. The study was supported by RFBR grant (14-04-97007_r_povolgie_a).

J02.18

Mutation screening in autosomal dominant retinitis pigmentosa family using targeted next generation sequencing

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Background: Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous disorder with an incidence of 1 in 3,500, or a total of 1.8 million people affected worldwide. So far at least 55 genes (RetNet) are known to be involved in the pathology among which 23 genes have been implicated in autosomal dominant RP (adRP). By using next generation sequencing (NGS) in combination with bioinformatic analysis, we aimed to identify the disease causing mutation in a large adRP-family of Gypsy origin.
Methods: Targeted NGS of 4,813 genes associated with known clinical phenotypes was performed on APEX-negative adRP-patient using the TruSightOne Sequencing Panel and MiSeq system of Illumina. Illumina VariantStudio software was used to filter single nucleotide variants (SNVs) and insertions/deletions (indels).
Results: We identified 9,330 SNVs and indels and after applying filtering criteria the numbers of remaining variants (in parentheses) were as follows: (i) exclude homozygous changes (5,857); (ii) exclude minor variant frequency <10% (5,846); (iii) keep nonsynonymous changes and splice variants (2,666); (iv) keep if same variant presents in <1% of the general population (608); (v) exclude dbSNP with minor allele frequency >1% (574); (vi) exclude if allele is present in at least one of 6,500 individuals of the Exome Variant Server database (527); (vii) disease phenotype consistent with autosomal dominant retinal degeneration (11). Additional exclusion criteria including lack of segregation of the mutant allele within the affected family members will be applied.
Conclusion: Our results suggest that new adRP-locus exists since no pathogenic changes were found in known adRP-genes.

J02.19

Deciphering the Genetic basis of Hearing Impairment in Iran; an Ethnic based Survey

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Hearing loss is the most common sensory disorder worldwide which affects 1 of every 500 newborns. At least 50% of cases can be attributed to genetics, most often resulting in nonsyndromic deafness (70%), which is usually autosomal recessive (80%). Despite the heterogeinity, mutations in *GJB2* at DFNB1 locus are the major cause of autosomal recessive nonsyndromic hearing loss (ARNSHL) in many populations, including Iran (20%). The fact that many loci are involved together with the heterogeneity of the status, necessitate studying further loci in various Iranian ethnic groups. In this study, after mutation screening of *GJB2* and *GJB6*, we used homozygosity mapping to identify regions of autozygosity-by-descent in 23 large pedigrees all originating from a single province of Iran (South Khorasan), using STR markers for STR markers for 7 loci.
In our study, four out of the 23 families showed *GJB2* mutations. Interestingly heterogeinity within a family were observed, even in large consanguineous pedigrees. *GJB6* deletions were not detected. One family showed linkage to DFNB3 and one family showed to be linked to DFNB7/11. The remaining did not show linkage to the studied loci.
Our results once again emphasize the heterogeneity of HL among different Iranian ethnic groups .These results could provide further insight into the etiology of HL and may lead to better genetic diagnostics & counseling.

J02.20

Targeted next-generation sequencing for identification of ABCA4 gene mutations in Polish patients with retinal dystrophies

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The ABCA4 gene is one of the most important genes of the human retina. It encodes an ATP binding cassette (ABC) transporter that is expressed almost exclusively in the retina. Mutations in the ABCA4 gene cause a wide range of retinal degenerations. Due to the advent of therapeutic options, identification of a genetic cause of retinal diseases is becoming increasingly important. The aim of the project was to implement next-generation sequencing (NGS) for identification of ABCA4 gene mutations in a group of Polish patients with Stargardt disease, fundus flavimaculatus or cone-rod dystrophy. Genomic DNA isolated from peripheral blood of 58 patients served as a template. The introduced variant of NGS is based on the preparation of an amplicon library containing all coding sequences of the ABCA4 gene (50 exons). Next, the amplicons were sequenced using the genomic sequencer GS Junior System (Roche). Presence of allelic variants identified by NGS was confirmed by Sanger DNA Sequencing. To predict possible functional consequences of the identified missense variants, two different computational methods, i.e. SIFT and PolyPhen-2 were used. In the studied group of patients, 32 different known mutations and 20 different novel potentially pathogenic variants were found. Our study enabled identification of a genetic cause of retinal di-

sease in 88% of patients. Three patients (5%) did not carry any mutation in the ABCA4 gene and in four patients (7%) only one mutation was found. Patients with an unknown cause of the retinal disease will be examined using whole genome sequencing.

J03.01

Predictive value of alpha-1 antitrypsin level for Z mutation detection in chronic obstructive pulmonary disease

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Alpha1-antitrypsin (AAT) deficiency is an under-diagnosed condition in patients with chronic obstructive pulmonary disease (COPD). The aim of our study was to evaluate predictive value of quantitative methods of alpha1-antitrypsin for Z mutation detection in patients with chronic obstructive pulmonary disease. Ninety-one AAT deficiency genotypes (40 MZ, 39 MS, 1 SS, 3 SZ and 8 ZZ) were analysed. Calculated sensitivity of quantitative alpha-1 antitrypsin measurement by nephelometry for heterozygous PI*Z allele was 45% and for homozygous ZZ genotype - 88%. Specificity of quantitative alpha-1 antitrypsin analysis for heterozygous deficiency was 98% and for homozygous deficiency - 100%. Thus sensitivity of quantitative alpha-1 antitrypsin analysis is higher than specificity for both - heterozygous and homozygous deficiency.
The results of the present study support the general concept of targeted screening for AAT deficiency with adequate laboratory methods in European countries with PI*Z high frequency and large population of COPD patients with highest diagnostic value - AAT genotyping. A case detection programme of alpha-1 antitrypsin deficiency in patients with chronic obstructive pulmonary disease using quantitative methods could be used only in screening programs and exact diagnosis must be confirmed by determining AAT genotype.

J03.02

Identification of a novel missense COL4A5 mutation in Russian family with X- linked Alport syndrome by next Generation sequencing

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Alport syndrome (AS) is a genetically determined glomerulopathy that is caused by mutations in type IV alpha collagen chain genes COL43, COL44 or COL45 and clinically characterized by hematuria, proteinuria and end-stage kidney disease (ESRD). The syndrome is often combined with sensorineural hearing loss and ocular pathology.
The coding exons and splice sites of the abovementioned genes were sequenced in four patients and two healthy controls from the family on Ion Torrent platform. Here we describe a novel dominant missense mutation c.G3098A, p.1033 G>D in the collagen type IV alpha-5 gene (COL4A5) that is present in all family members affected. The patients are characterized by early-onset hematuria, proteinuria (before 1 year of age), sensorineural hearing loss and the early age of ESRD onset. Neither of them demonstrates any ocular abnormalities.

J03.03

Mr. Lev Shagam

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Alport syndrome comprises ~3% of chronic kidney disease cases with estimated frequency of 0.02% in human population. It is a genetically determined glomerulopathy caused by mutations in genes encoding the alpha chains of type IV collagen. The protein protomers are most prevalent component of glomerular basement membrane (GBM). Among them, collagen α3α4α5 protomer is the most frequent in a mature GBM, its components being encoded by COL4A3, COL4A4 and COL4A5 genes. About 85% of all examined patients with Alport syndrome bear COL4A5 mutations (this type of nephritis is X-linked and dominant), whereas the remaining 15% demonstrate COL4A3 or COL4A4 mutations (autosomal form, usually recessive).
Genetic test is known to be the only direct diagnostic approach for Alport syndrome. Here we discuss the research and diagnostic prospects for the CDS sequencing of collagen type IV genes based on multiplex PCR followed by next generation sequencing on Ion Torrent platform. We designed a panel that covers 98% of the CDS with 5 bases of padding around targeted coding exons. This approach allowed us to identify both previously described and novel (including missense and frameshift) mutations that cause Alport syndrome in Russian population.
The problem of pathogenicity assessment and distinguishing between polymorphic variants vs malignant mutations is discussed. Different approaches exist including online prediction tools (e.g., PolyPhen, SIFT) and databases (pubmed, HGMD, LOVD, ClinVar etc.). We compare those tools for variant classification in Alport syndrome and other pathologies.

J03.04

Gene variant rs2305480 C>T in gasdermin B gene (GSDMB) and the risk of recurrent wheezing and asthma in Bulgarian infants

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Objective: The gasdermin B (GSDMB) gene is located at 17q21.2 and recent reports suggest that GSDMB is associated with childhood asthma in several populations. We investigated the association of a SNP in GSDMB (rs2305480C>T) with recurrent wheezing, severity of bronchial obstruction and family history of asthma and allergy.
Materials and Methods: Family history of asthma and allergy, recurrent wheezing and atopy were assessed in 93 infants admitted to the hospital for bronchiolitis. All children were genotyped for rs2305480C>T by PCR RFLP analysis. The presence of AvaII restriction site was indicated by C-allele and the absence - by T-allele.
Results: Data were analyzed as a recessive genetic model. Genotype frequencies did not deviate significantly from expected under the Hardy-Weinberg Equilibrium - T/T genotype was 18%, C/T - 47% and C/C - 35%. Our data show that the genotype T/T is a possible risk factor for recurrent wheezing (OR 3,68, 95%, CI= 0,98-13,76). Children homozygous for T-allele are more likely to have a family history of allergy and asthma (OR 5,41, 95%, CI= 1,43-20,47) and early age of first wheezing - 6,88 mo compared to 10,4 mo for C/C, p= 0,02.
Conclusions: Our results support the role of GSDMB SNP (rs2305480C>T) for determining asthma phenotypes in preschool children. The study was financially supported by research grant (2013), Medical University - Sofia

J03.05

Novel deletion in AVPR2 gene causing complete nephrogenic DI

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Background: Nephrogenic diabetes insipidus (NDI) is characterized by the inability of urine concentration leading to a high risk of dehydration. In this study, we report a novel deletion in *AVPR2* gene causing complete NDI confirmed by microarray analysis. **Methods:** A male patient was admitted to due to polydipsia and polyuria. NDI was suspected and molecular studies were performed to investigate these clinical problems. **Results:** Sequencing result showed a total deletion (11535 bp) in *APVR2* gene (NG_009645.2:g.5303_16835del) on X chromosome accompanying 7-bp microhomology at the breakpoint. Following microarray analysis reconfirmed this complete deletion of *APVR2* preserving *LCAM1* and *ARHGAP4* gene. **Conclusions:** With sequencing and microarray analysis, we found the novel deletion in *AVPR2* causing complete NDI. As shown in this study, molecular diagnosis has several advantages compared with conventional in vivo test. First, patients unable to conserve water may become critically dehydrated in water deprivation test. There are several contraindications of this test, especially in other causes of polydipsia and polyuria such as DM, hypoadrenalism, or CRF. Second, molecular studies can give more detailed information that cannot be provided by conventional tests. In this patient, the sole deletion of *APVR2* preserving *LCAM1* and *ARHGAP4* meant NDI excluding central type. In addition, since *APVR2* is completely deleted, it can only be complete NDI, not partial type. Therefore, this study suggests that genetic testing is a safer and more useful laboratory tool than the physiologic test in diagnosing and subtyping NDI.

J03.06

Reversibility of bronchiectasis: case report of Kartagener's syndrome

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Kartagener's syndrome is an autosomal recessive disorder primarily manifesting as ciliary movement disorder. Kartagener's syndrome is part of the larger group of disorders referred to as primary ciliary dyskinesias (PCD). Although the condition is usually inherited in an autosomal recessive pattern, and some specific gene defects have been recognized, it is clear that

the syndrome shows substantial genetic heterogeneity. The incidence of this genetic disorder is estimated to be between 1 and 2 per 30 000 births. Symptoms result from defective cilia motility in the airways. The recurrent pulmonary infections are caused by the grossly impaired mucociliary transport in the respiratory tract causing stasis of the mucus within the bronchi. Progressive and significant lung damage occurs up to the time of diagnosis. Although the management of patients with Kartagener's syndrome remains uncertain and evidence is limited, it is important to follow up these patients with an adequate and shared care system. This report presents a clinical case of Kartagener's syndrome in a 25-year-old woman. Computed tomography showed dextrocardia and bronchiectasis. After 7 years, good treatment results were achieved: radiological findings and lung function were improved. The present clinical case demonstrated reversibility of bronchiectasis even in congenital Kartagener's syndrome, thus indicating, that bronchiectasis progression is a complex interrelationship among genetic variation and a proper nonspecific management.

J03.07
The role of HLA typing for celiac disease diagnosis among children with autoimmune disorders

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Background:The association of celiac disease (CD) with several autoimmune conditions is well-known.Objectives:To determine the prevalence of CD among children with autoimmune thyroid disorders(AITD) and insulin-depended diabetes mellitus(IDDM) and to asses the diagnostic role of HLA typing among this patients.Methods:74 children with AITD(lot 1), 98 children with IDDM(lot 2) and 80 healthy children were screened for CD.In patients with at least one positive serologic test for CD, intestinal biopsy was performed.All children underwent HLA typing for DQ2/DQ8.Results:CD prevalence in lot 1 was 7%, in lot 2 was 6% and in control lot was 0%.All children diagnosed with CD presented DQ2/DQ8 haplotype.20% of the control subjects associated heterozygous DQ2 alleles.From 69 children with AITD/without CD,3 patients (4%) presented heterozygous DQ2 alleles.From 92 children with IDDM/without CD, 25 patients (27%) associated homo or heterozygous DQ2/DQ8alleles.There were significantly more cases with IDDM withoutCD but with predisposing haplotype for CD(27%) compared to the number of patients with AITD seronegative for CD and with DQ2/DQ8 alleles(4%) p<0,005.Conclusions: Recommending AITD and IDDM as selection parameters for CD screening in asymptomatic children is justified. HLA assessment cannot highlight a significant role of a certain allele in the pathogenesis of autoimmune comorbidity AITD/CD or IDDM/CD. DQ2 and DQ8 alleles are mandatory but insufficient for CD development.The intervention of environmental factors is very important.Performing as first line approaching HLA typing in asymptomatic at risk children is important.A negative result for DQ2/DQ8 alleles will render CD highly improbable and there will be no need for subsequent CD antibodies testing in such cases.

J03.08
NPHS2 and WT1 mutations in a romanian children Population with nephrotic syndrome

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Mutations in NPHS2 and WT1 genes are a frequent cause of steroid resistant nephritic syndrome (SRNS) and occur in 10-28% of children while mutations are absent from children with steroid-sensitive nephrotic syndrome (SSNS). The frequency and spectrum of mutations in these genes is unknown for the romanian population. **Material and methods** This study comprised 42 pediatric patients with NS. 50 healthy children were enrolled as a control group. NPHS2 R229Q polymorphisms were determined by the PCR and RFLP technique utilizing specific primers. Mutation analysis was performed in WT1 genes, in case of abnormality the corresponding sample was sequenced. **Results** In NS group 85.71% were SSNS and 14.29% were SRNS. Mutation of NPHS2 R229Q gene, was found just in two patients with congenital NS.Screening of WT1 gene showed one heterozygous mutation in the donor splice-site of intron 9:c.1228+5 G>A in one SRNS girl with normal karyotype. **Conclusion** The incidence of NPHS2 mutations in romanian children with NS is considerably lower than that among European childern (10-30%). WT1 mutation was present in girl with focal segmental glomerulosclerosis. Therefore screening of WT1 gene in all females with FSGS is necessary. Because NPHS2 R229Q gene mutation was found in all cases of CNS, a screening of this gene in children with CNS in the adjacent counties is required. Further studies with a larger number of patients are needed.

Acknowledgements This work was funded by Internal Research Grants of the University of Medicine and Pharmacy Tîrgu Mureş, Romania, contract no29/11.12.2013.

J03.09
The Polymorphisms in the IREB2, CHRNA5, CHRNA3, FAM13A and HHIP Genes and Risk of Chronic Obstructive Pulmonary Disease in Russian Population

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Chronic obstructive pulmonary disease (COPD) is a multifactorial inflammatory disease primarily affecting distal respiratory pathways and lung parenchyma.
Genome-wide association studies have iden¬tified genetic variants influencing the risk of COPD. The aim of this study was to investigate whether IREB2, CHRNA5, CHRNA3, FAM13A and HHIP polymorphisms would be associated with COPD susceptibility in Russian populations.
Methods: six single nucleotide polymorphisms: rs13180 (IREB2), rs16969968 (CHRNA5), rs1051730, rs6495309 (CHRNA3), rs7671167 (FAM13A), rs13118928 (HHIP) were genotyped in a case-control study (511 COPD patients and 508 controls from Russia). To estimate the strength of association, odds ratios were calculated and potential confounding variables were tested by using logistic regression analysis.
Results: Statistical analysis revealed that SNP rs13180 (IREB2) was associated with COPD (P= 0.0004). Analysis showed an association of rs16969968 (CHRNA5) (P=0.0034) and rs1051730 (CHRNA3) (P=0.0015) in additive model and the A-A-G haplotype of rs16969968 (CHRNA5), rs1051730, rs6495309 (CHRNA3) genes polymorphisms (P= 0.0078) with COPD. The relationship between the rs13118928 (HHIP) (P=0.0017 for AG genotype) and COPD risk was found. Significant association with sever COPD were observed for rs13180 (P=0.0009), rs16969968 (P=0.0071), rs1051730 (P=0.0013), rs13118928 (P=0.003) polymorphisms. Early onset COPD (before 40 yrs) were associated with rs13180 (P=0.000001), rs16969968 (P=0.0001), rs1051730 (P=0.0004). The SNP rs13118928 near HHIP locus was significantly associated with pack-years of smoking in COPD patients (P= 0.029). We demonstrated that rs13180, rs13118928 and rs16969968 polymorphisms were associated with COPD only in smoking subjects.
We confirmed that SNPs the IREB2, CHRNA5, CHRNA3 and HHIP loci were associated COPD in Russian Population.

J03.10
MTHFR polymorphysms role in diabetic neuropathy

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Diabetes Mellitus (DM), the most important public health problem of the 21th century, leads to significant mortality and morbidity due to underlying complications. C677T and A1298C polymorphysisms of MTHFR gene elevates plasma homocysteine levels, reduces plasma folic acid levels, and converts gene expression by altering methylation status in genome. These effects enable the MTHFR gene to be associated with multiple diseases such as cardiovascular diseases, cancers, and schizophrenia.
The present study looked for a possible association between C677T and A1298C polymorphysisms of the MTHFR gene in diabetic neuropathy development in diabetic patients and healthy volunteers. Thus a study group consisting of 103 diabetic peripheral neuropathy patients diagnosed via electrophysiological examination (ENMG) and a control group of 100 healthy was formed. C677T and A1298C polymorphysisms of MTHFR in the DNA samples were analyzed by pyrosequencing. MTHFR C677T genotype distribution was in study group CC: %43.7, CT: %42.7, TT: %13.6, and in the control group CC: %45.0, CT: 47.0, TT: %8.0 (p>0.05), MTHFR A1298C genotype distribution was in study group AA: %33.0, AC: %46.6, CC: %20.4 and in the control group AA: %43.0, AC: %44.0, CC: %13.0 (p>0.05). χ^2 test was used for statistical analysis and in the study group, the ratio of 2 or more mutant allele was found to be significantly higher compared to the control group (p=0.010). The data obtained from this study supports the view that the increase in the number of mutant alleles in MTHFR gene can increase diabetic neuropathy susceptibility in patients with diabetes.

J03.11
A novel (Q97R) MEFV gene mutation in a Turkish FMF patient

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Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disease and characterised by episodic fever, serositis, arthritis, abdominal and pleuritic pain. FMF predominantly affects populations surrounding the Mediterranean basin including Armenians, Sephardic Jews, Arabs, Turks, Greeks and Italians. The MEFV gene mutations have been detected in the majority of FMF patients. MEFV gene is located on (16p13.3) comprises of 10 exons and encodes a protein of 781 amino acids called pyrin. Pyrin is expressed at high levels in granulocytes, monocytes and dendritic cells. Mutant pyrin cause dysfunction in the inflammasome complex and leads to excessive activation of IL-1β.

A total of 289 mutations and polymorphisms in the MEFV gene have been reported in Infevers database. Compared to other cities of Turkey, the highest prevalence of FMF is seen in Sivas city (Central Anatolia). Due to the most frequent mutations are M694V, E148Q, M680I (G/C) and V726A in Turkish FMF patients, we screened the mutations of these exons (2 and 10) of the MEFV gene using DNA sequencing method. During our MEFV gene mutation screening among FMF patients, we detected a novel nucleotide change Q97R (c.290A>G; p.Gln97Arg) at exon 2 of MEFV gene that cause of missense amino acid mutation in pyrin protein in a 22 years old female patient with typical FMF criteria.

Turkish FMF patients have a unique mutation spectrum together with our novel Q97R mutation. It is very important to detect novel missense mutations in order to explain their crucial roles in FMF pathogenesis.

J03.12

Features of clorine ions concentration in suspected cystic fibrosis patients sweat test and its correlation with C reactive protein

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Backround: It is essential to confirm or exclude the diagnosis of cystic fibrosis in time and with accuracy in order to avoid inappropriate testing. The aims of this study was to determine features of Cl ions concentration in patients sweat test that were suspected having cystic fibrosis and to evaluate correlation between this concentration and C reactive protein levels in serum.

Methods: we investigated retrospectively case files of patients with performed sweat test in period of 2011-2012 in Hospital of Lithuanian University of Health Sciences Kauno Klinikos Paediatrics department. These patients were suspected having cystic fibrosis (n=48).

Results: 9 out of 48 patients (18,75 %) showed higher than normal Cl ions concentration values. 6 of them (12,5%) had a borderline concentration. Mean value of Cl ions concentration in males sweat was 25,37 mmol/l (p<0,05; PI=21,05-30,40) and there was no statistical significance compared to mean Cl ions concetration in females sweat which was 24,44 mmol/l (p<0,05; PI=19,96-28,91). In our study no mutations of CFTR gene were found. CRP test was performed in 17 cases (35,42%). No statisticly significant correlation between concentration of Cl ions in sweat and serum CRP was found (r= -0,16; p=0,531).

Conclusions: 18,75% of checked patients had a higher than normal Cl ions concetration in sweat test. No statisticly significant difference between Cl ions concentrations in sweat test according to gender was found in examined group.

J03.13

The prevalence of the cagA and vacA genotypes of H. pylori from patients with upper gastrointestinal diseases in Uzbekistan

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Helicobacter pylori colonizes the gastric epithelium of more than one half of the world's human population. This pathogenic bacterium plays a causative role in the development of chronic gastritis, peptic ulcers, and gastric adenocarcinoma. There is increasing evidence that the genetic variability of H. pylori have a clinical importance. Several virulence factors of H. pylori have been described, such as cytotoxin associated gene (cagA) and vaculating cytotoxin (vacA). This study aimed to investigate the prevalence of the cagA and vacA genotypes of H. pylori from patients with upper gastrointestinal diseases and the relationship with clinical outcome in Uzbekistan. A total of 72 patients who underwent endoscopy were included in this study. DNA was isolated from gastroduodenal biopsy samples and the presence of cagA and vacA genotypes were determined by PCR. All patients were found to be H. pylori-positive. The main virulence strain observed in our cohort was the cagA+ strain identified in 56 patients (77,8%). From the 52 patients with gastritis, 30(57,7%) harbored cagA+ strains and 12(42,3%) were infected with strains that lacked cagA. CagA positivity was observed in 26(86,7%) of patients with peptic ulcer pathology, whereas CagA negativity was observed in 4(13,3%) of these patients. Four VacA genotypes were found: s1/m1(25,8%), s1/m2(46,8%), s2/m1(14, 5%), s2/m2(12,9%). Comparative analysis of resulting genotypes showed a high percentage of s1/m2 with a distribution of 72,7% in patients with peptic ulcer pathology (P < 0.05). The results of this study might be used for the identification of high-risk patients harboring H. pylori in Uzbekistan.

J03.14

Methylation of miR-137 in gastric cancer and preneoplastic lesions

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INTRODUCTION: MicroRNA miR-137 is an important regulator of gene expression and functions as a tumor-suppressor gene. Expression of miR-137 is downregulated in glioblastoma and colorectal cancer (CRC) due to CpG island methylation, however, the role of miR-137 methylation in gastric carcinogenesis remains largely unexplored.

AIM &METHODS: The aim of our study was to characterize the epigenetic regulation of miR-137 in gastric carcinogenesis. We determined miR-137 CpG island methylation level in 81 pairs of primary gastric cancer tissues samples (T-GC) and corresponding adjacent normal mucosa (N-GC), 20 samples of normal gastric mucosa (N) and 23 gastric tissues from patients with chronic/atrophic gastritis ± intestinal metaplasia (CG) using bisulfite pyrosequencing and compared to 29 colorectal cancers (T-CRC) and corresponding adjacent normal colonic mucosa (N-CRC).

RESULTS: We confirmed the higher methylation level of miR-137 promoter in T-CRC tissues compared to adjacent normal colonic tissues (p=0.004). In similar fashion, but to a lesser extent, methylation of miR-137 promoter region was observed in T-GC tissues compared to adjacent non-cancerous tissues (N-GC) (p=0.045). When compared to the normal mucosa from controls and mucosa from patients with gastritis, we found gradual increase in miR-137 methylation (p<0.0001). In subgroup analyses of gastric cancer tissues, miR-137 methylation was more frequent in tumors localized in antrum compared to cardia and corpus (p=0.07).

CONCLUSION: MiR-137 methylation is a frequent event in gastric carcinogenesis. The gradual increase in miR-137 methylation, as demonstrated for normal mucosa, chronic gastritis and tumour tissues, may be an early event in gastric carcinogenesis.

J03.15

Neonatal permanent diabetes caused by mutation *INS*/Y50C: integrated system CGMS and insulin pump

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Case description: first born on term female, spontaneous delivery, SGA. No gestational diabetes, no familiarity for type 1 and 2 diabetes. Normal glycaemia at birth. Hospitalysed at 24 days for irritability and mild jaundice: blood glucose 509 mg/dl, normal venous hemogasanalysis. Transferred to our hospital in Bari, started rehydration and parenteral regular insulin (starting dose 0,02 IU/kg/h). **Diagnosis and therapy:** We positioned continuous glucose monitoring system (CGMS Medtronic Enlite 27 Gauge 6 mm), glycaemic range 80-200 mg/dl and insulin pump (Paradigm Veo, model 554C). Started therapy with Insulin Lispro (basal dose: 0,025 UI/h - 0,1 UI/h, pre-prandial bolus 0,025-0,1 UI with automatic suspension of basal insulin delivery for glycaemia <80 mg/dl). Exclusively breastfed, CHO calculation not easily performable. Negative IAA, GAD e IA2; C-peptide: 1,4 ng/ml - after few days 0,73 (0,46-3,5 ng/ml). Molecular analysis: *de novo* heterozygous mutation c.A149->G TAC (Tyrosin)>TGC (Cysteine), (p.Tyr50Cys; position 50 of proinsulin), described as INS/Y50C. Discharged, after 38 days, with CGMS and insulin pump (total basal insulin: 2,1 UI/day; 0,7 UI/kg/day), lower night basal dose, pre-prandial bolus (0,025 UI before every meal when glycaemia>150mg/dl). After 6 months of follow up, normal weight gain, good metabolic control (HbA1c: 6,4%). Cognitive development assessment: QI 141/100, age equivalent score 8 months and ¾ (Griffiths Mental Development Scale Revised 0-2). **Conclusions:** The integrated system (CGMS-insulin pump) is an elective therapy model in neonatal diabetes for personalizing insulin therapy, planning therapy modifications, studying glycaemic trend in real time, reducing glycaemic variability and avoiding psychological burden correlated to hypoglycaemia.

J03.16

Prevalence and spectrum of CYP21A2 gene mutations in women with symptoms of hyperandrogenism in Uzbekistan

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Worldwide prevalence of hyperandrogenism varied from 3% to 23%. This wide range of variation is assosiated with difficulties of differential diagnosis between polycystic ovary syndrome (PCOS) and nonclassic congenital adrenal hyperplasia (NCAH). Molecular-genetic analysis plays a significant role in this issue, because NCAH is a genetic disorder which developes due to mutations in the CYP21A2 gene.
Therefore, our study aimed to investigate the prevalence of CYP21A2 mutations in women with hyperandrogenism in Uzbekistan. Real-time PCR using allele specific primers and TaqMan probes were used to detect eight most frequent CYP21A2 gene mutations in 361 Uzbek women with hyperandrogenic symptoms. Our results showed that 11,7% of these women have mutations in the CYP21A2 gene. We found the following spectrum of mutations: C1994T-71,4%, T999A-9,5%, A/C655G-7,2%, deletion of 8 bp(707-714GAGACTAC)-4,75%, G1683T-4,75% and C89T-2,4%. In the investigated group of women we did not find T1380A and C2108T mutations. C1994T mutation was found to be the most common mutation in our cohort. Women who harbored this mutation had abnormal menstrual cycle and recurrent miscarriages. In conclusion, genetic screening for CYP21A2 gene mutations by means of real-time PCR is an effective method for differential diagnosis between polycystic ovary syndrome and nonclassic congenital adrenal hyperplasia at the patients with hyperandrogenic symptoms.

J03.17

Genetic factors of exercise participation and their association with basal metabolic rate and body mass index in overweight/obese Turkish women

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Introduction: Obesity is one of the fast spreading diseases. Insufficient physical activity is one of the main environmental factors in the etiology of the disease. Twin studies have revealed that genetic factors play an important role in physical activity levels. Although the underlying genetic factors are not clearly understood, studies have shown that the *LEPR* gene is among the candidate genes affecting physical activity level. In our study, we aimed to show the effect of *LEPR* gene mutations related with the physical activity in obese women. In addition, we evaluated the impact of *LEPR* gene on body composition parameters. **Material and Methods:** 66 overweight/obese women were included in our study. Patients were categorized into 3 groups due to physical activity index levels. After the extraction of genomic DNA from buccal cells, *LEPR* gene regions were amplified by PCR and PCR products were sequenced. Physical activity levels and body composition parameters were calculated by using actical accelerometer and impedance meter, respectively. **Results and Discussion:** Three reported polymorphisms**,** one in exon 6 and two others in intron 7 of the *LEPR* gene were detected. No relationship between physical activity levels and *LEPR* was observed. A correlation was found between resting metabolic rate and rs12405556 mutation. Furthermore, we determined a significant relation between different physical activity levels with fat mass, body mass index and total energy expenditure. Further studies are needed to make an interpretation about the relation between physical activity levels, body composition parameters and gene mutations.

J03.18

Association and gene-gene interaction analyses of genetic variants in IL1B, IL1RN, IL8, IL10 and TNFA genes for peptic ulcer disease in Volga-Ural region of Russian Federation

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A peptic ulcer disease (PUD) is an area of damage to the inner lining (the mucosa) of the stomach or the upper part of the intestine (duodenum). A bacterium, Helicobacter pylori, is the main cause of ulcers in this area. The genes that encode proinflammatory and anti-inflammatory cytokines are good candidate markers of host susceptibility to gastroduodenal disease.
The present study was performed to evaluate association and gene-gene interaction of polymorphisms of cytokines genes IL1B (rs1143634), IL1RN (rs71941886), IL8 (rs4073), IL10 (rs1800872) and TNFA (rs1800629) for PUD in Volga-Ural region of Russia.
This study enrolled 264 patients with gastric and duodenal ulcers (112 individual were H.pylori-infected), the control group included 277 unrelated individuals without gastro-duodenal pathology with different ethnic origins (Russians, Tatars, Bashkirs). Genotyping was performed by PCR-RFLP analysis. To investigate gene-gene interactions, we employed generalized multifactor dimensionality reduction (GMDR) method.
The analysis has revealed a strong association of C allele and CC genotype of the rs1143634 of the IL1B gene with PUD in Bashkirs ($\chi2$=7,61, p=0,006; OR=2,87 и $\chi2$=9,28, p=0,002; OR=4,49; 95%CI 1,78-11,35), confirmed by meta-analysis. We have also detected that H.pylori-positive PUD-individuals has significant lower frequency of AA genotype of rs4073 of the IL8 gene compared with healthy donors ($\chi2$=5,29, p=0,02; OR=0,46). The 3-locus GMDR-model of cytokine gene-gene interaction, including rs1143634 of IL1B, rs4073 of IL8 and rs1800872 of IL10 genes, was shown for male subgroup (p=0,0547).
Thus, we have determined that cytokine genes may contribute in genetic susceptibility for peptic ulcer disease.

J03.19

Rare germline MEN1 mutation increases susceptibility to pituitary adenoma

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Introduction. Pituitary adenomas are up to 15% of clinically active primary intracranial neoplasms. They are considered benign, non-metastatic tumors. Although pituitary adenomas have prevalence of 16.7% in general population, clinically significant tumors affect one individual out of approximately 1000 to 1300 people in general population. There is evidence that both genetic and epigenetic factors are important to development of human neoplasms.
Methods. Our study was carried out using 144 cases and 354 controls. Seven candidate genes (*AIP, MEN1, GNAS, SSTR2, SSTR5* and *DRD2* and *PRKar1a*) were selected based on literature research about pituitary adenoma genetics and 96 *tag* and non-synonymous SNPs genotyped using Illumina GoldenGate genotyping assay on Illumina BeadXpress system. Basic association test was used to test difference between cases and controls.
Results. Our data showed that non-synonymous (A→T) SNP rs2959656 in *MEN1* is strongly associated (OR=17.8 CI95%=2.2-145, P=0.0002) with development of pituitary tumor. Most patients were diagnosed while their adenoma were smaller than 10mm in diameter (microadenoma, stage I) as opposed to trend of pituitary adenomas being diagnosed in macroadenoma with extrasellar extension (stage II or III) (39 *vs.* 105 in our sample) suggesting increased secretory activity than typical adenomas of same stage. There was no association with particular hormone secreting profile. Another notable finding was SNP rs7131056 in *DRD2* gene which was associated with higher risk for adenoma extrasellar growth (OR=2.3 Ci95%=1.4-3.7, P=0.001) suggesting involvement of dopamine system in aggressiveness of pituitary adenomas.
Conclusion. Polymorphisms in *MEN1* and *DRD2* constitute to development and aggressiveness of pituitary adenomas.

J03.20

The molecular genetic research of chemical hypersensibility among women in the case of autoimmune thyroiditis

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Autoimmune Thyroid Disease (AITD) is heterogenetic inflammatory disease of thyroid. Pathogenesis of AITD apparents by intensity of destruction thyroid follicles. According to the data from literature and own epidemiological surveys carried out in OAO «Gazpromneftechim Salavat» it was shown that AITD is occupational disease. The goal of research was molecular genetic analysis cytokine genes, cytokine receptors, signal transduction and ranscription factors IL6 (rs1800795), IL17A (rs4711998, rs1974226), JAK1 (rs310216), JAK3 (rs3212780), STAT1 (rs12693591), STAT3 (rs2293152), NFKB1 (rs28362491) in women with AITD working under the conditions of occupational hazards. DNA samples of 236 women living in the Republic of Bashkortostan. 116 women were present AITD. The number of healthy women was 120. Polymorphisms of analyzed genes were done by polyme-

rase chain reaction (PCR) and next digestion restriction endonucleases RsaI, MspI, TaqI, Hsp92II. NFkB insertion-deletion polymorphism was analyzed by PCR. Calculations were carried out on the programme SNPStats. The deviation from Hardy-Weinberg equilibrium were not found.
A genetic association between AITD and three of the eight genotyped SNPs was found in this study among women under the conditions of occupational hazards. It was determinated the association with AITD polymorphism rs28362491 NFkB gene in overdominant model OR=0.55 (CI 95% 0.33-0.92), p=0.00001. Association with AITD was identified in polymorphism rs12693591 STAT1 gene OR=1.76 (CI95% 1.03-2.99), 0.04 in dominant model (AC + AA vs. CC). Associations were found between rs310216 Jak1 gene OR=2.06 (CI95% 1.17-3.61), p=0.012 in the dominant genetic model (AG+AA vs. GG).
The work was done under supporting Russian Humanitarian Scientific Fund №13-06-00101.

J03.21
Inherited alpha- 1 antitrypsin deficiency and spontaneous pneumothorax: possible causal relationship

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Background. An increased incidence of serum alpha1-antitrypsin deficiency has been reported in patients with chronic obstructive pulmonary disease but has not been well proven in association with spontaneous pneumothorax. The aim of our study was to evaluate frequency of alpha-1 antitrypsin deficiency in subjects with spontaneous pneumothorax.
Methods. 39 patients with the diagnosis of spontaneous pneumothorax and 100 age- and sex-matched control subjects were included in the study. Alpha-1 antitrypsin concentrations were determined by nephelometry, Serum qualitative Z antitrypsin variant was analyzed using commercial ELISA kits and alpha-1 antitrypsin phenotyping was carried out by means of isoelectric focusing.
Results. AAT deficiency phenotypes were detected in 3 (7.7%) patients with spontaneous pneumothorax, and only in 1 (1%) case in the control group. However, the observed differences did not reach statistical significance due to the considerable size disproportion between groups. The mean serum alpha-1 antitrypsin level was significantly higher in patients with spontaneous pneumothorax (1.53±0.23 g/l) than controls (1.34±0.31 g/l) (p=0.03).
Conclusions. Preliminary data confirm the clinical importance of alpha-1 antitrypsin deficiency genotypes in patients with spontaneous pneumothorax and the need to screen them for alpha1-antitrypsin deficiency.

J03.22
WT1 mutations in Bulgarian steroid-resistant nephrotic syndrome patients

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Steroid-resistant nephrotic syndrome (SRNS) is a chronic, progressive disorder which affects approximately 10% of all children with nephrotic syndrome. Approximately 10% of all infantile ESRD cases are a consequence of SRNS, which makes the disease one of the major causes of child morbidity.
The genetic background of SRNS is diverse. The most common mutations are found in NPHS2 and WT1 genes. NPHS2 codes the protein podocin, which is an essential structural element of the podocytes slit diaphragm. WT1 encodes a transcription factor with key role for the urinary tract differentiation.
We present here the results from genetic testing of Bulgarian patients with SRNS. We used direct DNA sequencing to screen 22 children form 21 families for mutations in the coding regions of WT1 and NPHS2. We found several polymorphisms in both NPHS2 and WT1. In one patient we found a heterozygous R229Q substitution in the podocin gene, while two children carried novel missense variants in WT1. One of the novel WT1 mutations is located in exon 8, the other in exon 9. Both affect the zinc-finger structures of the DNA binding domain. Further tests were carried out for determining the origin of the two novel mutations and proving their role in disease.
We can conclude that in Bulgarian SRNS patients there is a prevalence of WT1 mutations above NPHS2 gene defects. Additional investigations need to be carried out in order to determine the genetic cause of the disease in the remaining patients.

J03.23
Tight Junction gene expression in intestinal epithelial cell monolayers

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Introduction: Disrupted intestinal barrier function is observed during the development of autoimmune and inflammatory diseases like Crohn's and celiac disease, and several genetic associations have been detected (*PARD3* and *MAGI2*). Cell models are necessary for the investigation of the functional implications of associated candidate genes. The Caco-2 subclone, C2BBe1, are human derived brush border expressing cells that grow in homogenous monolayers that form tight junctions (TJ) and are potentially a good tool for this research. **Aim:** To determine the function of TJ-related genes during monolayer formation in C2BBe1 cells. Methods: C2BBe1 monolayers (300000 cells/cm^2) were grown in 0.4 µm pore size PET inserts in Dulbecco's Modified Eagle Medium (DMEM) with 4.5g/l glucose supplemented with 10% inactivated FBS and 1% non-essential amino acids, and transepithelial electrical resistance (TEER) was monitored every 24h for 6 days. RNA was extracted each day and the expression of the most relevant TJ genes for TJ formation (*TJP1, CLDN2* and *ACTB*) and associated *PARD3* and *MAGI2* was analyzed by RT-PCR. **Results:** All genes except *MAGI2* were expressed in C2BBe1 and mRNA levels increased during the formation of the monolayers. There was a significant positive being significantly correlation between TEER and *CLDN2* (r^2=0.76; p=0.005), *TJP1* (r^2=0.84; p=0.001) and *PARD3* (r^2=0.70; p=0.01) expression, but not in *ACTB*. **Conclusion:** Monolayer cultures are valid tools for functional analyses of TJ genes. However, studies to determine those genes that are expressed in each cell line must be performed.

J03.24
Detection of Turner Syndrome by PCR-based approach in patients from Uzbekistan

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Turner syndrome (TS) is one of the most common genetic disorder affecting females, occurring in approximately 1:2500 female births. TS occurs when an X-chromosome is completely or partially deleted or when X-chromosomal mosaicism is present. It is characterized by short stature, gonadal dysgenesis, primary hypogonadism, congenital heart disease, renal anomalies, and a variety of somatic features. Girls with TS benefit from early diagnosis and treatment with growth hormone. However, many girls with TS are not detected until after 10 yr of age, resulting in delayed diagnosis and treatment.
This study aimed to apply PCR-based approach for detection of Turner syndrome and elucidation the parental origin of the X chromosome in patients from Uzbekistan. We have amplified by polymerase chain reaction five polymorphic markers along the X chromosome (DXS1283E, DYS II, DMD49, AR and DXS52) and three markers along the Y chromosome (SRY, DYZ3 and DYZ1). In addition we analyzed patients DNA samples by SYBR Green and TaqMan probe based real-time PCR assay. The results of our study show that monosomy(45,XO) was present in 78% of cases, 45,X/46,XX mosaicism in 18,8%, and 45,X/46,XY in 3,2%. We also determined the parental origin of the X chromosome in the 2 patients. They had a paternal single X chromosome.
PCR-based approach can be recommended for the screening programs regarding detection of girls with TS in Uzbekistan.

J03.25
Average telomere length as a biomarker in children and adolescents with type 1 diabetes at the diagnosis

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Introduction: Subjects with type 1 diabetes (T1D) an autoimmune chronic disease are prone to oxidative stress, increased levels of advanced glycation end-products and other factors leading increased risk for T1D complications. Shorter telomeres are associated with T1D complications and lower serum vitamin D levels.
Methods: Average telomere length (ATL), nutritional status (BMI-SDS) and vitamin D level at the onset of T1D were determined in 53 Slovenian T1D children/adolescents (median age 8.7 years, 1:1.3 male to female ratio) whole blood DNA samples. The ATL was determined with qPCR method, vitamin D levels were determined with HPLC method.
Results: There was a tendency between a shorter ATL and a higher BMI-SDS (rs=-0.241; p=0.08). In addition subjects with ATL in the higher ATL tertile tended to have a higher BMI-SDS when compared to those in the lower ATL tertile (0.259 ± 0.457 vs. -0.583 ± 0.282 SDS; p=0.06). Subjects in the upper BMI-SDS tertile had a lower serum vitamin D levels when compared to those in the lower BMI-SDS tertile (40.66 ± 3.07 vs. 52.86 ± 4.85 µmol/L; p=0.045). Vitamin D serum levels did not significantly differ between sub-

jects with ATL in the higher tertile and those in the lower tertile (47.71 ± 5.35 vs. 41.09 ± 3.10 µmol/L; p= 0.296).
Conclusion: T1D children/adolescents with a shorter ATL tend to have a higher BMI-SDS. Lower serum vitamin D levels were determined in T1D subjects with a higher BMI-SDS. An association between vitamin D serum levels and ATL was not determined.

J03.26

Association of *MMP3*, *ITGB5*, *ITGA4*, *ADAMDEC1*, *LIG1* genes with type 1 diabetes

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The search for new genetic variants involved in type 1 diabetes and its complications gives the opportunity for personalized medicine. In our study, 58 SNPs were studied in 48 genes which were associated in GWA studies with one or more of the following phenotypes: fibrogenesis, endothelial dysfunction, diseases of the cardiovascular continuum, type 1 diabetes (T1DM), and type 2 diabetes. Specimens were evaluated using the Sequenom MassARRAY (USA). Case-control study included 285 patients with T1DM and 300 population controls. All subjects were ethnically Russians living in Siberian region of Russia (Tomsk and Kemerovo cities). Association with the disease was obtained for the following genetic markers: *MMP3* rs679620 (AA, OR=2,03 (95% CI: 1,19-3,47), p=0,008), *ITGA4* rs1143674 (GG, OR=2,03 (1,27-3,26), p=0,003; allele G, OR=1,68 (1,24-2,27), p=0,001), *ITGB5* rs1007856 (TT, OR=1,67 (1,10-2,54), p=0,015; allele T, OR=1,31 (1,02-1,70), p=0,037), *ADAMDEC1* rs3765124 (AA, OR=1,76 (1,18-2,65), p=0,005; allele A, OR=1,31 (1,01-1,70), p=0,040), *LIG1* rs20579 (CC, OR=1,95 1,26-3,02), p=0,002; allele C, OR=1,91 (1,29-2,81), p=0,001). The *MMP3*, *ITGB5*, *ITGA4* and *ADAMDEC1* genes are involved in the formation of extracellular matrix and collagen metabolism. These associations can be explained by the participation of these genes in the formation of fibrotic changes in the renal tubules and interstitium, which leads to progression of diabetic nephropathy and deterioration of the patient condition. The *LIG1* gene is involved in DNA repair and recombination. Perhaps this gene is associated with type 1 diabetes through some basic metabolic pathway. Altogether, our results suggest that T1DM development is influenced by genes involved into fibrogenesis.

J03.27

Association study of promoter polymorphisms of *nucb2* gene in Iranian patients with type 2 diabetes

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Type 2 diabetes mellitus (T2DM) is characterized by both insulin resistance (poor tissue insulin sensitivity) and impaired insulin secretion from the pancreatic β-cell. Global prevalence of type 2 diabetes is increasing rapidly. Substantial evidence suggests that T2D is a multifactorial disease with a strong genetic component. Nesfatin-1, derived from the nucleobindin2 (NUCB2) precursor, was recently implicated as a mediator of anorexia in the central nervous system. Plasma nesfatin-1 concentrations were found to be elevated in subjects with both Impaired Glucose Tolerance (IGT) and newly diagnosed type 2 diabetes (nT2DM) and to be related with several clinical parameters known to be associated with insulin resistance. The aim of the present study is to investigate the association of polymorphisms of promoter of nucb2 gene in Iranian population.
We performed a case-control study consisted of 100 T2DM and 100 healthy individuals. Blood samples were obtained from subjects after informed consent was achieved. Isolated genomic DNA amplified for five SNPs at promoter of nucb2 by Sanger sequencing and results analyzed by Laser gene V7.1 and online software SNPanalyzer2.
Statistical analysis revealed a significant association for major allele of rs214088 (G) with type 2 diabetes in receive model (p=0.03; OR: 3.109, 95%CI: 1.064-9.082). In haplotype analysis, the CAG haplotype of nucb2 (rs214088, rs4757506 and rs214087) ascertained significant association with type 2 diabetes (p=0.03).
Our study suggests that there is a significant association between rs214088 at the promoter of nucb2 gene and T2DM. This SNP may applied as biomarkers for susceptibility of T2DM in Iranian population.

J03.28

When speed matters: using rapid next-generation sequencing to confirm Wilson-disease in a patient with severe acute liver failure

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Acute liver failure (ALF) is a rare form of Wilson disease (WD) developing mostly in young female patients. Use of chelating agents and supportive therapies including MARS (Molecular Adsorbent Recirculating System) in time may result in a remission in some cases making liver transplantation unnecessary. We are presenting here a 47 year old male patient with no alcohol consumption, negative hepatits- and autoantibody markers and with elevated transaminase and ferritin levels. The patient was admitted to our institution with severe ALF and with the potential diagnosis of haemochromatosis, but the patients coeruloplasmin level was low with very high level of ferritin. The D-penicillinamin test supported the diagnosis of WD, but the result of the genetic analysis of the most frequent disease causing mutation in Hungarian population (H1069Q) was negative (wild type). Targeted NGS-based analysis of the entire coding region of the ATP7B gene showed two different disease-causing alteration in this patient and made the diagnosis clear, showing the clinical potential of semiconductor based next generation sequencing with 36 hours of turn around time.

J03.29

Mutations in genes *HFE, SERPINA1, CFTR* in Wilson's disease patients in Latvia

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Introduction Liver inherited diseases are a group of genetically determined clinical entities that appear with an early chronic liver involvement. They include Wilson's disease (WD), hereditary haemochromatosis, and alpha-1 -antitrypsin deficiency. In addition, cystic fibrosis may cause a severe liver involvement in a significant percentage of cases. Mutations in the genes *HFE, SERPINA1, CFTR* could modify the pathogenesis and clinical appearance of Wilson's disease. **Aim** To detect frequency of the most common mutations causing inherited liver disorders mentioned above in patients with WD suggestive symptoms. *Material and methods* The study included 115 patients with WD suggestive symptoms and 295 unrelated healthy individuals. DNA analysis: both groups were tested for mutation C282Y and H63D in the gene *HFE*; F508del in the gene *CFTR*; PIZ and PIS in the gene *SERPINA1.* **Results** Frequency of different alleles: allele F508del in WD patients - 0.009, in control population - 0.01 (p=0.446); PIZ in WD patients - 0, in control group - 0.018 (p=0.058); PIS in WD patients - 0.019, in control group - 0 (p=0.045); C282Y in WD patients - 0.02, in control group - 0.035 (p=0.475); H63D in WD patients - 0.188, in control group - 0.121 (p=0.019; OR=1.687). **Conclusions** 1) Alleles PIS and H63D were more frequent in patients with WD suggestive symptoms that could indicate its significance in more severe and better detectable liver disorder in case of WD. 2) But still the role of iron overload and alpha-1-antitrypsin deficiency in the pathogenesis of Wilson disease is not finally elucidated.

J03.30

Molecular characterization of *COL4A5* in young Thai males suspected for X-linked Alport Syndrome

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Background: Presently, a number of young males affected by end-stage renal disease (ESRD) of unknown causes have been registered for renal replacement therapy. Either genetic or environmental causes cannot be excluded. However, search for monogenic causes is challenging since genetic screening can give the information regarding the approach to other patient's family members such as carrier screening. X-linked Alport syndrome (XLAS) is one of the suspected monogenic causes in young male patients. This particular syndrome is collagen IV-related nephropathy caused by the mutation of *COL4A5* on Xq22. Approximately 80% of XLAS leads to end-stage renal disease by the age of 40 with variable association with sensorineural hearing loss and ocular abnormalities. **Objective:** To characterize *COL4A5* mutation in Thai male patients affected by ESRD of unknown causes and to develop the rapid and efficient molecular testing strategies for identifying XLAS in clinical practice. **Methodology:** DNA extraction was performed from peripheral blood leukocytes of Thai male patients, age 10-40 years, affected by ESRD of unidentifiable causes. Three common mutations of COL4A5, C1564S, L1649R, R1677Q, were detected using qPCR and melting curve analysis. **Results:** Twenty patients were recruited in the study. Of them, *COL4A5* mutation was detected in four patients (20%). C1564S is the most prevalent

in this group. **Conclusion:** XLAS is the hidden cause of ESRD in young male patients affected by ESRD. Genetic screening is proposed to be necessary, not only for definite diagnosis, but also for genetic counseling and carrier testing in the family.

J03.31
A Retrospective Audit into the Screening for Complications in Patients with Hereditary Haemorrhagic Telangectasia (HHT) in the North West of England

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Background: HHT affects approximately 1 in 5000 people and has been associated with mutations in ENG, ACVRL1 and SMAD4. Features of this disease are epistaxis, telengectasia, a family history and visceral arteriovenous malformations (AVMs). International guidelines on the screening of patients with HHT were published in 2011.
Method: Patients with HHT were identified using the Molecular Laboratory database at St Mary's Hospital, Manchester. Screening on mutation-positive patients was audited against the International guidelines.
Results: 54 patients were identified and 35 (65%) were found to have a mutation in either ENG or ACVRL1. Pulmonary and cerebral AVM screening was undertaken in 92% and 62% of patients respectively. Screening for anaemia in those over 35 years was undertaken in 21%. 2/19 (11%) eligible patients were tested for SMAD4 mutations (both negative). AVMs occurred in 29% ENG and 21% ACVRL1 patients. Lung AVMs were more common in the ENG group (24% vs 7%). One cerebral AVM was identified and this occurred in the ENG group. Liver AVMs only occurred in the ACVRL1 group (21% vs 0%). Overall, AVMs occurred more frequently in women than in men (38% vs 7%).
Conclusion: A consensus on screening for HHT patients is needed in the UK. It is globally accepted that screening for pulmonary AVMs and anaemia should be undertaken but cerebral screening is still controversial. SMAD4 mutations should be sought in those patients who are negative for ENG and ACVRL1. The breakdown of AVMs in this population adds further evidence to a genotype-phenotype correlation in HHT.

J03.32
Primary hyperoxaluria type 1: Identification of a double mutation in AGXT gene in Tunisian families

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Primary hyperoxaluria type 1 (PH1) is a severe autosomal recessive inherited disorder of glyoxylate metabolism caused by mutations in the AGXT gene on chromosome 2q37.3 that encodes the hepatic peroxisomal enzyme alanine:glyoxylate aminotransferase. These mutations are found throughout the entire gene and cause a wide spectrum of clinical severity. Rare in Europe, PH1 is responsible for 13% of the end stage renal failure in the Tunisian child. In the present work, we identified the double mutation c.32C>T (Pro11Leu) and c.731T>C (p.Ile244Thr) in AGXT gene in five unrelated Tunisian families with PH1 disease. Our results provide evidence regarding the potential involvement of c.32C>T, originally described as common polymorphism, on the resulting phenotype. We also reported an extreme intrafamilial heterogeneity in clinical presentation of PH1. Despite the same genetic background, the outcome of the affected members differs widely. The significant phenotypic heterogeneity observed within a same family, with a same genotype, suggests the existence of relevant modifier factors.

J04.01
A five case molecular study of 3M syndrome

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3M syndrome is a rare autosomal recessive disease characterized by severe prenatal and postnatal growth retardation, facial dysmophism, unaffected intelligence and normal endocrine profile. Mutations are reported in three genes. CUL7 is the major gene responsible for the 3M syndrome accounting for 70% of cases. This work aims to establish the molecular diagnosis of 3M syndrome by detecting the presence of a founder mutation, previously reported among Maghrebian patients, in exon 24 of the gene CUL7 of five Tunisian patients with the typical features of the 3M syndrome. If the founder mutation is not detected, the molecular diagnosis of hotspots in exons 4 and 19 of CUL7 gene will be performed. Following the extraction of genomic DNA from peripheral blood sample, after parental consent, we performed a PCR amplification step followed by analysis by direct sequencing of the coding regions of exons 4, 18, 19, 24 and 25 of CUL7gene. Using these methods, we confirmed the presence of the founder mutation, del TG 4451-4452, in three of our patients . Furthermore, we did not detect either mutation or polymorphism in the five targeted exons of CUL7 gene in the index case of a second family that encloses two 3M patients. 3M syndrome is characterized by genetic heterogeneity that contrasts with the clinical homogeneity of the syndrome. We plan to complete the molecular diagnosis in the other exons of the major gene CUL7 before switching to the other two genes (OBSL1 and CCDC8) for cases of the family 02.

J04.02
Inherited alpha-1 antitrypsin deficiency and chondrosarcoma - causal relationship?

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Alpha 1-antitrypsin deficiency is a genetic risk factor for manifestation of COPD and chronic liver diseases. There is an ongoing worldwide discussion concerning the role of serpins (serine protease inhibitors) in tumor genesis. Protease inhibitors such as alpha 1-antitrypsin have generally been considered to counteract tumor progression and metastasis because of their ability to inhibit proteases. In this case report we analyze relationship between inherited alpha-1 antitrypsin deficiency and chondrosarcoma. A 47-year-old woman was admitted to the hospital with relapse signs of humerus chondrosarcoma. The patient also had a history of COPD. After chest X-ray and CT, alpha 1-antitrypsin deficiency was suspected. Severe alpha-1 antitrypsin deficiency (PiZZ homozygous genotype) was confirmed. Alpha 1-antitrypsin deficiency might have facilitated the development of chondrosarcoma. Because of a small incidence rate of such diseases, we presume that there is a slight chance for such rare disorders to manifest concurrently in the same patient.

J04.03
The importance of MEFV gene mutations in HLA-B*27 positive Ankylosing Spondylitis patients

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Ankylosing spondylitis (AS) that is etiologically unknown, can cause back and lumbar pain, and environmental, immunological and genetic factors have a role in its pathogenesis, is a chronic inflammatory disease. HLA-B*27 gene, located on short arm of sixth chromosome, have significant role in susceptibility of AS disease. The predisposition of AS disease is higher in HLA-B*27 positive people than HLA-B*27 negative ones. Also, there are some patients who have both AS disease and MEFV gene mutation. In our study, association of MEFV gene mutations have been analysed in HLA-B*27 positive and negative patients who diagnosed as AS, and HLA-B*27 negative healthy controls. 80 patients including HLA-B*27 positive 36 male and 14 female AS patients, HLA-B*27 negative 11 female and 19 male AS patients have been studied in the research. 50 healthy controls including 17 female and 33 male HLA-B*27 negative healthy individuals have been studied. HLA-B*27 allele of both patient and control groups were determined by PCR-SSP method. Pyrosequencing method was used for detection of MEFV gene mutations. HLA-B*27 positive patient group and healthy control group were compared in terms of MEFV gene exon 2 and 10 mutations. Frequency MEFV gene exon 2 and 10 mutation is determined statistically higher in HLA-B*27 positive patient group than healthy control group (p= 0,012) We suggest that MEFV gene mutations may have a role in etiopathogenesis of HLA-B*27 positive AS disease. That hypothesis needs to be supported by further studies, involved in different populations including higher number of patient and control.

J04.04
Influence of sequence variations in MMP3 and GDF5 genes on risk of the anterior cruciate ligament rupture in the Russian population.

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Objectives: Anterior cruciate ligament (ACL) rupture is a severe multifactorial injury. A familial predisposition toward tearing ACL was demonstrated. Matrix metalloproteinases and growth differentiation factor 5 (GDF5) are important physiological mediators of extracellular matrix degradation, remodeling and chondrogenesis. The aim of this study was to determine impact of *MMP3* rs679620 and *GDF5* rs143383 variations on the risk of ACL

rupture in Russians.

Methods: 70 patients (18 women) with diagnosed ACL ruptures (ACL-group) and 247 asymptomatic controls were studied. Both groups consisted from similar on a sex, age, BMI, physically active unrelated Caucasians. gDNA was isolated from blood and buccal epithelium. Genotyping was performed by TaqMan® SNP Genotyping Assays (AppliedBiosystems, USA). The results were analyzed by using TaqMan® Genotyper Software (AppliedBiosystems). Hardy-Weinberg equilibrium (HWE) was checked by GDA software (http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php). Goodness of fit test was conducted using the IBM-SPSS v. 21.

Results:

Groups	*GDF5* rs143383				
	A	G	AA	AG	GG
ACL	0.671	0.329	0.386	0.571	0.043
Control	0.596	0.404	0.369	0.453	0.178
	MMP3 rs679620				
	C	T	CC	CT	TT
ACL	0.543	0.457	0.357	0.371	0.271
Control	0.550	0.450	0.312	0.477	0.211

Significant deviation of genotypic frequencies from HWE was detected only for *GDF5* rs143383 in ACL-group (P=0.017). MAFs of tested SNPs in controls were similar to other Caucasians. Goodness of fit test reveals significant difference of *GDF5* rs143383 genotypic frequencies between groups. GG genotype carriers had a significantly decreased risk of ACL ruptures versus AG+AA genotypes ($\chi 2$ 5.995, P=0.014, OR 0.196, 95% CI 0.040 to 0.763). This study suggests a relationship between *GDF5* rs143383 variation and risk of ACL rupture in the Russian population.

J04.05

Filaggrin mutations and atopic dermatitis in Volga-Ural region of Russia

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder and is often the first step in the atopic march. Mutations in the filaggrin gene (FLG), a key component of stratum corneum, have been identified as a strong predisposing factor for allergic diseases. In this study, we have screened three FLG loss-of-function mutations (c.2282del4, p.Arg501X and p.Arg2447X) in AD patients and controls. The AD group consisted of 448 AD patients (177 Russians, 126 Tatars, 145 individuals of mixed ethnic background). The control group included 408 non-atopic individuals (152 Russians, 109 Tatars and 147 individuals of mixed origin). Genomic DNA was isolated by phenol-chloroform extraction. The genotyping of SNPs was performed by PCR-RFLP. In our study the most prevalent FLG mutation was c.2282del4. The allelic frequency in AD patients was 6.61% in general group, 6.03% in Russians (p=2,3*10-4) and 9.35% in Tatars (p=1,6*10-5). In controls the frequency of c.2282del4 was significantly lower: 1.12% in total group, 0.97% in Russians and 0.46% in Tatars. Second mutation p.Arg501X in our groups was rarely found. In AD subjects and controls it was detected with following frequencies: 0.92% and 0.71% in general group, 0.90% and 1.36% in Russians, 0.85% and 0.47% in Tatars, respectively. The third FLG mutation p.Arg2447X was also found with low frequency: 0.82% in general group of patients and 0.31% in controls; in Tatars it was 1.75% and 1.33%, respectively. In Russians this mutation was absent. Our results show that FLG mutation c.2282del4 is strong predisposing factor for atopic dermatitis.

J04.06

Correlation between vitamin D receptor gene polymorphisms and atopic dermatitis in Canakkale population: a study based on, FokI and TaqI RFLP technique

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Aim:The pathogenesis of atopic dermatitis (AD) includes genetic and environmental factors leading to immunological and nonimmunological dysfunctions. The VDR single nucleotide polymorphisms (SNPs), FokI and TaqI have previously been associated with atopic diseases such as allergic asthma. Methods:In a total of 88 AD patients and 96 healthy controls were included in the current study. The genomic DNA was isolated from peripheral blood-EDTA, and target VDR gene was genotyped by PCR-RFLP technique after VDR-FokI (rs2228570) and VDR-TaqI (rs731236) restriction enzymes digestion. Results were compared statisticaly. Results: We use Arlequin ver 3.5 integrated software for population genetics data analysis. Current results showed lack of association for VDR-TaqI polymorphism in AD but showed association for VDR-FokI in current cohort of AD (P=0.0364), (OR: 1.9526. Conclussion: The current preliminary results identified the association between VDR- FokI gene polymorphism and AD in Canakkale population. Results need to be confirme by large scale of patient groups.

J04.07

Vitamin D receptor gene polymorphisms in Iranian Azary patients with Behçet's Disease

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The aim of our study was to investigate the association of four polymorphisms of the VDR gene (FokI, BsmI, TaqI and ApaI) with their susceptibility to Behçet's Disease (BD) and their clinical manifestations in respect to the Iranian Azari population. In this cross sectional study we considered the BsmI, FokI, ApaI and TaqI polymorphisms in 50 Iranian Azary patients with BD and 50 healthy controls, with the use of Polymerase Chain Reaction-restriction fragment length polymorphism. A significant difference was found for the FokI polymorphism between the case and control groups. The f allele frequency of 26% was present in BD patients, compared to only 13% in the control group. In addition, the f/f genotype was significantly associated with BD. We found no significant differences between the BD and control groups regarding the distribution of ApaI, BsmI, and TaqI genotype frequencies. We found no association between VDR polymorphisms and the clinical manifestations of BD. The VDR f allele and f/f genotype is associated with BD in the Iranian Azari population.

J04.08

Brooke-Spiegler syndrome: A rare association of thichoepithelioma, cylindroma and spiradenoma. Report of a familial mexican case

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Introduction:

Brooke and Spiegler, independiently described an ephithelioma adenoides cysticum and skin endotelioma, as distinct entities. Brooke-Spiegler syndrome (BSS, OMIM#605041) characterized by benign adnexal neoplasia. Predominat tumors trichoepithelioma, cylindroma and spiradenoma appear childhood and early adolescence. BSS is autosomal dominant entity. The tumors located on head and neck, and increase throughout life. Trichoepithelioma showed flesh-colored papules on face; cylindromatosis presents erythematous nodules on the scalp; and blue-colored-painful lesions on trunk suggested spiradenomas. Pathogenesis is considered a defect in the diferentiation of folliculo-sebaceous-apocrine unit. Mutations have been identified in CYLD tummor suppressor gene, mapped to chormosome 16q12-q13.

Case Report:

Case I: 9 years-old female, birth: weight 3,400gr, height 53cm. Between 5-6 years-old displayed flesh-colored papules on face. Showed clinical and hystopathological features indicating trichoepithelioma, with keratinizing cystic spaces.

Case II: 42 years-old female showed, flesh-colored-papules on scalp, face and trunk. Three biopsies were reviewed, 1) right preauricular region revealed trichoepithelioma, 2) scalp region conclusive cylindroma, 3) third lumbar región conclusive ecrine spiradenoma. Given cryotherapy treatment and cosmetic surgery with good results.

Discussion:

Brooke-Spiegler syndrome, sex ratio of F:3: M:1. include skin appendage tumors such cylindromas, thichoepitheliomas and spiradenomas, share a common genetic basis. May be associated with other skin disorders such, basal cell adenomas/carcinomas. Treatment included dermabrasion, cryotherapy and some cases radiotherapy. We present a Mexican family, mother and daugther similar affected, with the clinical features of the disease. Is the first mexican case reported with this entity. Molecular studies are needed to understand the genetic bases of the disease.

J04.09

First molecular analysis of Ehlers-Danlos kyphoscoliotic type in Slavic population

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The kyphoscoliotic type of Ehlers-Danlos syndrome (EDS) is a heritable connective tissue disorder characterized by a deficiency of collagen lysyl hydroxylase 1 (LH1) due to mutations in PLOD1 gene. According to Villefranche criteria this type of EDS is characterized by kyphoscoliosis and hypotonia at birth, severe joint hypermobility as well as skin extensibility and fragility. 2-year old girl examined by us was born by caesarean section because of pelvic longitudinal position (situs longitudinalis pelvicus). Directly after the birth, kyphosis, hypotonia, joint hypermobility and unilateral hip dislocation were observed. Parents of investigated child did not present any clinical features of EDS. The gDNA of affected patient was obtained by isolation from peripheral white blood cells. Direct sequencing of all PLOD1 exons was carried out, revealing the presence of two mutations. The first one, substitution of cytosine by thymine in position 1095 (c.1095C>T), was localized in the exon 10. It was a splice site mutation, described earlier. The second one, which turned out to be a missense mutation that had never been described earlier in patients with kyphoscoliotic type of EDS, was localized in exon 17.
Our investigations of genetic background of kyphoscoliotic type of Ehlers-Danlos syndrome were the first ones in East-Central Europe.

J04.10
Atypical Fibrodysplasia Ossificans Progressiva (FOP) phenotype in a girl carrying uncommon missense mutation (p.G356D) in ACVR1

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Fibrodysplasia ossificans progressiva (FOP, MIM #135100) is a rare genetic condition characterized by progressive transformation of soft tissue into bone. There are approximately 3 000 individuals living worldwide with this severely disabling disease, for which there is still no definitive treatment. Heterozygous mutations in ACVR1 (MIM# 102576) has been identified as a cause of this condition. All reported patients of various ethnic backgrounds with classic clinical presentation of FOP have previously been found with the identical heterozygous activating mutation 617G>A(R206H). Recently other types of mutations in ACVR1 gene have been described in patients with variant FOP.
We present a course of FOP in 3,5 years old girl carrying uncommon missense mutation 1067G>A(G356D) in the protein kinase domain of ACVR1. Identical mutation has been identified in several patients with atypical FOP among which at least in two with mild clinical symptoms. On contrary, our patient experiences a severe course of FOP with already significant restriction of movement. Though it should be emphasized, that retrospectively analyzing evolution of intense heterotopic ossification in our patient, we have discovered, that most of the lesions were directly triggered by soft tissue injuries resulting from diagnostic procedures and inadequate rehabilitation that she underwent prior to recognition of her genetic disease.
Our presentation may add to the understanding of the variability of clinical FOP presentation in patients with atypical ACVR1 mutations, as well as help spreading knowledge of this disease among health care professionals, in hope for the earliest possible diagnosis established in each newly affected child.

J04.11
Genetic characterization of a Portuguese patient with fibrodysplasia ossificans progressiva

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Fibrodysplasia ossificans progressiva (FOP) is a rare autosomal dominant disease with a prevalence of approximately 1 in 2 million worldwide. FOP is characterized by the presence of malformations of the big toes and of postnatal progressive heterotopic endochondral osteogenesis, especially in the presence of exacerbating factors such as trauma, surgical intervention, lesion biopsy, and intramuscular injection. FOP has been associated with a specific mutation on ACVR1 (c.617G>A; p.Arg206His), which encodes a receptor for bone morphogenetic proteins (BMPs). Our aim was to establish the molecular diagnosis by mutation screening of ACVR1. We report a male patient with progressive ossificans since childhood, showing calcification of the axial line with inability to perform flexion and extension of the scapular and pelvic girdle with neck stiffness. Surgical intervention in adolescence resulted in disease progression. At the moment, patient is bedridden and with partial jaw fixation. Mutation screening was performed by PCR amplification of all coding and flanking regions, followed by bidirectional direct sequencing. We have found one missense mutation in exon 6 (c.617G>A; p.Arg206His), previously described as a FOP disease-causing mutation. The codon 206 is at the end of the highly conserved glycine-serine rich (GS) activation domain at the junction with the kinase domain. To our knowledge, this is the first genetic study of FOP in a Portuguese patient. The mutation screening of ACVR1 distinguishing FOP from other disorders allows the correct clinical management of the patient. Molecular diagnosis will also allow appropriate genetic counseling to this patient and at-risk relatives.

J04.12
First Italian case of Crouzon-like Craniosynostosis and Dental Anomalies (CRSDA, #614188) with severe scoliosis

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Crouzon-like craniosynostosis and dental anomalies (CRSDA, #614188) is an autosomal recessive disorder due to homozygous mutations of the IL11-RA gene.
Our patient (a 16-years-old boy) was referred to our Clinic for oxycephaly with frontal bossing and biparietal narrowing. By the age of 4 years he underwent a surgical correction because of raised intracranial pressure with optic nerve atrophy. He had maxillary hypoplasia with malocclusion, persisting deciduous teeth and supernumerary teeth (1.3, 2.3, 3.1, 4.2) treated by surgical correction, severe dorsal scoliosis with right convexity and thorax asymmetry. Encephalus and spine MRI showed a Chiari I malformation, and enlarged dural sac.
His parents were first cousins hailing from Southern Italy (Naples); his father underwent scoliosis surgical correction by the age of 19 years.
Molecular analysis of IL11RA gene in the proband identified a homozygous mutation c.598C>A (p.Pro200Thr) in exon 6; bioinformatic analyses by PolyPhen2 and SIFT suggested that this mutation is harmful for the protein function.
The mutation has been previously described by Keupp et al. (2013) in a non-consanguineous Turkish family in two siblings compound heterozygous c.598C>A /c.710G>C. It is also present in the databases as a rare variant
To our best knowledge, this is the first description of an Italian patient affected by CRSDA. We speculate that severe scoliosis, not previously described in affected patients, could be included in the clinical spectrum of the syndrome.

J04.13
The role of polymorphisms of inflammatory mediators genes in pyoinflammatory diseases of maxillofacial area

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Despite advances in the treatment of pyoinflammatory diseases of maxillofacial area, the number of diseases is increasing from year to year. In this connection, the study of the pathogenesis of pyoinflammatory diseases is one of the most pressing issues in maxillofacial surgery. The purpose of this study was to investigate the role of the cytokines genes in the development of odontogenic inflammatory processes.
To estimate the role of polymorphisms of inflammatory mediator genes in genetic predisposition to pyoinflammatory diseases, the allele and the genotype frequencies distributions of IL1β, IL1RA, TNFα, TNFβ and IL10 genes were investigated. The studied groups included 189 patients with pyoinflammatory diseases of maxillofacial area divided into two groups: odontogenic phlegmon (141) and osteomyelitis (48) and 105 healthy controls. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform method. Genotyping was performed by the PCR-RFLP technique.
Studies have revealed that the C*A* genotype (OR = 1,83; 95% CI 1,08-3,10) of IL10 polymorphic locus 627C>A is associated with increased risk of odontogenic phlegmon, while the G*A* genotype (OR = 0,29; 95% CI 0,08-1,04) of TNFα polymorphic locus 308 G>A is associated with lower risk of osteomyelitis of maxillofacial area. It is possible to suggest that genotype C*A* of IL10 polymorphism (627C>A) and genotype G*A* of TNFα polymorphism (308 G>A) may be genetic predictor of odontogenic inflammatory processes. No significant differences were found between the groups of patients and

healthy control concerning the genotype frequencies of the polymorphisms IL1β (3953 C>T), IL1RA (VNTR) and TNFβ (1069 C>T).

J04.14
Icthyiosis, the XXI century pandemy of Ecuador

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Ichthyosis is a group of inherited keratinizing disorders. There are five different types described. This type of genetic disorder can be autosomal recessive or x-link. This disorder has been related with inbreeding and new mutations. More than 16000 babies are born each year with some form of ichthyosis. In Manabí province, Ecuador (population 1,369,780) there are more than 230 cases, compared with rates of other countries this is very high. But when put into context, Ecuador is a developing country, in more isolated areas marriage between closely related family members is still common place. We are presenting a case of twin brothers that suffer from ichthyosis.
Case report: Mother and father are first cousins. They are parents of three children, one girl and two tween brothers. The boys present the typical fish scales, alopecia, constant conjunctivitis and skin infections. We found isolates of different microrganisms in all samples taken from eyes, skin and wounds. They present constant weeping of the eyes and intense itching. They do not eat pork due metabolic disorders associated with this dissease. After living in several places in Ecuador, they moved to Banos a small city in the Andes (1,815 m) because the moderate climate (moisture and temperature) is more favorable to their condition. Their treatment is: Rocutan (isotretinoin) for 2 weeks and after that Neotigason (retinoids, 25 mg every day or two). Their pathology has improved a lot since then. They do not show signs of hyperactivity or any mental problems.

J04.15
Lacrimo-auriculo-dento-digital syndrome, case report

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Lacrimo-auriculo-dento-digital syndrome (Levy-Hollister syndrome) (MIM:149730), is a extremly rare genetic disorder (prevalence < 1/1 000 000) characterised by abnormalities affecting the lacrimal and salivary glands and ducts, ears, teeth and fingers. Levy-Hollister syndrome may occur sporadicaly or be inherited as an autosomal dominant trait. We present a 16 years old index male patient clinicaly diagnosed with this syndrome. Our patient present recurrent obstruction of nasal lacrimal ducts and bilateral agenesia of parotid glands. The auricular feature was cup-shaped pinnas. The dental features were represented by hypodontia, microdontia, spaced teeth, enamel dysplasis and early onset caries. The limb defects were fifth finger clinodactily, hipoplasia of thenar eminence and absence of thumb flexion creases. No family history was found in our case. Due to agenesia of parotid glands is possible that our case shuold be the result of FGF10 de novo mutation. We compare the phenotype and findings of our case to previuosly published cases.

J04.16
A Novel mutation in SHOX gene in four members with Langer Mesomelic Dysplasia and in three members with Leri-Weil Dyschondrosteosis of a family

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Leri-Weill dyschondrosteosis (LWD; #MIM 127300) is an autosomal dominant hereditary disease, which is characterized by short stature, mesomelic shortening of the limbs, and characteristic bilateral abnormality of the wrists known as Madelung deformity. LWD is caused by mutations in the Short Stature Homeobox gene (*SHOX).* The other diseases which are associated with *SHOX* gene mutations are Langer Mesomelic Dysplasia (LMD, MIM #249700) and Turner syndrome as well as nonsyndromic idiopathic short stature (ISS). The *SHOX* gene is localized within the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes. Both alleles of *SHOX* gene must be functional for normal growth. While heterozygous mutations of *SHOX* gene or its enhancer regions are responsible for both LWD and ISS syndromes, LMD is caused by homozygous or compound heterozygous mutations in this gene. Herein, four LMD and one LWD siblings and their consanguineous parents with LWD are presented. Mutation analysis revealed that the parents and one sibling were heterozygous, and the other 4 siblings were homozygous for the c.42delG (p.Q14QfsX15) in the *SHOX* gene.

J04.17
A novel mutation in LEMD3 gene in a Turkish family with Buschke-Ollendorf syndrome

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Buschke-Ollendorff Syndrome (BOS, OMIM#166700) is an autosomal dominant disorder of connective tissue with an incidence of 1/20000. Although It is characterized by multiple subcutaneous nevi or nodules and osteopoikilosis (OPK), some patients may also have melorheostosis. Expressivity of bone and skin manifestations differs between the cases. Mutations in LEM Domain-Containig Protein-3 (*LEMD3*, OMIM *607844, chromosome 12q14.3) have been implicated in BOS. 14-year-old girl was directed to our department for an incidentally realised osteopoikilosis in the X-Ray exploration. She also has yelowish plaques on her anterolateral thigh. Complete X-Ray study revealed multiple osteoclastic foci around the bones as implicated in te BOS. 35-year-old mother has been X-Ray examined and she also has ospteopoikilosis but she has no skin plaques. *LEMD3* gene was sequenced from the DNA of the patient and the mother. Both of them have a heterozygous c.2405_2406insAGTG mutation in the 12th exon of LEMD3 gene.This mutation results in a premature stop codon at the 802th position of the MAN1 protein encoded by *LEMD3* gene. The remaining parts of the gene were also sequenced and confirmed that there was not another mutation. We submit the mutation to MutationTaster and see that it causes loss of Interaction with SMAD1, SMAD2, SMAD3 and SMAD5. To our knowledge, this is the first study implicating the c.2405_2406insAGTG mutation in the cases of BOS.

J04.18
HRM scanning of mutations in human structural proteins

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Background: This project aims at the development of assays for detection of mutations in structural proteins. Both intracellular (cytoskeleton and nucleoskeleton) and extracellular proteins (extracellular matrix) are considered, as well as non-structural proteins which are closely functionally associated with these structural proteins. Mutations in the selected proteins are known to cause rare diseases (RD). The project resonates with current clinical need to improve diagnostics and prognostics of rare diseases as formulated in many running national and European programs (e.g. Czech National Action Plan for Rare Diseases, COST NANONET, ORPHANET, EURORDIS, ERNDIM, EUROCAT, ICBDSR, E-rare ERA). **Methods:** The main detection method chosen is the scanning high-resolution melting analysis. The primers were designed for 65 subselected genes. The amplicons are succesively optimized to fit maximally two major PCR/HRM conditions for each gene. The following assays have been technically finalized on control DNA and both major tested platforms (LightScanner 96 and LightCycler 480): LMNA, MYH7, DMD, ACTC1, MYH6, MYL2, MYL3, DES, MYLK2, MYH14, MYH9 and MYO6. LMNA, MYH7 and DMD is currently undergoing testing on positive patient and synthetic DNA samples. **Results:** Female patient, 35 years old, with suspected Emery-Dreifuss muscular dystrophy was scanned using the LMNA scHRM assay. The scanning revealed putative mutation in the exon 6 of the LMNA gene which was confirme by the Sanger sequencing. Additional reported cases with mutations in the DMD and MYH7 genes will follow. This work is supported by the FR-TI3-588 grant from the Ministry of Industry and Trade of the Czech Republic.

J04.19
A novel homozygote p.Met540Ile LMNA mutation causes mandibuloacral dysplasia type A

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Mandibuloacral dysplasia with type A lipodystrophy (MADA) is a rare genetic disorder inherited in an autosomal recessive fashion characterized by hypoplasia of the mandible and clavicles, acro-osteolysis and lipodystrophy due to mutations in LMNA or ZMPSTE24 gene. Very few families have been studied for the above genes alteration so far. We have investigated a consan-

guineous family with an affected boy for LMNA alteration. Isolated genomic DNA derived from subjects was amplified using intronic primers. The entire sequence of the LMNA gene, including coding regions and exon-intron boundaries were analyzed by PCR and Sanger sequencing. Molecular analysis ascertained a homozygote mutation c.1620 G>A (p.M540I) in the proband and heterozygote alteration in the rest of the family. We have also applied several online tools including PolyPhen2, Pmut, SIFT, Mutation Taster and phyre2 to predict the p.Met540Ile substitution effects. All these tools showed reduction the stability of the protein structure. We conclude that M540I mutation may causes disease in homozygous state.

J04.20
Atrophic skin patches with abnormal elastic fibers, as a presenting sign of MASS phenotype associated with mutation in the Fibrillin-1 gene

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Marfan syndrome (MFS) is a dominantly inherited disorder of connective tissue caused by mutations in the Fibrillin-1 (FBN1) gene. The most common skin finding in MFS is striae distensae. Particular individuals referred for suspicion of MFS whom do not completely fulfill the MFS diagnostic criteria are classified as having a MASS phenotype. The acronym represents the phenotype's apparent manifestations: a prolapsed Mitral valve, Myopia, Aortic root enlargement, Skeletal and Skin manifestations. Mutations in FBN1 have been shown to be associated in a few cases with MASS phenotype. Skin manifestations may be an important clue to the diagnosis of these disorders. We studied a case referred for unusual atrophic skin patches on the buttocks. Histopathology and electron microscopy demonstrated markedly abnormal elastic fibers. Consequent medical genetics evaluation led ultimately to the diagnosis of MASS phenotype, and to the discovery of an underlying FBN1 mutation.
Though the clinical suspicion and diagnosis of MFS and related disorders are usually established by its main associated clinical features including the eye, skeletal and vascular involvement, clinicians should be aware of the associated skin manifestations, including unusual atrophic patches with abnormal elastic fibers that can sometimes be the first noted sign of the genetic disorder.

J04.21
Novel *COL9A3* mutation in a family diagnosed with multiple epiphyseal dysplasia

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Background : The clinical and radiographic phenotypes of multiple epiphyseal dysplasia(MED) are heterogenous according to the genetic mutation. Mutations *COMP*, *MATN3*, *COL9A1*, *COL9A2* and *COL9A3* result in autosomal dominant MED, and mutations in the *DTDST* gene is associated with an autosomal recessive MED. Here in, we present a family with novel *COL9A3* gene mutation.
Case : The proband was a 12-year-old boy born from non-consanguineous parents. He was referred to the pediatric orthopedic clinic for the evaluation of intermittent knee pain that occurred from a few months. The radiographic phenotype of COL9-MED is that the epiphysis of the distal tibia, distal radius and distal ulnae showed lateral shortening, wedge shape and small, respectively. Direct sequencing of COL9-MED associated genes was performed and identified a novel mutation c.104G>A in exon 2 of *COL9A3*, which resulted in p.Gly35Asp. The same mutation was observed in the proband's father. The molecular dynamics (MD) simulation of wild type and mutant model structure were performed using AMBER11 program and demonstrated that the mutant type collagen was aggregated by themselves while wild type structure formed hydrogen bonds with its neighboring strand. In addition, the distances between the mutation site of the wild type and the mutant collagen are almost twice. The calculated energy state of the mutant is much lower, causing a self aggregation.
Conclusion : We report a family of MED with novel *COL9A3* mutation and propose that major structural change caused by the mutation of COL9A3 gene induce the malfunction of the type IX collagen.

J04.22
Neurofibromatosis-Noonan Syndrome - clinical and molecular studies of 4 patients

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Neurofibromatosis-Noonan syndrome (NFNS) (OMIM 601321) combines the variable clinical signs of both entities – Neurofibromatosis type I (NF1) (OMIM 162200) and Noonan syndrome (NS) (OMIM 163950). All these clinical disorders belong to the RASopathies caused by the dysregulation of the RAS/MAPK pathway. The phenotype of NS includes short stature, heart defects (particularly pulmonary stenosis), chest deformities, ptosis, bleeding abnormalities and dysmorphic features. The clinical features of NF1 are: café-au-lait (CAL) spots, neurofibromas, Lisch nodules, axillary and inguinal freckling, optic glioma and skeletal defects. In the majority of patients with NFNS the mutation in *NF1* gene is found. In few patients also the mutation in *PTPN11* was identified.
We present 4 patients with clinical features of NFNS with multiple CAL spots (all patients), relative macrocephaly consistent with NF1 (2 patients) and patchy depigmentation of skin (1 patient). None of them had neurofibromas. Dysmorphic features and heart defects (mitral or aortic valve prolaps, descrete pulmonary stenosis) specific for NS were present in all patients. The short stature and myopia were observed in 2 patients, speech delay, ptosis, occipital region cyst, submucous cleft palate, pectus deformity, unilateral cryptorchidism and bleeding abnormalities in 1 patient. The mutations in *NF1* gene were identified in all patients. No mutation was found in the *PTPN11* gene. This and previous studies indicate that there are significant phenotypic differences between NFNS and NF1 or NS. Detailed comparison of NFNS clinical symptoms in described group of patients with data from publications as well as genotype-phenotype correlation will be presented.

J04.23
Expression of the genes that are indicators of young bone growth and histological analysis in evaluation of osteogenic process

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Osteoreparation is a complex, dynamic and still incompletely revealed process. There are different approaches in evaluation of osteogenic process. The purpose of our research is evaluation weather and to what extent relative expression of genes that are indicators of young bone growth are in correlation with histological findings, as commonly used methods for evaluation of osteogenic process. In our research, subcutaneous implantation model on Balb/c mice was used. The implants were composed from deproteinized bone mineral matrix and bone marrow and/or different components of blood. The animals were sacrificed after 1, 2, 4, and 8 weeks after implantation. Analysis of implants included comparison of relative expression profiles of the gene for alkaline phosphatase, osteocalcin, osteonectin, osteopontin and collagen type I (RealTime PCR) and histological analysis of implants after staining with hematoxylin-eosin and Masson's trichrome method. Our results show: the most informative genetics markers are profiles of expression of genes for osteocalcin and alkaline phosphatase, as indicators of stimulation of young bone growth; histological picture was lagging in comparison to the findings of gene expression in all terms of sacrifice; valid assessment of osteogenic process requires a combination of different methods, because neither gene expression nor histological analysis are sufficient for complete evaluation of osteogenic process.
Key words: RealTime PCR, histology, ectopic osteogenesis

J04.24
Bone mineral accrual and fracture outcomes in children with osteogenesis imperfecta treated by pamidronate

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The purpose of this study was to evaluate the bone mineral accrual and fracture outcomes in children with osteogenesis imperfecta (OI) treated by pamidronate (PAM). Material and Methods: in our retrospective study 21 children with different types of OI were included: 7 boys and 14 girls. According to clinical OI classification proposed by D. Sillence, patients were divided in 3 types: I type - 11, III type - 7, IV type - 3. We divided patients in 2 groups: mild to moderate (OI I type) and moderate-to severe (III and IV types). The standard protocol with cyclic PAM infusions (3 consequent days 3-4 times in a year) was applied in annual cumulative 9-12 mg/kg. All children received vitamin D and calcium supplementation in physiological doses. Observation period was 36 months. Bone mineralization parameters were detected by dual-energy X-ray absorptiometry of lumbar spine L1-L4. Results: There were no differences in bone mineral accrual between types

of OI. The maximum efficacy in bone mineral accrual was observed in first year (+32.9%) and second year (+22.1%) and no real improvement in BMD in third year. Reduction of fractures in OI I types was from 0.87 (0.64; 1.08) to 0 (0.0; 0.5) fractures per year (p=0.09). In severe OI group fracture reduction was more impressive: from 28.5 (4.6; 56.2) to 1.1 (0.86; 2.6) fractures per year (p=0.02). Conclusion: PAM treatment was effective in bone mineral accrual and fracture reduction. The maximum efficacy in bone mineral accrual was observed in first two years.

J04.25
Medical approach in a severe case of osteogenesis imperfecta

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Osteogenesis imperfecta is one of the most common skeletal dysplasias and comprises a group of genetic disorders that are characterized by increased bone fragility, low bone mass and increased susceptibility to bone fractures. The inheritance is autosomal dominant, but it may also results from new dominant mutations. We present the case of a young woman, 27 years old, retired, diagnosed with osteogenesis imperfecta in early childhood. She has negative family history, thus, it was considered to be due to a new mutation. The first fractures, double fracture of tibia and femur occured during infancy, after minor trauma. Until now more than twenty bone surgical treatments were needed. The patient has very short stature, severe scoliosis, deformed thorax, dorsal kyphosis, muscular and bone pain, poor muscle tone, osteoporosis, dentinogenesis imperfecta, white sclera, signs that led to the clinical diagnosis of osteogenesis imperfecta type III. Functional independence is affected due to low muscle strength, especially in the lower extremities, with a tendency to fall, due to reduced joint mobility and not least because of the pain. Complex therapies include not only surgery, but also medication, secondary prophylaxis with healthy lifestyle and diet, and application of early rehabilitation programs. Management of subjects with osteogenesis imperfecta, especially severe forms, by a multidisciplinary team, increase psychomotor development, improve quality of life and prevent fractures with major vital risk.

J04.26
LRP5 gene polymorphism V667M (rs4988321) in man with osteoporosis and Polish population - a pilot study

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Many recent reports confirmed, in the disease onset, both genetic and environmental factors, may be different in osteoporosis for women and men. Mutations in the low-density lipoprotein receptor-related protein 5 (LRP5) gene cause rare syndromes characterized by altered bone mineral density (BMD). LRP-5 is a transmembrane protein encoded by gene located in 6q25.1. LRP-5 protein takes part in a proliferation and differentiation of osteoblasts via Wnt signaling. The Wnt/b-catenin signaling pathway stimulates bone formation through a number of mechanisms such as stimulation of preosteoblast replication, induction of osteoblastogenesis, and inhibition of osteoblast and osteocyte apoptosis. LRP-5 is widely expressed in most human tissues, with greater amounts in the liver and pancreas. In bone, it is mainly expressed by the bone-forming cells as osteoblasts, in the endosteal and trabecular bone surfaces. Common LRP5 variants were associated with the osteoporosis risk in males. The aim of this pilot study was to analyze occurrence of polymorphic variant c.2074G>A (p.V667M, rs4988321) in a group of 187 male patients with osteoporosis and 203 individuals from Polish population. Genotyping was performed by pyrosequencing technique. Hardy-Weinberg equilibrium (HWE) were examined for subjected groups by chi-square distribution and Fisher exact tests. The odds ratios (ORs), 95% confidence intervals (CIs), and p-values were calculated. Statistical significance was set at $p<0.05$. We observed statistically relevant higher frequency of minor allele c.2074A in male osteoporosis patients with p=0.01401 (OR=2.162, CI=[1.154-4.050). In conclusion we state that c.2074A in LRP5 gene variant may be one of the risk factor for osteoporosis in males.

J04.27
Association of gene variants in TLR4, TNF-α, IL-3 and IL-6 genes with Perthes disease

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Background: Perthes disease is idiopathic avascular osteonecrosis of the hip in children, with unknown etiology. Inflammation is present during development of Perthes disease and it is known that this process influences bone remodeling. Since genetic studies related to inflammation haven't been performed in Perthes disease so far, the aim of this study was to analyze the association of frequencies of genetic variants of immune response genes: toll-like receptor 4 (*TLR4*), tumor necrosis factor alpha (*TNF-α*), interleukin-3 (*IL-3*) and interleukin-6 (*IL-6*) with this disease.
Methods: The study cohort consisted of 37 patients with Perthes disease and 50 healthy controls from Serbia. Polymorphisms of *TLR4* (Asp299Gly, Thr399Ile), *TNF-α* (G-308A) and *IL-6* (G-597A, G-174C) genes were determined by polymerase chain reaction restriction fragment length polymorphism method, while *IL-3* gene polymorphisms (C-16T, C132T) were determined by direct sequencing of PCR product.
Results: *TLR4* polymorphisms (Asp299Gly, Thr399Ile) were in complete, while *IL-3* (C-16T, C132T), as well as *IL-6* (G-597A, G-174C) polymorphisms were in perfect linkage disequilibrium. A statistically significant increase of heterozygote subjects for *IL-6* G-174C/G-597A was found in controls in comparison to Perthes patient group (P=0.047, OR=2.49, 95% CI=1.00-6.21). Also, the patient group for *IL-6* G-174C/G-597A polymorphisms wasn't in Hardy-Weinberg equilibrium. No statistically significant differences were found between patient and control groups for *TLR4* , *TNF-α* or *IL-3* analyzed polymorphisms.
Conclusion: Our results suggest that children who are heterozygous for the *IL-6* G-174C/G-597A polymorphisms have a lower chance of developing Perthes disease than carriers of both homozygote genotypes.

J04.28
Medical Management in Pierre-Robin Sequence

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Background: Dysregulation of the genes SOX9 and KCNJ2 may be involved in Pierre-Robin Sequence, evidenced by a familial translocation with a breakpoint located in the gene empty region between SOX9 and KCNJ2, and by reduced expression of SOX9 and KCNJ2 in non-translocated patients. Pierre-Robin Sequence congenital craniofacial malformations associated with obstructive sleep apnoea, results in decreased pharyngeal airway, which, in severe cases, may lead to tracheostomy dependence. We aim to highlight management difficulties in such a case. Material and method: An 11 year old male diagnosed with Pierre-Robin sequence presented to our clinic for multidisciplinary management. Results: The child presents craniofacial anomalies associated with severe microretrognathia, protruding ears, hypotelorism, malocclusion, bilateral simian crease and sandal gap sigh, short stature, failure to thrive, severe difficulties in feeding and sleep apnoea. Blood work was unremarkable. Head CT revealed: severe microretrognatia and crowding of teeth (Angle type I- neutrocclusion). Sleep studies have demonstrated obstructive sleep apnoea. He did not require tracheostomy, but will undergo mandibular distraction in an attempt to relieve the severe upper airway obstruction and improve his sleep pattern and food ingestion and chewing. Conclusions: Infants with Pierre-Robin Sequence should be evaluated by a multidisciplinary team to assess the anatomic findings, delineate the source of airway obstruction, and address airway and feeding issues, but also genetic counceling for the family.

J04.29
First molecular-genetic analysis of Rothmund-Thomson syndrome in Serbia

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Rothmund-Thomson syndrome (RTS) is an autosomal recessive genodermatosis presenting with a characteristic facial rash (poikiloderma) associated with short stature, sparse scalp hair, sparse or absent eyelashes and/or eyebrows, juvenile cataracts, skeletal abnormalities, radial ray defects, premature aging and a predisposition to cancer. The prevalence is unknown but several hundred cases have been reported in the literature so far. Two clinical subforms of RTS have been defined: RTSI characterised by poikiloderma, ectodermal dysplasia and juvenile cataracts, and RTSII characterised by poikiloderma, congenital bone defects and an increased risk of osteosarcoma in childhood and skin cancer later in life. RTS is genetically heterogeneous: RTSII is caused by homozygous or compound heterozygous mutations in the RECQL4 helicase gene (detected in 60-65% of RTS patients), whereas the

aetiology in RTSI remains unknown. According to literature data missense mutations are rare, while frameshift, nonsense mutations and splice-site mutations prevail. We describe 5 years old girl with typical signs of RTSII and indication for molecular genetic testing of RECQL4 mutations. After extraction of genomic DNA from peripheral blood we performed direct sequencing of mutation prone exons of RECQL4 gene. Two different mutations have been detected in exon 9: c.1568G>C (p.Ser523Thr) and c.1573delT (p.Cys525AlafsX33). The observed frame shifting deletion is the most common RECQL4 mutation, while substitution is rarely described. This is the first case of RTSII from Serbia confirmed by genetic testing. Genetic testing of RTSII is important in the context of differential diagnosis and genetic counseling for patients and their families.

J04.30
A family with proximal symphalangism (SYM1)

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Background: A 33 year old woman was referred to genetic counselling with a family history of congenital stiffness of the digits. Absence of the proximal interphalangeal joints of all fingers except the pollex, and fusion of the calcaneus naviculare and calcaneus cuboideum had previously been diagnosed radiographically. At the physical examination, stiff digits and poorly discernable skin crease was found along with severe pes planus. The pedigree demonstrated autosomal dominant inheritance with four generations of affected family members presenting with stiff fingers, fusion of foot bones and at least one sister with impaired hearing as a result of stapes fixation. A review of the literature revealed that hereditary fusion of the proximal interphalangeal joints was first described by Harvey Cushing in 1916, followed by other case reports. This is - to the best of our knowledge - the first family to be reported in Denmark. The *NOG* gene (17q22) encodes noggin, a secreted polypeptide important for regulating multiple signaling pathways, particularly in cartilage and bone. A mutation herein is believed to cause Proximal Symphalangism (SYM1) which conforms to the above condition. **Methods**: Sequence analysis of *NOG1*. **Results**: Test results of *NOG1* mutation screening are pending.

J04.31
Investigation of Interleukin-10 (IL-10) family cytokines polymorphisms in patients with psoriasis

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Background: Interleukin (IL)-10 family cytokines IL-10, IL-19, IL-20, and IL-24 have been implicated in autoimmune diseases and we have previously reported that genetic variants in IL10 gene cluster were associated with psoriasis. Objective: To analyze the relationship of genetic polymorphisms in the IL10 gene cluster with psoriasis for Russian population. This study also explores whether there are gene-gene interactions among these genetic polymorphisms. Methods: A total of 273 patients with psoriasis and 298 matched healthy controls were enrolled to carry out a case-control study for 48 SNPs of IL10 gene cluster. Genotyping for the SNPs was conducted on the Applied Biosystems 3730 DNA Analyzer using SNPlex™ technology. Results: The results showed that the genotype distributions of IL20 T/T (rs1518108) and IL20 A/G (2232363) are significantly different between case and control groups (P = 0.034 and P = 0.047, respectively). Carriers of IL20 A (2232363) allele conferred risk to psoriasis (OR = 2.26, 95% CI = 1.05-4.88) while those of IL10 T (rs1554286) and of IL20 T (1400986) alleles conferred protection to psoriasis (OR = 0.71, 95% CI = 0.53- 0.94; OR = 0.69, 95% CI = 0.49-0.97). Conclusions: Our preliminary data suggest that four polymorphisms (rs1554286, 1400986, rs2232363, rs1518108) located in IL10 gene cluster related to inflammatory and immunity processes showed an association with protection or development of psoriasis in Russians.

J04.32
Mutations in COL2A1 in a Brazilian cohort of 15 patients with SEDC phenotype

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Type II collagenopathies are characterized by a spectrum of different conditions among which the most common phenotype is the spondyloepiphyseal dysplasia congenita - SEDC. More than 30 missense mutations, usually private and involving the change of a glycine, have been described in patients with SEDC. Unfortunately, little is known about severity and follow up of the patients with known mutations. Here we present the molecular results of 15 new patients with SEDC. The molecular analysis was performed by direct sequencing of the COL2A1. Thirteen mutations have been found until now, and the majority is located at the center of the triple-helical domain between exons 23-27. Among the 13 mutations, eight are novel (p.G516S, p.G528A, p.G570D, p.G708R, p.G498D, p.G687R, p.G1149R, and p.G1080V), and result in the glycine substitution by a bulkier amino acid. Two mutations were recurrent and previously described, p. R989C and p.G594E. In two patients with no mutations the sequencing of COL2A1 is still ongoing. Severe phenotype was associated with the following mutations: p.R989C, p.G570D and p.G687R. The patients with the following mutations G549E and G516S, all died in the first months of life. The mutations p.G1149R and p.G1080V were seen in less severe phenotypes. The remains mutations could not to be associated with severity because children are still very young. In conclusion, p.R989C mutation seems to produce a constant and severe phenotype. The two less severe phenotype were associated with mutations in the extremity of the triple-helical domain.

J04.33
Homozygous shox gene deletion detected by array CGH in a girl with langer mesomelic dysplasia

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Langer mesomelic dysplasia (LMD) is characterized by hypomelia with severe hypoplasia of ulnae and fibulae, and bowed, thickened radii and tibiae, causing deformities of the hands and feet. LMD is caused by homozygous mutations in the SHOX/SHOXY (short stature homoebox) gene, of which heterozygous mutations or deletions cause Leri-Weil Dysplasia (LWD). Phenotype of LWD can be incomplete between and within families. We present a 13 year old female with LMD, the second child of healthy first cousin parents. She had micrognathia, disproportionate short stature with various musculoskeletal findings (absence of the distal flexion creases of the 3rd, 4th, 5th fingers on the right, camptodactyly of the 3rd, 4th, 5th fingers on the left, tibial bowing). X-rays revealed hypoplasia of ulnae, fibulae and the mandible. Chromosome analysis and FISH investigation by using SHOX gene probe revealed normal results. Sequence analysis failed due to unsuccessful PCR amplifications. Array comparative genomic hybridization (a-CGH) study showed a 174 kb homozygous deletion, encompassing the SHOX gene. Proband's parents were heterozygous for the same deletion by a-CGH. FISH was uninformative, because there was no difference between the intensity of the signals on both chromosomes. Since the primers used were located within the deleted region, molecular studies could not be performed. A-CGH proved to be the most powerful diagnostic tool in this case.

J04.34
Papillon-Lefevre syndrome and an autosomal dominant form of palmoplantar keratoderma in the same Indian family: mutation screening and in-depth bioinformatic analyses of the cathepsin C gene

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Papillon-Lefevre Syndrome (PALS, MIM 245000) is a rare autosomal recessive disorder characterized by palmoplantar keratoderma and early onset severe periodontitis affecting both deciduous and permanent dentition. Several recessive families and sporadic cases with variable clinical presentation have been reported. Several loss-of-function mutations have been identified in the lysosomal protease cathepsin C gene (*CTSC*) in PALS individuals from various ethnic groups. We describe Papillon-Lefevre syndrome and an autosomal dominant form of palmoplantar keratoderma in the same Indian family (UR075) and present the mutation analysis of the cathepsin C gene. Sequence analysis of known exons and splice junctions revealed a homozygous nucleotide substitution G to A at nucleotide position 901, resulting in a change from glycine (GGC) to serine (AGC) at amino acid position 301 (G301S) was observed in homozygous condition in an affected individual, and excluded other affecteds with severe palmoplantar keratoderma from the same family. We re-evaluated family UR075 and excluded chromosome 11q14-q21 region by linkage analysis. This mutation was found to be at a

highly conserved residue in of *CTSC* gene. Structure prediction and energetic analysis of wild-type *CTSC*, comparison with mutant (G301S) revealed that this change in amino acid does not imply any secondary structural change. However, prediction of functional effect(s) of this mutation is possibly damaging the protein structure and/or function. Additionally, based on the energy calculation and the modeled protein structure of the mutant is expected to be energetically unstable.

J04.35

PROGINS progesterone receptor polymorphism in Systemic Lupus Erythematosus

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Background: Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease with an unexplained etiology. Several studies have investigated the role of steroid hormones and their receptor polymorphisms for the development of the disease. However, the clinical significance of the PROGINS receptor polymorphism of the progesterone receptor gene has not been studied yet, despite the known immunosuppressive actions of the progesterone. Therefore, the present study aimed to investigate the potential influence of the PROGINS haplotype on the SLE onset and clinical manifestations. Materials and methods: The PROGINS Alu insertion polymorphism was investigated in 122 Caucasian lupus patients and 105 healthy controls by PCR-RFLP analysis. Results: PROGINS variant allele (Alu ins) was found in 15.20% of the participants. No significant differences in the genotype frequencies of progesterone receptor PROGINS polymorphism in patients and controls were observed, although the prevalence of Alu ins/Alu ins genotype was more common in controls than in patients (4.76% vs. 0.82%, p=0.182). The progesterone receptor polymorphism did not influence the clinical manifestation, severity of the disease or the age of SLE onset (p>0.05). Conclusions: PROGINS polymorphism is not associated with a specific clinical phenotype in Bulgarian SLE patients. Further studies in other ethnic groups are needed to establish the influence of the PROGINS polymorphism on the SLE development as well as on the autoimmune disease susceptibility. The present study was financially supported by the Medical University Sofia (Grant 26/2010, Grant 55/2012).

J04.36

Detection of rs2073618 polymorphism in the Osteoprotegerin gene in Slovak post-menopausal women

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Osteoporosis is a common complex disease in post-menopausal women, which is characterized by decreased of bone mineral density (BMD) and detoration of skeletal microarchitecture leading to increased bone fragility and fracture. Several studies proved that genetic factors play an importatnt role in the pathogenesis of osteoporosis. Osteoprotegerin (OPG) is a recently discovered member of the TNF receptor superfamily that acts as important paracrine regulator of bone remodeling. In the present study, we screened rs2073618 polymorphism in the OPG exon 1. Study group included 200 post-menopausal women diagnosed as osteoporotic by clinicians based on clinical features and radiological evidence. Control group included 200 post-menopausal non osteoporotic women. Genotyping for the presence of rs2073618 polymorphism was performed using the Custom Taqman®SNP Genotyping assays. The frequencies of investigated genotypes for rs2073618 polymorphism in the group of patients with osteoporosis were as follows: GG (21.0%), GA (56.5%), CC (22.5%), the distribution in control groups was: GG (24.0%), GA (56.0%), CC (20.0%). Hardy-Weinberg equilibrium was tested for each group of participants using $\chi 2$ test. All statistical analyses were performed with SPSS 16.0. No differences in genotype or allele frequencies in OPG gene rs2073618 polymorphism between patients with osteoporosis and control subjects were found ($\chi 2=0.69$, p=0.405; $\chi 2=0.61$, p=0.436). Further studies are necessary for obtaining of more reliable results on the larger population for clarification molecular mechanisms with practical use in everyday practice.

This work was supported by project APVV-0716-10 and projects ITMS 2622120023 and ITMS 26220120041.

J04.37

Severe osteosclerosis in a patient with trichothiodystrophy

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Trichothiodystrophy (TTD) is rare autosomal recessive disease that affects DNA repair mechanism. TTD demonstrate great variability in severity and clinical involvement of different tissues and organs - skin and hair, bones, gonads, brain. Several attempts are made to classify TTD, according photosensitivity, age of onset and, more recently, genetic findings.

We report on a female patient with severe form of TTD. She is the first child in a family. The baby was delivered in 33 week of pregnancy and had low birthweight and poor accommodation during perinatal period due to the breeding and swallowing difficulties. Ichtiosis was present from the beginning, ranging from dry skin, scaling to freckling in different parts of the body. Photosensitivity was not clinically evident. Trichorexis and dystrophic nails were present, as well as typical facial appearance. Short stature and developmental milestones were noticed after the second year. Axial osteosclerosis started from her fifth year initially discovered with X-ray and densitometry, afterwards progressing rapidly and at the age of 9 year she experienced profound walking difficulties. DNA repair synthesis was moderately affected. A heterozygous mutation in ERCC2 gene was found - Y339X, 1017C>G and R722W, 2164C>T.

Patients with TTD have altered production of transcription factor II, due to the mutation in one of the three subunits: ERCC2, ERCC3 and GTF2H5. Osseous anomalies were seen in 20% of TTD cases and are often undescribed. Although reported to give a severe clinical presentation, disease specific causative allele R722W was rarely associated with the appearance of osteosclerosis.

J04.38

Association between vitamin D receptor gene polymorphisms and chronic periodontitis among Libyans

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Background: Chronic periodontitis (CP) is an oral disease resulting in inflammation within the supporting tissue of the teeth "the periodontium", progressive attachment loss, and alveolar bone loss, it has a microbial aetiology. Recent findings suggest that the genetic factors, such as vitamin D receptor gene polymorphisms have been also involved in the inflammatory disease aetiology of CP. **Aim of the research:** Investigation of the relationship between vitamin D receptor gene polymorphisms and CP among Libyans. **Materials and Methods:** In this study, we examined 196 unrelated Libyans between the ages of 25 and 65 years, including 99 patients and 97 controls. An oral examination based on Ramjford Index was performed at different dental clinics in Tripoli, and information's were obtained using a self-reported questionnaire. DNA was extracted from buccal swabs, the VDR ApaI, BsmI, and FokI polymorphisms were genotyped by using polymerase chain reaction (PCR), and were sequenced to read the nitrogen bases by using Sanger Method. **Results:** A significant association between ApaI SNP C/T rs#731236 and CP was found (P value = 0.022), while no significant associations were found between ApaI SNP G/T rs#7975232 , BsmI SNP A/G rs#1544410, and FokI SNP rs#2228570 and CP (P value = 0.939, 0.466, 0.239) respectively. **Conclusion:** Vitamin D receptor ApaI SNP C/T rs#731236 may be related to the risk of CP in the Libyan population.

J04.39

Frequent mutation Ala597Glu in Alox12B gene at autosomal recessive congenital ichthyosis in patients from Russian Federation

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of disorders of keratinization. It divides on several types the main of which are lamellar ichthyosis (LI) and nonbullous congenital ichthyosiform erythroderma (NCIE), although phenotypic overlap within the same patient or among patients from the same family. Mutation in several genes can lead to both LI and NCIE. We analyzed DNA samples from 11 patients with LI and 8 patients with NCIE on mutations in Alox12B gene. We found mutations in DNA from 11 patients (6 with LI and 5 with NCIE). One mutation Ala597Glu in exon 14 occurs on 11 chromosomes in 8 families: 3 in homozygous state and 5 in heterozygous state. So this mutation is responsible for 72% ARCI with mutations in Alox12B gene.

J04.40

Molecular-genetic analysis in patients with autosomal recessive osteogenesis imperfecta from Russia

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Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous brittle bone disorder. Whereas dominant OI is mostly due to heterozygous mutations in either COL1A1 or COL1A2 encoding type I procollagen, recessive OI is caused by mutations in genes encoding proteins involved in type I procollagen processing or chaperoning. We aimed to study mutations in CRTAP, LEPRE1, PPIB and SERPINF1 genes in OI patients. We examined 78 patients with OI and 100 healthy controls corresponding by age, gender, ethnicity and place of residence. We sequenced the coding and exon-flanking regions of CRTAP, LEPRE1, PPIB and SERPINF1 genes. We identified two distinct heterozygous mutations, undescribed before. For the first time previously unreported splicing mutation c.1724+4G>A in LEPRE1 gene was identified in one patient from Tatar population and the c.913C>G (p.Leu305Val) mutation in SERPINF1 gene was observed in patient from Bashkir population. We observed 12 SNPs: rs4234239 and rs586178547 in CRTAP, rs2307247, rs2253557 and rs4904 in PPIB, rs3738499, rs3738498 and rs3738497 in LEPRE1, rs58697961, rs2071022, rs1136287 and rs11658342 in SERPINF1 previously described; whereas, the c.1153-78G>A in intron 6 of CRTAP gene was novel identified in two patients from one family. Interestingly, rs4904 in PPIB gene was identified in a patient with c.1081C>T (p.Arg361X) in COL1A1; one patient with OI was characterized by rs2307247 in PPIB gene and c.579delT (p.Gly194ValfsX71) in COL1A1. Accordingly, for the first time two novel unreported mutations in LEPRE1 and SERPINF1 genes and one novel SNP in CRTAP gene was observed in Russian patients with OI.

J04.41

A clinical report on congenital joint dislocations: A new association

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Congenital joint dislocations are caused by structural abnormalities in joint development. The differential diagnosis is broad and involves recessive Larsen syndrome, diastrophic dysplasia, gPAPP deficieny and some others. Molecular studies have revealed several gene defects in proteoglycan biosynthetic pathway and have highlighted the importance of proteoglycans in the joint development. Loss of function mutations have been identified in the chondroitin sulfate synthase 1 (*CHSY1*; MIM 608183) gene which encodes a key protein in glycosaminoglycans biosynthesis and a secreted FRINGE enzyme required for NOTCH signalling to cause Temtamy Preaxial Brachydactyly syndrome (TPBS, MIM 605282). Congenital joint dislocations have not been reported in association with *CHSY1* gene mutations so far. We here present the first clinical report on two siblings with TPBS carrying a novel homozygous *CHSY1* mutation in whom one of the sibs had bilateral complete anterior knee and unilateral hip dislocations in addition. The *CHSY1* deficiency is yet another disorder of proteoglycan biosynthesis that can present at birth with congenital joint dislocations.

J04.42

Autosomal recessive congenital ichthyosis: identification of a Spanish family with a new PNPLA1 mutation

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PNPLA1 has recently been identified as one of the eight genes (ALOX12B, ALOXE3, CERS3, CYP4F22, NIPAL4 or TGM1) in which mutations can cause autosomal recessive congenital ichthyosis (ARCI), although only two families harbouring such mutations (both of North African origin) have been identified.
In a study of ALOX12B, ALOXE3, CYP4F22, NIPAL4 and TGM1 performed in 17 ARCI families in Galicia (NW Spain), about 80% of these families had mutant TGM1 or ALOXE3, while no mutations of NIPAL4, CYP4F22 or ALOX12B were found.
In 2012 a new locus of ARCI mutations, in PNPLA1, was published and we accordingly investigated the prevalence of PNPLA1 mutations in our series of 18 ARCI families, which constitute 95% of the 19 Galician ARCI families identified to date. Four Galician families known to have no deleterious mutations of ALOX12B, ALOXE3, CYP4F22, NIPAL4 or TGM1 were studied. The potential pathogenic effect of the variant identified was evaluated using the programs Align-GVGD, MAPP, PolyPhen and SIFT. One consanguineous family was found to harbour the missense substitution c.100G>A (p.Ala34Thr), located in the catalytic domain of PNPLA1. Cosegregation analysis indicating the deleterious nature of this variant was supported by the bioinformatic predictions.
We have identified the third known ARCI family with PNPLA1 mutations, and the first of European ancestry. Although mutant PNPLA1 was found in about 6% of our Galician ARCI series, the small number of Galician ARCI families prevents us from generalizing this figure.
This work was funded by a grant from the Ramon Areces Foundation awarded to AV.

J05.01

Simvastatin affects ABCA1 expression and cholesterol efflux in THP-1 macrophages by a ROR-Alpha-dependent pathway

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The ATP-binding cassette transporter A1, ABCA1, is a ROR-Alpha target gene that participates in the removal of cholesterol from lipid-laden macrophages, a crucial anti-atherogenic mechanism. Statins are currently the most efficacious therapy for the treatment of hypercholesterolemia and cardiovascular diseases. Some studies have shown that statins decrease ABCA1 expression and choleterol efflux from human macrophages. However, other studies have reported no change, or even a modest increase in this variables. In this study our aim was to investigate the ABCA1 expression and apolipoprotein AI (apoAI)-mediated cholesterol efflux after simvastatin treatment in THP-1 macrophages. Cholesterol is one known ligand of ROR-Alpha and is important in cardiovascular diseases like atherosclerosis. Thus, we further explored the effect of simvastatin on activation of the ROR-Alpha in human macrophages by studying the influence of cellular cholesterol efflux. We observed that simvastatin repressed the expression of ABCA1 gene, and that this repression was partially prevented by ROR-Alpha ligands (especially by SR1001). Furthermore, ligand induced activation of ROR-Alpha by CPG 52608 and SR 1001 increased apoAI-mediated cholesterol efflux in THP-1 macrophages. In conclusion, activation of ROR-Alpha not only increased ABCA1 expression and cholesterol efflux in the absence of simvastatin, but also restored these functions in the presence of simvastatin. With the demonstration of the ABCA1 that involve in the pathogenesis of atherosclerosis might be controlled by an inducible transcription factor, this study offers a potential therapeutic treatment for the disease.

J05.02

Expression of two ABCG1 transporter isoforms in macrophages of patients with atherosclerosis

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The ABCG1 transporter plays an important role in reverse cholesterol transport by mediating the efflux of cholesterol from macrophage foam cells to high density lipoproteins. Two major ABCG1 isoforms exist in humans, which differ by the presence or absence of twelve amino acids between the ATP cassette and the transmembrane domains (ABCG1(+12) and ABCG1(-12)). We have reported earlier that expression of the ABCG1 gene was reduced in macrophages of patients with atherosclerosis. However the role of the ABCG1 isoforms in human atherosclerosis is still undiscovered. The aim of this study was to evaluate the expression of the ABCG1 isoforms in macrophages of patients with atherosclerosis and in the control group. Human peripheral blood monocytes were obtained from 10 patients with angiographically proved atherosclerosis and 10 healthy blood donors. Monocytes were cultured with macrophage colony-stimulating factor (M-CSF) for 5 days to get monocyte-derived macrophages. Real time PCR system designed for this study was used for simultaneous detection of ABCG1(+12) and ABCG1(-12) mRNA levels. Mann-Whitney U-test was used for statistical analysis of the data. ABCG1(-12) mRNA levels were significantly reduced in group of patients when compared with the control group: medians were 0.70 (0.15 - 2.02) and 1.61 (0.51 - 2.98) ($p<0.05$). There were no differences in ABCG1(+12)/ABCG1(-12) mRNA ratio between patients and controls. ABCG1(+12)/ABCG1(-12) mRNA ratio tends to be 0.55 in human macrophages. In conclusion, while expression of the ABCG1 gene is reduced in macrophages of patients with atherosclerosis, the ratio of two main ABCG1 isoforms doesn't change during atherosclerosis.

J05.03

Chromosomal deletion in abdominal aortic aneurysms

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Abdominal aortic aneurysm (AAA) is an irreversible and progressive dilatation of the abdominal aorta. Known risk factors for AAA include smoking, male sex, increasing age, and family history, but no genetic markers have clearly been shown to be responsible for this pathology.
In order to assess the potential role of genetic rearrangements in the pathogenesis of AAA, we analyzed a cohort of 50 patients who had undergone surgical repair of AAA. DNA from peripheral blood and AAA wall specimens of each patient was analyzed by CGH array. A common deletion region on chromosome Xp22 was found in about half of AAA wall specimens and never in the corresponding peripheral blood. In order to demarcate the boundaries of the identified deleted region, a real time PCR was performed on some genes located within the deleted region: SHOX, CRFL2 and DHRSX. Specifically, samples deleted for SHOX, CLRF2 and DHRSX genes represented 41.8, 28 and 32.5% respectively.
These results allowed us to identify for the first time a common deletion region on chromosome Xp22 link to abdominal aortic aneurysm using CGH array technology. Future characterization of the involved genes within the deleted region may contribute to define the molecular mechanisms involved in the structural alteration of vascular wall tissue and predisposing to abdominal aortic dilatation.

J05.04

Mutations in ACTA2 are not a common cause of Bicuspid Aortic Valve associated with Thoracic Aortic Aneurysm

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Bicuspid aortic valve (BAV) is a common congenital heart defect, occurring in 0,5% to 2% of the population. Its clinical presentation is heterogeneous, but associated dilatation of the ascending aorta is often present. Familial studies showed that BAV with or without thoracic aortic aneurysm (TAA) segregates in an autosomal dominant manner with incomplete penetrance and variable expression, likely underlying a single common gene defect. Even if mutations in NOTCH1 and rarely in GATA5 and TGFBR2 have been linked to BAV+/-TAA, its molecular basis in most families remains unknown. Mutations in ACTA2 have been reported as major cause of familiar TAA (up to 14%), with associated BAV in some individuals.
We recruited 20 patients who underwent surgery for BAV and TAA to investigate the possible role of ACTA2 mutations in their phenotype, to evaluate possible common clinical signs correlating with the cardiovascular pathology, to determine the prevalence of familiarity of BAV+/-TAA (performing echocardiography in first degree relatives).
We did not identify ACTA2 mutations in our probands, nor common clinical signs possibly related to their heart disease. Furthermore, we found that the phenotype BAV+/-TAA segregates in 25% of our families, in accordance to literature data. Although our cohort is small, we conclude that ACTA2 does not seem to contribute significantly to the common phenotype of BAV+/-TAA, and that cardiac ultrasound screening is important in probands' relatives.

J05.05

Dysmorphic features and genetic counselling goals of germline Braf mutated patients: Two unrelated Tunisian children

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Germline heterozygous gain-of-function mutations of the BRAF gene that encode a downstream molecule of RAS in the RAS-MAPK signaling pathway have been identified in noonan syndrome and other RASopathy, essentially cardiofaciocutaneous (CFC) syndrome. Distribution of BRAF mutations in this syndrome was specific and nonrandom. Here, we report dysmorphic features of two unrelated Tunisian; BRAF mutated children and discuss difficulties of genetic counselling in our environment. The first case (P1) and second case are 7-years-old girls born to distant consanguineous parents from Bir Ali Ben Khalifa and no consanguineous parents from Sidi Bouzid, respectively. Pregnancies were complicated by hydroamniosis. P1 had a congenital severe valvular pulmonic stenosis detected immediately after birth, treated 4 years later but with a failed valve repair. P2 had hypertrophic cardiomyopathy detected 6 months after birth without any familial history of congenital heart diseases. Molecular analysis of genes involved in the RASopathies genes (PTPN11, KRAS, HRAS, NRAS, BRAF, RAF1, SOS1, MAP2K1, MAP2K2, SHOC2, and CBL) by HRM and direct sequencing revealed heterozygous mutations of the BRAF gene: 736G-C transversion in exon 6 (A246P) for P1 and 1501G-A transition in exon 12 (E501K) for P2. Cognitive impairment, speech delay and ectodermal abnormalities as well as a distinctive facial appearance similar for P1 and P2 were consistent with CFC syndrome characteristics. At the genetic counselling level, the management of the risk for haematological malignancies must be discussed with parents and clinical team. Furthermore, regarding the theoretic possibility of germline mosaicism, prenatal diagnosis must be offered.

J05.06

Sudden infant death syndrome and unexplained intrauterine fetal demise: role of calmodulin

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Background: Cardiac channelopathies are responsible for approximately 15% of sudden infant death syndrome (SIDS) and 9% of intrauterine fetal demise (IUFD). Recently, mutations in two genes encoding the calcium-binding protein calmodulin (CALM1 and 2), have been associated with recurrent cardiac arrest in infants with Long QT Syndrome, Catecholaminergic Polymorphic Ventricular Tachycardia and Idiopathic Ventricular Fibrillation. Calmodulin mutations disrupt Ca2+ signalling in the heart, affecting membrane ion channels' function and kinase-mediated signal transduction. Given the life-threatening arrhythmias described in infants, it seemed logical to expect that calmodulin could play a role in SIDS and in IUFD.
Methods: Genomic DNA was extracted from frozen tissue. All three genes encoding calmodulin (CALM1-2-3) were analysed with Sanger sequencing in a cohort of SIDS cases (n=46) and in a population of IUFD (n=44; gestational age at death >=20 weeks) classified as "unexplained" after a post-mortem evaluation.
Results: Mutational analysis of the 3 CALM genes did not identify any pathogenic variant. In addition, the CALM3-rs115265989 polymorphism (NM_005184.2:c.267G>A) was identified in one Black IUFD case, and is present in 1% of subjects of the Exome Sequencing Project Black population.
Conclusions: Surprisingly, even though calmodulin mutations have been described in severe and early-onset cardiac arrhythmic syndromes, no genetic variants were identified in our SIDS and IUFD populations. These data suggest that calmodulin mutations are not a major cause of SIDS and IUFD. Possible explanations include a combination of rarity of calmodulin mutations and small sample size of the population analysed.

J05.07

Beware at the Cardiac Genetic Clinic: things are not always what they may seem

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We report on two families seen at our Regional Cardiac Genetic Service in Glasgow. Both demonstrate clinical variability as well as the potential for incorrect or delayed diagnosis.
Family 1 was originally reviewed at another centre. The post mortem of a 19 year old male demonstrated features suggestive of arrhythmogenic right ventricular cardiomyopathy (ARVC). Subsequently, his 56 year old mother who has dilated cardiomyopathy (DCM) and had recently developed muscle weakness, was investigated locally. Her phenotype was consistent with a myofibrillar myopathy. A missense DES mutation (c.338A>C) was identified, confirmed in her deceased son (PM tissue sample) and her daughter who has conduction abnormalities. The mutation occurs at the 1A alpha helical domain. Similar mutations have been associated with ARVC.
Family 2 presented in 2008. A 26 year old mother and her 7 month old son were diagnosed with DCM. He had probable endocardial fibroelastosis. Investigations for Barth syndrome were negative. Subsequently, a 45 year old distant cousin was investigated for hypertrophic cardiomyopathy and found to have an MYH7 missense mutation (c.2441T>G). The mutation segregated across all affected family members, including another 1st cousin with left

ventricular non-compaction.
Clinical heterogeneity is an important feature of DES and MYH7 related inherited cardiac disorders. There is a need for careful assessment of individuals at the Cardiac Genetic Clinic to ensure recognition of these conditions, early diagnosis, appropriate treatment, cascade testing and surveillance for unaffected, at risk relatives.

J05.08
Chromosome 9p21 rs564398 variant is associated with the internal carotid artery stenosis severity

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Carotid atherosclerosis is atherosclerotic stenosis of proximal internal carotid artery (ICA), and is one of the main causes of stroke. Recent genome-wide association studies (GWAS) revealed chromosome 9p21 INK4b-ARF-INK4a is a novel locus for susceptibility to type-2 diabetes and coronary artery disease. INK4/ARF transcript, p16INK4a, arrests cell cycle progression by inhibiting the activities of CDK4/CDK6. Cell cycle regulation may be an important mechanism in vascular smooth muscle cells for atherosclerosis progression. Thus, we aimed to examine the frequency of the single nucleotide polymorphisms (SNPs) on chromosome 9p21 in carotid atherosclerosis (CA).
The study is composed of 50 symptomatic and asymptomatic CA patients and 53 healthy controls. Genomic DNA extraction was performed from peripheral blood leukocytes. Real-time polymerase chain reaction (RT-PCR) was used to analyze 4 SNPs (rs564398 A/G, rs10757274 A/G, rs2383207 A/G, rs10757278 A/G) in CA patients and controls.
Analysis of 4 SNPs revealed a significant difference in the genotype distribution for rs564398 and rs10757278 between CA patients and controls (p=0.027 and p=0.01). However no significant relationship was found in genotype frequencies of rs10757274 and rs2383207 when CA patients and controls were compared (p>0.05). There was also a significant relation in allele frequencies of rs564398, rs2383207 and rs10757278 polymorphisms between CA patients and controls (p=0.016, p=0.049, p=0.001). ICA stenosis severity was found to be associated with the AA variant of rs564398 polymorphism in CA patients (p=0.01).
These results indicate that, chromosome 9p21 rs564398 and rs10757278 polymorphisms may be associated with CA. These findings need to be confirmed by further studies.

J05.09
ADIPOQ variants in patients with coronary artery disease in Turkish population

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Adiponectin, a hormone produced predominantly by adipocytes, is an essential modulator of insulin sensitivity and has anti-atherogenic and anti-inflammatory properties. Several studies have been performed to investigate the association of genetic variations in the adiponectin with obesity, insulin resistance, and type 2 diabetes (T2D), but few studies were performed in association with coronary artery disease (CAD). Adiponectin is coded by ADIPOQ gene located on chromosome 3q27, consisting of 3 exons and 2 introns spanning a 17-kb region. Among the variations of the ADIPOQ gene reported, rs17300539 (−11391G>A), rs2241766 (+45T>G), rs1501299 (+276G>T), and rs2241767 (+349A>G) have been most extensively studied and are thought to be linked to CAD. In our study, the effects of these polymorphisms in ADIPOQ on CAD were investigated and 125 patients and 123 healthy controls were included. Polymorphisms were screened by PCR-RFLP technique. Genotyping data and demographic characteristics were analyzed by the SPSS18.0 program. p<0.05 was considered statistically significant. No effect of these polymorphisms on CAD was detected in association analysis under additive, dominant and recessive models (p>0.05). While Genotype distributions were in Hardy-Weinberg equilibrium (p>0.05) in patient and control groups, except a deviation observed in both groups for +276G>T (p<0.05). ADIPOQ and its variants, shown having role in common genetic background of insulin resistance, T2D and CAD in several populations, had no effect on CAD in our society. We note that this study was based on a relatively small sample size, which limits the power to detect association. These results need to be interpreted with caution.

J05.10
Investigation of association between Leu125Val polymorphism of PECAM-1 gene and coronary heart disease in the Iranian population

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Coronary heart disease (CHD) is a major cause of death in Iran and many other countries. CHD is a multifactorial disease, which is probably influenced by a combination of environmental and genetic factors. Several studies indicate that conditions leading to myocardial infraction and death can be prevented by controlling environmental factors and screening for genetic risk factors. Atherosclerosis is the most predominant coronary artery pathology and it seems that Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) plays a role in creation of atherosclerotic plaques. In this study, we have investigated the association between Leu125Val polymorphism of PECAM-1 gene and coronary artery disease in Iranian population. Blood samples were collected from 85 healthy controls and 126 angiographically confirmed CAD patients with stenosis in at least one coronary artery. After DNA extraction, the PECAM-1 Leu125Val polymorphism detection was carried out by PCR-RFLP method. Frequencies of GG, CC, GC genotypes were 17%, 35%, and 48% in patients and 20%, 35% and 45% in healthy controls, respectively. Data analysis (p-value= 0.815) revealed no association between Leu125Val polymorphism of PECAM-1 gene and coronary artery disease. Preliminary results do not reveal an association between Leu125Val polymorphism and coronary artery disease. This study is continuing.

J05.11
Association of rs7903146 polymorphism in TCF7L2 gene with myocardial infarction in T2DM patients in Uzbekistan

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The Coronary Heart Disease (CHD) has the similar risk factors with type 2 Diabetes Mellitus (T2DM). A polymorphism in the TCF7L2 gene has been found to be associated with type 2 diabetes in several ethnic groups. Possible relationship between the genetic polymorphism of the TCF7L2 gene and CHD was not clear.
We aimed to determine the association between rs7903146 (C/T) polymorphism in TCF7L2 gene with the development of myocardial infarction (MI) in patients with T2DM in Uzbek population. We genotyped 108 patients divided into 3 groups: I - patients without CHD (n = 26, control); II - patients with CHD without MI (n = 42); III - patients with MI (n = 40). All patients were unrelated, aged over 45 years, and had disease duration over 10 years.
Results of genotyping showed that the CC genotype frequency in I group was 15.4% and the CC genotype was present in 70% patients of III group. The frequency of the T allele (83,8%) in patients with MI was significantly higher compared to the group without CHD (65,4%, P <0.05, control). At the same, we found significantly lower prevalence of allele C (16,3 ± 4,1%) in patients with MI compared with the control group (34,6 ± 6,6%, P <0.05)
The results of our study suggest that TCF7L2 rs7903146 polymorphism is significantly associated with development of MI in patients with T2DM. TT genotype of TCF7L2 gene may be a predictor of the risk of myocardial infarction in patients with T2DM.

J05.12
GLA nonsense mutation (W162X) and cardiac involvement in heterozygous females

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Background. In the past, medical literature stated that Fabry disease affects only male. Based on X-linked pattern of inheritance the heterozygous females are usually asymptomatic. Recent studies describe Fabry disease in heterozygous females but manifestations tend to occur at a later age than in males and are often less severe. Objectives. The aim of our study was to detect the presence of GLA mutation in females of a family with Fabry disease and corelate with the severity of clinical phenotype. Subjects and methods. Five related females of the same family were enrolled and clinically assessed. Enzyme activity levels were evaluated too. Genetic testing included isolated DNA from blood samples and sequence analysis of all coding exons and all intron-exon boundaries of the GLA gene. Results. All five females were found to be heterozygous for a familial pathogenic GLA mutation (c.485G>A). The mutation caused different low levels of enzyme activity in grandmother, mother, daughter and the two fraternal nieces of the grandmother. None of

these females received enzyme replacement therapy prior to the study. Age-related variable phenotypic expression was noticed. Grandmother, now 62 year-old presented mild angiokeratoma and mild left ventricular hypertrophy. Her daughter, 31 year-old, presented mitral valve thickened with mild regurgitation. The granddaughter, 5 year-old, had no clinical manifestation. Both fraternal nieces of the grandmother, 20 year-old and respectively 22 year-old, presented dry skin and mild angiokeratoma but no cardiac involvement. Conclusion. Early genetic testing should be considered in younger female with a family history of Fabry disease.

J05.13
Polymorphisms in FII, FV and PAI-1 genes in Ukrainian patients with atherothrombotic and cardioembolic ischemic stroke

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Background and aim: Imbalance between coagulation and fibrinolysis factors is known to be a predictor of thrombosis and cardiovascular events. The aim of our study was to compare the genotype distribution of the coagulation factors FII and FV and fibrinolysis factor PAI-1 functional polymorphisms in patients with atherothrombotic ischemic stroke and patients with cardioembolic ischemic stroke with atrial fibrillation (AF).
Methods: 54 patients with cardioembolic ischemic stroke with AF and 60 patients with atherothrombotic ischemic stroke from Ukraine were included in this study. The genotypes of FII G20210A, FV G1691A (Leiden) and PAI-1 5G/4G polymorphisms were determined by PCR analysis based on the banding pattern on gel electrophoresis.
Results: No individuals with FII and FV homozygous mutations were found in both study groups. In cardioembolic stroke patients with AF, 1 (2%) FII and 3 (6%) FV heterozygous mutations were found. In patients with atherothrombotic stroke, 5 (8%) FII and 0 (0%) FV heterozygous mutations were found. Among patients with cardioembolic ischemic stroke with AF, PAI-1 5G/5G, 5G/4G and 4G/4G genotypes were observed in 9 (17%), 23 (43%) and 21 (40%), and in atherothrombotic stroke in 14 (24%), 19 (32%) and 26 (44%) patients respectively.
Conclusion: Our findings suggest that there is a tendency toward a higher frequency of FII G20210A heterozygotes in atherothrombotic ischemic stroke patients compared with cardioembolic ischemic stroke patients with AF. We also observed that FV Leiden heterozygotes are more frequent in cardioembolic stroke with AF. PAI-1 homozygous state is equivalent between the two study groups.

J05.14
The importance of genetic profile for thrombophilia detection in patients with pulmonary venous thromboembolism after operation for anomalous pulmonary vein return malformations

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Aim: To present two rare cases of pulmonary thromboembolism in vital vessels, after surgery for cardiac malformations, late discovered with positive genetic profile for thrombophilia.
Material and methods: A 3 mo old girl presented with signs of cardiogenic shock and myocardial iskaemia due to totally anomalous pulmonary venous return (TAPVR) in coronary sinus. The second case was a 2 yo girl, with recurrent wheezing, discovered with right pulmonary veins draining in superior vena cava, a partial anomalous pulmonary veins return (PAPVR). Both patients were operated.
Results: First case, two years after cardiac surgery presented with discrete bluish discoloration of the skin, but with normal O2Sat. The pulmonary venous return was redirected to the left atrium. Angio CT detected superior vena cava thrombosis, with reverse flow into azygos system, and complete thrombosis of the right venous brachiocephalic trunk. Second case, was operated, redirecting the right pulmonary vein collector to left atrium. After two years she presented with haemoptisia. Angio CT detected right pulmonary venous trunk embolism, with pulmonary edema in the right lung. She was treated with heparin. In both patients we detected positive genetic predisposition to thrombophylia, that changed the recommendation for aticoagulant therapy, for life long.
Conclusions: Patients with operated TAPVR and PAPVR have the risk for pulmonary thromboembolism, which is vital when the collector is redirected to left atrium.
Genetic profile for thrombophilia is important to be searched in this type of cardiac malformations. If positive, anticoagulant therapy is life long mandatory, to prevent thromboembolism and even death.

J05.15
Investigation on Mitochondrial DNA deletions in Iranian Dilated Cardiomyopathy and Hypertrophic Cardiomyopathy patients

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Hypertrophic cardiomyopathy is a genetic disorder with autosomal dominant inheritance. The disorder has been estimated to occur in 0.05%-0.2% of population. Recently mitochondrial DNA mutations have been associated with cardiomyopathies. Mitochondria are the major site of energy production in the cell. Thus it is reasonable to assume that energy dependant tissues such as heart, brain, skeletal muscle and endocrine system are affected by mitochondrial dysfunction. Many mitochondrial diseases arise form defects of the mitochondrial respiratory chain. mitochondrial disorders have maternal inheritance pattern. The mtDNA mutation rate is much higher than nuclear DNA due to lack of a repair mechanism and also having no intron. Recent studies have reported maternally inherited, non-x linked HCMs are associated with defects in mitochondrial oxidative metabolism. Methods: In this study we screened 52 Iranian hypertrophic cardiomyopathy's patients for mitochondrial DNA deletions. Results: Mitochondrial DNA deletions were detected by PCR using 6 paired primers. Five different deletions were found in 29 patients (55.8%). Eighteen patients (34.4%) showed 8.5 kb deletion. Twelve (23%) patients had 9 kb deletion. Seven patients (13.4%) had a 7.3 kb deletion. Eight patients (15.5%) had 4977 bp common deletion between nt8161-nt13640, and 11 patients had 7.4 kb deletion. Multiple deletions have been found in 11 patients (21.1%). Conclusion: Mitochondrial DNA deletions may occur as a result of aging; on the other hand mt deletions may affect myocardium and lead to secondary hypertrophy. However, the question regarding primary or secondary role of mtDNA deletions in hypertrophic cardiomyopathy remains unanswered.

J05.16
Novel SMAD4 mutation causing juvenile polyposis (JP) and hereditary haemorrhagic teleangiectasia (HHT)

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HHT is a genetic disorder with autosomal dominant inheritance pattern, causing abnormal blood vessel formation - multifocal vascular telangiectases and/or arteriovenous malformations(AVMs). The majority of patients harbor mutations in members of the transforming growth factor (TGF)-β pathway - either the endoglin (ENG) or activin receptor-like kinase 1 (ALK1) genes. Mutations in SMAD4 gene, encoding an intracellular mediator of TGF-β signals, are known to cause JP. Both HHT and JP are uncommon, but a combined syndrome JPHHT has been reported.
We present three generations of HHT family with three affected members. All of them were anaemic, and have been diagnosed with multiple pulmonary AVMs, cerebral cavernous haemangioma was documented in one of them. The proband had a history of gastrointestinal (GI) bleeding and few colon polyps documented at 9 year age. No GI bleeding/polyps were documented in remaining family members. No mutations were found in ENG/ALK1 genes on direct sequencing. We performed SMAD4 direct sequencing on the proband and found a novel frameshift mutation (p.R531GfsX536) in exon 11, leading to stop codon and synthesis of truncated protein.
The clinical importance of SMAD4 mutation finding in HHT has been widely discussed as these patients are likely to be at risk of JPHHT and developing gastrointestinal cancer. Based on our findings, ENG and ALK1 mutation negative HHT patients might benefit from SMAD4 genetic testing. We suggest that HHT patients with SMAD4 mutations should be regularly screened for colonic and gastric polyps associated with JP.

J05.17
Next generation sequencing - useful tool in molecular diagnosis of inherited cardiomyopathy

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Cardiomyopathy is characterized by mechanical or electrical dysfunction of cardiac muscle and it is a known risk factor of sudden cardiac death. More than hundreds of variant in 84 genes have been associated with inherited cardiomyopathy. A high number and variability of involved genes complicate diagnosis of cardiomyopathy. However, existence and availability of new

modern method - next generation sequencing enables analysis of high number of genes at the same time- in massively parallel approach. Sequencing DNA libraries in this fashion significantly shortens time and reduces costs of analysis, and makes previously cost prohibitive experiments possible.
Here we present our diagnostic NGS workflow for the analysis of 46 genes involved in pathogenesis of the inherited cardiomyopathy by the using TrueSight Enrichment technology (Illumina) followed by sequencing on the MiSeq (Illumina). This pilot study comprised group of 40 unrelated patients with DCM, ARVC and ventricular fibrillation. We detected several different variants, some of these variants were common and already published with proven association with development of cardiomyopathy and some of them were classified as new variants, where co-segregation analysis in pedigrees has to be performed. All detected variants classified as pathological, likely pathological and variants of unknown clinical significance (VOUS) have been confirmed by classical Sanger sequencing.
Our results demonstrate that this is a sensitive and robust assay with an average of 95 % of target regions consistently covered to x20 depth. This diagnostics approach can be a powerful tool for identifying presymptomatic individuals in families.

J05.18
The relationship between PIA1/PIA2 Glycoprotein IIIa genetic polymorphism and ischaemic stroke in Northern Romania

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Introduction: Ischaemic stroke is one of the leading causes of disability and death in Romania. Platelets play a key role in generating the protective hemostatic plug that prevents blood loss at sites of vascular injury. The identification of functional prothrombotic polymorphisms during the past decade encouraged the study of common polymorphisms affecting platelet glycoproteins (GP) in stroke. The aim of the present study is to evaluate the relationship between PIA/PIA2 Glycoprotein IIIa gene polymorphism and ischemic stroke in a Northern Romanian population group and to determine whether it has an influence on the risk of cerebral events. This is a cross-sectional, randomized, case-control study for the evaluation of PIA/PIA2 Glycoprotein IIIa gene polymorphism alleles frequency among patients with ischemic stroke.
Material and method: The study included 131 patients diagnosed with ischemic stroke (neurological and CT scan examination), and 110 healthy unrelated controls. PIA/PIA2 Glycoprotein IIIa genotyping was carried out using PCR-RFLP technique. The amplification of the relevant gene fragment was subjected to restriction enzyme digestion, followed by gel electrophoresis.
Results: Molecular analysis did not reveal an increased frequency of A1A2 mutant genotype in the study group compared to the control group (p = 1.000, OR = 0,951, CI = 0.504- 1.797).
Conclusions: We found no significant differences in distribution of the PIA/PIA2 Glycoprotein IIIa gene polymorphism between ischemic stroke patients and controls.

J05.19
Noninmune hydrops fetalis atypical presentation of Milroy disease

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Milroy disease, is an autosomal dominant disorder characterized by typical phenotype of infantile onset lower-limb lymphedema accompanied by variable expression of recurrent episodes of cellulites, toenail changes, and papillomatosis. Mutations in the vascular endothelial growth factor receptor 3 (VEGFR3) have been identified as a genetic cause of Milroy disease.
We report a case of 22 week old foetus with bilateral pleural effusion, that although in uterus thoracocentesis, oedema progressed and ended in severe polyhydramnios and massive hydrothorax. Fetal karyotyping and array-CGH after amniocentesis were normal. Anatomopathological examination identified bilateral pedal lymphedema with pretibial extension.
The mother reported a personal history of bilateral lymphedema of the feet from birth, which improved during the first year of life. No lymphedema in other first degree family members. A paternal cousin of the patient had a child with bilateral lymphedema of the feet from birth. The diagnosis of Milroy disease was considered highly likely in view of the family history of lower limb lymphedema of congenital onset. Genetic analysis revealed novel missense mutation in VEGFR3 that cosegregates with the disorder in the family.
Clinical presentation ranges from asymptomatic individuals through classic features to rare prenatal life-threatening conditions, even in patients bearing the same mutation. Although in utero hydrothorax and hydrops fetalis is a rare manifestation of Milroy syndrome, should be taking into account when facing a case of in utero noninmune hydrops fetalis. A detailed family history could be the clue to reach de diagnosis, although de novo cases have been described.

J05.20
MTHFR and NNMT gene polymorphisms and conotruncal heart diseases

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Congenital Heart Defects are the most common congenital anomalies with a worldwide 1 % prevalence and are a big part of childhood mortality and morbidity. The etiology of conotruncal heart diseases is complex, with both environmental and genetic causes. Hyperhomocysteinemia which is greatly accompanied with the defects of folic acid metabolism, is known to cause conotruncal heart anomalies. In this study we have evaluated three polymorphisms in two hyperhomocysteinemia related genes, such as Methylene Tetrahydrofolate Reductase (*MTHFR* C677T and A1298C) and Nicotinamide N-methyl Transferase (*NNMT* rs694539) in 79 children with conotruncal heart disease (CHD) and 99 children without CHD. We found no association in case and control groups for *MTHFR* C677T and *NNMT* rs694539 polymorphisms, while for *MTHFR* A1298C polymorphism we found a significantly higher frequency of C allele, suggesting that C allele might be a risk factor for CHD.

J05.21
Mutation analyses of PTPN11 and SOS1 genes in Belarusian patients with clinical features of Noonan/LEOPARD syndrome

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Noonan syndrome (NS) and LEOPARD syndrome are genetically heterogeneous autosomal dominant disorders associated with gain-of-function mutations in various genes encoding proteins of the RAS/MAPK signaling pathway, mainly in PTPN11, SOS1 and RAF1 genes.
8 Belarusian patients with Noonan/LEOPARD syndrome were screened for mutations in PTPN11 gene: 6 patients with clinical features of NS (2 family cases with mother-daughter pair, 2 sporadic cases) and 2 patients with clinical features of LEOPARD syndrome (a family with father-daughter transmission). All of the patients had cardiac diseases (pulmonary valve stenosis, septal defect, cardiomyopathy) and characteristic minor abnormalities.
Leukocyte genomic DNA was amplified for the 15 exons and flanking intronic sequences of PTPN11 gene by PCR. The PCR products were sequenced from both directions on an ABI3500xl autosequencer.
Four different PTPN11 mutations were identified in 7 out of 8 patients with clinical features of Noonan/LEOPARD syndrome (4 probands, 3 relatives available for testing). All mutations were heterozygous missense mutations: Asn58Lys (new genetic variant c.174C>A), Asp61Gly, Asn308Asp, Thr468-Met, and clustered either in exon 3 encoding the N-SH2 domain or in exons 8 and 12 encoding the PTP domain.
One out of 8 patients does not carry mutation in PTPN11 gene. Therefore, we searched for mutations in the second frequently mutated gene SOS1 - undescribed heterozygous mutation (c.797_798delCAinsAAGTA) was found in exon 6 of SOS1 gene encoding the DH-domain. At the age of 1.6 years old patient showed typical NS phenotype without ectodermal abnormalities. Detection of new mutation is important for further delineation of genotype-phenotype correlations.

J05.22
IL-6 gene polymorphism (-174G/C) in Romanian patients with ischemic stroke -results from a pilot study

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Stroke, mostly of ischemic origin, is considered a major cause of mortality worldwide. Interleukin-6 (IL-6) is demonstrated to be associated with atherosclerotic disease, also is considered a key mediator of inflammation in cerebral ischemia. IL-6 single nucleotide polymorphism (SNP) -174G/C was found to be associated with several atherosclerotic diseases; its relation to ischemic stroke is conflicting.
We aimed to investigate the role of -174G/C IL-6 SNP in ischemic stroke sus-

ceptibility in Romanian population. To our knowledge, this is the first study of IL-6 polymorphisms in Romanian patients with stroke.
We genotyped 208 subjects (60 patients, 148 unrelated controls) by Real-Time PCR technique (Taqman_SNP_Genotyping Assays_ rs1800795, Applied-Biosystems, USA) for the -174G/C IL-6 polymorphism. Statistical analysis was performed by using the SNPStats program for genetic association studies [1]; p-values ≤0.05 were considered significant.
All studied groups were in Hardy-Weinberg equilibrium (HWE) for -174G/C IL-6 polymorphism. The frequencies of minor allele -174C were similar in stroke patients and controls (0.28/0.25). There was no significant difference in SNP distribution regarding the genotype frequency between stroke and control subjects before (CC/GC/GG: 0.08/0.35/053 and 0.09/0.41/0.51, respectively) and after adjustments by age, sex and body mass index.
The present study shows no association of -174G/C polymorphism with the susceptibility to ischemic stroke in patients from Romania. In order to improve the statistical power of this study, a larger number of patients may be required to verify this conclusion.
References:
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J05.24
Allelic variants of IL8 and IL10 genes influence ischemic stroke risk and prognosis

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Ischemia-related inflammatory signaling is involved in all stages of ischemic cascade. Pro- and anti-inflammatory cytokine balance is altered by changes in gene expression due to polymorphisms in promoter and intron regions. This study aimed to evaluate the role of IL8 gene C-781T, and IL10 gene C-592A polymorphisms as genetic markers of acute ischemic stroke (AIS) risk. Case group included 183 AIS patients. Control group - 88 individuals older 65 years without AIS history. Genotyping was performed using PCR followed by RFLP analysis. Significantly (P<0,05) higher frequency of IL8 -781T allele carriers in case group (81,6%) comparing to control (70,1%) was revealed. -781C allele carriers have 2-fold increased AIS development risk (OR=1,886; 95% CI: 1,041-3,417). Significantly (P<0,05) higher frequency of IL10 gene -592C allele carriers was observed in patients with AIS (98,2%) comparing to control (90,7%). AIS development risk in such individuals is 5-fold increased (OR=5,71; 95% CI: 1.48-22.11). These genotypes may result in aberrant initial inflammatory ischemia response. Genotype role in post-stroke improvement (state severity assessed using RANKIN scale on 3rd and 14th treatment day) was evaluated. -592C allele homozygotes have more than 2-fold higher improvement chances during the first fortnight of treatment (OR=2,76; 95% CI: 1.26-6.07). Pro-inflammatory cytokines induce expression of inflammation-specific molecules, and cerebral infarction zone expands. High levels of anti-inflammatory IL-10 may prevent from this outspread. Concluding, IL8 gene -781C and IL10 gene -592C variants may be considered the genetic markers of AIS development risk. Unlikely IL10 gene -592CC genotype is associated with better post-stroke improvement prognosis.

J05.25
Identification of Plasminogen as a pleiotropic susceptibility factor of coronary artery disease and periodontitis

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Periodontitis (PD) is a chronic inflammatory disease of the oral cavity, which affects human populations worldwide at prevalence rates of 11% for the severe forms. PD and coronary artery disease (CAD) share several risk factors such as obesity, smoking and sex, and there is consent of an association between both diseases, but a causative relationship is not supported. In the past, we identified several shared genetic risk alleles in the genetic regions of ANRIL and CAMTA1/VAMP3, which indicate a link of glucose and fatty acid metabolism and immune response in the shared etiology of both diseases. We aimed to identify further shared genetic risk factors to better understand conjoint disease mechanisms and genotyped all 46 published CAD risk loci of genome-wide significance in a large case-control sample of aggressive periodontitis (AgP), with the Illumina genotyping array Immunochip and Affymetrix 500K Arrays. We identified and replicated the same CAD risk allele within plasminogen (PLG) to be significantly associated with AgP upon correction for multiple testing, independent of smoking and sex (rs6923419, Ppooled =2x10-5, odds ratio (OR)=1.28, 95% confidence interval (95%CI)=1.1-1.4, 858 cases, 3,597 controls). A subsequent combined analysis of several genome-wide data sets of CAD and AgP suggested TGFBRAP1 to be associated with CAD (rs2679895, Ppooled=0.00003, OR=0.86, 95%CI=0.8-0.9, 4,117 cases, 5,824 controls) and AgP (rs2679895, Ppooled=0.00021, OR=1.29, 95%CI=1.2-1.5, 703 cases, 2,143 controls). We also give molecular biological evidence that in addition to PLG, and TGFBRAP1, all currently known shared susceptibility loci of CAD and PD, are members of TGF-β signaling.

J05.25
Detection of a new silent mutation, antitrhombin Ala382Ala, in the analysis of antithrombin Cambridge mutations type I and II

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Background: Antithrombin (AT) is a serine protease inhibitor that inactivates Factor IIa and Factor Xa. Patients with AT deficiency are at an increased risk for venous thromboembolism (VTE) and pregnancy loss. Our unit is developing a study to determine risk factors in healthy women who are taking contraceptive hormone therapy. The case that we present is a 26 years old healthy woman without a history of thrombosis. *Methods:* We performed a genetic analysis of AT Cambridge II (*SERPINC1 G13268T*; p.Ala384Ser) using PCR and digestion with *PvuII* (Biolabs) and was confirmed by sequencing to distinguish it from the AT Cambridge I (*G13268C;* p.Ala384Pro). AT plasma levels were normal (108%). Protein C, Protein S, antiphospholipid antibodies, lupus anticoagulant, Factor V Leiden mutation, *F2G20210A* and *F12C46T* were normal. *Results:* The results showed a heterozygous pattern of AT Cambridge mutation. However, sequencing analysis revealed a new genetic variant within exon 6 of the *SERPINC1* gene, A*13264T.* This variant is a synonymous mutation and causes a codon change (GCA à GCT) but does not alter the sequence of the gene product of residue Ala382. *Conclusions:* This new synonymous mutation Ala382Ala was not detected in 100 healthy control subjects, suggesting that it is a rare nucleotide variation. Although the prevalence of this new polymorphism *(A13264T*) is low, its position adjacent to the *G13268* implies a possible interference with the genetic diagnosis of AT Cambridge II (*G13268T*) and AT Cambridge I (*G13268C*).
Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III, RETICS (RD060014/0016)

J05.26
DNA methylation patterns in coronary heart disease

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Epigenetic heterogeneity of arteries can contribute to regional vascular bed differences concerning susceptibility to atherosclerosis. We analyzed DNA methylation in atherosclerotic lesions of right coronary artery (AL-RCA) and plaque-free samples of internal mammary artery (IMA) harvested from coronary heart disease patients during surgical treatment. The first step included genome-wide DNA methylation comparison of more than 27K CpG-sites in AL-RCA (n=6) and matched IMA (n=6) samples in order to avoid the effect of interindividual variation. We identified 178 CpG-sites of 156 genes to be hypomethylated and 201 CpG-sites of 190 genes to be hypermethylated significantly (pFDR-adjusted < 0.05) in AL-RCA group compared to IMA, respectively, with the difference in methylation level of as minimum as 20%. Gene-annotation enrichment analysis revealed hypomethylated gene set implicated in immune processes (GO:0002376), response to stimuli (GO:0050896) and embryonic skeletal system development (GO:0048706). The next step was to validate and replicate results concerning DNA methylation of some relevant loci from received list in a larger cohort of vascular tissue samples (n=42). We confirmed frequent hypomethylation at the homeobox gene *HOXD4* and imprinted gene *MEST* by bisulfite pyrosequencing in AL-RCA compared to IMA tissues. Our observations suggest an atherosclerosis-related reduction in DNA methylation around genes that associated with inflammation in response to stimuli and related to developmental processes. This research was supported by President Grant for the Leading Scientific Schools of Russian Federation (5096.2014.4).

J05.27

Prevalence of copy number changes and loss of heterozygosity in atherosclerosis

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Numerous studies have relied on the idea that atherogenesis has molecular similarities with cancer. It is possible that genomic microstructural alterations, characteristic of various
neoplasms, are also present in atherosclerotic lesions. In this study we used, for the first time, array-CGH for detection of DNA copy number variations (CNVs) and copy-neutral loss of heterozygosity (cnLOH) patterns in patients with coronary heart disease undergoing coronary artery bypass graft surgery. The SurePrint G3 Human CGH+SNP 2×400K microarrays were used for DNA testing from patients' white blood cells (WBC, n=5), right coronary arteries in the area of atherosclerotic plaques (CAP, n=5), and internal mammary arteries (IMA, n=5). Polyploidy was observed in CAP of one patient. We detected the multiple CNVs and cn-LOH in all analyzed tissues from patients with atherosclerosis. Right coronary arteries in the area of atherosclerotic plaques presented a higher average CNVs length and number of genes located in their vicinity in comparison with other tissues that may support the notion of higher genomic instability. The majority of CNVs (68-91%) were identified in disease affected tissues, suggesting that some variations might represent somatic events. The gains in 3p21.31 (*CACNA2D2*), 7q32.1 (*FLNC*), 19p13.3 (*PIP5K1C*), and 21q22.3 (*COL6A1*) were detected in the vascular tissues but not in WBC. We identified gain 7p15.2 (*SKAP2*) in all tissues that not overlapping with any CNV regions currently reported in the Database of Genomic Variants. CnLOH were detected in 12 out of 13 chromosomal regions involving tumor-suppressor genes, such as *SFRP1, CEBPD, RB1CC1, DIRAS3, TUSC3* and *ZDHHC2.*

J05.28

The effect of endogenous opioids on apoptosis of cardiomyocytes in a rat model of cirrhotic cardiomyopathy

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Background: Cirrhosis is known to be associated with various manifestation of cardiovascular dysfunction (cirrhotic cardiomyopathy). Some possible pathogenic mechanisms has been reported and still more details should be explored.
Aim: To explore the contribution of endogenous opioids in the apoptosis process in a rat model of cirrhotic cardiomyopathy.
Material and Methods: Cirrhosis was induced in rats by bile duct ligation (BDL) and resection. Cardiomyopathy was confirmed using trichrome staining for fibrosis. Naltrexone, an opioid antagonist was administered for 29 ± 1days. Apoptosis was detected using TUNEL assay. For molecular analysis, expression of BCL2, Caspase3, Fas and FasL was explored using reverse transcriptase real-time PCR.
Results: Left ventricular (LV) wall thickness was significantly ($p<0.001$) lower in the BDL group than the sham group, either receiving naltrexone or saline. Apoptosis density was significantly increased in BDL-saline group ($P<0.001$) vs. sham-saline group. Cardiomyocyte apoptosis was significantly decreased in the BDL-naltrexone group compared to BDL-saline group ($P<0.001$). There was no significant change in apoptosis density in sham groups receiving either naltrexone or saline. BDL-saline group showed significant over-expression of BCL2 and FAS and down regulation of caspase3 by a factor of 1.44 ($p<0.001$) compared to sham-saline group, 1.3 ($p< 0.001$) compared to BDL-naltrexone group and 0.77 ($p< 0.001$) compared to sham-naltrexone group, respectively. No significant change was observed in the other 4 analysis for BCL2, caspase3 and FAS. **Conclusion:** Apoptosis occurs during cirrhotic cardiomyopathy through both intrinsic and extrinsic pathways activation and endogenous opioid receptors blockade using naltrexone decreases its amount.

J05.29

Association of CELSR2 gene with Coronary artery disease, replication in Tehran Lipid and Glucose Study (TLGS)

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Background: Coronary artery disease (CAD), is a leading cause of death worldwide and also have high health care cost in Iran. However environment and lifestyle are major risk factors of CAD, genetic susceptibility plays a major role in its pathogenesis. As association of cadherin, EGF LAG seven-pass G-type receptor 2 gene (CELSR2) and CAD was reported in other population including European and Asian-Indians, here we report a replication study to determine association of rs646776 polymorphisms (near CELSR2 gene) and CAD in Iranian population using samples from the Tehran lipid and glucose study (TLGS), a large population-based cohort study.
Methods: 600 cases with coronary artery disease and 1050 age and sex matched healthy control was selected from participant of TLGS cohort and enrolled in the study and rs646776 has been genotyped using the Centaurus (Nanogen) platform in DeCODE genetics. Association of G-allele with coronary artery disease, lipid related variables (e.g. triglyceride, cholesterol, HDL and LDL) and anthropometric index was tested using plink software after age and sex adjustment.
Results: G-allele of rs646776 was associated with higher HDL levels ($p <10–4$), lower waist circumference (WC) levels ($p < 0.029$) and lower prevalence of coronary artery disease ($p < 0.018$) (OR: 0.841).
Conclusion: Our findings showed the association between the presence of G allele in rs646776 and coronary artery disease and related traits among Iranian population and confirmed previous result in other ethnicity.

J05.30

DNA methylation profile of coronary artery bypass grafts

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Human blood vessels vary in their predisposition to develop atherosclerosis. The internal mammary arteries (IMA) are commonly used as the conduit to bypass major coronary artery stenosis, and have shown greater long-term resistance to atherosclerosis as compared to saphenous vein (SV) grafts. To explore differential DNA methylation profile in IMA and SV will help to further elucidate the mechanism of graft atherosclerosis. Vascular tissues were obtained from six patients undergoing coronary artery bypass grafting. Genome-wide DNA methylation analysis in IMA versus matched SV was performed using the Illumina HumanMethylation 27K BeadChip, which contains 27,578 probes within 14,475 genes. Using our criteria of pFDR-adjusted < 0.05 and a minimum median β-value difference of 20%, we identified 337 probes (290 genes) that were significantly differentially methylated. Gene ontology analysis revealed enrichment of biological processes associated with the development. There were four genes (*ALDH1A3, DAB2IP, DLX5, WT1*) with significant differences in methylation levels of three and more CpG-sites located inside CpG-islands. In conclusion our analysis lays the groundwork for further molecular studies of graft atherosclerosis by identifying novel pathways and genes potentially involved in pathology.
This research was supported by President Grant for the Leading Scientific Schools of Russian Federation (5096.2014.4).

J06.01

Link of the PPAR gamma and Omentin-1 gene expression in visceral adipose tissue

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Adipose tissue is the source of soluble mediators (adipokines), secreted mainly by adipocytes. The adipokines could play a central role in the development of insulin resistance and type 2 diabetes (DM2), and the increased risk of cardiovascular disease associated with obesity. Omentin-1 is one of the adipokines that is down-regulated in association with obesity-linked metabolic disorders including insulin resistance, glucose intolerance and DM2. The nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-dependent transcription factor that acts as primary regulator of adipogenesis, adipocytes metabolism, insulin action. The link between PPARγ and Omentin-1 is unclear today. The aim of our work was to estimate

the Omentin-1 gene, PPARγ mRNA levels in visceral fat of individuals without DM2 and cardiovascular dysfunction as well as serum Omentin-1 levels. We generated the visceral fat of 30 individuals without DM2 and cardiovascular dysfunction (mean age 45±9, 9 males, mean BMI 32±9). Visceral fat was received from gastrocolic omentum during laparoscopic cholecystectomy in non-acute period of gall-stone disease. PPARγ, Omentin-1 mRNA levels were estimated by RT-PCR with TagMan Probes. G protein mRNA levels (GNB2L1) was used as internal control. Serum Omentin-1 levels were determined by ELISA. Using Spearman correlation analysis positive correlation between PPARγ mRNA levels and Omentin-1 mRNA levels (r= 0,443; p=0,044) and PPARγ mRNA levels and serum Omentin-1 levels (r = 0,493, p=0,027) were found. This is first the report about the link between gene expression PPARγ and Omentin-1gene in visceral adipose tissue of individuals without DM2 and cardiovascular dysfunction.

J06.02
Molecular analysis in X-linked adrenoleukodystrophy patients: Identification of a novel mutation

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X linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disease characterized by progressive demyelination of the central nervous system, adrenocortical insufficiency and elevated levels of very long chain fatty acids (VLCFAs). It is caused by mutations in ABCD1 gene located at Xq28. More than 1300 mutations have been identified to date without any genotype-phenotype correlation. In this study we report the mutational analysis of 3 X-ALD patients (1 male and 2 females) showing variable clinical spectrum. In one of the female patients previously reported heterozygous p.W132X mutation was detected. In the other female patient showed IVS5-6delC (c.1489-6delC) and p.P543L variations in compound heterozygous state. The male patient was found to be hemizygous p.R104P mutation that was not reported previously. In conclusion the cases presented in this paper may contribute to the mutation and clinical spectrum of X-ALD while defining a novel mutation and a female case presenting cerebral symptoms.

J06.03
Nutritional variations in pcu children

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We have invested in the biological diagnosis of Phenylketonuria (PKU) for many years.
We performed nutritional blood tests on 30 children with PKU, who had been placed on a diet poor in Phenylalanine for at least 12 months. The age of these children was between 15 months and 10 years old.
Following our nutritional study, which included determination of the calcium phosphate product, determination of iron balance and the dosage of vitamins B9, B12 and D parallel to a determination of phenylalanine concentration in serum, we concluded that in the patients that presented consistently a high concentration of phenylalanine in serum there was a direct correlation to a nutritional imbalance.

J06.04
Rare association between thyroid tumor and Anderson-Fabry disease

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Background: Anderson-Fabry disease (AFD) is a rare, X-linked, inborn error of glycosphingolipid catabolism, resulting from mutations in the alpha-galactosidase A gene (GLA) at Xq22.1. The disease usually starts in early childhood with acroparesthesia and angiokeratomas. Untreated patients have poor prognosis and usually die in their third or fourth decade of life from stroke or uremia. Associations between AFD and some kinds of neoplasm are known. Data about AFD and neoplasm in children are absent for our experience. **Methods:** 15 year old boy with positive family history of AFD has low limbs acroparesthesias since 11 years. Presence of mutation R301L in the GLA gene confirmed the diagnosis. Family members are affected by AFD: mother has acroparesthesias, hypertrophic cardiomyopathy (HCMP) and angiokeratomas, uncle from mother side has HCMP and end stage renal disease. Also 2 family member (men) died early through unknown reason. No neoplasm in the affected family members known. Later patient developed spread acroparesthesias, pain in legs, fever and erythrocyte sedimentation rate elevation. Enzyme replacement therapy with Fabrazyme started in 15.5 years. **Results:** At the age of 15 years old boy presented the clinical signs suspected to hypothyroidism. Ultrasound has revealed a single nodule in thyroid. Follicular adenoma was diagnosed on ultrasound-guided needle biopsy and also confirmed after successful hemythyroidectomy. Currently he receives hormonal replacement therapy. Interestingly, the boy's grandmother had the same adenoma of thyroid gland. **Conclusion:** in our case we report about rare association between thyroid tumor and AFD in childhood age.

J06.05
Study of EPCR gene A1 and A3 haplotypes' in Iranian patients affected with combined hyperlipidemia

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Disorder of lipid metabolism, especially hyperlipidemia is among the major human health problems. Hyperlipidemia is a disorder of lipid metabolism leading to abnormal rise in fatty substance in circulating blood lipids. The studies that have been done in our country in the recent decade indicate a high prevalence of hyperlipidemia and resulting clinical complications including cardiovascular diseases. Hyperlipidemia broadly occurs for two reasons; genetic disorders and different environmental factors. Today, various genes have been identified in which mutation can cause hyperlipidemia disease among which reference can be made to clotting-factor genes such as EPCR. EPCR gene, as one of the major factors in the control path of thrombosis, is considered as a receptor of protein C in endothelial cells, and its connection with cardiovascular diseases has made us embark on the possible survey of EPCR in hyperlipidemia diseases given the relationship between hyperlipidemia and cardiovascular diseases. The samples includes 100 infected and 100 normal individuals from among the patients who have come to the Center for Endocrine Research of Shahid Beheshti Medical University of Tehran. After genetic counseling, samples are taken from the individuals in question and the samples are sent to the laboratory. Primer design is performed to determine the genotype of the samples based on the molecular methods study of ARMS PCR. The results indicated significant difference between the infected and control groups in A1 Haplotype and in A3 Haplotype($p<0.05$). Therefore, EPCR gene polymorphisms is irrelevant with susceptibility to family combined hyperlipidemia in the Iranian population.

J06.06
Distribution of I172N missense mutation in Macedonian and Serbian simple virilizing 21-hydroxylase deficiency patients

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Background: Steroid 21-hydroxylase deficiency is an autosomal recessive disorder, present in 90-95% of all cases with congenital adrenal hyperplasia (CAH). Its classical simple virilizing (SV) form leads to virilization of external genitalia in newborn females and pseudoprecocious puberty in both sexes, due to reactive androgen overproduction. Mainly associated with SV CAH is I172N missense mutation at codon 4 of the CYP21A2 gene that produce enzyme retaining 1-2 % of normal activity.
Method: In 16 Macedonian and 5 Serbian patients suffering from SV CAH, we have performed molecular detection of the I172N mutation, using the PCR/ACRS method. Patients were diagnosed according to standard clinical criteria at the Department of Endocrinology and Genetics, University Children's Clinic, Skopje, Republic of Macedonia and Institute for Mother and Child Health, Belgrade, Serbia.
Results: The I172N mutation was observed in 25% (4/16) of Macedonian SV patients on 18.8% (6/32) of the alleles. Two of them were homozygotes for I172N, one compound heterozygote with P30L on the second allele and one was heterozygote without observed other mutation among tested nine most common point CYP21A2 mutations. The I172N mutation was detected in only one Serbian SV patient (20%) in homozygous state.
Conclusion: The I172N mutation distribution in Macedonian and Serbian SV patients was slightly lower than reported in the European population of the simple virilizing patients. However, our findings suggesting that this mutation indeed acts as a simple virilizing deficiency allele.
Key words: Congenital adrenal hyperplasia, CYP21A2 gene, I172N mutation.

J06.07

The effect of Sodium Butyrate on the expression of liver specific urea cycle genes (CPS 1, OTC) in human lymphocytes

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Introduction: At present the only method for enzyme assay and confirmation of mutations that causing splicing error in CPS 1 and OTC urea cycle genes is liver biopsy. Therefore developing a noninvasive method for evaluation of gene expression in lymphocytes can be an efficacious method in diagnosis of urea cycle disorders. Induction of gene expression using Histone deacetylase inhibitors is a usual method. we have assessed the OTC and CPS 1 expression level after treatment of human lymphocyte cell line with sodium butyrate.
Material and Methods: MTT assay was done to check cell cytotoxicity. Then Quantitative assessment of gene expression was done at non-cytotoxic time-concentrations using SYBR-GreenI Real-time RT-PCR method.
Results: After 48 hours, treatments more than 10 mM, lead to cell injury in human lymphocyte cell line by increasing cell granulation and decreasing cell adhesion. MTT assay also confirmed that 1 and 5 mM concentrations are not cytotoxic. Sodium butyrate significantly increased CPS 1 gene expression (p-value <0.05). This increment was near the expression level in Hep-G2 cell line specifically at 5 mM concentration after 48 hours. In addition sodium butyrate treatment induced OTC gene expression in human lymphocyte cell line.
Conclusion: This study showed that sodium butyrate can induce and or increase expression of two urea cycle specific genes (OTC and CPS 1). The most induction effect occurred in 5 mM treatment after 48 hours. Key words: CPS 1, OTC, Sodium Butyrate
Key words: CPS 1, OTC, Sodium Butyrate, HDACi

J06.08

Cystic fibrosis liver disease-ultrasound evaluation

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Objectives: Cystic fibrosis (CF) is characterized by clinical polymorphism impressive , whether manifested by chronic obstructive pulmonary disease and pancreatic insufficiency or chronic liver disease that is associated with secondary diabetes . Early diagnosis and proper monitoring of all diseases and aims to extend the quality of life of these children. Ultrasound is a very useful method for the diagnosis neivazivă these complications.
The purpose of this paper is to evaluate hepatopathy associated with cystic fibrosis using ultrasonography .
Methods: 158 patients who were registered with the National Center for Mucoviscidoza (Cystic Fibrosis) Timisoara were evaluated by ultrasound in addition to clinical examination and biological assessment .Ultrasonography Williams score has been used for the assessment of liver and in some cases hepatic transient elastography .
Results: The prevalence of liver disease was 32.27 % (51 patients) . Most patients 62.74 % (32 patients) had moderate liver disease , a rate of 9.8% associated multilobular cirrhosis.There was a good correlation between Williams ultrasound score and the detection of fibrosis by transient elastography , with a better sensibility of the last method.
Conclusion: The frequency of cystic fibrosis associated liver disease is significant. Ultrasound is an extremely effective method for the diagnosis and monitoring of hepatobiliary disease in cystic fibrosis . Early detection of the disease allows the establishment of an appropriate treatment options with improved life expectancy of these patients.

J06.09

Cystic fibrosis and cow`s milk allergy

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Background: In cystic fibrosis an important feature is the pancreatic insufficiency expressed by steatorheea; in some cases, despite enzyme supplementation the chronic diarrhea is poorly controlled. Aim study was to evaluated the presence of cow`s milk allergy among cystic fibrosis children.
Methods: In one year, sixty seven children with cystic fibrosis, aged 1month -3 years (19 infants), followed in our center were observed for CMPA. Cow`s milk allergy was suspected in the presence of specific clinical manifestation(gastrointestinal symptoms, cutaneous signs, respiratory features), in cases with a suggestive medical history(failure to thrive, colics etc). In addition CMPA-specific IgE(α andβ lactoglobulin, casein) and diagnostic elimination test + food challenge test.
Results: Among infants, CMPA was diagnosed in 47.36% (9 patients) of children, predominantly in pancreatic insufficient children (77% -CF infant with pancreatic insufficiency). Toddlers (1-3 years) were diagnosed in a smaller percent, only 16.6 %(8 children) proved to have CMPA, although in more than 35% of toddlers, a positive history for CMPA diagnosis was found. Cystic fibrosis patients with pancreatic insufficiency associated more frequently CMPA(88.23%) than cystic fibrosis patients pancreatic sufficient. The prevalence of cow`s milk allergy was important, cumulating a 25.37% of CF patients.
Conclusion: Cow`s milk allergy was frequently found in CF children, especially associated with pancreatic insufficiency. The „ combined" enteropathy could influence the disese`s outcome and should be considered especially in persistent diarrhea of CF children with correct enzyme supplementation.

J06.10

Heme oxygenase microsatellite polymorphism, oxidative stress, glycemic control in type 2 diabetes Iranian patients

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Heme oxygenase-1 (HMOX-1) is activated by oxidative stress, and gene responsiveness is reportedly determined by the number of dinucleotide (GT(n)) repeats in its highly polymorphic promoter region. „Short" (S; GT(n)<25) alleles reportedly associate with higher response, lower oxidative stress, lower risk of type 2 diabetes mellitus (type 2DM), and better glycemic control and outcome, but data are conflicting. We investigated GT(n) in type 2DM subjects (all ethnic Iranians) in relation to basal glycemic control, oxidative stress, and outcome during up to 3 years' follow-up. Fasting blood from 418 type 2 DM subjects was collected at entry for GT(n) genotyping, glycated hemoglobin, glucose, lipids, and biomarkers of oxidative stress and antioxidants. A subset (n=368) was followed for up to 3 years for incident complications or death. GT(n) genotype distribution was 128, 182, and 108 for, respectively, S/S, S/L, and L/L. No significant differences in glycemic control, lipids, or oxidative stress were seen across genotypes. During follow-up, 168/368 subjects developed complications. No association was seen with GT(n). Glycated hemoglobin and lymphocytic DNA damage was higher (p<0.05) at entry in the incident complications group. No other significant differences were seen in oxidative stress or antioxidants. Data do not support the postulated link between HMOX-1 microsatellite polymorphism and type 2 DM or the putative beneficial effect of the S allele on glycemic control, oxidative stress, or outcome in type 2 DM patients, at least in this particular population.

J06.11

Association study of 844ins68 CBS gene mutation in patients with PKU from Republic of Moldova

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Inherited nonsyndromic sensorineural hearing loss (NSHL) is characterized by a high level of genetic heterogeneity, making extremely challenging to obtain a molecular diagnosis with traditional screening methods. Whole exome sequencing (WES) has been recently introduced as an alternative approach to search for alleles underlying Mendelian disorders and has been successfully applied for gene/mutation discovery. In this study, we used WES to identify the pathogenic mutations responsible for NSHL in three families (NSHL6, 11, and 12), with a recessive inheritance pattern and at least two affected siblings. A total of 9 individuals were subjected to WES using the SeqCapEZ Exome capture kit (Roche) and the HiSeq 2000 sequencer (Illumina). In particular, the NSHL6 patients were compound heterozygous for two novel mutations within the TMPRSS3 gene (DFNB8/10 locus). Both variants segregate with post-lingual, bilateral, high-frequency NSHL and affect evolutionary conserved amino acids located within the TMPRSS3 catalytic domain. In the NSHL11 family, two novel missense variants were found in the heterozygous state in OTOGL, a gene that was only recently associated with NSHL (otogelin-like protein, DFNB84 locus). Finally, in the consanguineous NSHL12 pedigree, WES coupled with homozygosity mapping analysis poin-

ted out a known missense variant (rs74315438) in the CLDN14 (DFNB29 locus) gene, coding for the claudin 14 protein. In conclusion, we provide evidence of the usefulness of WES for the diagnosis of NSHL and increase the knowledge on the genetic defects underlying this disease. This study was supported by: Italian Telethon Foundation (grant#GGP11177) and Fondazione Cariplo, grant N°2013-0825.

J06.12
Adipocytes mitochondrial DNA copy number variations in metabolic syndrome patients

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Defects in mitochondrial functions play an important role in the metabolic syndrome pathogenesis. Using droplet digital PCR method we showed different fat tissue (greater omentum, hypodermic adipose tissue and mesostenium) mtDNA copy number variations in metabolic syndrome patient ranked by body mass index (BMI). One hundred subjects were recruited from Kaliningrad Regional Hospital, Kaliningrad, Russia during 2012-2013. This group consisted of 20 subjects without metabolic syndrome (BMI ≤ 24.9 kg/m^2) and 80 subjects with metabolic syndrome. The last one was ranked by BMI into four subgroups: pre-obese (BMI 26-30 kg/m^2), obesity first degree (BMI 31-35 kg/m^2), obesity second degree (BMI 36-39.9 kg/m^2), and obesity third degree (BMI ≥40 kg/m^2). The adipose tissue was taken from the patients during scheduled laparoscopic operations. We showed a tendency to reduce the mtDNA copy number, which was observed with increasing body mass index.

J06.13
Identification and functional analysis of a novel mutation in methylmalonyl CoA mutase gene (MUT gene) causing methylmalonic acidemia

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Methylmalonic acidemia (MMA) is one of the inborn errors of metabolism, caused by either deficiency of the enzyme methylmalonyl CoA mutase, or a defect in the biosynthesis of its cofactor, adenosyl-cobalamin. At least 200 mutations in the methylmalonyl CoA mutase gene (MUT gene) have been identified in patients with methylmalonic acidemia. Here we present a novel mutation in the MUT gene of a 3 years Old Iranian girl with metabolic acidosis.
The patient had history of recurrent attacks of severe metabolic acidosis. Tandem mass spectroscopy showed elevated propionyl carnitine (C3) and the urine organic acid analysis confirmed the diagnosis of MMA. She was screened for mutations in the MUT gene. A novel C to G nucleotide change was found in position -3 of the acceptor splice site in intron 12. The heterozygosity of both parents was confirmed.
In order to demonstrate the possible effect of this nucleotide change, an Insilico analysis was done using CBS prediction site (http://www.cbs.dtu.dk/services/SignalP). This analysis suggested that the reported nucleotide change can create abnormal splicing pattern.
To confirm abnormal splicing from the mutant allele, total RNA was extracted from patients' father peripheral blood. cDNA synthesis was done. Appropriate primers were designed to amplify fragments from the region representing normal and aberrant variants of mRNA. Amplified products were sequenced. This experiment, clearly confirmed the retention of intron 12, caused by the reported nucleotide change, in sample obtained from patients' father, consistent with the role of reported change in causing MMA.

J06.14
Study of nuclear gene POLG with mitochondrial function in mitochondrial diabetes

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Mitochondrial diseases are highly variable; they can affect several organs such as the heart in cases of cardiomyopathy, ears deafness, brain in case of neurodegenerative diseases, and the pancreas in diabetes.... The common denominator of these diseases is the dysfunction of the respiratory chain, plays an essential role in the stimulation of insulin secretion and the regulation of blood glucose levels. This deficiency may be due to abnormalities related to mitochondrial DNA or nuclear DNA. Indeed, the mitochondrion is semiautonomous which 20 % of the proteins are encoded by the mitochondrial DNA, and 80% by nuclear DNA. We noted the nuclear gene *POLG*, involved in mitochondrial respiratory chain and responsible of mitochondrial DNA replication.
In this context, a Tunisian patient with mitochondrial DNA deletions, suffering from mitochondrial diabetes was studied: searching for mutations and polymorphisms in *POLG* gene, by PCR using specific primers followed by sequencing.
The results showed the complete absence of mutations in exons 13, 14, 19, 20 and 22, however, we note the presence of a heterozygous profile (10/ 11) of the CAG repeat different cases of normal control, insertion of four bases c.2734 +37-2734 +38 ins AGGT at intron 17 and a polymorphism described c.3708 G> T in the heterozygous state (rs3087374) at exon 23.
In this study we want to look if there is any correlation between mutations found at the nuclear POLG gene and mutations and deletions already found at the mitochondrial DNA of the patient.

J06.15
Mucolipidosis II presenting with rickets-like features in a newborn

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Mucolipidosis II (I-cell disease) is a progressive inborn error of metabolism with clinical onset at birth and fatal outcome most often in early childhood. The incidence is approximately 1 of 500,000. Urinary excretion of oligosaccharides are excessive, and high levels of lysosomal enzymes, such as β-hexosaminidase, β-galactosidase, α-mannosidase and arylsulfatase A, are detected in serum or cultured fibroblasts. GNPTAB is the only gene in which mutations are known to cause Mucolipidosis II. Here, we presented a female infant born as the second child of first degree cousin marriage. On her physical examination; course facies, craniotabes, long eyelashes and eyebrows, broad nasal bridge and tip, low set ears, gingival hyperplasia, short neck, rizomelic shortness of extremities, width of the wrists, pes cavus, hepatosplenomegaly, and abdomen distention were detected. Skeletal survey showed ovoid spine and scoliosis. Limb radiographies showed bilateral shortness of the humerus and femur, metaphysial dysplasia, wide metaphysis, osteopenia, sclerotic and lytic areas. Patent ductus arteriosus and secundum atrial septal defect were noted on echocardiography. Cranial magnetic resonance imaging revealed wide encephalomalasic areas on cerebral parenchyma and hydrocephaly. The urine tests for mucopolysaccharides detected elevated glucosaminoglycans (3.37mg/dl, normal ranges:0-3mg/dl). Plazma beta-hexosaminidaz A+B was 9223 umol/l/h (normal:600-3500, control: 922), alfa mannosidaz was 3069 umol/l/h (normal:20-100, control:43). This result was consistent with Mucolipidosis type II.
Although Mucolipidosis II is a rare disorder, it is important to consider in the differential diagnosis of newborns with Rickets-like radiological and biochemical features. Early diagnosis allows more effective medical management and genetic counseling.

J06.16
Association study of ACE gen I/D polymorphism in Iranian affected patients with multiplex hyperlipidemia

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Hyperlipidemia disease is the main causes of dangerous disease such as atherosclerosis, CVD, and ischemic heart disease. high cholesterol and other lipids in the blood is a susceptible Such disease in individuals patients hyperlipidemia. The main objective of this study is to Association study of ACE gene I/D polymorphism in Iranian affected patients with multiplex hyperlipidemia. This is a Case-control study based on population and 100 cases (56 men and 44 women) between 9-66 yearsold, in affected patients with multiplex hyperlipidemia Referred to the endocrinology clinic of Taleghani hospital and 100 healthy subjects have been implemented. All samples were measured cholesterol and triglycerides and completed questionnaires were obtained. Cholesterol above 200 mg/dl and triglycerides above 150 mg/dl, were classified as hyperlipidemia. Than we used GAP-PCR technique. Results showed that there is significant between patients and control group (P=0.004). So, ACE gene I/D polymorphism has association with hyperlipidemia disease in Iranian population.

J06.17
Testing the impact of genetic and non-genetic factors on common forms of obesity from Romania

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Obesity is one of the greatest medical challenges of the 21st century and its prevalence is continuously increasing. It has a complex determinism that involves genetic and non-genetic factors.
The aim of this study was to evaluate the impact of several genetic and non-genetic factors on common forms of obesity from Romania.
Unrelated obese patients (n=80) and healthy normoponderal subjects (n=80) were selected. Biological samples and clinical information was collected after informed consent was obtained. The genetic markers tested were Insulin -23Hph, IGF2 Apa I, SELL P213S, TGFb C-509T, HSPG BamH1 and IL6 G-174C.
Multiple linear regressions showed a relationship between obesity, cholesterol levels and triglyceridemia. The infection with Torque teno virus did not show a significant association with obesity. Genetic polymorphisms did not significantly alter the risk of obesity. However, statistical analysis showed an increased association between several genetic markers and teeth damage risk (HSPG BamH1, TGFb C-509T, SELL P213S, and IGF2 ApaI) and 2D/4D ratio (IGF2 ApaI) in our subjects.
The complex interplay between genetic and non-genetic factors plays an important role in modulating obesity risk. Nonetheless, further information is needed to accurately assess the impact of these factors on common forms of obesity.

J06.18
The importance of NMR Spectroscopy in diagnosis of some inborn errors of metabolism: lessons from hyperammonemia condition, galactosemia, and alkaptonuria

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Metabonomics is a powerful tool for identifying any disturbances in normal homeostasis of metabolic processes and this emerging fields, in which a large number of small-molecule metabolites are detected quantitatively in a single step, promises immense potential for early-diagnosis, monitoring, and understanding the pathogenesis of many diseases. In many errors of inborn metabolism, the relationship between disease state, metabolic biomarker and genetics is easily understood, but the clinical/ biochemical findings in some inborn errors of metabolism (IEM) are often nonspecific; an early differential diagnosis made in a single urinary sample it gives an important advantage. We present the spectrum of metabolites of urine from 1 year-old girl with stroke-like episode, elevated transaminases, coagulopathy, being first interpreted as encephalitis. Beside the clinical presentation and the hyperammonemia, the fast results gave by urinary NMR-spectrum showing a high concentration of orotic acid indicates the OTC (ornithine transcarbamilase) deficiency diagnosis. Beside this, we present our results and the utility of this method for rapid diagnosis and monitoring steps for galactosemia and alkaptonuria. The level of excretion of the metabolites in these three IEM has been well within the range of NMR detection. In the critical care setting, IEM that were not diagnosed through the neonatal screening should be considered as cause of acute neurologic, hepatic/ renal decline, rapid diagnosis being essential. We demonstrate the effective use of NMR-spectroscopic-profiles of urine in differential diagnosis for Urea Cycle Disorders and the possibility of management in other IEM.

J06.19
A novel mutations in Iranian family with Phenylketonuria

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Phenylketonuria ((PKU; MIM# 261600) is an autosomal recessive inborn of metabolism resulting from a deficiency of PAH.
Since 2005, PKU screening program was performing in Iran. According to the national databases a number of mutation has been identified throughout the PAH gene.
We have investigated a consanguineous family referred to our center, as a center for national PKU screening program. Blood sample were collected after informed and written consent was obtain. Isolated genomic DNA derived from subjects was amplified using intronic primers. The entire sequencing of the PAH gene including coding region and exon-intron boundaries were analyzed by PCR and Sanger sequencing.
Molecular analysis revealed a heterozygote mutation (ex3 del. 4765) in parentsand homozygote mutation in the proband.This is the first report of this mutation in Iranian population.

J06.20
Phenylalanine hydroxylase (PAH) gene mutation spectrum in patients with PKU in Karachay-Cherkessia Republic, Russian Federation

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Phenylketonuria (PKU) is the most common inborn error of amino acid metabolism in Europeans. PKU is classified by the severity of hyperphenylalaninemia in patients. Phenylketonuria is caused by a high variety of mutations in the PAH gene (up to date more than 550 mutations is known).
We have studied 28 PKU-patients from Karachay-Cherkessia Republic, Russian Federation. For screening of 10 PAH gene mutations (IVS2+5G>A, IVS2+5G>C, p.L48S, p.E280K, p.R261X, p.R243X, p.R243Q, p.E390G, p.A403V, p.Y414C) the multiplex system for MLPA PCR-analysis was created. Using this method we detected mutation p.R261X on 45 (80,3%) chromosomes and mutation p.L48S on 1 (1,8%) chromosome. The remaining uncharacterized PKU chromosomes were analyzed by scanning the 2, 4, 5, 6, 7, 10, 12 exons and intron/exon junctions of PAH gene by automated sequencing. In addition three different mutations were found: p.R413P on 5 chromosomes (8,9%), p.F331S on 2 chromosomes (3,6%) and novel mutation p.P211L on one chromosome. Mutation p.P211L was not detected in 50 normal controls. A mutation detection rate 96,4% was achieved.
The p.R261X, p.R413P and p.F331S mutations account 92,8% PAH mutations detected in PKU patients from Karachay-Cherkessia Republic Russian Federation.

J06.21
A Whole mitochondrial genome and POLG gene screening in a tunisian patient with mitochondrial myopathy and Diabetes

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Inherited mitochondrial diseases can be caused by mutations of mitochondrial DNA or of nuclear genes that encode mitochondrial proteins. Although many mitochondrial disorders are multisystemic, some are tissue specific .Mitochondria-related myopathies (MM) are a complex and heterogeneous group of neuromuscular disorders defined by a varying degree of dysfunctions of the mitochondrial respiratory chain (OXPHOS). In the present study, we performed the clinical, genetic, and molecular characterization of a Tunisian patient with clinical features of mitochondrial myopathy associated with Diabetes. The analysis of the mtDNA extracted from the blood leucocytes in the studied patient revealed various reported polymorphisms in the D-loop region, the ribosomal and transfert RNAs but also the coding genes The detected missense substitutions, especially the 8701A>G(T59A),the 8860A>G (T112A) ,the 10086A>G(N10D),the 13105A>G(I257V) ,the 14766C>T(T7I) were reported in the literature in patients with LHON, Mitochondrial diabetes , Leigh syndrome, cardiomyopathy,muscle pathology and unaffected individuals .Indeed, we detect the 15940delT in the MT-ATT was previously described in association with Mitochondrial myopathy .In addition,we carried out a mutational analysis of POLG1 gene encoding the mtDNA polymerase gama ,to look for an evnetual implication of nuclear gene in the mitochondrial diseases .The results of direct sequencing of all the POLG exons show the presence of the known heterzygote variation c.2492A>G in exon16 which substitutes the conserved amino acid Tyrosine to Cysteine (Y831C) described in associtaion with mitochondrial myopathy The described proband suffers from multisystemic disorders affecting neuromuscular and endocrine organs which could be caused by the association of these variations.

J06.22
Clinical complexity of Prader Willi Syndrome- genotype-phenotype correlations

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Background: The genetic variability of Prader Willi Syndrome (PWS) has a high impact for the phenotypic features.
Aims:. We aim to evaluate the genotype-phenotype correlation of a Romanian PWS patients group.
Methods: The study group had 34 PWS patients, boys and girls: subgroup A- 21 patients with chromosome 15 deletion and subgroup B- 13 patients with positive methylation test. The mean age was 10.731±8.478 and 8.553± 5.458 years. We compared the PWS characteristic features between the two subgroups.
Results: The clinical score mean was 9.083±1.717 for subgroup A compared with 10,045±1.738 for subgroup B. All patients from deletion subgroup had facial dysmorphism. Hypogonadism and hair and skin hypopigmentation were

more frequent in subgroup A. Both groups presented sleep disorders (75%). The hyperphagia onset was almost the same for both groups (around the age of 2 years) with a similar fat mass distribution. All patients from subgroup B and 80.95% from subgroup A had small hands and feet; thick saliva and verbal coefficient were more frequent in subgroup B. Skin picking and ocular abnormalities had a higher prevalence in subgroup A. We didn't identify significant differences between the two groups regarding the spine and bone density.
Conclusions: The results highlight the clinical variability of PWS. The differences can be caused by qualitative change of paternal gene expression located outside the critical region and missing in patients with uniparental disomy. A reduced life expectancy is correlated with a severe phenotype.

J06.23
Familial hyperoxaluria and infertility: is there a correlation?

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Hyperoxaluria (PH) is an autosomal-recessive disorder of endogenous oxalate synthesis characterized by accumulation of calcium oxalate primarily in the kidney; and leads to nephrocalcinosis and end-stage renal disease. A wide spectrum of associated anomalies may be present (cardiovascular; skeletal; neurological), and there are three forms of primary hyperoxaluria in which the underlying defects and clinical onset and severity have been identified.
It's about a 31-years-old man, with history of recurrent urolithiasis for who chromosomal investigation was carried out to explore a male infertility of 1 year and half related to azoospermic profile at the semen level, varicocele and microlithiasis on testicular ultrasound. Cytogenetic analysis carried out using RHG banding, disclose the presence of klinefelter syndrome (47,XXY) despite the normal morphotype. At the genetic counselling, the patient had non consanguineous parents and familial history showed similar recurrent lithiasis in his mother and brother, who was infertile since 2002.
To my knowledge; this is the first case of familial hyperoxaluria; especially the type IIII (HP3; OMIM #613616); a rare and middle entity with mitochondrial transmission, which is associated with klinefelter syndrome. The last is often associated with testicular microlithiasis; a rare entity; which may be the consequence of overproduction of oxalate and the cause of infertility. Thus, delineation of diagnostic both by genetic and histopathological screening is mandatory in order to clarify the genotype phenotype correlation.
Therefore; we insist in the comprehensive evaluation of infertility at cytogenetic (chromosomes abnormalities?) and molecular level (mosaicism?), since it's a heterogeneous and complex disease.

J06.24
Mutations of mitochondrial genome and atherosclerosis of coronary and carotid arteries.

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Aim. To evaluate the association between the level of heteroplasmy for mitochondrial mutations C3256T, G13513A, G14846A, G12315A in human white blood cells and presence of atherosclerosis in coronary and carotid arteries.
Methods. We included 130 patients (mean age 55±9 years, 116 men) with coronary heart disease (CHD) verified by angiography. Control group consisted of 63 subjects without angiographic coronary artery disease. DNA samples were obtained from whole venous blood using commercially available kits for DNA extraction. For the amplification of fragments of mitochondrial DNA by polymerase chain reaction method followed by pyrosequencing, the corresponding primers and conditions were used. On the basis of pyrosequencing data analysis, the level of heteroplasmy for C3256T, G13513A, G14846A, G12315A mutations in DNA samples were calculated.
Results. The level of G13513A and C3256T heteroplasmy was significantly higher (p=0.03; p=0.01, respectively) and level of G12315A heteroplasmy was lower (p=0.004) in CHD patients versus control group. There was significant correlation of carotid atherosclerosis severity and levels of C3256T (r=0.49, p=0.0001), G14846A (r=0.48, p=0.0001) and G12315A heteroplasmy (r= -0.32, p=0.01). Level of G14846A heteroplasmy was higher in subjects older than 45 years. There was no any relation of mitochondrial genome mutations and smoking, hypertension, CHD family history. Presence of hyperlipidemia was positively related to C3256T heteroplasmy (r=0.18, p=0.01) and negatively associated with G12315A heteroplasmy (r= -0,2, p=0,005). There was positive significant correlation between lipoprotein(a) level and G14846A (r= 0,23, p=0,01).
Conclusion. We found independent positive correlation of mutations C3256T, G13513A and G14846A with both coronary and carotid atherosclerosis.

J06.25
Assessing the implication of genetic and non-genetic factors in type one diabetes mellitus

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Type 1 diabetes mellitus (T1DM) is a multifactorial disease with a high rate of incidence in the Romanian population. The purpose of this case-control study was to test the possible association of genetic and non-genetic factors with type 1 diabetes mellitus. For this study, eighty T1DM patients and eighty healthy subjects were selected. Blood samples along with clinical, anthropometric and lifestyle data were collected after informed consent. The genetic factors tested were polymorphisms of insulin, IGF2, SELL, TGFb, HSPG and IL6 genes. Total cholesterol levels were higher in T1DM group (p<0.0001), regardless of gender. Triglyceride levels were higher in diabetic women than in same sex controls (p=0.0003), and also in healthy men compared to healthy women (p=0.001). Men had fewer visits to the dentist in the last 12 months (p=0.001). Insulin -23Hph polymorphism was significantly associated with T1DM (OR AA = 4.26, p<0.0001). HSPG BamH1 polymorphism (OR TT = 0.22, p=0.0006), as well as the combination of IL6C allele and SELL CC genotype (OR=4.69, p=0.0004) were associated with dental damage. Our data showed that insulin -23Hph polymorphism is associated with T1DM in our patients. Dental damage risk may be modulated by genetic factors such as HSPG BamH1 polymorphism in our cohorts.

J06.26
Late-onset of Wilson's disease (case report)

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Wilson's disease is a rare inherited metabolic disease that leads to copper accumulation mainly in the liver and brain. The clinical symptoms and age at onset of Wilson's disease (WD) are highly variable.
Most patients with WD are diagnosed between the first and the fourth decade of life, although the age at presentation can vary from 3 to 70. Wilson's disease has many symptoms. The broad spectrum of clinical manifestations can be divided into neurological (brain-related) and non-neurological. The primary consequence in about 40 percent of patients with Wilson's is liver disease.
We report a case in which the diagnosis was made late in a man of 67 years. This patient suffered from macroglobulinemia Waldenstrom and had standard specific scheme with chemotherapy treatment. After a course of chemotherapy the patient had an acute attack of jaundice.
To establish the correct cause of jaundice more tests were done: MRI cholangiography, endoscopic retrograde cholangiography. Since these investigations were normal mechanical jaundice was excluded. Because jaundice progressed were performed and other tests, including ceruloplasmin, plasma copper and urine copper. Upon these determinations Wilson's disease was suspected because were found low level of serum ceruloplasmin (12 mg/dl), increased serum copper (187µg/dl), increased urinary copper excretion (97 µg/24 hours). FibroMax test revealed a moderate liver fibrosis denoted by F2. Comprehensive evaluations of clinical signs, liver biopsy and gene analysis are helpful for a correct diagnosis. Late-onset WD is a frequently overlooked condition.

J06.27
Increased level of oligomeric plasma alpha-synuclein in patients with different lysosomal storage diseases

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Lysosomal storage diseases (LSD) are class of inherited disorders caused by mutations in genes, encoding proteins critical for lysosomal function. Alpha-synuclein is a presynaptic neuronal protein that can form neurotoxic oligomers and has been implicated in Parkinson's disease and other neurodegenerative diseases. Clearance of alpha-synuclein is mediated by ubiquitin-proteasome system and autophagy-lysosomal pathway so lysosomal dysfunction may play a role in accumulation and aggregation of alpha-synuclein.
The aim of our work was to estimate the level of oligomeric plasma alpha-synuclein in LSD patients.

We generated plasma of 46 patients with different LSD: 41 - Gaucher disease (GD; median age 15, range 1-71, 17 males); 5 children with other LSD (2 - Niemann-Pick type C, 2 - Krabbe disease, 1 - Wolman disease; median age 4, range 1-18, 2 males) and two control groups: 41 healthy individuals (median age 16, range 3-70, 17 males) and 21 healthy children (median age 10, range 3-16, 9 males). Oligomeric alpha-synuclein plasma level was measured using sandwich ELISA (Human Synuclein OLIGO kit Roboscreen, Germany).
The level of alpha-synuclein oligomers was significantly elevated as in GD (patients: median 22,9 pg/ml, range 1,57-444,58 pg/ml; controls: median 6,08 pg/ml, range 1,05-439,43 pg/ml, p = 0.0001) so in others LSD (patients: median 126,35 pg/ml, range 5,42-378,50 pg/ml; controls: median 8,95 pg/ml, range 2,29-103,14 pg/ml, p = 0.021).
This is the first report of elevated oligomeric alpha-synuclein level in plasma of different LSD patients. Our results allow to suggest that mutations in lysosomal proteins genes promote alpha-synuclein aggregation in LSD.

J06.28
Functional activity of lymphocytes mitochondria in children with genetically diagnosed gycogen storage disease of type I

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Introduction:Glycogen storage disease(GSD) is a metabolism disorder resulting in glycogen accumulation in hepatocytes and in development of the secondary mitochondrial disfunction.
Aim:to evaluate functional activity of lymphocytes mitochondria in children with genetically diagnosed GSD of Ia and Ib type.
Materials and methods:23 patients with GSD were examined,9 of them-with Ia type,14-with Ib type.34 nominally healthy children composed the control group.
The functional activity of mitochondria was estimated by succinatedehydrogenase(SDH) activity, which was identified in the general lymphocytes population,-in T-lymphocytes,B-lymphocytes and Nk-cells by flow cytometry method(FC500).
Results:In patients with GSD of Ia type there were determined mutations:c.247C>T, c.883C>T of G6PC gene. In patients with Ib GSD, mutations of the gene SLC37A4:c.1042_1043delCT , c.345insG, c.411G>A,c.413G>A,c.528delG, as well as mutations non-reported before:c.1016G>T(in 3 patients),c.817G>A(in 1 patient) were identified.
In all children with I type GSD,decrease of SDH activity in the general lymphocytes population in comparison with the norm was revealed(p<0,02,Kolmogorov-Smirnov test).
In patients with Ib type, characterized by more severe disease course, there was detected greater decrease of mitochondria functional activity than in children with Ia type(p<0,05).
SDH activity analysis in lymphocytes population showed 40% decrease in B-cells and 18 % in T-lf regarding the norm, with 20 % SDH activity increase in Nk-cells and NkT-lymphocytes compared with the norm.
Conclusions:Disfunctions of mitochondrial apparatus, more prominent in patients with Ib type, were revealed in patients with Ia and Ib GSD. SDH lymphocytes activity analysis may be used as additional diagnostic criterion for evaluation of the condition severity of children with GSD.

J07.01
The Importance of Test Variability in Acute Myeloblastic Leukemia and the Comparison of Different Parameters in Follow-up of Minimal Residual Disease

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Acute myeloid leukemia (AML), phenotypically and genotypically is a quite heterogeneous disease. A patient who applied to hematology polyclinic of our hospital with various complaints was diagnosed with AML. Following this, in genetic analyses that were performed, t(8;21) and FLT3-ITD were found as positive. Additionally, in chromosome analysis trisomy 8 was observed. After remission was ensured in the patient, allogenic stem cell transplantation was made. By performing t(8;21), FLT3, and trisomy 8 analyses of the patient within regular intervals, it is aimed to provide prior information about relapse and minimal residual disease. In this patient, our follow-up parameter for minimal residual disease with RT-PCR was t(8;21). FLT3-ITD was our follow-up parameter with conventional PCR. Additionally in our patient, the chimerism follow-up was done in capillary electrophoresis by the analysis of short tandem repeats (STR). From the aspect of MRD follow-up, concordant results were obtained from both conventional PCR and RT-PCR and also from the capillary electrophoresis. From these three methods the one which has the highest sensitivity is RT-PCR, then in the second place capillary electrophoresis comes and after that conventional PCR comes. From the point of laboratory since the sensitivities of the methods are different, studying with several methods for the follow-up of minimal residual disease might be required. Additionally with this case, it is attempted to emphasize the importance of examination of all chromosomal anomalies along with known gene mutations in newly diagnosed AML patients from the aspect of diagnosis, follow-up and prognosis of the patients.

J07.02
BIOMED-2 Protocols: The Best Diagnostic Tool for Suspicious Lymphoma Malignancies

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B cell non-Hodgkin lymphomas (B-NHL) are generally considered as aggressive malignancies which originate from of the transformed B cell. BIOMED-2 multiplex PCR has been suggested as a gold standard method for the detection of monoclonality in the immune cell system in hemato-malignancies and lymphoproliferative disorders. Molecular clonality (assays?) were performs based on the patterns of gene rearrangements in the immunoglobulin chains loci. During B cell malignancies, immunoglobulin recombinant constructions are produced exclusively, and are applied as diagnostic biomarkers in B-NHL. Here, we used the BIOMED-2 protocol to reveal the diagnostic value of immunoglobulin light chains (Igκ, Igλ) and incomplete IGH D-J monoclonal gene rearrangements on FFPE samples. The study was performed on 70 patients with B-NHL which were previously assessed for IGH monoclonality and failure to clarify rearrangements. Our results revealed positive clonality in 62 out of 70 (~89%) cases in the Igκ and Igλ analysis. The samples with positive clonality included 41.4% for Igλ and 47.2% for Igκ. However, our investigation on FFPE tissue revealed that EuroClonality BIOMED-2 protocols could be considered as a valuable and reliable method for clonality detection especially in failure of the IGH analysis. In general, clonal Ig gene rearrangement assays are applicable for the diagnosis of lymphoproliferative disorders and are a distinguishable method for differentiating between malignant and benign lymphoma disorders. Furthermore, we are able to implement the BIOMED-2 protocol as a routine diagnostic tool for hemato-malignancies.

J07.03
IGH Clonality Detection in Lymphoma Malignancies of an Iranian Population Using BIOMED-2 Protocols

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The B-cell differentiation procedure is coordinated with the expression of particular cell surface antigen receptors, the so-called immunoglobulins. Lymphomas are mostly characterized as clonal prolif¬erations of specific tumour cells. Routinely, the detection of malignant lymphomas are largely evaluated by their morphological features, immunohistochemistry and flow-cytometric im¬munophenotyping. However, these conventional methods cannot be relied upon to distinguish between certain types of lymphomas. BIOMED-2 multiplex PCR has been suggested as a gold standard method for differentiating between malignant and benign lymphoma disorders. Here, we used BIOMED-2 protocols to determine the clonality of IGH gene arrangement in patients with lymphoma. PCR amplification was performed on FFPE of 50 patients with B-cell lymphoma, which consisted of 11 cases of with HLs, 25 cases of B-NHLs and 14 cases of B-LPD (lymphoproliferative disorders) with an unknown subtype. Positive clonality was detected in 76% of patients with B-NHLs, with 24% of the cases illustrating clonality showing a polyclonal pattern. In B-HLs, 63% of the cases showed clonality and 36% of the cases showed polyclonality. In addition, positive clonality was observed in 42.8% of cases with B-LPD, with clonality not observed in 57% of cases in any of the immunoglobulin gene family (FR1, FR2, FR3. In the DLBCL groups, clonality was detected 75% of the cases. Patients which were diagnosed with FL and MALTs showed 100% clonality for complete IGH. However, our investigation on FFPE tissue revealed that EuroClonality BIOMED-2 protocols could be considered as a valuable and reliable method for clonality detection, especially in IGH analysis.

J07.04

Decreased IL-17A gene expression and decreased interleukin-17A on T-cells in children with Down Syndrome

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Down syndrome is by far the most common best known chromosomal disorders in human. It expresses multiple systemic complications with both structural and functional defects as a part of the clinical manifestation. Mechanism of immune changes occurring in Down Syndrome is complex and include extra gene copy of chromosome 21 and secondary dysregulation of numerous intercellular interactions. Recent studies suggest a role of IL-17 proinflammatory cytokine located on 6p12 chromosome in the pathogenesis of autoimmune diseases. Here we aimed to analyze IL17A gene expression in peripheral white cells and interleukin-17A intracellular expression on CD4+ T-cells. The research was carried out on the group of 58 children aged 6 to 12 including the group of 30 children with Down Syndrome (the simple trisomy of chromosome 21 only) and the reference group of 28 healthy children. We evaluated gene IL17A expression using real-time PCR and intracellular IL-17A analyzed by flow cytometry. We revealed significantly decreased gene expression on white cells and significantly decreased expression on IL-17A levels on CD4+ T-cells in Down Syndrome. Our data indicate that decreased IL-17A expression may play significant role in etiology of autoimmune diseases in Down Syndrome. Moreover, we demonstrated that in Down Syndrome the other gene located outside the extra chromosome 21 is also affected.

J07.05

Familial autoimmune Lymphoproliferative Syndrome caused by homozygous FAS Ligand (FASLG)mutation mimicking Familial Hemophagocytic Lymphohistiocytosis(FHL)

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Familial Hemophagocytic Lymphohistiocytosis (FHL) is a fatal disease of children. Genetic defects have been identified in the majority but not all cases. Lack of a familial marker impairs counseling and appropriate treatment strategy, also allowing misdiagnosing.
We describe the example of a consanguineous family in which three children died of complications associated with FHL-directed therapies, which turned out to be undue in the light of the recently defined diagnosis.
Given the incomplete fitting of the clinical pictures with FHL diagnostic criteria, and lack of mutations in the FHL-related genes, whole exome sequencing of constitutional DNA of both consanguineous parents was performed. The novel c.A773G p.Y258C mutation was identified in both carrier parents and confirmed by Sanger sequencing, which showed it at the homozygous state in one affected child. The diagnosis of FHL, made elsewhere in the early '80s based on the clinical picture only, drove the decision to treat the child with an aggressive approach including chemotherapy. Recurrence of the disease and the fatal outcome of the initial case supported the choice of an aggressive therapeutic approach in this family. Identification of homozygous mutation of the FAS ligand gene allowed to redefine the diagnosis, from FHL with unknown genetic defect to FASLG. Definition of genetic diagnosis of congenital immune deficiencies may have not only counselling but also therapeutic implications and should be thus pursued, even retrospectively. Exome sequencing could be a valid tool to help defining genetic bases of difficult clinical pictures.

J07.06

Molecular analysis of Haemophilia A in a Colombian family with Haemophilia A and von Willebrand disease diagnosis

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The von Willebrand factor circulates in plasma in a complex with coagulation factor VIII joined by noncovalent bonds. This interaction prevents enzymatic degradation of factor VIII (FVIII) and ensures its transport to the place of the fibrin clot formation. The factor VIII is transformed to factor VIIIa (activated) and acts as a co-factor for Factor IX activated (FIXa), which will continue activating next step in the blood clotting cascade. Because of their close relationship, decreasing activity of one or another factor may be affected, or they may also affect the other. The late generates a clinical diagnosis not very accurate, sometimes for Haemophilia A or von Willebrand disease. We report here a Colombian family that suffers the two diseases according to clinical diagnosis. However, the lack of a genetic study to verify and contrast the diagnosis made by health institutions which is based on phenotype only, can lead to a wrong classification of von Willebrand disease as a type of mild Haemophilia. The aim of this study was to confirm the clinical diagnosis in this family by molecular analysis. To achieve this, we identify the presence of the most common inversions of FVIII gene in introns 22 and 1 by LD - PCR and a general scan of the gene for frameshift mutations or stop codons through three family generations. The use of molecular techniques to confirm the clinical diagnosis for bleeding disorders will improve adequate treatment and patient prognosis in Colombia.

J07.07

Association between vitamin D receptor gene polymorphisms and Hashimoto's thyroiditis in Serbian population: a pilot study based on FokI, ApaI and TaqI RFLP technique

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Aim: Hashimoto's thyroiditis (HT) is the most prevalent autoimmune thyroid disorder caused by an interaction between genes and environmental triggers. Intra-thyroid lymphocytic infiltration may lead to progressive destruction of thyroid tissue and consequently hypothyroidism. Many studies in other populations have shown association between vitamin D receptor (VDR) gene polymorphisms and various autoimmune diseases, including HT. ***Methods:*** The study included 44 female patients (mean age ± standard deviation 38±5.4) with Hashimoto's thyroiditis and 32 healthy controls of age-, sex- and geographically matched adult without personal history of autoimmune and endocrine diseases. Genomic DNA was isolated from peripheral blood-EDTA, and target VDR gene was genotyped by PCR-RFLP technique after VDR-*Fok*I (rs2228570), VDR-*Taq*I (rs731236) and VDR-*Apa*I (rs7975232) restriction enzymes digestion. ***Results:*** We use Arlequin 3.5 integrated software for population genetics data analysis and found significant difference in the genotype distribution of VDR *Fok*I polymorphism between HT patients and controls (P=0,00465). For *Apa*I and *Taq*I we found the higher frequency of variant allele but not significantly different compared to control women (p>0,05), which is consistent with previous studies. ***Conclusion:*** The current first and preliminary results identified the association between VDR- *Fok*I gene polymorphism and Hashimoto's thyroiditis in the Serbian population. Results need to be supported by further investigations that define haplotypes patterns for VDR gene polymorphisms in a larger group of HT patients of both sexes.

J07.08

A Rare Alpha-Globin Mutation is Associated with Early-Onset Hemochromatosis with HFE C282Y Homozygosity, but not C282Y/H63D Compound Heterozygosity

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A 30 year-old Caucasian man presented with significant iron overload (ferritin 3287, transferrin saturation 87%) and was found to be homozygous for the C282Y HFE mutation. He concomitantly had mild microcytic anemia, and was found to have a missense mutation in HBA2 (c.242T>G). This mutation is known to produce an unstable hemoglobin (called Hemoglobin Ann Arbor) and cause mild, chronic hemolytic anemia in the heterozygous state.
The patient's mother also carries the Ann Arbor mutation. Interestingly, she is a C282Y/H63D HFE compound heterozygote. However, at age 55, she showed no signs of hemochromatosis (ferritin 197, transferrin saturation 40%,).
Severe iron overload has been reported as a complication of specific erythroid disorders (including sideroblastosis and porphyrias) in the context of HFE homozygosity (and questionably C282Y/H63D compound heterozygosity). However, to our knowledge, no cases of HFE mutations in combination with alpha-globin mutations or deletions have been reported.
We propose that the instablility of hemoglobin Ann Arbor leads to increased erythropoetic activity and enhanced iron absorption, which predisposed this C282Y HFE homozygote to atypically early-onset hemochromatosis. The mother's normal iron profile is consistent with the weakly-penetrant C282Y/H63D genotype, however it is noteworthy that in contrast to her C282Y homozygous son, HFE compound heterozygosity in combination with the Ann Arbor mutation did not result in early-onset hemochromatosis in this individual.

J07.09

Large deletions of SERPING1/C1NH gene in Russian patients with hereditary angioedema

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Hereditary angioedema (HAE) is caused by defects in the C1 inhibitor gene (SERPING1/C1NH). Point mutations and large rearrangements have been found in HAE patients. Previously we have screened the entire C1 inhibitor coding region to identify point mutations in 53 Russian patients with HAE and have registered mutations in 29 patients. In this work, DNA samples from other 24 patients were examined by using MLPA MRC-Holland kit to detect large deletions/duplications in SERPING1/C1NH gene. Large deletions were revealed in 6 patients, in 25% of patients without point mutations, and have constituted 17% of all mutations in Russian HAE patients. Large deletions were including ex 1 del, two cases of ex 4 del, ex 7 del, ex 1-3 del and ex 3-7 del.

J07.10

Single nucleotide polymorphisms in cytokines genes promoters are associated with the susceptibility to HIV-1 infection in the Ukrainians

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Background. Cytokines genes single nucleotide polymorphisms (SNPs) involved in the vulnerability to HIV infection in different population groups. We aimed to determine whether the carriage of cytokines genes allele variants influence the risk HIV-1 infection in Caucasian Ukrainians.
Methods. We examined promoter SNPs in IL-4 (rs 2243250), IL-10 (rs 1800872), TNF-α (rs 1800629) among 78 HIV-1 infected European Ukrainians (68 % male, 32 % female; age at diagnosis (33,35±0,76) years), 22 HIV-negative persons from the high risk infection group and 100 healthy controls using PCR-RFLP.
Results. The dominant cytokines genes variants among HIV-1 infected Ukrainians were major allele homozygotes that correspond to controls and the high risk infection group (C/C IL-4 - 62.82 %, C/C IL-10 - 53.85 %, G/G TNF-α - 62.82 %). T/T IL-4 and G/A TNF-α genotype variants significantly overrepresented in people with HIV-1 (p≤0.01-0.05); susceptibility to HIV-1 infection does not depend on gender. IL-10 minor allele distribution showed the difference among study groups: A/A variant was associated with the disease in men (p≤0.05). We found that carriage of IL-10 homozygous major allele genotype had protector effect on risk of HIV-1 infection among male population (p≤0.05).
Conclusions. The first report of cytokines genes allele frequencies in Ukrainian population shows their association with susceptibility to HIV-1 infection and suggests further research in the field of host genetic risk factors.

J07.11

Association between interleukin-1 type I receptor gene polymorphisms and the expression level of membrane-bound receptors

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Interleukin-1 (IL1) is a pro-inflammatory cytokine involved in a wide range of physiological processes, including a central role in the regulation of acute and chronic inflammation. The biological effects of IL1 (IL1α and IL1β) are realized upon binding of the cytokine to the membrane-bound IL1 type I receptor (IL1RI). The biological activity of IL1 is dependent on the expression level of its membrane-bound receptors. The aim of this study was to determine if there is an association between single nucleotide polymorphisms (SNPs) in the *IL1RI* receptor gene with the expression level of membrane-bound IL1RI on subpopulations of peripheral blood mononuclear cells (PBMC) and on serum levels of soluble IL1 receptors (sIL1RI) in healthy individuals. Serum levels of sIL1RI were determined via ELISA. Expression levels of membrane-bound IL1RI were determined by flow cytometry and genotyping was carried out using PCR-RFLP. This study did not reveal any association between the serum levels of sIL1RI and the SNPs examined. Healthy individuals with genotype GG in SNP rs3917225 and genotype CC in SNP rs2234650 in *IL1RI* displayed increased levels of membrane-bound IL1RI on intact $CD14^+$ cells. Individuals with genotype TT in SNP rs2234650 showed a lower percentage of cells expressing IL1RI in populations of intact $CD14^+$ monocytes and in a population of $CD14^+$ monocytes from mock-stimulated cultures of PBMCs. In summary, this study found that polymorphisms in the IL1 type I receptor gene can influence the expression level of membrane-bound receptors on immunocompetent cells.

J07.12

ITPA gene variant protects against combination treatment-induced anemia in Ukrainian patients with chronic hepatitis C

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Standard chronic hepatitis C (CHC) antiviral treatment includes pegylated interferon-alfa (PEG-IFNα) and ribavirin (RBV) combination therapy. The most common RBV treatment side effect is hemolytic anemia - the major RBV dose reduction cause. The aim of this study was to clarify the association between the inosine triphosphate pyrophosphatase (ITPA) gene variants and PEG-IFNα/RBV combination treatment induced anemia in CHC Ukrainian patients. The data were collected from 80 CHC patients with HCV genotype 1 infection. All study participants received standard doses of PEG-IFNα and RBV. According to the Hb level changes patients were distributed into: case group - 42 patients with combination treatment induced anemia, and control group - 38 patients with no signs of anemia. Genotyping for ITPA gene rs1127354 and rs7270101 variants was performed using PCR followed by RFLP assay. Fisher's exact test was used to estimate the difference in genotype and allelic distribution. Distribution of rs7270101 genotypes was not significantly different between groups of CHC patients with RBV-induced anemia and without it. The frequency of rs1127354 A allele carriers was significantly higher (P<0,05) in group of CHC patients without anemia (23,7%) comparing to the group of patients with anemia (7,3%). The respective allele frequency in control group (13,2%) was almost 3-fold higher (P<0,05) comparing to the case group (4,9%). In conclusion, significant association of ITPA gene rs1127354 with protection against RBV-induced hemolytic anemia was found in Ukrainian patients with CHC infection. Rs1127354 variant may assist as a pharmacogenetic marker in HCV antiviral therapy correction for side effect avoidance.

J07.13

Combination gene therapy with Mcl-1 and survivin siRNA inhibits HL-60 malignant leukemia cell growth in vitro

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The over-expression of the myeloid cell leukemia-1 (Mcl-1) gene and survivin gene are associated with the survival and progression of various malignancies including leukemia. The aim of this study was to explore the effects of Mcl-1 and survivin small interference RNA (siRNA) on the proliferation and apoptosis of HL-60 acute myeloid leukemia (AML) cells. siRNA transfection was performed using a liposome approach. Relative mRNA and protein expressions were quantified by quantitative real-time PCR and Western blotting, respectively. Trypan blue assay was performed to assess tumor cell proliferation after siRNA transfection. The cytotoxic effect of siRNAs on leukemic cells was measured using MTT assay. Apoptosis was also detected by the annexin V/PI double-staining method. siRNAs clearly lowered both Mcl-1 and survivin expression levels in a time-dependent manner, resulting in marked inhibition of cell survival and proliferation. Furthermore, siRNA co-transfection significantly enhanced the extent of HL-60 apoptotic cells relative to single transfection.Our study demonstrated that Mcl-1 and survivin siRNAs could exert antileukemic effects in vitro. It suggested that combinatory gene therapy targeting Mcl-1 and survivin could be used as a new strategy in the gene therapy of AML.

J07.14

Beta-defensin targeting by using anti-cytokine antibodies as a therapeutic approach in psoriasis

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Psoriasis is one of the most prevalent chronic inflammatory disorders which affect patient lifestyle severely. Psoriasis causes are unknown but many studies suggest that a sophisticated interplay between genetic and environmental factors are involved that trigger an excessive inflammatory response in the skin. Dendritic cells and effector T-cells are critical in the development of the psoriastic lesion and production of cytokines by these cells increase keratinocytes proliferation and stimulate the migration of inflammatory cells into the skin, promoting epidermal hyperplasia and inflammation. Previous studies have extensively documented chemotactic activities of beta-

defensin. Human beta-defensin is strongly expressed in lesional psoriatic epidermis. New findings suggest that systemic levels in psoriasis are largely determined by secretion from involved skin and not by genomic copy number. In this review, we showed that inhibition of beta-defensin stimulation by using anti-cytokine antibodies can decrease inflammatory response and plaque formation. Application of new immunologic therapy like anti-cytokines for psoriatic plaque can lead to new therapeutic approaches and introduces novel candidates for immunomodulation.

J07.15
Tenfold expansion of regulatory T cells homozygous for the CCR5 gene variant Δ32 after CD3/CD28 activation in the presence of exogenously added recombinant IL-2 in vitro

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The unprecedented power of the hematopoetic stem cell transplantation of the CCR5Δ32/Δ32 cells resistant to HIV has been proved to cure HIV infection in the case of patients from Berlin, Brigham and Women Hospital in Boston (1,2,3). Viral entry into $CD4^+$ T cells is mediated by the interaction with a cellular chemokine receptor, the most common of which are CCR5 and CXCR4 (1). Cells of persons homozygous for the CCR5 gene variant Δ32 (CCR5Δ32/Δ32) are naturally resistant to infection with CCR5-tropic HIV strains (R5 HIV) because of the lack of functional CCR5 cell-surface expression (2). Our data based on the general population in Czech Republic show a frequency of approximately 20% heterozygous persons. Four homozygous persons bearing Δ32 mutation (CCR5Δ32/Δ32) were identified out of 709 individuals tested. Here we report a more than tenfold increase in the frequency of regulatory T cells (Tregs) following CD3/CD28 co-stimulation within a week of *in vitro* cultivation of human Tregs, irrespective of their genotype. Importantly, similar the treatments, which lead to the activation of Treg function in humans - *e.g.* anti-CD3/CD2/CD28 stimulation (4) simultaneously drove expansion of Tregs *e.g.* using anti-CD3/CD28 and/or IL-2. Our study demonstrates a useful tool for *in vitro* evaluation of Treg function and facilitates further understanding of the mechanisms of immunological self-tolerance, which may also provide insights into how strong immune responses, such as graft rejection, can be restrained and engraftment of HIV resistant cells in HIV patients with AIDS lymphoma or leukemia can be augmented.

J07.16
Inherited thrombophilia in women with Systemic Lupus Erythematosus (SLE).

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by different organ damages and increased mortality. The potential synergic influence of inherited thrombophilias on the SLE clinical manifestation has not been completely clarified. Therefore the present study aimed to investigate the role of several common thrombophilias in SLE patients.
Materials and methods: Factor V Leiden (FVLeiden), prothrombin G20210A mutation (FIIG20210A), MTHFR C677T and ACE 287 I/D mutations were investigated in 112 Caucasian women with lupus by PCR-RFLP analysis. Age of SLE onset, the presence of different ACR criteria, SLICC and SLEDAI indices were registered in all patients.
Results: FVLeiden, FIIG20210A and ACE 287 I/D polymorphisms were not significantly related to the clinical characteristics of the investigated patients (p>0.05 for all). MTHFRC677T TT carriers were more susceptible to photosensitivity than the others (100% vs. 70%, p = 0.034), while the presence of at least one T allele was associated with increased prevalence of anemia (27.6% vs. 8.3%, p=0.026) as well as a tendency for more active disease (SLEDAI [median] 6 vs. 4, p=0.084).
Conclusions: FVLeiden, FIIG20210A and ACE 287 I/D polymorphisms did not aggravate the clinical phenotype of Bulgarian SLE women. The MTHFR C677T polymorphism could modulate the SLE characteristics in affected patients and further studies are needed to establish it precise significance for the disease onset, activity and severity. The present study was financially supported by the Medical University Sofia (Grant 26/2010, Grant 13/2013).

J07.17
Hereditary thrombophilia and pregnancy. Impact on quality of life of patients and their families

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Purpose: Hereditary thrombophilia is a disease characterized by a group of various mutations leading to vascular microthrombosis. It is now considered to be one of the multiple causes of pregnancy loss.The purpose of this study is to determine the impact of reproductive failure in the quality of life , both of patients and their families.
Methodology: During 2010-2014 , we evaluated 145 women with reproductive failure , with the ages between 23 and 42 years old.The time of abortion was between 6 and 36 weeks of pregnancy. 86 patients received treatment with Low Molecular Weight Heparin and Folic Acid , since the confirmation of pregnancy until delivery and 6 weeks after giving birth.The rest of the patients are not yet pregnant and they receive only Aspirin and Folic Acid. We used WHOQOL and HADS scales , witch were applied both to patients and their family members.
Results: We have found an increased rate of both depression (85%) and anxiety (80%) , loss of self esteem (65%) , negative feelings (70%) and insomnia (66%).There were significant differences in the quality of life ($p<0.5$) between the lot with multiple pregnancy loss and the lot with only one pregnany loss, but with significant improvement in quality of life for those who delievered healty babies after treatment.
Conclusions : There is need for a multidisciplinary approach (psychologist; gynecologist; haematologist and geneticist) in order to help the patients and their families to better accept the diagnosis and treatment.

J07.18
Using linkage analysis to determine responsible genes for Glanzmann syndrome: Reporting two large deletions in an Iranian population

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Glanzmann thrombasthenia (GT) is a rare abnormality of platelet aggregation with quantitative and/or qualitative abnormality of αIIbβ3 integrin that is encoded by ITGA2B and ITGB3 genes. It is more prevalent in some Middle East population including Iraq, Jordan and Iran. Many different mutations including point mutations, deletions, duplications and in some cases large deletions have been reported in these genes.
In this study we screened the responsible genes (ITGA2B or ITGB3) by linked STRs that were followed by direct sequencing of the entire coding region and exon-intron boundaries of the candidate gene and /or deletion investigation by Long PCR.
From 12 families with an affected child who were referred for mutation detection to our Lab, after initial screening with STRs, 2 cases that were linked to ITGA2B gene showed no point mutation. Investigating the region by Long PCR revealed a homozygous large deletion in exon 2 and 3 of both unrelated affected cases that has not been reported yet. Deletion was present in the affected children parents in heterozygote state.
As GT is a rare disorder and direct sequencing of two genes with 45 exons could increase the test cost, screening with indirect methods like STR can be useful for more rapid and cost-benefit diagnosis.

J07.19
SYBR GreenI RT-PCR for Quantitative Detection of TLR4 Gene Level in Colorectal Cancer Cells

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Background: TLR4 is expressed in normal intestinal epithelial cells which has important role in homeostasis, furthermore current studies suggest that TLR4 on immune cells may actually have anticancer properties but aberrant TLR4 expression can promote certain types of cancer. So in order to determine the TLR4 gene expression variations and to facilitate detection

of cut off for pathogenic TLR4 gene level in future research, we developed a quantitative and accurate method by real time (RT PCR).
Method: We used SW480 and HCT116 as the TLR4 gene high and low expressing colorectal cancer cell lines respectively. Designed primers of this gene do not amplify genomic DNA, while recognize functional transcripts specifically. For real time RT PCR, SYBR Green I was the florescent dye and β-actin were used as the house keeping gene for normalization in relative quantitation (RQ) of TLR4 gene.
Result: Designed primers well amplified cDNAs of low and high expression of TLR4 in 2 mentioned cell lines. TLR4 gene expression in SW480 was 28 times more than HCT116 cell line.
Conclusion: These preliminary results indicate that real time PCR may be employed to detect TLR4 level in CRC, successful development of real time quantitative PCR (RQ PCR) by SYBR Green I resulted to an easy method with lower cost in comparison to probe based method.
Key Words: TLR4, RQ PCR, Colorectal Cancer cells.

J07.20
Lymphoid enhancer-binding factor 1 (LEF 1) a possible immunomodulator in triple negative breast cancer

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Wingless-Int (Wnt) signaling pathway role in embryonic development, proliferation, survival and differentiation of hematopoietic stem cells is already established. There is strong evidence regarding involvement of defects in the Wnt signaling pathway in several solid tumors such as breast, colorectal and prostate cancer. Wnt receptors promote the activation of canonical Wnt /β-catenin and downstream Lymphoid enhancer factor 1/T-cell factor (LEF1/TCF) pathway influencing genes expression of cell proliferation, differentiation and apoptosis. Our proposal was to examine gene expression levels of LEF 1 in peripheral blood of triple negative breast cancer (ER negative, PR negative, Her2 negative) patients by qRT-PCR in association with immunohistochemistry and clinicopathological data in order to establish the modulation effect of LEF 1 in triple negative breast cancer circulating immune cells. Our study included analysis performed on peripheral blood leukocytes collected in EDTA tubes of 16 triple negative breast cancer patients and 7 healthy donors correlated with immunohistochemistry and clinicopathological data. After analysing qRT-PCR data of the expression level of LEF 1 gene, we obtained significant statistical results that revealed LEF 1 downregulation in peripheral blood immune cells of triple negative breast patients compared to control healthy donors peripheral blood. There are evidence-based data that reveals elevated levels of LEF 1 in breast cancer cell lines but considering our analysis describes LEF1 downregulation, a difference of expression signature may arise due to the peripheral blood immune cells different environment implication.

J07.21
Elucidating the microRNA transcriptomic footprint in chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) has a heterogeneous clinical evolution. While some patients have an indolent stable disease, others suffer from a rapidly progressing disease. This difference can partly be explained by immunoglobulin gene mutation, cytogenetic abnormalities, and mutations in genes that contribute to the development and course of CLL. Despite progress on the elucidation of driver mutations and genes for CLL malignancy, the precise pathways responsible for the heterogeneous evolution of CLL are currently unknown.
MicroRNAs are small non-coding RNAs that function in regulation of gene expression. Aberrant microRNA expression has been linked to CLL, affecting major oncogenic pathways. We have explored microRNA expression in a well-annotated cohort of 97 CLL patient cases by small RNA sequencing. Data were analyzed using our in-house SMARTAR pipeline. In addition, DNA (whole exome sequencing, WES and whole genome sequencing, WGS) and messenger RNA sequencing (mRNAseq) data was obtained for 88 cases. Integration of mRNA and small RNA sequencing data reveals differential expression of several microRNAs with CLL-related targets. An interesting candidate is miR-150, implicated in hematopoietic differentiation. We found an anti-correlation in expression levels of miR-150 and its targets, suggesting that deregulation of the miR-150 pathway could be implicated in disease evolution of CLL.
Moreover, GES and WES data are used to study the effect of SNVs and CNVs on microRNA expression and processing. Interestingly, expression levels of microRNA pairs within clusters are correlated, suggesting that structural variations in the CLL genome might indeed comprise the loss of microRNAs located in this region.

J07.22
Hemophagocytic lymphohistiocytosis developed in visceral leishmaniasis may have genetic etiology

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Hemophagocytic lymphohistiocytosis (HLH) is a complex immune disregulation disorder developed either by genetic defects in Perforin, UNC13D, Syntaxin 11, STXBP2 genes or secondary to various infections, other disorders, drugs. Distinction is complicated since infections; viral in particular, can trigger genetic HLH. HLH has overlapping clinical symptoms with visceral leishmaniasis (VL) and may remain under-recognized because of difficulties in diagnosis and rapidly fatal outcome. Genetic HLH triggered by VL has not been described. One-year-old girl from a consanguineous family diagnosed as VL by showing Leishmania amastigots in bone marrow aspirates (BMA). Symptoms also fulfilling HLH diagnostic criteria were resolved by therapy for VL initially but reappeared subsequently despite clearance of amastigots in BMA. HLH2004 protocol therapy achieved remission however recurrence observed afterwards. Mutation analysis in HLH genes revealed homozygous 627delT frameshift mutation in exon 8 of UNC13D gene leading to frameshift and premature termination of translation 40 amino acids downstream. Parents were heterozygous for this single nucleotide deletion. Stem cell donor was not available. Until she died at age four, remissions were followed by HLH reactivations and/or five central nervous system relapses under continuous protocol therapy. In conclusion, HLH developed in VL may have genetic etiology and VL may trigger underlying genetic HLH, which may make diagnosis and therapy even more complicated and mortality rate very high. Awareness created among clinicians might thereafter have important public health impact in lifesavings, disease surveillance and eradication especially in endemic tropical and subtropical countries. This study was supported by TUBITAK (Project No: 105S386-SBAG 3193).

J07.23
Identification of two novel missense mutations on C2 and A3 domain of factor VIII

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Hemophilia A, resulting from coagulation factor VIII deficiency is caused by a large variety of disease-causing mutations in FVIII gene and point mutations are the most prevalent and deleterious mutation. Active form of factor VIII includes A1/A2/A3-C1-C2 domains. Each domain plays a critical role in FVIII function and structure. The A3 domain near the phospholipid membrane is in close association with the C1 and C2 domains. Many moderate hemophilia phenotypes are caused by missense mutations that alter the binding of FVIII to VWF. These mutations are clustered within the C1 and C2 domain.
In this study two novel point mutations were investigated form a patient with serum factor VIII level less than 1% and a moderate bleeding phenotype. He has no history of bleeding diathesis and/or coagulation factor transfusion in recent 5 years. Entire coding region and exon-intron boundaries were investigated using direct sequencing method. Two novel point mutations (c.5359G>A and c.6521A>G) were detected which lead to amino acid substitution E1787K and H2174R respectively. These nucleotide variations also present in patient's mother in heterozygote state in Cis. In silico analysis with SIFT, Polyphen 2, PANTER and I-Mutant 2 soft wares showed moderate effect of these mutations on protein structure and function that could explain the moderate phenotype of disease.
According to the finding it seems using bioinformatics in silico analysis of the novel mutation could help to predict functional effect of nucleotide alteration especially in milder clinical presentation.

J07.24

Homozygosity for a null allele of SMIM1 defines the Vel-negative blood group phenotype

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This abstract describes the discovery of the gene underlying the Vel blood group system, the last clinically important unresolved human blood group system. The Vel antigen is present on red blood cells (RBCs) from all humans except rare Vel-negative individuals who can form antibodies to Vel in response to transfusion or pregnancy. These antibodies may cause severe hemolytic reactions in blood recipients. We combined SNP profiling and transcriptional network modeling to link the Vel-negative phenotype to SMIM1, located in a 97-kb haplotype block on chromosome 1p36. This gene encodes a previously undiscovered, evolutionarily conserved transmembrane protein expressed on RBCs. Notably, 35 of 35 Vel-negative individuals were homozygous for a frameshift deletion of 17 bp in exon 3. Functional studies using antibodies raised against SMIM1 peptides confirmed a null phenotype in RBC membranes, and SMIM1 overexpression induced Vel expression. Genotype screening estimated that ~1 of 17 Swedish blood donors is a heterozygous deletion carrier and ~1 of 1,200 is a homozygous deletion knockout and enabled identification of Vel-negative donors. Our results establish SMIM1 as a new erythroid gene and Vel as a new blood group system. (Nature Genetics 2013; 45(5):537-541)

J07.25

De novo homozygous mutation of the C1 inhibitor gene in a patient with Hereditary Angioedema

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Background: Hereditary angioedema (HAE; OMIM #106100) is a rare autosomal dominant disease resulting from congenital deficiency of C1 esterase inhibitor protein (C1-INH) that controls complement, contact-kinins, coagulation and fibrinolytic cascades. HAE is due to mutations in the C1 inhibitor gene (*C1NH*) that affect its protein synthesis (HAE type I) or function (HAE type II). **Objective:** We characterized the biochemical profile of a patient with HAE and a homozygous *de novo* null mutation (c.646_647insTCAGTGTCGTGdelA, p.Lys216Serfs*4) in the exon 4 of *C1NH* gene. **Methods:** Biochemical diagnosis of HAE was confirmed by using direct DNA sequencing and western blot analysis on the proband and her family. Furthermore, long range PCR, RFLP, SNP genotyping and real-time PCR were performed to identify the possible mechanisms that could explain the homozygous *de novo* mutation in the patient. **Results:** The patient showed very low antigenic and functional levels of C1-INH and C4, with normal levels of C3 and C1q. Western blot analysis confirmed the absence of native and cleaved forms of the protein. Her parents showed no alterations in complement parameters and did not present the mutation. By using different approaches we could exclude the deletion of exon 4 as the cause for the homozygous *de novo* mutation. **Conclusions:** This is the first report of a patient homozygous for a *de novo* null mutation affecting the *C1NH* gene probably resulting from a small size event of gene conversion. In contrast with the previous homozygous cases, clinical phenotype of the patient resembled typical heterozygous form of HAE.

J08.01

A new case of 2p15p16.1 microdeletion syndrome

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2p15p16.1 microdeletion syndrome is a recently identified disorder of which at least 11 cases have been reported so far. Developmental delay, intellectual disability, delayed language skills, microcephaly, structural brain abnormalities, characteristic facial dysmorphism, short stature, feeding and behavioral problems are the major features of this syndrome. Some of the reported patients also displayed craniosynostosis, genitourinary abnormalities, and vision and hearing disturbances. All reported cases have overlapping deletions, varying from 570 kb to 6.9 Mb. Most of them have single 3.6 Mb overlapping region (chr2: 58,747,888-62,363,205 Hg19) that contains 23 genes. Here we describe an Estonian patient carrying a 1.2 Mb deletion inside this critical region, who presented milder phenotype of the syndrome. Detected *deletion* (chr2: 61,178,171-62,376,313 Hg19) contains 14 of 23 genes from the critical region. Unlike previously described cases, our patient is of normal height, and does not have microcephaly, craniosynostosis, structural brain abnormalities and vision or hearing problems. At the age of 4 years, he is slightly dysmorphic without characteristic facial appearance and has moderate delay in intellectual and language development. Also, h*e is slightly spastic and up to age three, he had problems* with chewing solid foods. We thus assume that genes in this deleted region (AHSA2, C2orf74, CCT4, COMMD1, FAM161A, KIAA1841, LOC100130280, LOC339803, LOC647077, PEX13, PUS10, SNORA70B, USP34, XPO1) could not be responsible for growth retardation, microcephaly, skull and brain abnormalities in the patients with 2p15p16.1 microdeletion syndrome and may be candidates for developmental and language delay.

J08.02

Deletion of 3q11.2 region detected by arrayCGH in a girl with an Angelman syndrome-like phenotype

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We present a girl with a de novo q11.2 deletion of chromosome 3, with the phenotype reminiscent of Angelman syndrome (AS). At the age of 2 she was evaluated for motor delay, mental retardation and seizures. The girl showed obvious retardation: sitting at 12 months, walking at 22 months with unstable gait and balance problems. During assessment the height was 85 cm, the weight 10.5 kg, and head circumferences 42 cm. She had microcephaly, epicantus, almond shaped eyes, large nasal root, arched upper lip, blond hair and fair skin.
Behavior: she spoke only 3 words and exhibited an excessively happy demeanor; the resemblance to a high functioning AS child was striking. Ophthalmologic examination revealed: optic atrophy, nystagmus convergent strabismus on the left, myopia, astigmatism. MRI of the brain showed: parietal-occipital atrophy and enlargement of lateral ventricles.
Based on the above findings we suspected AS. The routine GTC chromosome and FISH analysis showed no evidence of specific microdeletion.
ArrayCGH was perfomed and a deletion of 292 kb in 3q11.2 region was found. This microdeletion includes 5 known genes: PROS1, ARL13B, STX19, DHFRL1 and NSUN3. The parental arrayCGH analysis were normal.
Interesting is that although haploinsufficiency of two genes in the deleted region (PROS1 and ARL13B) are responsible for specific diseases namely the thrombophilia RA and DA and Joubert syndrome, the clinical features we observed in our case do not meet these conditions.

J08.03

Phenotypic variability in a Hungarian patient with the 4q21 microdeletion syndrome

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Interstitial deletions of 4q21 have been reported in about a dozen patients (Bhoj et al, 2013) with deletions ranging from 2 to 15.1 Mb delineating a common phenotype including marked growth restriction, hypotonia, severe developmental delay with absent or delayed speech and distinctive facial features. A minimal critical region of 1.37 Mb accounting for the common features with 5 known genes (*PRKG2, RASGEF1B, HNRNPD, HNRPDL, ENOPH1*) has been described so far (Bonnet et al, 2010). Here we report on a 5 year-old Hungarian girl presenting with severe psychomotor delay, absent speech, short stature, dystrophy, hypotonia, distinctive facies including broad forehead, frontal bossing, downward slanting palpebral fissures, hypertelorism, hypoplastic ear-lobes, anteverted nostrils, short philtrum, small mouth, high-arched palate, short, small hands and feet, distally narrowing fingers and clinodactily. Cerebral MRI showed ventricular dilation and an increase in periventricular signal intensity. After extensive metabolic tests and exclusion of subtelomeric deletions array CGH analysis was performed using the Agilent Human Genome G3 Sureprint 8x60K Microarray (Agilent Technologies, USA), which detected a 4,8 Mb de novo interstitial de-

letion of 4q21.21-4q21.23. The clinical symptoms only partly overlap with so far reported 4q21 microdeletion cases. Among multiple annotated genes our patient is also haploinsufficient for the genes: *RASGEF1B* being a strong candidate for the neurodevelopmental features and *PRKG2* for severe growth delay. The first Hungarian case of 4q21 deletion adds to the phenotypic spectrum of this novel microdeletion syndrome and underlines the importance of array CGH to uncover the heterogeneous causes of ID.

J08.04
Application of Microarray-based Comparative Genomic Hybridization in pediatric patients with developmental delay and dysmorphic features

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The array Comparative Genomic Hybridization technology (aCGH) is intensively used in research and clinical diagnosis for the detection and identification of genome rearrangements in patients with different physical/intellectual development anomalies.
The aim of our study was to apply the aCGH technology in evaluation with high resolution of the entire genome in pediatric patients with developmental delay (DD), intellectual disabilities (ID) and dysmorphic features (DF) for identifying the genetic causes responsible for the
clinical observation.
Twenty patients with DD, ID and DF from Romania, aged between 4 months and 14 years, were included in high resolution aCGH analysis with NimbleGen ISCA Plus 3x1.4M Platform (Roche). All patients were previously assessed by conventional cytogenetic analysis.
The aCGH analysis detected clinically relevant chromosomal abnormalities in seven of the patients analyzed, as follows:
- micro-deletions ranging between 2.5 Kb-6 Mb, associated with Pitt-Hopkins syndrome (18q21.2), obesity, ID/DF (6q16.1-q16.2-q16.3), DiGeorge syndrome (22q11.2);
- micro-duplications ranging between 12.5 Kb-1.9 Mb, associated with ID (17q23.2), the Potocki-Lupski rare syndrome (17p12-11.2) and autism (Xp22.11);
- a 30 MB duplication (8q13.3-q22.3), leading to the identification and exact characterisation of a marker chromosome indicated by conventional cytogenetic analysis.
Genomic anomalies identified and characterized by aCGH provided accurate diagnosis of unidentified or unexplained diseases suspected to have a genetic cause, contributing to appropriate clinical management of the affected patients.
Our study demonstrated that aCGH technology can play a very important role in identification and characterization of cryptic and/or complex chromosomal rearrangements, being a valuable tool in postnatal diagnosis.

J08.05
Novel alteration in AMPD2 gene segregates with non-syndromic intellectual disability linked to MRT4 locus, conjointly responsible from Pontocerebellar hypoplasia

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Non-syndromic autosomal recessive intellectual disability(NS-ARID) with genetic loci are listed with MRT numbering by Mendelian Inheritance of Man (MIM). Since the discovery of the first gene in MRT1, PRSS12, in 2002, to date a total of 34 loci and 17 genes are identified. Only few of these genes are published causative in more than one family, while the rest are identified in a single family that are characterized, disclosing the high heterogeneity of the genetic basis. MRT4 was published in 2007 in an examination of a large consanguineous family with four affected members. The linked region at 1p21.1-1p13.3 was 6.6 megabase commencing 78 genes. Exome sequencing of family members and filtering variations according to the pedigree data revealed a single point mutation c.1526C>T, in AMPD2 gene, located at 1p13.3, altering uncharged polar amino acid threonine, at position 509, to nonpolar methionine (p.T509M), in evolutionally conserved adenosine deaminase domain. This variation was not found in our in house exome sequencing of 150 Turkish individuals or in publically available SNP databases. Furthermore, this variation is assigned to be damaging by diverse prediction software analysis. AMPD2 plays a critical role in energy metabolism, functioning in purine metabolism by converting AMP to IMP via salvage pathways. Recently, deleterious mutations in AMPD2 gene are reported in five families with Pontocerebellar hypoplasia (PCH) with characteristic brain imaging. Affected individuals in our family do not carry progressive context. We conclude that our case will expand the phenotypic spectrum of damaging AMPD2 mutations.

J08.06
Array CGH findings in 280 cases with intellectual and developmental delay, multiple congenital anomalies and autism spectrum disorders in Iran

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Copy number variations have gained increasing recognition in the etiology of disease. Many cohorts have established the significance of genomic imbalances in intellectual/developmental delay, multiple congenital anomalies and autism spectrum disorders. Most of these studies have been conducted in Western populations and have predicted an overall detection of 15-20% when using whole genome arrays.
We have performed 1000 array CGH studies in the past three years. 280 of these cases have been referred for ID/DD, MCA or ASD. We detected a pathogenic CNV in 45 of these cases, with an overall detection rate of 16%. Considering that 13 of the cases with CNVs were referred following aberrations detected in cytogenetic analysis and for confirmation of these abnormalities, the rate of detection among unselected cases is 12% (32/263).
As this is to the best of our knowledge among the first case series of patients from Middle Eastern populations, we would like to postulate the significance of consanguinity in the possible detection rate of genomic imbalances. First, there is an increased possibility of homozygosity for CNVs as a result of consanguinity, as detected in one of our cases with homozygous deletion of *GRID2* gene. Second, the role of consanguinity and consequently increased possibility of autosomal recessive conditions and the way it will decrease the frequency of pathogenic CNVs in the etiology of ID/DD, MCA, ASD in unselected cohorts of patients.

J08.07
9q34.11-q34.12 deletion associated with autism and dysmorphic features: a case report

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The use of high resolution array-based comparative genomic hybridization (array-CGH) in whole genome investigation led to an unprecedented rate of discovery of clinically relevant microdeletions/microduplications in neurodevelopmental disorders. We report on a 12-year-old boy with facial dysmorphic features, autism (Asperger syndrome), motor and vocal tics, speech disorder in early childhood presenting a 9q34.11 - q34.12 deletion. Cytogenetic investigations included array-CGH investigation (105K platform, Agilent Technologies) and FISH (BAC probes). Array-CGH revealed a de novo 2.4 Mb deletion spanning cytogenetic bands 9q34.11-q34.12 (genomic coordinates, hg19: 131485878-133931680). Approximately 50 genes are located within the deleted region, some of these being disease-causing OMIM genes: AGM5, CDG1M, TOR1A, ASS1, ABL1. Deletions of genomic region 9q34.11-q34.13, proximal to EHMT1, are rarely described. Few patients with deletions similar to our case have been reported in the literature and centralized in databases (e.g. DECIPHER). Haploinsufficiency of genes located in this region might be responsible for the clinical findings in the reported patients (such as autism, intellectual disability, epilepsy, movement disorders etc). Thus our case adds to the body of knowledge and will help in establishing genotype-phenotype correlations. Acknowledgments: Professor Jean-Michel Dupont from Cochin Hospital, Paris, France for kindly providing the BAC-FISH probe; Project PN 09.33.02.03.

J08.08
A clinical case with autism and 22q13.3 deletion or Phelan McDermid syndrome

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The 22q13.3 deletion is a contiguous gene deletion syndrome (MIM 606232) with gene locus SHANK3/ProSAP2 on the terminal end of chromosome 22. The syndrome is characterized by neonatal hypotonia, mental retardation, speech delay, autistic behaviour, normal to accelerated growth, minor facial dysmorphism.
The authors present a boy who was successfully treated for VSD at 6 months of age with cardiosurgical operation. After the procedure the child started

losing interest for toys and relatives. At age of 11 years, he was consulted for autistic behaviour. He presented with facial and cranial dysmorphysm (hypertelorism, epicanthic folds, ptosis, dolichocephaly, fleshy hands, severe mental retardation, severe speech delay. The diagnosis of DiGeorge syndrome was suspected at the base of history for VSD, mental retardation and facial dysmorphysm. Heparine blood of the child was investigated with Fluorescent in situ Hybridization (FISH). Test VYSIS - locus specific for 22q11.2 and test ARSA - control of locus 22q13 were applied. In all 30 investigated interphase nucleus and metaphases were found one green signal for ARSA and two red for TUPLE-1. The result showed nuc ish (22)q11.2 (TUPLE-1x2), 22q13 (ARSAx1) which confirmed the diagnosis Phelan McDermid syndrome. The recommended treatment included a free of milk and gluten diet, special education and logopedic therapy. The family attended genetic conseling.

The presented clinical case confirmed the association of 22q13.3 deletion with autism (Itsara et al., 2009. The authors proposed for a first time a new symptom - congenital heart malformation in Phelan McDermid syndrome.

J08.09

Exome sequencing reveals a novel mutation in BBS2 gene in an Iranian family diagnosed with Bardet-Biedl Syndrome

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Rare autosomal recessive Bardet-Biedl Syndrome (BBS) is a kind of pleiotropic ciliopathies which is more common in developing countries. BBS is characterized by symptoms including obesity, retinitis pigmentosa, polydactyly, learning problems, hypogonadism and kidney abnormalities that vary both within and between families. Nineteen disease-causing genes are involved in 80% of BBS cases and code proteins localized to the cilia which each function affect different parts of a cilium. *BBS2* in contribution with six most conserved BBS genes, form BBSome complex of the cilium.

Here, we report an Iranian family with a ciliopathy disorder ascribing BBS. Whole exome sequencing (WES) was performed for proband which revealed a novel homozygote splice mutation c.535-1 G>C in *BBS2* gene that would probably lead to skipping of exon 5. Using bioinformatics software, Mutation Taster, predicts c.535-1 G>C as a disease causing variant. WES data were confirmed by Sanger sequencing and co-segregation was performed for his family.

Estimating about 8% of BBS reports, *BBS2* is among one of the common genes in BBS (same results in Iranian population-unpublished data). This result was in accordance with our expectations. In conclusion, due to the clinical and genetic heterogeneity observed in Bardet-Biedl syndrome WES could be considered as the method of choice for clinical genetics practice.

J08.10

Further characterization of the 16p11.2 microdeletion phenotypes

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Introduction: Recurrent rearrangements of 16p11.2 are increasingly recognized as one of the most common structural chromosome disorders. Reciprocal 16p13.1 rearrangements predispose to developmental delay. Besides, 16p11.2 rearrangements have been identified in up to 1% of autistic individuals. These deletions have also been associated with obesity. A ~550-kb deletion is commonly reported; and a smaller deletion of an adjacent region on 16p11.2 has also been found in patients with overlapping clinical features. The phenotypic spectrum of rearrangements in this genomic region remains to be fully characterized. **Methods:** We describe two new unrelated cases with 16p11.2 microdeletions diagnosed by whole genome oligonucleotide array-CGH. We also compare the phenotype of our patients with other cases described in the literature. **Results:** One patient, referred to our clinic with moderate developmental delay, significant behavioral problems and unspecific dysmorphisms, has the „typical" ~550-kb deletion. There is a known family history of learning disabilities. However it was not possible to test his relatives because he was placed in institutional care. The second patient presents developmental delay and unspecific dysmorphisms. He has a maternally inherited 187-kb 16p11.2 deletion. His mother has mild cognitive difficulties and obesity. **Conclusions:** Our patients present adjacent, nonoverlapping 16p11.2 microdeletions, and they share some similar clinical features. They also share characteristics with other patients described in the literature. However, it should be underlined that variable expressivity has been described associated with these rearrangements, even between members of the same family and therefore testing of relatives at risk is important for accurate genetic counseling.

J08.11

A phase III clinical trial to test the effectiveness of Ascorbic acid and Alpha-tocopherol on the Fragile X Syndrome

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Introduction and Objectives: Fragile X syndrome (FXS) is a neurodevelopmental disorder affecting intelligence and behaviour. Current treatments are unable to normalize these symptoms. We propose a combination of Ascorbic acid and Alpha-tocopherol to improve learning abilities in young patients.

Material and Methods: 100 FX patients (50 in placebo): A) 3 to 6 years old (N= 19), B) 6 to 17 years old (N= 64), C) older than 18 years old (N= 17).

A clinical questionnaire and neuropsychological tests was performed at the beginning of the trial (T0) and 12 weeks later (T1). Variables: Wechsler intelligence scale for children (WISC-R), manipulative and verbal subscales and Peabody Picture Vocabulary Test (PPVT-R). The percentage of change were tested by U Mann Whitney Test ($p<0.05$)

Results: Significant improvements were detected in group B (6 to 17 years old) with 65% potency. The percentage of change in the Verbal WISC-R scales were: 30.8 in the treated group versus 13.7 in the placebo group ($P< 0.05$). The percentage of change in the manipulative WISC-R scales were: 35.4 in the treated group versus 10.9 in the placebo group, $P= 0.01$. A significant improvement was observed in the percentage of change in the Peabody median scores: 24.4 in the treated group versus 6.8 in the placebo group ($P<0,05$)

Conclusions: Clinical trials for FXS are necessary. Our results demonstrate improvement in learning and receptive language in children after 12 weeks of treatment with a combination of antioxidants: Ascorbic acid and Alpha-tocopherol.

J08.12

Submicroscopic chromosomal alterations in individuals with idiopathic intellectual disability

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Etiological studies of intellectual disability (ID) show that chromosomal alterations are factors which significantly contribute to this condition. Recently, the techniques of MLPA (Multiplex Ligation-dependent Probe Amplification) and aCGH (Array Comparative Genomic Hybridization) have allowed advances in clinical cytogenetics, enabling the detection of submicroscopic chromosomal abnormalities, ensuring a better knowledge about the causes of ID. We investigated the presence of submicroscopic chromosomal abnormalities by MLPA and aCGH in 50 patients with idiopathic ID that were selected in three institutions of the state of São Paulo - Brazil, after clinical and laboratorial evaluation. All 50 individuals with ID were investigated by MLPA with the kits P036-E1, P070-B1 (subtelomeric rearrangements) and P245-A2 (microdeletion syndromes). The P036-E1 identified one subtelomeric deletion in 4p16.3 (1/50) (2%), confirmed by P070-B1. While the P245-A2 detected two alterations (2/50) (4%), the deletion already identified 4p16.3 and one microduplication in region 22q11.2. The aCGH was performed in only 25 of the 50 subjects with idiopathic ID. Genomic alterations were identified in 14 of them (14/25) (56%), including 07 deletions (04 considered non-pathogenic CNVs) and 09 duplications (two considered non-pathogenic CNVs and two defined as false positive), totaling 08 (08/25) (32%) clinically relevant genomic changes. This research shows the importance of the application of new technologies, especially the aCGH, in the study of individuals with idiopathic ID, moreover should be fundamental part in the investigation of the possible causes of this condition due to the accentuated frequency of submicroscopic chromosomal alterations observed in these cases.

J08.13

Patients with Prader - Willi and Angelman syndrome in Latvia

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Introduction: Prader - Willi syndrome (PWS) and Angelman syndrome (AS) are imprinting disorders affecting growth, psychomotor development and metabolism. PWS is caused by lack of expression of the paternally contributed 15q11-q13 genes, while lack of expression of maternally contributed 15q11-q13 genes causes Angelman syndrome. **Aim:** To analyze clinical symptoms, age at diagnosis of patients with PWS and AS in Latvia. **Methods:** We analyzed clinical data of 19 patients (12 boys and 7 girls) with PWS and 5 patients (3 boys and 2 girls) with AS who were consulted by clinical geneticists. The diagnosis was confirmed for patients with FISH analysis or DNA methylation analysis or both. **Results:** The age of diagnosis for PWS was in range from 2 weeks till 13 years of age with great difference between girls and boys. The median age of confirmation the diagnosis for boys was 2,4 years, for girls - 8,6 years. All patients had hypotonia and feeding problems during infancy and excessive weight gain later. Patients had characteristic craniofacial features and hypogonadism was early finding in boys. Mental retardation ranged from mild to moderate. The age of diagnosis for AS was in range from 1 year till 7 years of age with median age of 4,4 years. All patients had severe developmental delay: late and jerky gait, 2 patients had absent speech, 4 patients had epilepsy. **Conclusions:** The diagnosis for Prader - Willi syndrome and Angelman syndrome is late and it is necessary to improve knowledge about these disorders among neonatologists, pediatricians, neurologists.

J08.14

Homozygosity mapping of families with intellectual disability and examination of WWOX gene

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Intellectual disability is a common disorder affecting all populations of the world. A majority of genetically caused intellectual disability phenotype follows an autosomal recessive mode of inheritance. Consanguineous marriage is prevalent in Jordan and other Arab countries. Consanguinity increases the prevalence of rare autosomal diseases. Trying to find out genetic causes for intellectual disability in Jordanian patients we set to collect samples of consanguineous families with the phenotype. We collected samples of 4 families. Extracted DNA of members of those families had undergone genome wide SNP genotyping to identify regions of homozygosity and follow-up with disease causing gene identification. We identified multiple regions of homozygosity and analyzed data for linkage. An area of overlap was identified in two families and the region contained a gene connected to epilepsy and seizures which were found in patients of those families. WWOX gene was sequenced in members of the two families but no pathologic mutations were found. The homozygosity regions identified in all the families provide a narrowed areas in the genome which can be used later to identify disease causing genes, maybe by whole-exome gene sequencing.

J08.15

Application of array-based comperative genomic hybridization in 251 patients with intellectual disability: Our experience in 2009-2011.

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Intellectual Disability (ID) is one of the most common developmental disorders and its prevalence is considered to be approximately 1-3%. Array-Based Comparative Genomic Hybridization (aCGH), also known as molecular karyotyping, is a high resolution technique developed to scan the segmental genomic copy number variations (CNV) in the genome level. Here, we review our experience with determining CNV's using both oligo- and BAC-based comperative genomic hybridization arrays for patients with ID between 2009 and 2011. In a cohort of 251 patients with ID with or without dysmorphic features, additional neurodevelopmental abnormalities, we found 68 different chromosomal aberrations in 56 patients (22.3%). The most common aberrations were 8p23.1 and 16p11.2 deletions. Our results showed that aCGH is a powerfull tool to detect CNV's causing intellectual disability and is usefull for detection novel candidate genes for neurodevelopmental abnormalities. According to the available literature, this is the first comprehensive aCGH study of a Turkish cohort of patients in this spesific population.

J08.16

Molecular karyotyping by array CGH in an Israeli cohort of children with intellectual disability and autism

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BACKGROUND: Chromosomal microarray (CMA) has become the first-line diagnostic test for individuals with intellectual disability (ID) and autism spectrum disorders ASD), with a 10-20% diagnostic yield. Here, we introduce a cohort of children with ID and ASD in Israel, who have undergone genetic testing by CMA.
METHODS: 203 children with ID and ASD of unknown etiology were prospectively tested between 5/2013 and 2/2014. All children have undergone genetic counseling prior to genetic testing. Oligo's microarray (Bluegnome-Cytochip ISCA 8x60Kv3.3) was used.
RESULTS: Normal array was reported in 132 (65%) cases. 25 cases (12%) were defined as pathogenic. All other probands (23%) were considered as having variants of unknown significance (VOUS) that further subcategorized as likely pathogenic (4), unknown (10), or likely benign (32). Pathogenic alterations included unbalanced sub-telomeic translocations (3), dup19q11-q13.2 mosaic, 12 micro-deletions and 8 micro-duplications. Pathogenic findings included PTEN, deletion syndromes of 5p-, 22q11.2, 16p11.2, Potocki-Lupski, Greigcephalopolysyndactyly and Aarskog; Aarskog syndrome was caused by a 3.5kb intronic deletion, proven as pathogenic by a typical phenotype. Parental origin was evaluated in 11 pathogenic cases; 4 were inherited and 7 were de-novo. Only 3 of the pathogenic cases could be identified by conventional cytogenetic.
CONCLUSIONS: Incidence of pathogenic findings in the Israeli cohort is similar to this reported elsewhere. Accurate clinical report allowed greater diagnostic sensitivity. Our data provides further evidence of the high diagnostic yield of CMA for genetic testing in children with ID and ASD. It confirms its value as first-tier tool regardless of the high prevalence of VOUS.

J08.17

Identification of a novel variant in TMEM67 gene responsible for JBTS6 by whole exome sequencing

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Joubert syndrome (JBTS) is a clinically and genetically heterogeneous disorder with autosomal recessive pattern of inheritance. The disorder is characterized by cerebellar hypoplasia, intellectual disability (ID), ataxia and oculomotor apraxia. Other clinical features include retinal degeneration, renal anomalies, hepatic fibrosis, and skeletal involvement. The hallmark of JBTS is a radiological pattern at magnetic imaging resonance (MRI) named "molar tooth sign".
In 2007 Baala et al. identified a new form of Joubert syndrome designated JBTS6 with causative mutation in TMEM67 gene. To now only 5 mutations in this gene has been detected responsible for JBTS6.
Due to high heterogeneity of ID in Iran,ourstudy launched to find more causative genes and their mutations in Iranian families affected with autosomal recessive IDusing whole exome sequencing (WES).One of these families has two affected with profound ID, seizure, strabismus and renal failure in one affected. On WES data a number of variants detected in thisfamily and with respect to the clinical features,a novel variant in TMEM67 gene chose as candidate. Because of suspicion of JS, MRI was performed and molar tooth sign was observed. The variant co-segregated in the family and was absent in normal population.
Underlying causes of ID remain unknown in many cases because of clinical and genetic heterogeneity;therefore, exome sequencing is an effective and helpful technique in detection of de novo mutation in this type of disorders. This approach results in more precise genotype-phenotype correlation and clinical diagnosis.

J08.18

Novel MECP2 gene mutation (472 T> G) identified in Ukrainian patient with Rett syndrome

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Rett syndrome is a neurodevelopmental disorder that occurs almost exclusively in females with a frequency of 1:10,000. It is characterized by arrested development between 6 and 18 months of age, regression of acquired skills, loss of speech, stereotypical movements (classically of the hands), microcephaly, seizures, and intellectual disability. Most cases of Rett syndrome are sporadic. It was established that the development of Rett syndrome due to the presence of mutations in the MECP2 gene. To date, 315 mutations in MECP2 gene have been identified in patients with Rett syndrome. Because of such a significant number of identified mutations in MECP2 gene and mostly the sporadic origin of the disease, we have developed denaturing gradient gel electrophoresis (DGGE) method for mutant variants screening in exons 2 and 3. Using this method we have analyzed exons 2 and 3 of MECP2 gene in 14 patients with clinical signs of Rett syndrome. In five cases abnormal migration patterns of MECP2 exon 3 PCR products have been shown. The results of Sanger sequencing has revealed 4 known for Rett syndrome mutations R168X (502C> T), A140V (419C> T), T158M (473C> T), R133C (397C> T). In one case (a patient with severe Rett syndrome symptoms) we have identified a novel mutation 472 T> G which led to substitution T158P.

J08.19

Analysis of chromosomal aberrations in patients with mental retardation using the array-CGH and MLPA technique: a single Czech centre experience

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Submicroscopic structural chromosomal aberrations (microduplications and microdeletions) are believed to be common causes of mental retardation. Patients with idiopatic mental retardation in our laboratory are examined according to an algorithm based on experimental and economical efficiency. At first, MLPA technique is applied (P245, P297, P036, P070, P250, MRC Holland) as a screening method of the main microdeletions and microduplications detection. Whole genome screening using array-CGH follows in cases of negative results or as an alteration size specification (4x180K). Afterwards, FISH is usually used for both methods confirmation. Since 2007 to 2013, 1030 patients were examined. Chromosomal aberrations were detected in 124 patients (12%). Using MLPA, 931 patients were examined and alterations were found in 90 cases (9.7%) - 43 subtelomeric changes (P036, P070), 14 DiGeorge syndrome (P250) and 33 another changes including 4 Williams-Beuren syndrome, 3 Slavotinek syndrome, 3 cases of 1q21 microduplication syndrome (P245, P297). There were examined 99 patients by array-CGH and chromosomal aberrations were observed in 32 patients (35.5%). These findings can be devided into pathogenic (11), benign (3) and variation of unknown significance (18). According to the origin, we found 6 de novo cases, 6 cases with familial origin and 20 cases of unknown significance. World databases of patients (e.i. DECIPHER) were used for better case clarification. The suitability of all these methods combination is confirmed by a case report of a girl with unbalanced translocation t(17;22). This aberration simultaneously causes Phelan-McDermid syndrome in this patient. Supported by OP VK CZ.1.07/2.4.00/31.0155 and CZ.1.07/2.3.00/20.0183

J08.20

Range of mutations of *PAH* gene at patients with a classical form of the phenylketonuria, living in the Novosibirsk region

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Molecular-genetic inspection of patients PKU carried out throughout 1991-2012, living in the Novosibirsk region, has shown that frequency R408W of PAH gene among all mutations of this gene makes 0,653. These mutation are revealed both in the homozygous form, and in a compound with other rare mutations. For the purpose of identification of a range of mutations of PAH gene with use of a complex of molecular-genetic technologies research of exons 5-12 of PAH gene at 62 patients with PKU, among which 48,38% had easy, 45,17% - the moderate and 6,45% heavy degree of mental retardation was carried out. As a result of the carried-out inspection (besides a major mutation of R408W) in structure investigated exons 5-12 of PAH were identification thirteen more rare mutations and their frequency characteristics - R158Q (0,0323) are established, to E221D222fsdelAG (0,0081), by R243Q (0,0081), R243X (0,0161), R252W (0,0161), R261Q (0,0484), E280K (0,0081), P281L (0,0403), S349P (0,0081), IVS10nt-11g→a (0,0081), A403V (0,0081), Y414C (0,0242), IVS12nt+1g→a (0,0323). The analysis of association of genotypes with features of a clinical picture at the surveyed patients with PKU showed communication existence between moderate and heavy forms of mental retardation and a homozygous genotype of mutations of R408W and IVS12nt+1g→a and their compound homozygous genotype with mutations of R158Q, E221D222fsdelAG, R261Q of PAH. The received results testify to essential clinical and molecular heterogeneity of PKU among inhabitants of the Novosibirsk region and allowed to develop optimum algorithm of molecular and genetic diagnostics of this disease in burdened families.

J08.21

A rare case of microduplication Xp11.23-11.22

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Over the last few years, array-comparative genomic hybridization array (CGHa) has considerably improved our ability to detect cryptic unbalanced rearrangements in patients with mental retardation. We report on a young female patient, aged 18, suffering from moderate mental retardation, subjected to CGHa. She was previously studied by conventional cytogenetic techniques, and the karyotype was 46, XX. In addition, Williams syndrome and Fragile X syndrome were excluded. The subject came to our attention had clinical symptoms such as EEG abnormalities (irregular paroxysmal discharges), moviments clumsiness for fine and coarse gesture, flat foot, delayed language development, mild mental retardation, overweight. The array CGHa analysis revealed the presence of a microduplication of the short arm of the X chromosome (Xp11.23-11.22), whose size was 4.6 Mb, and excluded the presence of additional rearrangements along the X chromosome. Only a small number of individuals with duplications within the proximal short arm of the X chromosome have been so far reported, whose clinical findings correspond to that of the subject under consideration. Our findings suggest that overexpression of genes from proximal Xp is likely to have contributed to her clinical phenotype.

J08.22

An Additional Case of Temple-Baraitser Syndrome: The Sixth Case in the Literature

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Temple-Baraitser syndrome (TBS) is a very rare disorder characterized by severe mental retardation and anomalies of upper and lower limbs with absence/hypoplasia of the nails. Various dysmorphic facial features may accompany with the syndrome. Only 5 cases have been reported in the literature. Genetic etiology of TBS is still unknown. We report a 13-year-old female who was admitted to the hospital because of severe mental retardation, history of epileptic seizure and facial dysmorphic features. She was born to consanguineous parents at term. On physical examination at admission, her weight was 83 kg (>97p), height: 163 cm (75-90p) and head circumference: 58 cm (>97p). She had broad forehead, myopathic facies, hypertelorism, upslanted palpebral fissures, long and trapezoid philtrum, downturned corners of the mouth, broad thumbs, broad toes, absent nails of upper and lower limbs. X-ray showed central translucency of distal phalanges of thumbs. Cranial MRI was normal. Karyotype and subtelomeric FISH were normal. Temple-Baraitser syndrome should be in the differential diagnosis for cases presenting with mental retardation particularly accompany abnormal thumb with great toes and hypoplastic/absent nails.

J08.23

Recurrent findings of microduplications in the pseudoautosomal region 1 at Xp22.33 - with unknown phenotypical significance

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Microduplications in the PAR 1 region at Xp22.33 when found by SNP-array analysis, poses a challenge to interpret. As yet no certain clinical significance has been shown.

However, it has been proposed, that the *ASMT* gene, located within this area, can be linked to autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD). *ASMT* encodes the enzyme acetyl serotonin methyl transferase, which catalyzes the last step in converting serotonin to melatonin. Melatonin is synthesized in the pineal gland during the night, and is crucial for sleep induction and circadian rhythm regulation.

In both ASD and ADHD sleep alterations has frequently been reported and it has been shown that there is a decreased level of melatonin in these patients

and that administration of melatonin greatly improves sleep patterns.
Recently we noted frequent findings of microduplications within the area, which gives rise to the question: could there be a link between a dose related activity in the *ASMT* gene (microduplications at Xp22.33) and the phenotypical presentation in our cases.
To investigate this further we wish to present our cases with microduplication at Xp22.33, to show if an association can be established.

J08.24
A novel mutation in the endosomal Na+/H+ exchanger NHE6(*SLC9A6*) causes Christianson syndrome with electrical status epilepticus during slow-wave sleep (ESES).

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Mutations in the solute carrier family 9, subfamily A member 6(*SLC9A6*) gene, encoding the endosomal Na+/H+ exchanger 6 (NHE6) are associated with Christianson syndrome, a syndromic form of X-linked intellectual disability characterized by microcephaly, severe global developmental delay, autistic behavior,early onset seizures and ataxia.
In a 7-year-old boy with characteristic clinical and neuroimaging features of Christianson syndrome and epileptic encephalopathy with continuous spikes and waves during sleep, we identified a novel splice site mutation (IVS10-1G>A) in *SLC9A6*. These findings expand the clinical spectrum of the syndrome and indicate NHE6 dysfunction as a new cause of electrical status epilepticus during slow-wave sleep (ESES).

J08.25
Cost-effectiveness of using array CGH for diagnosing learning disability

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Whilst chromosomal imbalance has traditionally been diagnosed using karyotype analysis, the advent of array-based comparative genomic hybridisation (array CGH) has provided a more powerful diagnostic tool for clinical genetics services. We present an economic evaluation conducted in a retrospective cohort of patients with undiagnosed learning disability and developmental delay referred consecutively to the regional clinical genetics service at Guy's and St Thomas' NHS Foundation Trust. We compare the use of array CGH as a first-line diagnostic tool versus as a second-line diagnostic tool following karyotype analysis from a clinical genetics service perspective. Data were extracted for 848 patients in the first-line test arm and 742 patients in the second-line test arm. The mean incremental cost of testing patients was -£241.56 which means that using array CGH as a first-line was cost saving. The mean incremental gain in the percentage diagnoses was 0.39% which meant that an additional 0.39 patients would gain a diagnosis for every 100 patients tested. We calculated both the incremental cost-effectiveness ratio and the net monetary benefit and found the first-line testing strategy to dominate the second-line testing strategy. Sensitivity analyses conducted support these conclusions. Based on these findings, we estimate that moving to a first-line testing strategy will have saved in excess of £3 million based on 14,000 patients tested whilst also improving the number of diagnoses identified at Guy's and St Thomas' NHS Foundation Trust.

J09.01
DISC1 polymorphisms are associated with ADHD in Iranian population

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Attention deficit hyperactivity disorder (ADHD) is a common heritable psychiatric disorder with a worldwide prevalence of 5%. The etiology of ADHD is still incompletely understood, but several studies, consistently indicate the strong role of genetic factors on this disorder. ADHD is known to have polygenetic nature with multiple genes involved in its genetic basis. Disrupted-in-Schizophrenia-1 (DISC1) has been identified as a susceptibility locus for several psychiatric disorders and some of its polymorphisms are studied in many neurological disorders, but are not included in ADHD studies as much. In this study we investigated the association of four SNPs (rs11122330, rs6675281, rs11122319, rs1417584) in the DISC1 gene with ADHD in Iranian population. 600 subjects composed of 300 patients and 300 normal controls were included and tetra-primer ARMS PCR technique was used for genotyping all SNPs. We found differences in genotype distributions of rs11122319 (p = 0.01) and rs6675281 (p = 0.009) variants between patients and controls. Our findings strengthens the role of disc1 gene as a susceptibility locus for ADHD and indicate that rs11122319 and rs6675281 variants are strong risk factors for ADHD in Iranian population.

J09.02
Genetic analysis of Aicardi-Goutières Syndrome in an Italian cohort

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Aicardi-Goutières Syndrome (AGS) is a rare genetically determined encephalopathy that may overlap with the phenotype of congenital infection. Mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C ,SAMDH1 and ADAR1 genes have been found in the majority of AGS cases .TREX1 gene codes for 3'->5' DNA exonuclease with specificity for ssDNA. AGS2, AGS3, AGS4 patients have mutations in genes coding for the three subunits of the Human Ribonuclease H2 enzyme, complex implicated in ribonucleotides removal from RNA:DNA duplex. The AGS5 gene, SAMHD1, encodes for a triphosphohydrolase that converts deoxynucleoside triphosphates to deoxynucleoside and inorganic triphosphate. AGS6 can be caused by mutations in the ADAR1 gene traslating into an adenosine deaminases acting on RNA which catalyzes the hydrolytic deamination of adenosine to inosine in ds RNA In the last years, IRCCS "C. Mondino" Foundation and International Aicardi-Goutières Syndrome Association (IAGSA) were involved in many projects. An AGS biobank to collect serum, plasma, lymphoblastoid cell lines and PBMCs has been established. .We recruited samples of 23 patients with clinical diagnosis of AGS and their parents.. We collect two AGS1/TREX1 case, 15 AGS2/RNASEH2B cases, one AGS3/RNASEH2B case, one AGS4/RNASEH2C case ,one AGS5/SAMHD1 case and two AGS6/ADAR1 cases. Although one of the screened patients did not show mutations in the mentioned genes above, we may still consider the possibility of the presence of new mutations yet to be identified in other genes. The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement number 24177.

J09.03
Evaluation of PAI-1 gene 844G/A polymorphism in Iranian patients affected by Alzheimer and Normal Individuals

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It has extensively established that PAI-1 gene is involved in Alzheimer pathogenesis. Numerous genetic risk factors have been related with Alzheimer, but no study has unraveled a possible association between Alzheimer and PAI-1 gene 844G/A polymorphism.There are many common risk factors such as 844G/A polymorphism that play key roles in the development of Alzheimer disease.Polymorphisms in PAI-1 gene have been associated to Alzheimer in some populations. However, other groups failed to replicate this finding in other populations. Evidence suggests that PAI-1 gene 844G/A polymorphism might play a role in Alzheimer, as a result we Studied PAI-1 gene 844G/A polymorphism common polymorphisms in Iranian with Alzheimer.**Materials and methods:**We conducted study including a clinically well-defined group of 52 Alzheimer patients to test the association between 844G/A polymorphism and Alzheimer in Iranian population. In the present case control study, the PAI- gene 844G/A polymorphism has been investigated in 52 patients with Alzheimer and 112 healthy subjects by using ARMS-PCR methods. Then, the data were analyzed by pasw statistics 18 (SPSS) software.**Results:** In patients samples the genotype distribution for PAI-1 844G/A showed 52% , 46%, 2% for AA, AG and GG respectively. In control samples 96% of the cases were AA,4% were AG and 0% were GG.**Conclusion:**The results of this study show significant association between Alzheimer and PAI-1 gene 844G/A polymorphism in Iranian population. ($P < 0.05$)

J09.04

Apoe Allele frequency in Alzheimer's disease in turkish population

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Alzheimer's disease is an irreversible, progressive brain disorder and the most common cause of dementia in older adults. Apolipoprotein E (APOE) is a protein involved in the transport of lipids that is strongly linked to Alzheimer's disease. The APOE gene has three common alleles (epsilon 2, epsilon 3, and epsilon 4) that determine six genotypes in the general population. Especially frequency of the allele for apolipoprotein E type 4 (epsilon 4) is increased in late-onset familial and sporadic Alzheimer's disease (AD). In this study, we have examined APOE4 frequencies in a total of 481 patients and 101 controls for an association with the APOE-epsilon 4 allele. DNA samples of patients were isolated from peripheral blood with standart methods. PCR-Reverse Hybridization (254 patients) and Real-Time PCR (227 patients) was used for APOE genotyping. The allele frequencies obtained in patients and controls, respectively, as follows; e2=55 (5.71%) and 13 (6.43%), e3=750 (77.96%) and 163 (80.69%), e4=157 (16.32%) and 26 (12.87%). E4 allel and E4/E4 genotype is a risk factor for Alzheimer's disease in Turkish population.

J09.05

The association of the rs121918398 with Alzheimer's disease in Iran northern population

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Introduction and Aim: Alzheimer's disease (AD) is the most common type of dementia characterized by memory impairment and alteration of diverse cognitive abilities. Pathologically, it is the formation of amyloid plaques and neurofibrillary tangles in the brain. The most common form of AD is Late-onset AD (LOAD), and is usually sporadic. Although several susceptibility genes for AD have been reported, by far the strongest genetic risk factor for LOAD is Apolipoprotein E that located on chromosome 19q13.2 and universally recognized as a major disease susceptibility gene for LOAD. APOE has three common alleles, APOE-2, APOE-3, APOE-4. In this study we examined association of the APOE gene polymorphism (rs121918398) with Alzheimer disease in Iran northern population.
Methods: Study included 50 patients with AD and 50 healthy volunteers. An informed consent was obtained from all participants. Genomic DNA was extracted from peripheral blood leukocyte. Genotypes determined by PCR and restriction fragment length polymorphism (RFLP). Statistical analysis was performed using the MedCalc program for windows version 12.
Discussion and Conclusion: The prevalence of genotype frequencies of the APOE A/A, A/G, G/G were 16%, 34% and 50% respectively, in AD subjects, and in healthy volunteers were 10%, 64% and 26% respectively. Statistical analysis has not emerged significant difference from the comparison of either genotype (p>0.05). There was no evidence that APOE variants were associated with AD in this population. However our results may be different by selecting different geographical sites or bigger size population.

J09.06

Replicative association analysis of Alzheimer's disease in Russian population of Siberian region

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder observed in the elderly. This pathology is the most common cause of dementia and accounts for 50-80% of dementia cases. The World Health Organization estimated that currently about 25 million people worldwide have Alzheimer's disease. It is a complex disorder with genetic, environmental and lifestyle factors. Common neurological disorders including AD are the subject of intensive genetic research based on genome-wide association studies (GWAS). Genetic variants associated with cognitive impairments, which are an important endophenotypes for AD, also have been revealed by GWAS. The aim of this study was to analyze associations of 15 SNPs reported in GWAS with Alzheimer's disease in Russian population of Siberian region. SNPs were genotyped by real-time PCR in 108 AD patients and 285 age-matched controls of Russian origin. The association of rs2616984 located in the region of CUB and Sushi multiple domains 1 (*CSMD1*) gene, previously reported in GWAS by Cirulli et al. (2010), was confirmed in Russians (OR = 1,5, p = 0,01). Genetic markers in loci for *VRK2, SLCO6A1, NOTCH4, TCF4, ZNF804A, AGBL1, TLR4, RELN, ZFP64P1, KCNB2, CPVL, NRGN* and *NRIP1* previously reported in GWAS was not associated with the disease in Russian population. This work is partially supported by RFBR grant #12-04-00595.

J09.07

Association study of BDNF polymorphism Val66Met in Bulgarian patients with Alzheimer disease

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Background: Alzheimer disease (AD) is the most common form of dementia characterized by cognitive impairment, memory and personality disorders caused by progressive degeneration of the brain neurons. Brain-derived neurotrophic factor promotes neuronal survival and growth processes during development. The Val66Met (rs6265, G>A) polymorphism in BDNF gene has been extensively studied for association with various neuropsychiatric and neurodegenerative diseases. Several studies show that Val66Met polymorphism is implicated in cognitive impairment, depression, intracellular trafficking, reduced hippocampal volume etc. Materials and methods: In this study 202 patients with AD and 97 healthy controls, matched to the patients by age, gender and ethnicity (NC) were included. Genotypes were determined using TaqMan assay (Applied Biosystems). Statistical analysis was done using Plink toolset and chi square test. Results and discussion: The Val66Met polymorphism showed significant difference in allele frequency distribution between the AD and NC group. The common Val allele was more frequent among AD patients, while the rare Met allele was associated with decreased risk of AD (P=0.01, OR=0.58), When the sample was analysed in sub-groups according to the age of onset, significant association was found in the early onset group (EOAD) (P= 0.01, OR= 0.51), but not in the group with late onset (LOAD) (P= 0.07, OR= 0.62). Conclusions: Our findings show association and increased risk effect of the common Val66 BDNF allele (P= 0.02, OR= 1.732) in Bulgarian patients with early onset AD. Acknowledgements: This work was supported by Infrastructural Grant: DUNK01/2/2009 funded by National Science Fund, Ministry of Education and Science, Bulgaria

J09.08

Brown-Vialetto-Van Laere syndrome: a case report of a family from Italy

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Background. Brown-Vialetto-Van Laere syndrome (BVVLS) is a rare motor neuron disease (MND) characterized by childhood onset, neurosensory deafness and bulbo-pontine paralysis. Most familial cases are recessive and genetic studies have demonstrated mutations in several riboflavin transporter encoding genes (i.e. SLC52A3 [hRFT2], SLC52A2 [hRFT3] and SLC52A1 [hRFT1]) and the UBQLN1 gene.
Objective.To describe the phenotypic and genotypic characteristics of an Italian family with autosomal dominant BVVLS.
Patients and methods. The proband is a 16-years-old girl affected by BVVLS since she was 3. A second case in the family is the proband's maternal aunt, who was diagnosed with BVVLS at age of 27; however, deafness and bulbar weakness had started during adolescence. No other family members are affected.
DNA was extracted from blood of the proband and several relatives. A screening for patogenic mutations in riboflavin transporter genes and UBQLN1 was performed.
Results. No pathogenic mutations in all three riboflavin transporter genes were detected. We found that the proband was bearing a heterozygous substitution E54D in exon 1 of the UBQLN1 gene, previously suggested to be implicated in the disease. This polymorphism was also present in a healthy adult family member.
Conclusions. We identified a rare BVVLS family with an autosomal dominant pattern of inheritance with incomplete penetrance and variability of age at onset. The UBQLN1 gene might represent a genetic risk factor for this rare motor neuron disease.

J09.09

Repeat expansion in C9ORF72 is not a common cause of Parkinson's disease among Iranian patients

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Expansion of the hexanucleotide repeat (GGGGCC) in intron 1 of the C9ORF72 gene is the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). C9ORF72 expansions have also been observed in some Parkinson's, Alzheimer and essential tremor patients. The mechanism by which the mutation causes disease is definitively unknown. During a recent screening of only 80 Iranian ALS patients, ALS, FTD, and PD was each observed in three individuals of a single family. The hexanucleotide repeat in C9ORF72 was observed in all three patients. This finding prompted screening of repeat in a cohort of 170 Iranian PD patients by the repeat primed polymerase chain reaction protocol. LRRK2, PRKN, DJ-1, and PINK1 had earlier been screened in many of the patients and those with known mutations in these genes were excluded from the C9ORF72 screening. No pathogenic expansion was found in any of the cases. We conclude that abnormal C9ORF72 repeat expansions are not a common genetic cause of PD in Iranian. The same finding has been reported in C9ORF72 repeat expansion screenings of cohorts from other populations.

J09.10
Preimplantation diagnosis of CADASIL (cerebral arteriopathy with subcortical infarcts and leukoencephalopathy)

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Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominant progressive disorder of the small arterial vessels of the brain manifested by migraine, strokes, white matter lesions, diffuse myelin loss, with resultant cognitive impairment in some patients. It is caused by heterozygous mutation in the NOTCH3 gene on chromosome 19p13.The case report presents the preimplantation diagnosis of CADASIL family. The proband had severe transient neurological symptoms (immobility, cognitive impairment) after childbirth. Her sister is clinically healthy, but a MRI scan shows supratentorial white matter changes. Their father has minimal clinical symptoms. All three family members have NOTCH3 gene mutation.

J09.11
Identification of two novel mutations in ERCC6 and ERRCC8 genes in two mildly affected patients with Cockayne syndrome

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Cockayne syndrome is clinically heterogeneous condition in which postnatal growth delay, microcephaly and neurological dysfunction are the cardinal features. Characteristic dysmorphic findings like deep-set eyes, triangular face, aged appearance can be seen in various degrees. Photosensitivity, demyelinating peripheral neuropathy, cataract, cachexia, dental abnormalities and sclerotic epiphyses are also the features of this syndrome. It should be noted that brain calcifications are observed but not a must. ERCC6 and ERCC8 mutations are responsible for the clinical findings of these patients. Here we report three patients with Cockayne Syndrome and two novel mutations in ERCC6 and ERCC8 genes. Two of them had a mild clinical course and very mild dysmorphic findings and the other was severely affected. Insight of these findings we discuss the phenotypic heterogeneity of this condition and aimed to underline the possibility to overlook in the absence of some cardinal criteria and mild facial features.

J09.12
One novel SCN1A mutation in a Bulgarian patient - case report

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Dravet syndrome (DS) is an early-onset epileptic encephalopathy characterized by polymorphic seizure types. At onset, they are usually induced by fever and often present as tonic-clonic or hemiclonic status epilepticus during the first year of life. Later, patients also manifest other seizure types, including absences, myoclonic, and partial seizures, all being refractory to treatment. The EEG is often normal at onset, but later characteristically shows generalized spike-wave activity. Psychomotor development stagnates around the second year of life, and affected individuals show subsequent mental decline and other neurologic deficits. DS is caused by mutations of the SCN1A gene in more than 80% of patients. Here we report a 1-year old boy with seizure onset at 5 months, presenting by series of febrile or afebrile tonic-clonic or hemiclonic seizures up to status epilepticus, some with postictal hemiparesis. Several interictal EEGs were normal or with background slowing only. Four antiepileptic drugs had no effect and long-lasting seizures occurred every 10-20 days. Based on clinical data, at the age 10 months the diagnosis of DS was proposed. The molecular genetic testing of the SCN1A gene detected a novel de novo mutation - c.3587_3588insCTTC in exon 18 of the gene. The mutation causes frameshift and the shifted mRNA is most probably degraded through nonsense-mediated mRNA decay mechanism.

J09.13
A novel mutation in SLC1A3 gene associated with episodic ataxia

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Episodic ataxia (EA) is an autosomal dominant genetically heterogeneous group of disorders characterized by episodes of incoordination and imbalance. Mutations have been mainly associated to KCNA1 and CACNA1A genes, causing EA1 and EA2. Mutations in other genes, CACNB4 and SLC1A3, have been identified in very few cases, causing EA5 and EA6. Here we describe a patient with episodic ataxia phenotype carrying a novel mutation in SLC1A3 gene, encoding for a glial glutamate transporter (EAAT1). The patient had episodes characterized by vertigo, intentional tremor, gait imbalance, dysarthria lasting from few days to a week, since the age of 30. At examination, when she was 50, she presented with gait ataxia, dysmetria, dysarthria, abnormal eye movements and cerebellar atrophy. DNA mutation screening for all four known EA genes revealed a heterozygous nucleotide substitution (c.227G>A) in SLC1A3 leading to a replacement of a highly conserved arginine to a glutamine (p.R76Q) located between two transmembrane segments. The pathological effect of this missense substitution on EAAT1 structure have been bioinfomatically evaluated. This variant was present in the proband's son showing nistagmus and reporting mild vertigo episodes, at age 40. The proband's daughter, normal at the clinical examination, doesn't carry the nucleotide change.
This variant was not detected in 418 Italian control chromosomes and it is reported in dbSNP with a very low frequency (2/13000 random chromosomes).
This is the third EAAT1 mutation so far reported for SLC1A3 gene associated to EA.
Supported by "NANOMAX PB.P04.006.005" to LV

J09.14
Does the imbalance between agonistic and antagonistic IL-1 play a role in progression of febrile convulsions?

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Objective: Inflammation may play an important role in the etiopathology of febrile convulsions (FC). IL-1 β is an important mediator of inflammation and fever is also important in formation of FCs. It is suggested that there may be a relationship between polymorphisms of IL-1 β and FC. The aim of the present study is to investigate the polymorphic stituation of promoter region of IL-1 β in two sites (-31 and -511) and assess the IL-1 RA VNTR polymorphisms in FC patients in comparison with healthy control groups.
Materials and Methods: Fifty FC patients and 50 healthy controls (HC) were included in the study. DNA extraction was performed by QIAamp DNA Mini Kit from peripheral blood lymphocytes of all subjects. IL-1β promoter polymorphisms were analyzed by PCR-RFLP, IL-1 RA VNTR polymorphisms were analyzed by PCR-agarose gel electrophoresis.
Results: Genotype distribution of IL-1 β promoter region in position -31 was statisticaly different between FC patients and control groups. Allele I and allele II of IL-1 RA distribution were also statisticaly different in FC patients and healthy controls.
Conclusion: We have found a significant association between IL-1 RA allele distribution and FC and a poor correlation of T/C substitution at the -31 position of IL-1 beta promoter in FC. Further studies are needed to investigate the gene expression levels and polymorphic situation in same samples.

J09.15
The emerging phenotype of FOXP1 haploinsufficiency

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Background The *FOXP1* gene, located on 3p13, has recently been involved in neurodevelopment. *FOXP1* haploinsufficiency has been reported in about 10 patients with specific facial features, global developmental delay, intellectual disability, and speech impairment, where expressive skills are more severely affected than receptive skills. **Case description** We report on a 2-year-old Caucasian boy, born at term with relative macrocephaly. He developed neonatal cholestasis for which an extensive etiological assessment was performed, including *JAG1* (Alagille syndrome) and *ABCB4* (PFIC3) gene analysis. Ductular paucity was objectified with progressive improvement and genetic work-up was normal. Progressively, global developmental delay was observed. He had axial hypotonia, dyskinetic movements and is still not able to walk. Expressive language has not yet been acquired, though receptive language appears more advanced. Growth is normal. He has frequent respiratory tract infections, bronchial hyperreactivity and gastro-esophageal reflux. Facial features include prominent and broad forehead, bilateral palpebral ptosis, broad nasal tip and arched palate. Hypoplastic nails are also observed. Chromosome molecular analysis using CytoScan HD-array (Affymetrix) revealed a *de novo* ~120kb heterozygous interstitial deletion at 3p13 covering exons 7 to 11 of the *FOXP1* gene. **Conclusion** Global developmental delay, expressive language delay, relative macrocephaly and specific facial features are associated with *FOXP1*. Our patient presents with bilateral ptosis, a sign previously reported in the literature in at least two other patients, and could be a clue for the diagnosis when present. Whether or not the liver involvement is an associated feature remains to be studied in larger series of patients.

J09.16
DXS998-DXS548-FRAXAC1 represents a novel informative haplotype at the FMR1 locus in the Iranian population

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Fragile X syndrome which is caused by mutation in the FMR1 gene region is one of the most prevalent forms of mental retardation. Direct diagnosis of the disease is based on PCR and southern blot analysis, but because of technical problems, use of polymorphic DNA markers can be helpful for carrier detection and prenatal diagnosis in families with an affected individual. The polymorphic markers usually show a population-based haplotype frequency and heterozygosity. In the present study, genotyping and analysis of haplotype frequency of three microsatellite markers including DXS998, DXS548 and FRAXAC at the FMR1 gene region were carried out in 140 unrelated healthy women and 26 families from the Iranian population. The data indicated the presence of a novel allele for DXS998 in the Iranian population. Estimation of haplotype frequency using Arlequin program showed 50 different DXS998-DXS548- FRAXAC1 haplotypes for the input date of 5, 7 and 4 alleles, respectively. Among these haplotypes four of them showed relatively high frequencies (≥0.05). Analysis of linkage disequilibrium (LD) for the unrelated individuals using the Powermarker computer program, showed that this haplotype combination can be an informative haplotype for linkage analysis in carrier detection and prenatal diagnosis of fragile X in the Iranian population.

J09.17
Effect of GAA repeat on CpG methylation at first intron of frataxin gene in Indian patients of Friedreich's ataxia

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FRDA is an autosomal recessive neurodegenerative disease characterized by progressive gait and limb ataxia, dysarthria, lower limbs areflexia. The onset of symptoms usually occurs before the age of 25 years, and typically around puberty. The expanded GAA repeat in intron 1 of the frataxin gene is the common cause of FRDA. Unaffected individuals have at least one allele with 8-33 repeats, while most individuals with FRDA have 90 or more repeats in both alleles resulting in deficiency of frataxin, an essential protein of mitochondrial iron metabolism. We studied the epigenetic alterations at first intron in the present study which is one of the mechanisms hypothesized to hinder the frataxin synthesis.
The study was conducted in 10 healthy controls and 15 Friedreich ataxia cases. Two microgram of extracted DNA was bisulfite treated and amplified with nested primers followed by standard Sanger's sequencing procedure and results were evaluated.
Average age of onset of patients was 22±5.5 years. Average size of alleles of patients and controls were 1200±400 and 12±3.5 respectively. No difference in methylation patterns were observed between the cases and the controls of Indian patients. The CpG islands methylated irrespective of GAA repeat number. However this study is limited in sample size which could be one of the factors that may over-represent the methylation phenomenon. We plan to further extend our study on more Friedreich ataxia patients to draw a strong conclusion along with methylation sites at the promoter region as they are more important for gene expression.

J09.18
Atypical parkinsonism and very early-onset dementia in a patient carrying two GBA gene in cis mutations

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Heterozygous glucocerebrosidase (GBA) gene mutations are a common risk factor for Parkinson Disease (PD). GBA mutation carriers display cognitive and neuropsychiatric disorders more frequently than PD patients without GBA mutations.
We describe a male patient carrying two GBA *in cis* mutations and affected by a severe early-onset parkinsonism associated with behavior disorders and cognitive decline.
The patient had been presenting apathy and memory loss since the age of 33. One year later he developed a bradykinetic parkinsonism partially responsive to levodopa therapy. SPECT study showed mild striatal presynaptic dopaminergic dysfunction. The neuropsychological assessment performed at 34 years of age showed deficits in all tested skills with worse performances in executive function and attention and relative sparing of visuospatial abilities; Mini Mental State Examination (MMSE) score was 26/30. At 37 years of age MMSE score was 22/30.
Brain MRI showed diffuse and symmetric brain atrophy with major involvement of the temporomesial lobes and the cerebellar vermis.
Testing of PARK2, LRRK2, DJ1 and IT15 genes was normal. GBA molecular analysis detected two *in cis* mutations in exon 10 (L444P and A456P) inherited from the father. These mutations are probably due to a recombination event between GBA gene and its pseudogene.
Clinical and neuropsychological examination of the father and family history were unremarkable.
Reports on GBA mutation carriers with parkinsonism may contribute to defining the cognitive phenotype of these patients, the possible genotype-phenotype correlations and the clinical markers that may be useful in identifying candidate subjects for GBA genetic testing.

J09.19
Next Generation Sequencing And Data Interpretation In Recessive Hereditary Spastic Paraplegia Patients From Turkey

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Hereditary Spastic Paraplegia (HSP) comprises a group of clinically and genetically heterogeneous neurodegenerative disorders. In 'pure' HSP, lower limb spasticity and progressive weakness are observed. 'Complicated' HSP additionally include neurological and non-neurological symptoms. HSP can be inherited in autosomal dominant, autosomal recessive, or X-linked manner. Thirty-two loci and 23 genes are associated with autosomal recessive form of HSP (ARHSP). One patient was selected for whole exome sequencing (WES) from each of six ARHSP families from Turkey. After determining candidate variations, segregation analyses were performed in the families to confirm that the variation can be responsible for the HSP phenotype. In two of the families mutations were identified in known HSP genes. First mutation is the c.4321C>T (p. A1394X) variation in Spastizin gene (SPG15) in family H61. Spastizin is the second most common causative gene for ARHSP with thin carpus callosum that is also observed in our patient. The other mutation is c.325_326insTGTC insertion in ALS2 gene in family H59. ALS2 gene is responsible for juvenile amyotrophic lateral sclerosis (JALS) and infantile onset hereditary spastic paraplegia (IAHSP). Four families were found to be negative for mutations in known HSP genes. In two of the families, novel HSP candidate genes were identified and segregation with the disease was shown in the families. For the remaining two families, WES data is still under analysis. Our WES analysis gives promising preliminary data for identification of novel genes in families from Turkey and it suggests evidence for further genetic heterogeneity in ARHSP.

J09.20

Novel mutation in SPG4 gene in three families with autosomal dominant hereditary spastic paraplegia from Bashkortostan Republic (Russia)

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Hereditary spastic paraplegias (HSP) - is a genetically and clinically heterogeneous group of neurodegenerative disorders characterized by progressive spasticity of the lower-limbs. The major pathological feature of HSP is a degeneration of the pyramidal tracts. The HSP frequency in Bashkortostan Republic (BR) is 3,5:100000. Autosomal dominant (AD), recessive (AR), or X-linked inheritance were described. To date, 30 causative genes and 50 loci have been identified. Mutations in the SPG4 gene are responsible for about 45% of the pure AD type of HSP and for 12-18% of the sporadic cases. SPG4 codes for spastin - AAA (ATPase associated with diverse cellular activities) family protein. Disorder onset ranges between 1 and 63 years. Over 300 mutations have now been described, with the majority of them being located in the AAA domain.
We examined HSP patients from BR and detected one rearrangement of DNA sequence in 12-th exon in patients from three unrelated families. This previously not described mutation is inversion translocation, starts at c.1469. Twelve exon codes the region that corresponds to a highly conserved ATPase domain, which is also called AAA cassette. This region is responsible for ATPase activity of the spastin protein. New polymorphism in 11-th intron (c.1413+43_46dup (TATA)) and polymorphisms (c.1098+118 A>G (rs 12617289) and c.1098+127 A>G (rs 12617290)) in 7-th intron were revealed in the same patients.
Large chromosomal rearrangements are frequent cause of SPG4 HSP. The mutation we found is among them. We supposed that this mutation was spread in BR by the founder effect.

J09.21

Two novel compound heterozygous mutations in ROBO3 gene in a sporadic case of Horizontal Gaze Palsy with Progressive Scoliosis

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The syndrome of horizontal gaze palsy with progressive scoliosis (HGPPS) is characterized by absence of conjugate horizontal eye movement and scoliosis. HGPPS is an autosomal recessive neurologic disorder caused by homozygous or compound heterozygous mutations in the ROBO3 gene on chromosome 11. Here we report a case of a 34-year young woman from non consanguineous parents. No history of scoliosis or gaze palsy in the other family members. At six months was noticed a fixation in her eyes. At the age of 9 years a dorsal-lumbar scoliosis was diagnosed and a physical examination showed absence of the horizontal eye movements and absence of nystagmus. The morphologic MR examination, the colour map DTI and the f-MRI showed typical signs of HGPPS. Similar results have been confirmed by neurophysiological tests (PESS, PEM). So we perform a molecular screening of the ROBO3 gene. Sequencing of the all 28 coding exons and relative intron/exon boundaries revealed two novel mutations: a heterozygous missense mutation and a splice-site mutation. The information provided by a multimodal approach (conventional RM, DTI, fMRI, neurophysiology, and molecular genetics) are crucial in confirming the clinical diagnosis, and when integrated with each other allow to clarify the possible different expressions of the lack of decussation of the cortical spinal tracts in the brainstem.

J09.22

Holoprosencephaly complicated by neurogenic hypernatremia

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Holoprosencephaly is defined as a structural anomaly of the brain in which there is failed or incomplete separation of the forebrain early in gestation. It occurs in 5-12/10,000 live births. Classic holoprosencephaly is classified as alobar, semilobar, lobar and middle interhemispheric variant typeș. It can result from environmental causes, an inherited or de novo chromosome abnormality, an inherited monogenic syndromic disorder, an inherited or de novo mutation for a gene associated with nonsyndromic autosomal dominant holoprosencephaly, copy number variations or unknown causes. We report the case of a 3 month old infant admitted for to oure clinic for sepsis, with characteristic clinical features of HPE. Lab tests revealed severe incoercible hypernatremia alternating with three episodes of severe hyponatremia. During admission,under the established treatment, the sepsis was remitted; nevertheless high natrium levels were maintained. In this case HPE was complicated by neurogenic hypernatremia. Genetic testing revealed a normal cariotype and the uncharacteristic phenotype of the mother ruled out autosomal dominant transmission. In the presented case, neurogenic hypernatremia was the consequence of impaired osmoregulation of ADH release due to malformations involving midline structures of the brain.

J09.23

Homozygosity in Huntington's disease

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Huntington disease is caused by a dominantly transmitted CAG repeat expansion mutation that is believed to confer a toxic gain of function on the mutant protein. Huntington disease patients with two mutant alleles are very rare. In other poly(CAG) diseases such as the dominant ataxias, inheritance of two mutant alleles causes a phenotype more severe than in heterozygotes. We identified a homozygous female patient, 89 years old and compared clinical features with those of a group of heterozygotes. The age of onset was within the range expected for heterozygotes with same CAG repeat lengths. However, she showed a rather different clinical course with emotional instability and behavioral disorders. Cerebral magnetic resonance imaging showed no evident atrophy, notably not in the cerebral cortex or the striatum. Clinical examination revealed she had chorea predominating on the upper limbs and the trunk. She was partially unable to control the speed and the force of her movements and mild dysarthria was noted. Severe memory deficits associated with a severe frontal deficit were found, all symptoms suggesting Huntington disease. Mutation analysis revealed two HTT alleles with CAG repeats of 43 and 45. This suggests that although homozygosity for the Huntington disease mutation does not lower the age at onset of symptoms, it affects the disease phenotype and progression. These data, once confirmed in a larger series of patients, will point to the possibility that the mechanisms underlying age at onset and disease progression in Huntington disease may differ.

J09.24

Gene expression profile in fibroblasts of Huntington's disease patients and controls

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Huntington's disease is an inherited disorder caused by expanded stretch of consecutive trinucleotides (CAG) within the first exon of the huntingtin gene (HTT) on 4p16.3. The mutated huntingtin (mHTT) gains toxic function, probably through mechanisms that involve aberrant interactions in several pathways, causing cytotoxicity. Pathophysiology of disease involves several tissues; indeed it has been shown that there is a broad toxic effect of mHTT in the peripheral tissue of patients with HD. In this study we compared gene expression profiles of HD fibroblasts and matched controls using microarray technology. We used RT-PCR to test the consistency of the microarray data and we found four genes up-regulated in HD patients with respect to control individuals. The genes appear to be involved in different pathways that have been shown to be perturbed even in HD models and patients. Our study shows that gene expression profiles seem to be altered in the fibroblasts of HD patients. Validation of the differential expressions at the protein level is required to ascertain if this cell type can be considered a suitable model for the identification of HD biomarkers.

J09.25

Identifying the genomic imbalance of individuals with mental retardation in Taiwan with G-banding, MLPA, and aCGH

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Mental retardation (MR) occurs in 1-3% of the general population and about one-half of the cases their etiology is still elusive, thus appropriate treatment and genetic counseling remains as great challenges. About 50% of idiopathic MR with IQ less than 50 is likely to have genetic defects. Some of them are involving genomic disorders. However, submicroscopic genomic disorders can not be detected by conventional cytogenetic analysis. With the recent advances in molecular cytogenetics analysis and genome-wide array-based comparative genome hybridization (aCGH) technology, submicroscopic genomic disorder bases of MR can be identified and characterized.
The array CGH profiles of 204 idiopathic MR patients in Taiwan have been

obtained. 28 MR patients were found to have genomic imbalance (microdeletion/microduplication) with a detection rate of 13.86%. Our finding is within or in agreement with the most recent multiple centers studies on chromosomal microarray study of MR. The identification of genomic imbalances in our study could lead to the identification of some pathogenic CNVs for MR. This could lead to the discovery of a network of neuro-developmentally associated genes. The informed network of neurodevelopmentally-associated genes will help us understand the etiology of MR disorders and will assist in diagnosing, managing and treatment of the disorders.

J09.26

Deletion in exon 15 of KCNQ2 gene responsible of Benign Familial Neonatal Seizures in a large Spanish family.

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Benign familial neonatal convulsion disease (BFNC, OMIM #121200) is a rare autosomal dominant inherited epilepsy characterized by unprovoked generalized or multifocal tonic-clonic convulsions. The convulsions typically start around the third day of life and resolve spontaneously after several weeks or months. In the majority of BFNC families the disease is linked to chromosome 20q13.3, and mutations of the voltage-gated potassium channel gene KCNQ2 have been identified as the underlying defect.
The proband is the first daughter of healthy non consanguineous parents. She was born by normal vertex delivery at full term after an uneventful pregnancy. Her birth weight, head circumference, and length were in a standard range. Her Apgar scores were 8 and 10 at 1 and 10 minutes, respectively. On the fourth day she manifested convulsions with apnea and clonic movements. The attacks were of short duration and ceased spontaneously. Clinically, the patient demonstrated no abnormal facial features, with normal skin, power, tone, and reflexes. Basic laboratory investigations produced normal results. Cranial ultrasound and magnetic resonance imaging also produced normal results. After interrogation, there were members of the family with a history of repeated seizures in their childhood,
Direct DNA sequencing of the KCNQ2 gene exons and their flanking intronic sequences, of the proband and members of her family, revealed a deletion in exon 15 of a cytosine nucleotide; c.1960 in the amino acid 595 (NM_172107), that is predicted to cause a frameshift in the protein. This mutation has not been previously described in literature.

J09.27

Mutational analysis of cathepsin F and CLN6 genes in a Japanese patient with Kufs disease, an adult onset neuronal ceroid lipofuscinosis

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Kufs disease is an adult onset neuronal ceroid lipofuscinosis, which is autosomal recessive progressive lysosomal disorders. Kufs disease are clinically divided into two types; Type A and B. Type A presents with progressive myoclonus epilepsy, whereas type B presents with dementia and motor abnormalities. Recently, the genes responsible for Kufs disease are discovered. Mutations in CLN6 are the major cause of recessive type A, whereas mutations in cathepsin F is the cause of type B.
In the past, we have reported a type B Kufs patient, whose parents were consanguineous marriage (Sakajiri K et al. Intern Med, 1995;34:1158-63). Here we present the first mutational report from Japan. In this study, we performed mutational analysis of responsible genes for this Kufs patient. We found several SNPs in cathepsin F gene, and a known but homozygous mutation (c231C>G, pN77K in exon 3) in CLN6 gene. PCR-RFLP analysis revealed that the mutation was not a SNP. The amino acid is perfectly conserved between human, mouse and rat. Furthermore in silico analysis using PolyPhen2, the mutation is predicted to be probably damaging. These data suggest that the mutation must be pathogenic one. Since we found a known but homozygous mutation in CLN6 gene in a type B patient, genotype-phenotype correlation seems to be complex than we expected.

J09.28

The Association of Migraine and Endothelial Nitric Oxide Synthase Haplotypes

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Migraine is a complex and debilitating neurovascular disorder predominantly affecting women. It recurs as attacks of severe headache associated with nausea, vomiting, phonophobia, and photophobia. Despite its high prevalence, migraine is a disease involving complex pathogenetic mechanisms that remain to be clarified. As other multifactorial diseases migraine also has a genetic basic combined with triggering environmental factors. There is strong evidence implicating nitric oxide (NO) in the pathophysiology of migraine. Therefore, genetic polymorphisms in the endothelial NO synthase (eNOS) gene have been studied as candidate markers for migraine susceptibility. NO plays an essential role in the control of cerebral blood flow and may be involved in the activation of nociceptors in the trigeminovascular system and release of vasoactive neuropeptides during neurogenic inflammatory response. There are several studies on the association of eNOS gene variations and the risk of migraine in different populations. In the present study, we focused on one of the most common single nucleotide polymorphisms (SNP) of the eNOS gene. The prevalence of the rs1799983 in Iranian population has been evaluated by ARMS-PCR method. We have not found interaction between the selected SNP and the risk of the migraine in 90 patient samples compared to 113 normal individuals.

J09.29

Plasminogen Activator Inhibitor Haplotypes Associated with Migraine

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Migraine is a common disorder affecting approximately 16-10% of the world population, almost three times more women than men. It is characterized by recurrent moderate to severe headaches often in association with a number of autonomic nervous system symptoms. Studies of twins indicate a 34 to 51% genetic influence of likelihood to develop migraine headaches and a number of specific variants of genes have been established to increase the risk by a small to moderate amount. Like other multifactorial diseases genetic basics of migraine are combined with triggering environmental factors.
There are some data supporting the involvement of endothelial, haemostatic and haemorheological functions in the pathogenesis of migraine. Plasminogen Activator Inhibitor subtype 1 (PAI-1) gene encodes a member of the serine proteinase inhibitor (serpin) superfamily, concentrations of the gene product are associated with thrombophilia. Inasmuch as it's plasma level decreases in migraine, PAI-1 seems to play a determinant role in vascular diseases related to migraine.
In the present study, we focused on one of the most common single nucleotide polymorphisms (SNP) of the PAI-1 gene. The prevalence of the rs2227631 in Iranian population has been evaluated by ARMS-PCR method. We have not found a significant association ($P<0.05$) between the selected SNP and the risk of the migraine in 98 patient samples compared to109 normal individuals.

J09.30

Identified a neonatal Spanish patient with Miller-Dieker Lissencephaly Syndrome by MLPA

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Miller-Dieker syndrome (OMIM #247200); 17p 13.3 deletion with haploinsufficiency of PAFAH1B1) is a contiguous gene deletion syndrome that is characterized by lissencephaly, facial dimorphisms, growth restriction and seizures. PAFAH1B1 encodes LIS1, a protein essential for neuronal migration, proliferation and survival. It participates in a complex to regulated spindle orientation during neuronal progenitor divisions.
The patient is the first child of healthy non consanguineous parents. He was born at full term after an uneventful pregnancy. His birth weight, head circumference, and length were in a standard range with Apgar index of 8/10. Clinical examination at birth only showed undescended testicles as remarkable aspect. At 4 months old male referred for evaluation because of hypotonia, microcephaly and developmental delay. Cranial sonography sho-

wed agyria-pachygyria with thick cortex, a less severe form of lissencephaly. Electroencephalographic monitoring showed a diffuse dysfunction for the age of the patient.
DNA was isolated from EDTA-blood collection by standard protocol and the diagnosis of deletion of exon 3 and exon 5 of the PAFAH1B1 gen was established by MLPA. The array-CGH cytochip focus constitutional array (Cambridge Bluegnome) showed no evidence of a clinically significant deletion or duplication (result: ISCN: arr (1-22)x2, (XY)x1) with a medium resolution of 1Mb in the whole genome (backbone) and 100Kb in 97 regions associated with birth defects OMIM of 143 genes. In our case phenotype was mild than in a classical Miller-Dieker syndrome probably due to a small deletions in PAFAH1B1 that are not detected by array analysis.

J09.31
Association of HLA-DRB1 alleles with Multiple Sclerosis in patients from Bogotá, Colombia

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Multiple Sclerosis (MS) is a chronic autoinmune inflammatory disease that attacks the central nervous system. Multiple factors are associated with the development of this disease, many of them related to the environmental and to the personal genetics. It has been shown a closed relationship between the HLA-DRB1 variant, HLA-DRB1*1501, and the pathology. In fact, the presence of HLA-DRB1*1501 increases the risk to present it. The aim of this study is to find the association of HLA-DRB1 alleles with MS in patients from an heterogeneous population as the one we have in Bogotá, Colombia. The study population is composed of 99 patients diagnosed with MS and 198 healthy controls. Every participant signed an informed consent and the inclusion and exclusion criteria were confirmed by a professional. Peripheral blood lymphocytes were used to obtained DNA, and the variant determination was done using a PCR-SSP. We found that the HLA-DRB1*1501 allele confers a significant higher risk of suffering MS (OR= 2,89; 95% CI= 1,60 - 5,19); in contrast, HLA-DRB1*1401 allele exhibit a protector effect (OR=0,29 ; 95% CI= 0,11 - 0,76) in our population. The association of HLA-DRB1 alleles with MS will enrich our knowledge of the disease and will focus new approximation to a better diagnosis and treatment.

J09.32
Genetic characterization of narcolepsy patients from Estonia

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Human narcolepsy is a complex genetic disorder, characterized by excessive daytime sleepiness, cataplexy, hypnagonic hallucinations, sleep paralysis, and disturbed nocturnal sleep. Most cases are sporadic and approximately 0.02% of the population is affected worldwide. An autoimmune mechanism for the disease has been suspected based on its strong association with the genetic marker - HLA DQB1∗06:02. Recent studies support this hypothesis. To date thousands of patients and matched controls have been genotyped from different populations and genome-wide association studies (GWAS) carried out. Significant associations with narcolepsy include polymorphisms in the *TCR* alpha, *CTSH*, *TNFSF4*, 3´untranslated region of *P2RY11*. Nevertheless, the overwhelming portion of risk and protection is still found within HLA region. Other common variants found through GWASs have little contribution.
Narcolepsy in Estonia is presumably underdiagnosed disorder. Patients are diagnosed by standard protocols, including assessment of excessive daytime sleepiness, polysomnography and test for Multiple Sleep Latency Time. Hypocretin levels in CSF were not measured.
Estonian Genome Center has an is ongoing project to gather narcolepsy patients all over Estonia. To date we have 17 patients, sent by sleep doctors: age 19-66, 10 men and 7 women. In these patients *DQA1*01:02-DQB1*06:02* variants were tested and 50% of patients with cataplexy were *DQB1*06:02* carriers.
Simultaneously, we are using control individuals from our database, who were genotyped by either Illumina ImmunoChip (1000 individuals) or Illumina HumanExome Beadchip (4400 individuals), to identify the DQB1variants and assess the frequency of *DQB1*0602* in general population. Distribution of other significantly associated SNP alleles will also be presented.

J09.33
Two novel mutations in NF1 identified in Mexican patients with neurofibromatosis type 1

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Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder with worldwide incidence of 1 in 2500 to 1 in 3000 individuals. It is caused by mutations in neurofibromin 1 (*NF1*), located on chromosome 17q11.2. The main signs and symptoms are café-au-lait spots, Lisch nodules in the eye, and fibromatous tumors of the skin. Other clinical manifestations that have been reported include bone dysplasia and an increased risk of malignant tumours.
The aims of this study were to describe the clinical manifestations of the disease in 22 patients with neurofibromatosis type 1, and to identify the underlying *NF1* mutations, by sequencing all 60 exons of this gene. Five different mutations were identified in 7 patients. Two of these mutations have not been previously described (Table 1).
Given the clinical variability of this disorder, and the small sample size, no statistical genotype-phenotype correlations were preformed. However, the prevalence of clinical manifestations and the type of mutations seen are in agreement to what has been reported in other populations.

Sample	Exon	Mutation	Mutation Type
25	28	c.3810_3820delCATGCAGACTC	Deletion (new)
29	30	c.4077_4078insG	Insertion (new)
39	24	c.3194_3195insA	Insertion
38	37	c.5257_5258delG	Insertion
40	51	c.7549C>T	Nonsense
41	51	c.7549C>T	Nonsense
42	51	c.7549C>T	Nonsense

J09.34
Mutational spectrum of the NF1 gene in Greek patients with Neurofibromatosis Type 1

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Neurofibromatosis Type I is one of the most common autosomal dominant disorders with a birth incidence of one in 3,500 individuals worldwide. It is caused by mutations of the NF1 tumor suppressor gene, located at 17q11.2. Neurofibromin, the NF1 gene product, is an important negative regulator of cellular Ras signaling pathway. The main clinical features of the disease include café-au-lait spots, skinfold freckling, benign cutaneous neurofibromas, plexiform neurofibromas, optic gliomas and Lisch nodules of the iris. Disease penentrance is about 100%, while expressivity is extremely variable.
In our study we focused on the elucidation of the genetic causes underlying the disease. A multi step protocol based on genomic DNA has been established for molecular diagnosis in Greek patients fulfilling the NHI diagnostic criteria. This protocol includes multi-step PCR, sequencing of all NF1 gene exons and multiplex ligation-dependent probe amplification for deletions/ duplications. The protocol was validated in a cohort of 34 NF1 patients and 20 relatives, identifying the germline mutations in most cases.
Our results include 13 genetic variants, in coding and non-coding regions of NF1, most of them already reported in Human Gene Mutation Database. Among the novel variants the majority were stop codon mutations or variants which may affect the splicing process. Some novel mutations were found in patients with unusual phenotypes. All novel variants were analyzed with the use of bioinformatic tools. In an attempt of possible phenotype/ genotype correlations, we detected different mutations in patients within the same family, indicating the genetic complexity of the disease.

J09.35
Missense mutation (Met1035Arg) of the NF1 gene revealed through NGS

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Neurofibromatosis type 1 (NF1) is an autosomal dominant condition, with a high allelic heterogeneity of the *NF1* mutations and variable expressivity. We present a case of a male, with a diagnosis of the NF1, first made on clinical grounds by observation of his phenotype at age of 3, although the causal mutation was identified much later. Family history was negative. The patient has had hypotonia since neonatal period. By the time the boy was one year old first café-au-lait spots were developed and more than 11 spots came into being till adolescence. Freckles, neurofibromas, glioma, Lisch nodules were not present. He was shorter than average. Later he has had learning difficulties (ADHD) and sporadic migraine. He was referred to a geneticist to specify the diagnosis at the age of 14. The young patient has been tested through Next-Generation-Sequencing (NGS), which allows detection of a much higher level of detail of the NF1 gene. NF1 diagnosis was confirmed by using the MiSeq system and the TruSight Cancer sequencing panel (Illumina). Sequencing of exon 23 revealed the missense mutation c. 3104T›G (p.M1035R). The mutation was absent in the parents. Although missense mutation can be associated with a mild progress of NF1 condition the effect

of apparent *de novo* mutation is difficult to predict. Confirmation of NF1 diagnosis may provide a possibility of more specific health supervision for the patient's condition and monitoring of potential long-term complications.

J09.36

Influenza A virus infection changes the expression of NMDA receptor subunits in mice

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N-methyl-D-aspartate receptors (NMDARs) play an essential role in the process of synapses formation, neuronal differentiation, brain plasticity as well as participate in the molecular response to the neurotoxic substances action. The NMDAR dysfunction is associated with schizophrenia, Alzheimer's disease, and Huntington's disease. The influenza virus infection is also one of the potential negative factors influencing NMDAR function, but the molecular mechanisms of neurological complications of influenza are not well understood. The aim of our research was to study the influence of influenza A virus infection on the expression of NMDAR subunits (NR1, NR2(A-D), NR3(A-B)) in mice. We analyzed the expression of seven genes, coding NMDAR subunits, in brains and lungs on the 1, 3, 5 and 10 days post infection using real-time PCR. Two groups of mice were infected with H1N1 and H3N2 strains at 0,2 LD50 respectively. The expression of all seven NMDAR transcripts was detected only in brains of non-infected animals. The infection with influenza A virus strain led to the changes in expression levels of NR2A, NR2B, NR2D and NR3B mRNAs in brains. In lungs NMDAR mRNAs were not detected. Further we are going to make the more detailed analysis not only of NMDARs expression but also of other genes involved in the regulation of the central nervous system during influenza A virus infection in mice.

J09.37

New insights in non-syndromic intellectual disability: a case report

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A 15 years old girl with obesity, dysmorphic features (down-slanting palpebral fissures, short nasal bridge; short philtrum; micrognathia), moderate mental retardation, psychosis, dyslalia, hyperkinesis, aggressivity, bilateral hypoacusis was referred for genetic investigations. aCGH on an 105K Agilent platform revealed arr 5p13.2(37,479,936-38,118,402) x3,11q13.5q14.1(76,158,015-83,043,032)x1, 17q25.3(79,829,409-79-,910,442)x1. Interestingly, the ~6.88 Mb deletion on 11q includes 21 OMIM genes, among which two disease-causing entities: *MYO7A*, and *ALG8.* The first one encodes for an unconventional myosine, a motor molecule with structurally conserved heads moving along associated actin filaments and highly divergent tails suggested to act as carriers of various macromolecular cargo molecules; *MYO7A* is known to carry heterozygous mutations in nonsyndromic neurosensorial deafness and homozygous mutations in most cases of Usher syndrome. The second gene encodes for alpha-3-glucosyltransferase which, when mutated to compounded heterozygosity, produces the congenital disorder of glycosylation, a severe phenotype resulting in multiple dysmorphisms and defects leading to early infant death. The 0.63 Mb duplication on 5p harbors only one OMIM gene, *GDNF*, encoding for a specific dopaminergic protein associated with motor neuron function and Hirschsprung disease. Finally, the 0.08 Mb deleted fragment on 17q contains 8 OMIM genes, among which pyrroline-5-carboxylate reductase 1 that causes cutis laxa disease when it losses functions. The correlations of the identified genetic defects with the observed phenotype will be discussed, as well as their impact upon patient management.

Acknowledgements: project PN 09.33.02.03.

J09.38

Report of a novel compound heterozygous mutation in a mexican family with Niemann Pick Type C disease

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Introduction. The disease of Niemann Pick type "C", is an alteration in storage lisosomal, characterized for decline neurological progressive and death premature. He has un pattern of recessive autosomal inheritance, caused by mutations in the NPC1 and NPC2 genes. Clinical manifestations include, mental decline, ataxia, cataplexy, dystonia, vertical supranuclear gaze palsy(VSGP) and clumsiness. Case report. Female patient of 29 years old, daughter of the fifth pregnancy of non-consanguineous healthy parents. Family history: One sister died at age of 25-years-old with the same disease. The patient initiate at 17-years-old with, loss progressive of memory, cognity disorders, disartria, and dysphagia . At the 22-years-old, she presented coreics and dystonics movements of upper and lower extremities and gait problems. Phisical exploration: she had ataxia and dystonics and coreoatetosics movements and VSGP. The score performed for NPC was 201 points. The abdominal ultrasound showed splenomegaly and the magnetic resonance cortical atrophy. Methods. It was performed PCR and sequencing of 25 exons of the NPC1 gene and the five exons of the NPC2 gene. This study was carried out in parents and health sister of the patient. Results: We detected two different pathogenic mutations: [c.2604+1G>A] + [c.3630T>G], compatible with compound heterozygous genotype. The NPC2 gene sequencing was normal. Conclusion: This is the first case informed in Mexican population of Niemann-Pick type C. Miglustat is the important therapy in NPC disease.

J09.39

Identification of The Human Natural Resistance-Associated Macrophage Protein 2 (NRAMP2) Gene Polymorphism in Schizophrenia Patients

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Natural resistance associated macrophage protein 2 (NRAMP2), also known as divalent metal transporter 1 (DMT1) is responsible for the uptake of iron and iron translocation from the endosome. The regulation of metal ion transport within neurons is critical for normal brain function. Iron defciency is in charge of long-term results of behavioral abnormalities especially in fetal hippocampus and striatum. Five single nucleotide polymorphisms are identified within the NRAMP2 gene. One of them 1303C/A occurs in the coding region of NRAMP2 and results in an amino acid change from leucine to isolecine. Mutations or polymorphisms of NRAMP2 gene may affect the iron metabolism and have an impact on human health. So, we were investigated of one-single-nucleotide mutation (1303C/A) polymorphism in schizophrenia firstly by us.

The NRAMP2 1303C/A polymorphism was analyzed by PCR restriction fragment length polymorphism analysis (PCR-RFLP) in 100 subjects (scizophrenia patients: n=50; healthy controls: n = 50) of Turkish origin. PCR products were digested with SfaNI restriction enzyme. The 362 bp PCR product is digested to a 197 and 165 bp fragment when the normal 1303C is present. Comparisons of the data were performed by chi-square test. We were found that no significant differences in allele (for C allele χ^2=1.01, P=0.315; for A allele χ^2=1.77, P=0.018) and genotype (χ^2=3.95, P=0.138) frequencies. The study was supported by ESOGU (Grant no:201211007)

J09.40

PAC1 gene associated with PTSD: preliminary data

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The study tested the association between the development of PTSD and genetic variants of ADYAP1R1 (PAC1): C/C vs C/G vs G/G in survivors to 6 April 2009 L'Aquila earthquake. The sample examined so far consists of 103 subjects. Cells of the buccal mucous were collected to obtain DNA and Clinician-Administered PTSD Scale (CAPS) test is administered.

It has been conducted an analysis of variance (ANOVA) with LSD Post Hoc test to assess differences between groups in terms of CAPS scores compared to the three polymorphic variants. In line with the literature, the results suggest a strong role of SNP rs2267735 of PAC1 C/C genotype in the regulation of physiological response to stress, showing as a risk factor for the development of post-traumatic symptoms after a natural disaster. Further genetic investigations are still ongoing concerning: Val66Met BDNF allele and the S allele of 5HTTLPR , in addition to evaluation of various psychometric scales : the Hamilton Depression Scale (HAM-D), Hamilton Rating Scale for Anxiety (HAM-A), Mini-International Neuropsychiatric Interview (MINI), Temperament and Character Inventory (TCI-R), Toronto Alexithymia Scale (TAS -20) and Childhood Trauma Questionnaire (CTQ). These data will be subsequently analyzed in order to verify other possible associations in the development of PTSD. These data to shed light on the role of genetic factors in the formation or modulation of the stress response.

J09.41

The role of Vitamin D and FokI Variant in the Vitamin D receptor Gene among Iranian Parkinson's Disease Patients

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Introduction: Today, A role for vitamin D and it's receptor (VDR) in Parkinson's disease (PD) has been proposed. Several studies concerning the association between the VDR gene polymorphism and PD have been published. In this study, firstly we determined the correlation between 25 hydroxy vitamin D (25 OHD) and severity of PD then accessed frequency of FokI polymorphism in PD patients and healthy Iranian population.
Methods: The severity of PD was evaluated by using Hoehn & Yahr (HR) stages and Unified Parkinson's Disease Rating Stage (UPDRS) Part III. After amplification of DNA samples from 62 unrelated normal individuals and 60 PD patients by PCR, polymorphism was genotyped by RFLP according to FokI site.
Results: Our study revealed that 25OHD level in patients with PD was not associated with HR stage and UPDRS scores . The most frequency of genotype in PD group and normal individuals was FF (51.7%) and Ff (51.6%), respectively. Protection against the development of PD was applied when F allele be present, but it was not significant (OR= 0.7, 95%CI: 0.44-1.28, p=0.29). In contrast, insignificant susceptibility to PD (OR= 1.3, 95%CI: 0.437-1.283, p=0.29) was associated to f allele.
Conclusion: Our finding does not show correlation between vitamin D and HR stage and UPDRS scores during the early disease stages of PD in Iranian patients. Furthermore, after genotypes analysis, no significant association was seen for FokI polymorphism with Parkinson . Further studies are needed to reveal the main role of vitamin D and VDR gene in PD progression.

J09.42

Alpha-synuclein 3´UTR polymorphism is a risk factor for Parkinson's disease in Iranian population

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders and both genetic and environmental factors are involved in its etiology. The incidence of PD differs by race and ethnicity, is greater in men than women, and increases markedly with age. One of the well-known genes involved in PD development, is alpha-synuclein (SNCA) gene and several polymorphisms in this gene have been identified to be associated with PD susceptibility. In this study, we investigated the association of four SNPs (rs2301134, rs2301135, rs356221, rs11931074) in the promoter region and 3´UTR of the SNCA gene, with Parkinson's disease in Iranian population. A total of 960 subjects (480 PD patients and 480 normal healthy controls) were included and genotyped for all four polymorphism. The method used for genotyping was tetra-primer ARMS PCR. We found significant differences in genotype distributions of rs11931074 variant in patients compared to controls (odds ratio = 7.6970, 95% CI = 5.7514-10.3006, p = <0.0000001). Our findings indicate a significant association of rs11931074 SNP with PD in Iranian population.

J09.43

Association analysis of Parkinson's disease with polymorphic variants in SNCA, LRRK2 genes and MAPT-region (17q21.31) in three ethnic groups from Bashkortostan Republic of Russia (GWAS replication results)

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We performed replication of genome-wide association analysis (GWAs) with Parkinson's disease (PD) in three ethnic groups from Bashkortostan Republic (BR) on 550 PD patients (Russians - 215, Tatars - 243, Bashkirs - 90) and 622 controls (Russians - 190, Tatars - 338, Bashkirs - 94). The study included analysis of 5 polymorphic loci: rs653219 in SNCA gene, rs1491942, rs1907632 in LRRK2 gene, rs1981997 and rs12373139 in chromosomal region of MAPT gene (17q21.31).
The results of GWAs received on the indicated polymorphic loci of SNCA gene and MAPT-region were confirmed only for Tatar ethnic group. We found that genetic markers for PD development were genotype C/T (p = 0,002; OR = 1,73; CI = 1,21 - 2,46) and allele T (p = 0,04; OR =1,4; CI=1,01 - 1,8) of SNCA gene (rs653219); genotype G/G (p=0,001; OR=1,84; CI=1,16 - 2,92) and allele G (p = 0,01 ; OR=1,72; CI =1,11 - 2,65) of MAPT gene (rs1981997); genotype G/G (p=0,02; OR=1,7; CI=1,09 - 2,64) and allele G (p=0,04; OR=1,53; CI=1,02-2,3) of SPPL2C gene (rs12373139, MAPT-region). There was no association of PD with rs1491942 and rs1907632 in LRRK2 gene in the investigated ethnic groups.
The possible reasons for the discordance between results in different ethnic groups and previous GWAs may include effects of population structure and population-specific environmental interactions. Our findings suggest that additional studies are necessary to establish the role of these loci in modifying risk for Parkinson's disease in different populations.

J09.44

Molecular basis of mechanism of gene expression modulation induced by hypericin in genes involved in the pathogenesis of Parkinson's disease

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Parkinson's disease is a multifactorial heterogeneous neurodegenerative disorder manifested particularly in the elderly people. Although originally thought not to be hereditary, recent studies have identified several genes that are responsible for the manifestation of the disease. Clarification of the molecular basis of disease would allow more accurate diagnosis of the disease and would also help in treatment and prevention. The aim of our work was to study the effect of hypericin, a substance isolated from the plant Hypericum perforatum L., on expression modulation in genes involved in the pathogenesis of Parkinson's disease. Experiments were carried out on the A549 (alveolar adenocarcinoma), 42-MG-BA (glioma) and MSC (mesenchymal stem) cell lines using microarray and qPCR. Our results show that hypericin can modulate expression of more with Parkinson's disease associated genes. We focused on 8 genes, which play main roles in Parkinson's disease pathway. We detected prolonged changes in expression of DDC, LRRK2, CYCS and short term changes of other genes during 96 hours of monitoring. Our results may help in understanding the molecular mechanism of hypericin action in various neurodegenerative diseases as Parkinson's and Alzheimer's disease. Our results may help to indicate a potential of hypericin to be implemented as a medicine or prevention of major neurodegenerative diseases.

J09.45

RIT2, a susceptibility gene for Parkinson's disease in Iranian population

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Parkinson's disease (PD) is the second most common and a complex neurodegenerative disorder arising from a complex interaction between genetic and environmental factors. Determining the pathogenic gene contributed to Parkinson's disease have always been controversial. In a large meta-analysis study on Caucasian populations another novel PD susceptibility locus, RIT2, on chromosome 18, was identified. A genetic variant in RIT2, rs12456492, was found to be associated with risk of sporadic PD. In the present study, we investigated the association of the rs12456492 variant with Parkinson's disease in Iranian population. A total of 960 subjects (480 PD patients and 480 normal healthy controls) were included and genotyped for rs12456492 polymorphism (A/G) of RIT2 gene. Genotyping was done by tetra-primer ARMS PCR technique. We found significant differences in distributions of GG genotype in patients compared to controls (odds ratio = 1.60, 95% CI = 1.14-2.23, p = 0.005). Our findings increase the likelihood of association between PD and RIT2 variant in Asian populations.

J09.46

Partial duplication Xq27.1 in a dysmorphic and mentally retarded girl

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Background. Duplications of the long arm of chromosomes X have been related to mental retardation, different dysmorphic features, hypopituitarism and short stature.
Aim. We aim to examine the relation between the partial duplication of the Xq27 region and the presence of clinical manifestation in in a dysmorphic and mentally retarded girl.
Methods. We report on a 3 year old girl associating facial dysmorphism, hypotonia, delayed milestones, mental retardation. Proposita is the first child of a young couple, born at 39 weeks of gestation by caesarean section due

to pelvic presentation. Birth parameters were: weight 2850 g, length 52 cm, cranial circumference 31 cm.
Results. A good evolution was recorded after birth, with no sucking or swallowing difficulties. Craniofacial dysmorphisms includes: microcephalia, hypertelorism, astigmatism, wide nasal base, low set ears, carp shaped mouth. At age of 3 she cannot walk without support, respond to simple commands, says only 2-3 words. The girl is sociable and presents a happy attitude. EEG, EMG and MRI were normal. Conventional cytogenetic investigation showed a normal female karyotype. Revealed a 408 kb duplication on chromosome Xq27.1. The duplication encompasses SOX3 and CDR1 genes.
Conclusion. Further studies including the analysis to prove the X inactivation pattern will be done, knowing that a preferential inactivation of the aberrant chromosomes X in female preventing genetic imbalance is often reported. Array CGH was an essential tool for molecular characterization of this case and for establishing the recurrence risk for a future pregnancy.

J09.47
A 9 year-old boy diagnosed with Pantothenate Kinase-Associated Neurodegeneration after a 6 year history of psychiatric disturbances

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Pantothenate Kinase-Associated Neurodegeneration (PKAN) is a form of neurodegeneration with brain iron accumulation (NBIA) formerly called Hallerworden-Spatz disease. PKAN is an autosomal recessive condition characterized by iron deposition in the basal ganglia leading to progressive extrapyramidal dysfunction as dystonia, rigidity and/or choreoathetosis, pigmentary retinopathy, psychiatric disturbances and speech defects. PANK2 is the only known gene related to PKAN. MRI shows the highly specific "eye of the tiger" sign in almost all affected individuals with at least one PANK2 mutation. We report a Danish family in which a 9 year-old boy, of a sibship of 4 children, has been diagnosed with PKAN after a 6 year history of psychiatric disturbances. At the age of 2-3 years the patient developed behavioral problems, and was diagnosed with Attention Deficit/Hyperactivity Disorder (ADHD), and the first MRI was performed. At the age of 3-4 years the psychomotoric development slowed combined with some speech problems, and the patient was diagnosed with mild mental retardation. At the age of 7 he is diagnosed with infantile autism and the second MRI was performed, where the "eye of the tiger" sign was reported. At the age of 9 the patient developed choreoatic dystonia in the right arm and the right side of the neck, and the third MRI was performed confirming the "eye of the tiger" sign. Genetic analysis was performed and revealed compound heterozygotic for a pathogenic mutation in exon 5 and a deletion of exon 2 of the PANK2 gene, confirming the diagnosis of PKAN.

J09.48
Whole exome sequencing analysis in a large Primary Angle Closure Glaucoma (PACG) pedigree

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Glaucoma is a heterogeneous group of optic neuropathies characterized by progressive degeneration of the optic nerve head and visual field loss. It is sub-grouped according to the anatomy of the anterior chamber angle into two main forms: Primary Open Angle Glaucoma (POAG) and Primary Angle Closure Glaucoma (PACG). POAG is the more predominant form of glaucoma in Europeans, Africans, and possibly most populations. PACG is most prevalent in countries of the far East; 80% of PACG affected individuals live in Asia. PACG is characterized by a narrow iridocorneal angle which obstructs normal aqueous humor outflow and causes increased Intraocular Pressure (IOP). Although epidemiological studies have suggested a genetic basis for PACG, a causative gene for the disease has not been identified.
A relatively large pedigree with at least nine members diagnosed with PACG or PACG suspect all of whom presented with a closed iridocorneal angle, was introduced to us. Manifestation of PACG in multiple family members suggested a strong genetic contribution for the disease in the family. With the objective of identifying a causative gene, whole exome sequencing was performed on three affected members of the family. Output data were analyzed with appropriate softwares. After filtering against existing SNP databases, 72 novel, non-synonymous variants common among the three patients were identified. The variations were distributed in 68 genes. Some of these genes have defined functions related to eye and glaucoma pathology. Screening of the variations in control individuals and segregation analysis in the pedigree are in process.

J09.49
Sequence variants of PRNP gene in probable prion disease patiens

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Prion diseases are a family of rare progressive neurodegenerative disorders, caused by abnormally conformed infectious proteins, called prions. The functions of these normal prion proteins are still not completely understood. The abnormal folding of the prion proteins leads to brain damage and the characteristic signs and symptoms of the disease. There are 4 types of this disease: the sporadic (Jakob-Creutzfeldt disease, 85-90%), familial/genetic (10-15%), iatrogenic (1%), and variant types. Genetic form of prion diseases are caused by mutations in the prion-related protein gene (PRNP), and classified based on the phenotype, mutation and neuropathological findings. In this study, we analyzed PRNP gene to evaluate the frequency of PRNP sequence variants and their genetype-phenotype correlation in 60 probable Prion disease patients. Genetic analysis of the PRNP gene was performed on peripheral blood samples using polymerase chain reaction and direct-sequencing. We found four different PRNP sequence variants in thirty-six patients (G54S, M129V, D178N, octapeptide repeat deletion: c.247_270del). The rate of PRNP sequence variant was 60% in our samples. PRNP screening may be useful for genotype-phenotype correlation in Prion disease.

J09.50
Eight new cases of PEHO or PEHO-like syndrome?

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PEHO syndrome (**P**rogressive **E**ncephalopathy with oedema, **H**ypsarrhythmia and **O**ptic atrophy) (OMIM#260565) belongs to a rare neurodegenerative disorders of unknown etiology and probable autosomal recessive inheritance. Only 50 patients have been described worldwide so far, with the vast majority in Finland. We present eight new patients who fulfill diagnostic criteria of PEHO or PEHO-like syndrome. Patients were selected from a group of patients with a diagnosis of "Progressive encephalopathy of unknown etiology" or "Cerebral palsy". In all of them onset occurred during the first few weeks or months of life. The leading symptoms were hypotonia, poor feeding, drowsiness, infantile spasms, seizures with hypsarrhythmia in EEG, absent eye contact, optic atrophy, no any progress in psychomotor developmental. Dysmorphic features were peculiar and consisted of: microcephaly, "pear-shaped" face, narrow forehead with prominent metopic ridge, full cheeks, receding chin, epicanthic folds, an open mouth with a curved upper lip, hypertrophic gums, protruding ear lobes, a short nose with anteverted nares, edema of upper and lower extremities and tapering fingers.
In six patients cerebral, cerebellar and/or brainstem atrophy were found by MRI. This group was named as PEHO syndrome and rest two child's without any abnormalities in MRI as PEHO-like.

J09.51
Association of GRM3 gene polymorphic loci with schizophrenia

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Schizophrenia is a devastating psychiatric disorder with a morbid risk of 7.2 per 1,000 which is affected by genetic and environmental factors. The disruption of the glutamatergic system is becoming recognized as an essential component of the pathogenesis of schizophrenia .The type-three metabotropic glutamate receptor gene (GRM3) which codes for the mGluR3 protein localized to the periphery of pre- and post-synaptic neurons is essential for optimal signaling of glutamate in the brain.
The subject of the present study was the research of the association of two polymorphic loci rs274622 and rs187993 of GRM3 gene with the development of schizophrenia in a sample of 338 cases (50% Russians and 50% Tatars) and 350 controls (50% Russians and 50% Tatars) from Volga-Ural region of Russia. The genotyping of these polymorphic loci was carried out by PCR-RFLP. All observed genotype frequencies were consistent with Hardy-Weinberg equilibrium. It was found by analysis of variance that genotype GRM3*G/*G of polymorphic locus rs187993 was a statistically significant risk marker of schizophrenia development in Russian ethnic group (OR=2,16; P=0,043). However polymorphic locus rs274622 of GRM3 gene was not shown statistically significant differences between case-control groups of Russians and Tatars.

In addition this study can be useful for understanding of the pathogenesis of schizophrenia and is required to continue.

J09.52
Effect of PAI-1 gene on predisposition of schizophrenia in Turkish population

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PAI-1 is a protease belonging to the family of serine proteases. It regulates proteolysis and fibrinolysis via inhibition of tissue type plasminogen activator (tPA) and urokinase (uPA). It has a important potency in cerebrospinal fluid homeostasis. Fibrinolytic system in responsible in CNS affects long-term synaptic plasticity and remodelling. Activity of tPA is neurite outgrowth, neuronal migration and learning. Polymorphisms in this gene may influence disease development. Therefore, we aimed to investigate the effect of the 4G/5G insertion / deletion polymorphism localized in -675 upstream promoter region of PAI-1 gene and can modify the expression of protein levels, on predisposition of disease.
The presence of the 4G/5G polymorphism in the PAI-I gene was determined using the polymerase chain reaction (PCR) method. Samples identified according to whether they have 139 bp band or not. Samples produced 139 bp band with 4G primer were identified as homozygous 4G genotype, samples produced 139 bp band with 5G primer were identified as homozygous 5G genotype, samples produced 139 bp band with both 4G and 5G primer were identified as heterozygous 4G/5G genotype.
In our study, we invastigate the frequency of genotype and allele of PAI-1 gene 4G/5G polymorphism among schizophrenic patienst and healty controls. Significant correlation was found between genotypes ($p<0,05$), but no statistically significant differences were found in allele frequencies ($p>0,05$). Studying of more larger population may help to explain the effect of disease-related gene.The study was supported by ESOGU (Grant no:201211007)

J09.53
A new mutation at the SLC20A2 gene in an italian family with idiopathic basal ganglia calcification

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Idiopathic basal ganglia calcification (IBGC), also known as "Fahr disease (FD)", is a rare neurological disease characterized by symmetric and bilateral calcifications mostly located in the basal ganglia, but often involving also the thalami, the cerebellar hemispheres and subcortical white matter. Familial IBCG (fIBGC) is genetically heterogenous and typically transmitted in an autosomal dominant fashion, sometimes displaying anticipation. In a recent study SLC20A2 gene mutations were found to be responsible for 40% of the IBGC families studied. We describe a novel SLC20A2 variant in exon 9 (G1618A), consisting in a gly-to-arg substitution at position 540 of the protein highly conserved consensus region, in two family members clinically diagnosed with IBGC. This variant was not found in 200 unrelated Italian controls. The index case was a 67-year-old male patient who presented with a mild parkinsonism and a more relevant reduction in spontaneous speech, which evolved steadily over a few years to an almost complete inability to communicate. The proband's daughter developed symptomatic epilepsy at 19 years of age as the only clinical manifestation of IBCG, suggesting an anticipation of the disease over generations and striking intrafamilial phenotypic heterogeneity, as previously observed. This is the first SLC20A2 mutation associated to fIBGC reported in the Italian population. This mutation is damaging according to all prediction programs and it suggest a lost of function of the protein. In conclusion, the discovery of deleterious mutations in SLC20A2 as a cause of fIBGC greatly advances our understanding of this complex disease.

J09.54
Somnambulism in a Colombian family with dominant inheritance

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Sleepwalking and night terrors are sleep disorders classified into the group of excitement Parasomnias. These disorders generate a considerable decree in the individual's quality of life. However the inheritance patterns for these disorders are poorly known and there is a lack of knowledge of the responsible genes for these disorders. We report a three-generation Colombian family with 18 affected individuals, showing a dominant inheritance pattern with reduced penetrance. There is only one study that previously reported the same inheritance pattern in an American family demonstrating linkage with a region between the SNP type markers rs728331 and rs286819 (Licis, et al. 2011). However, there are some mayor differences between the reported family and ours. Onset prevalence and sex-affected ratio are the mayor phenotypic distinctions. We propose that we may have a new condition that is caused by different genes.

J09.55
The clinical phenotype of Polish patients with SPG11 gene mutations - preliminary description

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Hereditary spastic paraplegias (HSP) are a group of clinically and genetically heterogeneous, neurodegenerative disorders characterized by progressive spasticity of the lower limbs. The most common causes of autosomal recessive HSP with thin corpus callosum (TCC), associated with complicated form of HSP, are mutations in the SPG11 gene.
In a group of 159 index patients clinically diagnosed as HSP (criteria according to Fink), in whom SPAST and ATL1 mutations were previously excluded, MLPA analysis revealed homozygous mutation and compound heterozygous mutation in the SPG11 gene, confirming definitive diagnosis in two probands. Moreover, in three patients (from other families) the only one mutation was found. Direct sequencing analysis will be necessary to confirm diagnosis. Individuals were assessed by Spastic Paraplegia Rating Scale and classification used by Dürr.
In a 4 patients from two families with definitive diagnosis, age at onset ranged 10-16 years (mean 13±3), disease duration 9-23 years (15±6), SPRS scores 42-49 (mean 45±3). All patients had fast progression of symptoms and severe phenotype. One of them could not move without walking aids, three were wheelchair-bound. Brain MRI showed TCC (100%), cortical (100%) and cerebellar (75%) atrophy. Nerve conduction study revealed sensorimotor neuropathy axonal type in three individuals. All patients presented mild to moderate cognitive impairment.
The present study confirmed early onset, remarkable progression and severe clinical phenotype of all 4 individuals with mutations in SPG11 gene. The clinical picture in our patients was consistent with typical manifestation of HSP-TCC. Among five index patients only one had family history of AR-HSP.

J09.56
Setting up a registry and database for the purpose of genetic studies of stroke in the northeast of Iran

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Background: Based on the first population-based study of stroke during a 12-month period (2006-2007) in a north east region of Iran (Mashhad), the incidence of stroke was reported considerably greater than it was in neighboring countries where it could affect people at least one decade earlier. In line with the International Stroke Genetic Consortium, we aimed to build up a local database (ISD) to register all new cases of stroke for further research on dissecting genetic basis of stroke. **Methods**: Consecutive patients presenting to the Hospital in Mashhad with first ever stroke were recruited. Diagnosis of stroke was made based on clinical presentation and confirmed by imaging including CT and MRI. Control subjects without any stroke background, age and sex matched with patients, were also recruited. This will remain an ongoing project to allow researcher carry out further clinical and genetic studies. **Results:** The age of patient was ranged 18 to 85 years (both sexes). So far, data on 2000 patients with acute stroke has been collected. 99% of patients have complete follow-up. Association studies of 20 different genes involved in pathogenesis of stroke has already been done and the results are due for publication. **Conclusions:** The ISD dataset provides a source of primary data which could be used for planning further trials, genetic and clinical investigations.

J09.57

Metal levels in blood as biomarkers for ataxias.

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Metals are crucial for synaptic transmission, enzyme activity, proteins folding/conformation. Copper, zinc, iron and manganese are all localized to various subcellular compartments and their regulation is controlled by a diverse range of co-factors and chaperones. Loss of homeostasis of some of these elements is present in neurological disorders including Alzheimer, Parkinson and Huntington diseases, amyotrophic lateral sclerosis and Friedreich Ataxia. Metals are also key factors for ATP generation and to control oxidative damage in mitochondria.

We selected a cohort of spinocerebellar ataxia (SCA, n=20) and Ataxia-Telangectasia (A-T, n=20) patients, and we measured copper, zinc, iron, manganese and selenium levels in their blood samples, using atomic absorption spectrometry.

We found a significantly reduced manganese levels in SCA patients and increased copper levels in A-T patients compared to controls ($p<0.01$). Cu and Mn have essential roles in mitochondria: the former is a component of complex IV; manganese has a central role in maintaining antioxidant enzyme function and it is the superoxide dismutase co-factor (MnSOD; SOD2).

We evaluated in patients vs. controls LCLs mRNA levels of catalase-CAT, glutathione peroxidase-GPX1 and SOD2, enzymes involved in antioxidative response. We found SOD2 and CAT up-regulation in A-T patients and a SOD2 down-regulation in SCA patients.

We considered SOD enzymes activity and showed a reduction of MnSOD-activity in SCA LCLs, whereas Cu/ZnSOD-activity was halved in A-T LCLs.

These data, although preliminaries and restricted to a small number of samples, suggest a role for metals in ataxias and may represent biomarkers of pathology.

J09.58

Screening of TARDBP in Iranian amyotrophic lateral sclerosis (ALS) patients

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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disorder characterized by dysfunction and degeneration of both upper motor neurons in the cortex and lower motor neurons in the brainstem and spinal cord. Genetic analysis of familial ALS (FALS) pedigrees has led to the identification of at least 21 loci and 18 ALS-causing genes. After C9ORF72 and SOD1, mutations TARDBP gene are the most frequent cause of disease in FALS families. TARDBP encodes transactive response DNA-binding protein-43 (TDP-43). In addition to its prominent role in ALS, TDP-43 is important because of its potential involvement in the etiology of various neurodegenerative diseases. TDP-43 is a major component of aggregates of misfolded proteins in neurons of ALS, frontotemporal dementia, Alzheimer's and Parkinson's disease patients. As C9ORF72 and SOD1 have already been screened in cohorts of Iranian ALS patients, we here screened TARDBP in 90 unrelated patients. Fifteen were FALS and 75 were sporadic ALS (SALS) cases. The coding exons of the gene were sequenced by the Sanger protocol. A single missense mutation was observed in exon 6 in one FALS patient; no putative disease causing variation was found among the SALS patients. Although the sample size was relatively small, it appears that the frequency of Iranian ALS patients and specifically FALS patients (6.7%) who harbor mutations in TARDBP is similar to frequencies reported for other populations.

J09.59

Genetic alterations of postsynaptic NMDA receptor related complex are associated with autism spectrum disorder

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Autism spectrum disorder (ASD) is an early childhood neurodevelopmental disorder characterized by a significant genetic aetiology and prevalence currently estimated at 1/100. Several biological pathways have been highlighted, particularly the excitatory synapse, affected by copy number variations (CNV) and enrichment in mutations in genes belonging to the NMDA receptor complex (NRC). We performed a global genetic study of 100 French families including at least 1 individual with autism, who were included in the research project (ClinicalTrials.gov NCT01770548).

Our project aimed to evaluate both the contribution of CNV in ASD, and secondly the presence of mutations in the NRC complex. For each family, we firstly performed a high-resolution pangenomic comparative genomic hybridization (CGH) analysis with 1M CGH Agilent Array format to identify rare or de novo CNVs. In parallel, a high-throughput targeted sequencing of 216 genes mostly belonging to the NRC complex (179) was carried out with the SureSelect Agilent strategy in order to assess the contribution of gene mutations in our cohort.

The first results of our study allowed us to characterize candidates and likely pathogens genetic alterations in at least 10% of patient. We have found mutations or CNV in genes encoding receptors (*NLGN4X, GRM5*), and in cytosolic (*UPF3B*), nuclear (*MACROD2*), and synaptic (*NXPH3*) proteins. They also emphasized that the NRC is targeted both by mutations and CNV, inherited or *de novo*.

These results underscore the fundamental role of this multiprotein network in the process of neuronal communication and learning and its pathophysiological impact in autism.

J09.60

The study on the effect of fibrillation inhibitory compounds on the depolymerization of amyloid fibrils of alpha-synuclein using fluorescent-labeled protein.

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Parkinson's disease (PD) is the most prevalent movement disorder in the world which is caused by dopaminergic cell degeneration in substantia nigra pars compacta of midbrain. One of the major explanations for occurrence of this cell death is the aggregation of a protein called alpha-synuclein which produces cytotoxic aggregates and amyloid fibrils in brain. Many studies have done to find fibrillation inhibitory compounds for preventing and treating PD patients. But studying on concealed dimensions and side effects of these compounds is a great concern. In this research we studied on the depolymerization of alpha-synuclein firbils under induction of fibrillation inhibitory compounds include Curcumin, Cuminaldehyde and Baicalein by using fluorescent labeled alpha-synuclein protein. Our result shows that these compounds induce depolimerization of alpha-synuclein fibrils that is highly related to their cytotoxicity. Some studies on alpha-synuclein fibrillation revealed that intermediate aggregates include oligomeric species may play a greater role than amyloid fibrils in dopaminergic cell death. This depolimerization phenomenon can produce some intermediate aggregates with cytotoxic properties that can worst the patient's condition. Among these compound Cuminaldehyde shows lower depolimerization induction that define this natural organic compound as a valuable candidate for PD therapy.

J09.61

5-HT2A and Body Dissatisfaction involved in the development of Eating Disorders

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Despite numerous studies about Eating Disorders (EDs) their etiopathogenesis is currently debated and poorly understood. Studies carried out on families and twins have highlighted genetic factors' role; molecular genetic studies have identified the candidates genes potentially involved in ED's etiology. To date research has not produced conclusive results, nevertheless genes of serotonergic and dopaminergic neurotransmitters systems seem to be promising candidates. Among these important candidate genes in the EDs susceptibility, various studies have evaluated the possible role of the −1438 G/A polymorphism within the 5-HT2A. In this study 202 Eating Disorder patients (EDp) and 150 control subjects were analyzed for distribution of the 5-HT2A receptor promoter polymorphism and we shown that the AA genotype is more frequent in patients ($\chi2=6.69$; $p=0.01$) than in control suggesting an association of 1438 G/A polymorphism within the 5-HT2A with EDs, in agreement with the past literature. Moreover within our healthy sample subjects with a AA genotype reported higher Body Dis-

satisfaction subdimension of Eating Disorder Inventory - 2 (EDI-2; Garner et al., 2009), as compared with other subjects (F=3.73; p=0.02), demonstrating the important role of endophenotype concept. Both results show the involvement of a genetic component in the development of EDs.

J09.62

Red blood cells hemolysis influences the level of total but not oligomeric plasma alpha-synuclein in Parkinson's disease patients and controls

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Parkinson's disease (PD) is the second of most common neurodegenerative disorders. Oligomeric alpha-synuclein is the principal neurotoxic agent of PD pathogenesis. Plasma alpha-synuclein has been suggested as biomarker for PD with inconsistent results. As red blood cell (RBC) are the main source of plasma alpha-synuclein the level of total plasma protein are influenced by the degree of RBC contamination and hemolysis in plasma. The correlation between plasma hemoglobin and oligomeric alpha-synuclein levels remains unknown.

The aim of our investigation was to establish if RBC contamination and hemolysis in plasma influences the level of total and oligomeric plasma alpha-synuclein in PD patients and controls. The dataset was composed of 18 drug-naïve PD patients (the mean age 67±8.7 years) and 23 control individuals (mean age 65,67±11,27). All subjects were residents of the North-Western region of Russia. The total, oligomeric forms of alpha-synuclein as well as hemoglobin levels were estimated by means of ELISA (Human alpha-synuclein ELISA kit (Invitrogen, USA) and Hemoglobin (Human) ELISA kit (Abnova, USA), correspondingly).

Measured levels of blood plasma hemoglobin vary from 12614 ng/ml to 591041 ng/ml. The correlation between the total alpha-synuclein and hemoglobin level has been shown (N=39, r2=0.426, p=0.0001). However, there were no significant differences in the levels of the total and oligomeric plasma alpha-synuclein between groups.

Therefore our data suggest that hemolisys has effect on a total but not oligomeric alpha-synuclein levels in peripheral blood plasma. At the same time both total and oligomeric alpha-synuclein could not be used as a suitable marker of PD.

J09.63

A key element of Endocannabinoid System PPARγ2 and leptin are associated in turkish schizophrenia patients

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Background: Because the endocannabinoid system(eCB)is deeply involved in body weight regulation,other than the obesity genes,endocannabinoid genes may also have a role in the antipsychotic-induced weight gain in schizophrenia(SCH) patients.**Aim**:To further investigate this hypothesis,we performed an association study with PPARγ2 gene codifying for a key element of the eCB system and obesity related genes, leptin, leptin receptor and MC4R,in a sample of 320 SCH patients and 237 controls.**Methods:** Biochemical analyses, the effects of LEP c.-2548G>A, LEPR c.668A>G, PPARγ2 c.-2-28078C>G, MC4R c.307G>A polymorphisms and the impact of those genes mRNA and serum levels on metabolic adversities in SCH patients and control groups were studied.**Results:**Significantly higher BMI and fasting blood sugar and significantly lower HDL levels were present in SCH patients compared to controls(p<0.001).PPARγ2 and LEP polymorphisms were significantly different between SCH and control groups, while a significant difference was present in PPARγ2 polymorphism between male SCH patients and male controls(p<0.005)and LEP polymorphism between male and female SCH patients(p<0.005).Interestingly,MC4R c.307G>A(G/A+G/G)carriers showed significant differences in BMI and LEP mRNA levels compared to wild type SCH patients(A/A).LEP, LEPR, PPARγ2 mRNA levels and leptin serum levels were also significantly higher in SCH patients compared to controls(p<0.001, respectively).Leptin serum and mRNA levels were positively correlated in PPARγ2 c.-2-28078C>G carriers(p<0.001)and LEP c.-2548G>A carriers have significantly higher PPARγ2 mRNA levels(p<0.001).**Conclusion:** Our findings suggest a strong association between PPARγ2 and leptin genes in Turkish SCH patients which might indicate their potential role in the antipsychotic-induced weight gain,but further studies are needed in order to elucidate their involvement in the pathophysiology of SCH.

J09.64

Linkage of a locus for autosomal recessive familial spastic paraplegia to chromosome 8q24

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Hereditary spastic paraplegia (HSP) is characterized by insidiously progressive lower-extremity weakness and spasticity. HSP being a group of genetic disorders, they follow general inheritance rules and can be inherited in an autosomal dominant, autosomal recessive or x-linked recessive manner. The mode of inheritance involved has a direct impact on the chances of inheriting the disorder. This neuronal degeneration is thought to be caused by mutations at specific genes. Genetic mapping has identified at least 52 different HSP loci, designated SPG (Spastic Paraplegia) 1 through 52. We studied An Iranian family with autosomal recessive HSP with three affected and four unaffected siblings of two consangeous parents. Clinical, neurophysiologic, and neuroradiologic studies were undertaken. Genetic linkage analyses were carried out with polymorphic DNA markers. The candidate gene within the linked region was sequenced in order to verify the observation. Homozygousity mapping showed linkage to 8q24. As a result the first candidate gene for screening was SPG8 also known as KIAA0196 because this gene is located in the linked region and is one of HSP related genes. The KIAA019 contains 29 exons. Precise screenings of all 29 exons of the gene with their boundaries were carried out using sequencing but all patients were negative for *SPG8* mutations. In conclusion, these data confirm the presence of another SPG gene on chromosome 8q24 related to autosomal recessive HSP.

J09.65

The role of estrogen receptor alpha (ESR1) and oxytocin receptor (OXTR) genes in personality traits variation

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Estrogen and oxytocin integrate many processes including social behaviors, cognitive function and activity in the autonomic nervous system. We aimed to assess the main, haplotypic and GxE effect of ESR1 (rs9340799, rs2077647) and OXTR genes (rs4686302) on personality traits variation. We recruited 1018 healthy individuals (68% women) of Caucasian origin (Russians-409, Tatars-290, Bashkir-130, Udmurts-189) from Russia (mean age: 19.81±2.65 years) without any history of psychopathologies subjected to personality assessment (TCI-125). Genotyping of SNPs was performed using PCR-RFLP. Statistical analysis was conducted with PLINK v.1.07, Haploview 4.1 followed by correction for multiple testing under FDR-procedure. Subsequent haplotype analysis revealed an association of ESR1 A*C-haplotype (rs9340799, rs2077647, D'=0.76) and A*T-haplotype (PFDR=0.008) with high and low self-transcendence (ST) (PFDR=0.042), respectively, in Bashkir group. GxE analysis revealed the model ESR1*ethnicity explained variations in ST (PFDR=0.036) and self-directedness (SD) (PFDR=0.036). Accordingly, Udmurts carrying rs9340799 G-allele demonstrated decreased SD and Bashkirs with rs2077647 C-allele reported increased ST. Moreover, association of OXTR rs4686302 A-allele and lower Persistence (PFDR=0.031) was observed only in non-smoking group. Additionally, OXTR rs4686302* ethnicity model determined variations in SD (PFDR=0.021): Russian individuals with rs4686302 A-allele scored higher on this trait. Reported findings suggest that ethnicity and "smoking status" modulate the association between ESR1 and OXTR gene polymorphisms and personality traits. Thus, ESR1 and OXTR genes might be responsible for mental health and neuroprotection, since character traits (ST and SD) are predictive for personality disorders development. Study was supported by Russian foundation for humanities grant (№ 13-06-00583a).

J09.66

Exome sequencing reveals two compound heterozygous DDHD2 mutations in a non consanguineous Italian family with ARHSP-TCC

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Autosomal recessive spastic paraplegia with thin corpus callosum (ARHSP-

TCC) is a common form of complex HSP, representing about one third of the autosomal recessive forms. Eight genetic loci for ARHSP-TCC have been identified: SPG11, SPG15, SPG21, SPG32, SPG35, SPG46, SPG47 and most recently SPG54. DDHD2 gene, encoding one of the three intracellular phospholipases A1, has been recently identified as cause of the SPG54 form, characterized by intellectual disability, developmental delay and onset in childhood.
We identified two compound heterozygous mutations in the DDHD2 gene by using whole exome sequencing in a non consanguineous family with ARHSP-TCC. The family includes two affected sibling and unaffected parents. The coding region of one patient was enriched and captured by SureSelect Human All Exon V4, and exome sequencing was performed using the Hiseq2000 instrument. BWA and GATK software packages were used to align sequence reads to the reference. The data were then imported into Gem.app, a web-based database and analysis tool for next generation sequencing data. Exome sequencing data revealed two compound heterozygous point mutations in the index case in the DDHD2 gene: the novel c.307T>C and the already described c.1978G>C. Both mutations were validated by Sanger sequencing and they were confirmed in the second affected brothers.
In conclusion, we report the first Italian patients with two compound heterozygous point mutations in the DDHD2 gene. This finding enlarges the clinical spectrum related to DDHD*2* mutations, providing to determine the worldwide distribution and the diverse clinical features of SPG54 form.

J09.67
Mutational screening of GJB1, MPZ and PMP22 genes in a cohort of CMT patients from Southern Italy

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Charcot-Marie-Tooth (CMT) is the most common inherited neuromuscular disorder. To date, mutations in more than 40 genes are responsible for CMT disease. We have analyzed 500 patients coming from Southern Italy, referred to our centre with a suspected diagnosis of CMT. Firstly, all patients have been screened for the 17p.11.2 duplication. The non duplicated cases were further investigated for point mutations in the GJB1, MPZ and PMP22 genes. Among the 500 unrelated CMT patients, 209 of them (41.8%) harbored the CMT1 duplication. A subsequent mutation analysis of GJB1, MPZ, and PMP22 genes in 291 CMT patients, revealed 26 different mutations in 48 cases: 14 in GJB1, 7 in MPZ, 5 in PMP22. Out of 14 GJB1 missense mutations identified in 16 patients, seven are novel (Ser49Phe, Ala88Val, Ser128Leu, Leu131-Arg, Val148Phe, Phe153Leu and Arg164Leu). Out of 7 mutations in the MPZ gene identified in 27 patients, two are novel (Trp57X and Gly93Arg). Noteworthy, in 14 patients from Apulia the known Val102fs mutation has been detected, whereas seven patients from Sicily carried the known Ser78Leu mutation, confirming our previous results of a founder effect for the Ser78-Leu. Finally, in the PMP22 gene we detected two novel variants (Ala35Tyr and Pro122Leu). In conclusion, our data confirm that the duplication is the most common genetic cause of the CMT, followed by mutations in GJB1 and MPZ. Furthermore, this study recommends that for the patients from Apulia and Sicily the diagnostic test, after the exclusion of the duplication, might be start from MPZ gene.

J09.68
Variation in the miRNA-433 binding site of *FGF20* is a risk factor for Parkinson's disease in Iranian population

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DNA variation at the *FGF20* gene has been associated with Parkinson's disease (PD). Specially, single nucleotide polymorphism (SNP) rs12720208 in the 3' untranslated region (3' UTR) was linked to PD-risk through a mechanism that would implicate a differential binding to microRNA-433 (miR-433). In this study, we genotyped the rs2720208 SNP in a total of 480 PD patients and 480 healthy controls from Iran. We found significant differences in allele and genotype frequencies between patients and controls (Fisher exact $p<1\times10^{-7}$). The results obtained in this study revealed that the rs12720208 (C/T) polymorphism is a strong risk factor for late-onset PD in Iranian population.

J09.69
Evidence of Dysbindin gene promoter hypermethylation in peripheral blood lymphocytes as a potential biomarker in major psychosis

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DTNBP1 is the gene encoding Dysbindin or Dystrobrevin binding protein. DTNBP1 is established as a genes associated with certain psychotic disorders. Genetic variations of DTNBP1 have been found in association of Schizophrenia, Major Depressive Disorder, Bipolar Disorder, Substance induced Psychosis and certain cognitive properties. Functional studies have concluded that reduction of expression is the likely mechanism of effect of DTN-BP-1 in pathophysiology of mental conditions. Moreover, due to dysbindin protein functions in neurotransmission, dysfunction mechanisms have been postulated in cognitive and psychotic conditions.
In an EWAS on methylome of post-mortem brain of major psychoses patients, hypermethylation of an intron, promoter and an upstream region of DTNBP1 gene was found to be FDR-significant in association with bipolar disorder. Furthermore, variation of DTNBP1 mRNA levels in post-mortem cerebellum has been shown to be concordant with it in peripheral blood lymphocytes (PBL) of the individual in expression profiling studies. In addition, the expression reduction of DTNBP1 has been demonstrated in PBL of psychotic patients.
Here, we investigated DTNBP1 promoter methylation variation in psychotic bipolar disorder patients. Preliminarily, methylation level of a promoter CGI of DTNBP1 gene in 46 cases against 85 age and sex-matched controls was measured with Quantitative Methylation-Specific High-Resolution Melting(MS-HRM). We observed a statistically significant hypermethylation in psychotic bipolar cases against normal samples. Provided the results be replicated in broader investigation, PBL DTNBP1 promoter hypermethylation could be considered a potential biomarker of psychotic bipolar disorder.

J09.70
Clinical, Neuroimaging, and Genetic Characteristics of Megalencephalic Leukoencephalopathy With Subcortical Cysts in Egyptian Patients

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare cerebral white matter disease. Clinically, it is characterized by macrocephaly, developmental delay, and seizures. We explore the clinical spectrum, neuroimaging, and gene involvement in the first MLC patients described from Egypt. Patients: Six patients were enrolled from three unrelated families. Patient inclusion criteria were macrocephaly, developmental delay, normal urinary organic acids, and brain imaging of diffuse cerebral white matter involvement. Direct sequencing of the MLC1 gene in patients' families and GliaCAM in one questionable case was performed. Results: Clinical heterogeneity, both intra- and interfamilial, was clearly evident. Developmental delays ranged from globally severe or moderate to mild delay in achieving walking or speech. Head circumference above the ninety-seventh percentile was a constant feature. Neuroimaging featured variability in white matter involvement and subcortical cysts. However, findings of posterior fossa changes and brain stem atrophy were frequently (66.6%) identified in these Egyptian patients. Discrepancy between severe brain involvement and normal mental functions was evident, particularly in patients from the third family. MLC1 mutations were confirmed in all patients. Deletion/insertion mutation in exon 11 (c.908-918delinsGCA, p.Val303 Gly fsX96) was recurrent in two families, whereas a missense mutation in exon 10 (c.880 C > T, p.Pro294Ser) was identified in the third family. CONCLUSIONS: This report extends our knowledge of the clinical and neuroimaging features of MLC. It confirms the apparent lack of selective disadvantage of MLC1 mutations on gamete conception and transmission as supported by the presence of multiple affected siblings in Egyptian families.

J09.71
Association and Gene-Gene Interactions of the HPA Axis Genes with Suicidal Behaviour

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The hypothalamic-pituitary-adrenal (HPA) axis is activated in different ways

during chronic stress and involved in the neurobiology of different mood disorders including suicide behavior. The CRHR1 gene controle the activation of HPA axis via expression and functionality. Immunophillin FKBP51 is expressed in cortical neurons and regulate the function of the glucocorticoid receptor which regulates HPA axis. Genetic variants in the FKBP5 gene encoding FKBP51 are linked to suicide.

The aim of our study was to examine association of rs878886 polymorphism of CRHR1 gene, polymorphisms rs4713902 and rs7757037 in FKBP5 gene with suicidal behavior in Russian and Tatar patients from Bashkortostan. We genotyped DNA samples of 312 cases (101 - Male, 152 - Female, 150 - Russian, 120 - Tatar) who had suicide attempts and 346 control subjects (194 - Male, 152 - Female, 263 - Russian, 248- Tatar) from Russia using PCR-RFLP and PCR with fluorescent detection (FLASH/RTAS) techniques.

We observed a strong association between CRHR1 rs878886 and suicide: allele C was significantly overrepresented both in Russian (P=0,013, OR=1,79, 95%CI 1,13-2,86) and Tatar ethnicity groups (P=0,00001, OR=3,06, 95%CI 1,99-4,71).

No significant differences in either allele, genotype or haplotype frequencies of FKBP5 gene SNPs were found between suicidal and control groups. However, gene-gene interactions showed strong association in Tatar patients between investigated genes: rs7757037*A/G - rs878886*C/G (P=0,0012, OR=4,03, CI95% 1,75-9,35) and rs4713902*C/T - rs878886*C/G (P=0,0009, OR=4,84, CI95% 1,92-12,41).

Our results show contribution of investigated genes in predisposition to suicidal behavior, and confirms ethnic specifity of this association.

J10.01

Phenotype-genotype correlation in patients with dysferlinopathy in families from Daghestan

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Dysferlinopathies are autosomal recessive muscular dystrophies caused by mutations in dysferlin gene (*DYSF*, MIM# 603009). Dysferlin deficiency leads to two main phenotypes: limb girdle muscular dystrophy (LGMD) 2B and Miyoshi myopathy (MM). Dysferlin is located on the plasma membrane of skeletal muscle and is deficient in patients with MM and LGMD2B.

We've retrospectively reassessed the disease history (onset and progression). Earlier the phenotype genotype correlation was established in patients with dysferlinopathy variants in Daghestan families. Illarioshkin et al. in 1996 examined a large 6-generation family, which include 12 patients whom 9 were manifestations LGMD 2B and 3 were MM.

Clinical assessment was performed with a standardized protocol, including the Medical Research Council scale, a serum creatine kinase level, electromyography, and a muscle computed tomographic scan, sequencing DNA.

11 patients from 7 families, originally from a mountain village (9 males and 2 women), were examined. All of them had severe symptoms of progressive muscular dystrophy and the same mutation in exon 3 of the *DYSF* (c.573-574 TG>AT transition, in the homozygous state with replacement p.Val67Asp).

During the period 2013 we have been re-examining 5 patients and detailing disease history (onset and progression). Five elderly patients of 11 were clinically and molecular examined. Two of 5 patients over the age of 40 years had proximal pattern of muscle involvement (the hip and shoulder belts), 2 others had distal variant Miyoshi myopathy.

J10.02

Homozygosity mapping in an Iranian pedigree affected with muscular dystrophy limb girdle (LGMD) reveals linkage to chromosome 2p12-14 and a mutation in Dysferlin gene

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Homozygosity mapping is a powerful gene mapping method applicable to rare recessive disorders in inbred populations. Limb-girdle-muscular-dystrophy (LGMD) is heterogeneous group of disorders affecting the voluntary muscles, mainly around the pelvic and shoulder. Mutations in different genes can cause different types of LGMD. To date, at least 15 different genes have been identified as LGMD causative genes. Because, there are many genes for this group of disorders, mutation screening of all these genes is cumbersome and costly. Linkage analysis can be used for selection of the appropriate candidate gene. An Iranian family with four sibling affected with LGMD was identified. Genome-wide SNP genotyping was carried out on DNAs of three affected and one unaffected individuals using HumanCNV370-Quadv3_C BeadChips. Subsequently, homozygous regions common to affected individuals and absent in the unaffected sibling were sought. Disease status in the family linked to a homozygous region on chromosomes 2. The DYSF gene that associates with LGMD type 2B was positioned within the linked region. DYSF encodes dysferlin, a protein believed to be involved in the maturation of new muscle fibers and in repairing damage to muscle cells. Sequencing of DYSF exons in the proband of the Iranian revealed a novel insertion-deletion that is the likely cause of LGMD in the family. The phenotypic features of the patients harboring the mutation are uncommon because of prominent proximal presentations. To date, over 400 mutations disease in DYSF have been reported. The novel mutation reported here represents only the second mutation observed among Iranian LGMD patients.

J10.03

A multi-exonic *RYR1* deletion identified in individual with confirmed malignant hyperthermia

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Malignant hyperthermia susceptibility (MHS) is an autosomal dominant pharmacogenetics disorder of skeletal muscle calcium regulation associated with uncontrolled skeletal muscle hypermetabolism. The MH crisis is induced by exposure of the predisposed patients to halogenated volatile anaesthetics. Molecular genetic testing indicates that mutations in *RYR1* are identified in up to 80% cases of MHS. More than 400 mutations in *RYR1* have been associated with MHS. Most of the *RYR1* mutations are private. Almost all MHS-causative mutations are missense; however, an in-frame deletion of a single amino acid in the central region of *RYR1* and a single-nucleotide deletion at the extreme C-terminal end of the protein have also been reported. Large-sized genomic deletion of 6 520 bp was identified as the first genomic rearrangement in the *RYR1* gene.

Here we report the detection of large-sized deletion in the C-terminal end of the RYR1 in the MHS confirmed individual. At first, the deletion of exons 90 and 94 was identified using MLPA. In the second step, the deletion from exon 90 to 98 was determined using quantitative fluorescent PCR (QF PCR). We described novel multi-exonic deletion of 2 kb that belongs to new class of mutation of the *RYR1* gene. This is the second published multi-exonic deletion identified in the *RYR1* gene to our knowledge. This will clearly have consequences for the molecular investigation of RYR1-related diseases.

J10.04

Detection of deletions and duplications in the Duchenne muscular dystrophy in Russian patients

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DMD gene duplications detection and analysis of deletions at female relatives of the patient has not yet been carried out in Russian patients. Thus, the aim of this work is the development of methods of quantitative analysis of DMD gene duplications and deletions and determining the frequency of duplications in the selection of patients with DMD/BMD from Russia. To perform this task, we have developed a quantitative method based on specific ligation reaction (MLPA). Using this method, we analyzed 237 patients with Duchenne/Becker muscular dystrophy, which didn't reveal deletions of „hot exons". Duplications have been detected among 37 patients. Deletions not in „hot exons" have been found among 9 patients. Thus duplications are found in 6% of cases, which is supported by the literature data. In addition, this method allows detecting deletions / duplications in female genome, which allows to determining mutations in DMD gene in carrier.

J10.05

A Novel POMT2 mutation defined in a girl with Walker Warburg syndrome.

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Disorders disrupting normal brain development represent a clinically and genetically heterogeneous group. Genomic technologies are being used widely, especially in recent years, and enabling the discovery of many novel genes that are involved in brain development. However, many more are likely waiting to be brought to light. Here we present a girl with hydrocephaly which was diagnosed during antenatal period. She had hypotonia, occipital encephalocele, dandy walker malformation of the brain, pachygyria, agenesis of corpus callosum, microophtalmia and corneal opacity on the right

eye, retinal and choroid coloboma on the left eye, slight desquamation of the skin. Her creatinine kinase and lactate dehydrogenase levels were high. Whole exome sequencing revealed a homozygous novel mutaion (c.T431G:p.M144R) on Protein O-Mannosyltransferase 2 (POMT2) gene. Walker-Warburg Syndrome (WWS) is a rare form of autosomal recessive congenital muscular dystrophy associated with brain and eye abnormalities. Until now several mutations were found in the POMT1 and POMT2 genes. Increasing use of genomic sequencing approaches, along with other genome-wide interrogation technologies are helping to identify causative rare variants not only in disorders of brain development but also in other disorders showing Mendelian inheritance.

J10.06

Clinical characteristics and genotype-phenotype correlation of Korean patients with spinal and bulbar muscular atrophy

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Introduction Spinal and bulbar muscular atrophy (SBMA) is slowly progressive and adult-onset motor neuron disease caused by a CAG repeat expansion in the AR gene. Few data are available for clinical characteristics and genotype-phenotype correlation in Korean SBMA patients. **Methods** Forty consecutive patients diagnosed by genetic testing were included in this study. The age of onset was defined as the time of the patient's initial recognition of symptoms and disease duration was represented by the time taken from onset of symptoms to genetic diagnosis. The severity of symptoms was assessed by nine ADL milestones. Rate of disease progression was expressed as a number, differences of ADL scores between at onset and diagnosis/disease duration. **Results** The median age at onset and diagnosis was 44.5 and 52.5 years, respectively. The median disease duration was 5.00 years, median rate of disease progression was 0.2 score/year and median number of CAG repeats in the AR gene was 44. The number of CAG repeats showed significant inverse correlations with the age of onset of muscle weakness and the age of onset of any symptoms. Interestingly, a statistically significant correlation between rate of disease progression and age at onset was observed while an inverse correlation between rate of disease progression and disease duration was noticed. **Discussion** This study reaffirms the inverse correlation between the age at disease onset and the number of CAG repeats. It is of note that the rate of disease progression is influenced by age at disease onset in Korean SBMA patients.

J10.07

Molecular analysis of mutations smn1, smn2 and naip genes by multiplex ligation-dependent probe amplification (mlpa)

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Spinal muscular atrophy (SMA) is autosomal recessive neurodegeneration disease. The cause of the disease is mutations in the SMN gene. SMN1 gene has high homologue copy - SMN2 gene, the number of copies depends on the type of severity of the current disease and the patient's quality of life.

To identify mutations in the SMN1, SMN2 and NAIP genes, MLPA analysis was performed by using 3730xl DNA Analyzer. Molecular study of 43 patients was conducted in unrelated families with suspicion of spinal atrophy. The diagnosis was confirmed in 21 cases.

In three patients with SMA type I identified deletion of exons 7 and 8 of SMN1 and not marked increase in the number of copies of SMN2, two patients identified deletions of exons 7 and 8 and a deletion of the SMN1 gene and NAIP gene. In patients with SMA type II in 3 cases met deletion of exons 7 and 8 of SMN1 gene and increases the number of copies of the SMN2 gene and only in one case the deletion of exons 7 and 8 of the SMN1 gene. At the same time, in nine patients SMA type III - 8 cases of predominate deletions 7 and 8 exons SMN1 gene and increase the number of copies of the SMN2 gene, in one case revealed a deletion of 7 and 8 SMN1 gene.

Thus, these studies show that the number of copies SMN2 gene is not always associated with the severity of clinical manifestations in patients with SMA.

J10.08

Definition breakpoints in the dystrophin gene for heterozygous carrier revealing

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Duchenne muscular dystrophy - a disease caused by the presence of deletions, duplications and point mutations in the dystrophin gene (DMD), destroying one to several exons. An important aspect in the diagnosis of the disease is to determine the break points for the diagnosis of carriers of the abnormal gene. Objective: to develop a method of determining breakpoints of deletions of the intron of DMD gene to identify heterozygous carrier. Material research was genomic DNA of two patients suffering from Duchenne muscular dystrophy and their parents. DNA was extracted from whole blood using a kit Wizard ® Genomic DNA Purification Kit (Promega). Diagnosis of Duchenne muscular dystrophy was carried out using the MLPA, followed by capillary electrophoresis. Design of primers was performed using the program Primer 3 (http://primer3.ut.ee/). Determination of break points in the region of exons 45-50 was performed using PCR RT, the 7300 „Applied Biosystems" (USA). MLPA- analysis revealed a deletion of a segment encompassing exons 45-50 of the gene. The first break point occurred between exons 44-45, the second - between exons 50-51. Using genomic databases (http://www.genome.ucsc.edu) defined size between exons: 44-45 includes ~ 250,000 nucleotides 50-51 includes ~ 45 500 nucleotides. Using several sets of primers, these areas have been divided into several parts, followed by PCR RT, to identify the region deletions. At the last stage of the experiment break point was determined by sequencing. As a result, this study developed a method to identify heterozygous carrier in preclinical diagnosis of Duchenne muscular dystrophy.

J10.09

Novel pathogenic mechanism of a mid-intronic mutation in DMD gene

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Duchenne and Becker muscular dystrophy are X-linked allelic disorders caused by mutations in the *DMD* gene. The majority (65%) of these mutations are intragenic deletions/duplications that often lead to frameshift errors. Among the remaining ones, we find the mid-intronic mutations that usually create cryptic exons by activating potential splice sites. In this report, we identified, in a Becker Muscular Dystrophy patient, a mid-intronic mutation that created two ESE sites in intron 26 of DMD gene resulting in the insertion of a new cryptic exon in mRNA.

To our knowledge, this is the first case report describing this novel pathogen mechanism of mid-intronic mutations of *DMD* gene.

J10.10

Analysis of subtle mutations in SMN1 gene in non-deleted SMN1 gene Russian SMA patients

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SUMMARY: The proximal spinal muscular atrophy (SMA) is a severe autosomal recessive neuromuscular disease characterized by degeneration of alpha motor neurons in the spinal cord, resulting in progressive proximal muscle weakness and paralysis. 95% SMA patients have homozygous deletion of *SMN1* gene. The remaining 5% cases are caused by compound heterozygous mutation: a *SMN1* deletion on the one allele and a subtle mutation on the other allele.

OBJECTIVE: To identify the intragenic mutations in *SMN1* gene in the 11 unrelated SMA patients with the one copy of *SMN1* gene.

METHODS: MLPA was carried out to measure the copy number of *SMN1* and *SMN2* genes. The subtle mutation analysis of *SMN1* gene was performed by direct sequencing.

RESULTS: We have identified seven mutations in eight patients with proximal SMA.(Tab.1) Mutation c.824G> C (p.Gly275Ala) has been met twice.

In six of eight cases mutations are inherited from the father.

Mutations are well correlated with the type of disease.

It has been found no mutations in three patients. Possibly, in these cases, mutations are located outside the investigated sequences, f.e. at promoter region. Or these patients are carriers having another SMA-like disorder.

CONCLUSION: Analysis of subtle mutations in *SMN1* gene is essential to genetic counseling in non-deleted *SMN1* gene SMA families.

Patient	Type SMA	Genotype	Mutation		Exon/ Intron	Origin
			cDNA	Protein		
1	I	1T / 2C	c.43C>T	p.Gln15X	E1	de novo
2	II	1T / 3C	c.684dupA		E5	F
3	I	1T / 2C	c.815A>G	p.Tyr272Cys	E6	F
4	III	1T / 3C	c.821C>T	p.Thr274Ile	E6	F
5	II	1T / 2C	c.824G>C	p.Gly275Ala	E6	F
6	II	1T / 3C	c.824G>C	p.Gly275Ala	E6	F
7	I	1T / 2C	c.835-2A>T		I6	F
8	I	1T / 2C	c.836G>T	p.Gly279Val	E7	M
9	III	1T / 4C	n.d.	n.d.	n.d.	n.d.
10	I	1T / 2C	n.d.	n.d.	n.d.	n.d.
11	I	1T / 2C	n.d.	n.d.	n.d.	n.d.

Table 1. The results of current study (T, C - telomeric and centromeric copies of *SMN* gene).

J10.11
Phenocopies as a possible cause of molecularly unconfirmed cases of Emery-Dreifuss muscular dystrophy

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Emery-Dreifuss muscular dystrophy (EDMD) is genetically heterogeneous muscular disease; 6 genes have been identified by now: EMD, LMNA, FHL1 causing major forms and very rare SYNE-1, SYNE-2, TMEM43. However, in >60% patients with EDMD phenotype no mutations are found. Common opinion about this issue is existence of other causative genes not recognized yet. In our sample of 102 unrelated patients (74 men, 28 women) with EDMD phenotype tested for mutations in LMNA (all patients), EMD and FHL1 (men only) EDMD was confirmed in 38 (37.2%); 22 patients (21,6%) were heterozygous for LMNA mutations, 15 (14.7%) hemizygous for EMD mutations and one (0.9%) hemizygous for FHL1 mutation. Patients with no mutations and pedigrees compatible with autosomal recessive inheritance were additionally tested for common mutations in CAPN3, FKRP, SGCA, ANO5, DYSF genes responsible for limb-girdle muscular dystrophies 2A, 2I, 2D, 2L, 2B respectively. Four patients were found homozygous or compound heterozygous for CAPN3 mutations and one - heterozygous for ANO5 mutation (allelic mutation is in search); the five patients had typical EDMD features including primary contractures and cardiomyopathy with arrhythmia. Thus, part of molecularly verified diagnoses in the initial sample made up 42.1%. Our findings show that EDMD can be clinically mimicked by mutations in genes responsible for other muscular dystrophies. It explains, to some extent, high percentage of cases with EDMD phenotype but no mutations in EDMD genes. EDMD phenocopies (as well as rare clinically atypical EDMD cases) should be taken into account in practical diagnostics and genetic counseling in families.

J11.01
17q22 microdeletion syndrome; a case report

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The recognition of microdeletion/duplication syndromes have been facilitated by the development of array comparative genomic hybridization techniques. One of the microdeletion/duplication syndromes recently identified is '17q22 microdeletion syndrome' which has been defined in 2013. This syndrome presents with intellectual disability, conductive hearing loss, facial dysmorphism and skeletal anomalies. Only nine patients have been reported previously. The common deleted region includes NOG gene which is essential for joint formation and the phenotype assosiated with 'NOG-related symphalangism spectrum disorder' is characterized by symphalangism, joint contractures and conductive hearing loss.
Here, we present a new case with 17q22 microdeletion syndrome. She was refered to genetics department due to facial dysmorphism, long and tapering fingers and preauricular skin tags. On physical examination at the age of seven, she showed narrow forehead, midfacial hypoplasia, hemicylindrical nose, short and flat philtrum, thin border of upper lip, joint contractures and proximal symphalangism. She had severe intellectual deficit. Since she displayed symphalangism, NOG gene was sequanced and no mutation was revealed. Further testing by array-CGH showed a de novo 3,1 MB deletion on 17q22. The deleted region contains 36 genes, including NOG gene. The case will be discussed in view of the literature.

J11.02
TripleX Syndrome with short stature: a case report

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Triple X syndrome (47,XXX) is a sex chromosomal aneuploidy characterized by tall stature, microcephaly, hypertelorism, epichantal folds, congenital abnormalities and motor and language delays. We present a rare phenothype of the syndrome: a 10 years-old girl admitted to our hospital for short stature. She was born from non consanguineous parents at term of uneventful pregnancy weighting 2500 gr and measuring 47 cm. The girl was 117.7 cm (height SD score -2.87)< to the parental target eight of 154.5 cm, with a weight of 19Kg (SD score -2.13) and head circumference of 45.5 cm (below the 3rd percentile). The girl's growth rate was of 4-5 cm annually. The genitalia were of normal female phenotype with Tanner 1 stage of breasts. In addiction she presented clinodactyly, ogive palate and mild cognitive and speech delays. Karyotype was 47,XXX; FISH (probe CEP-X) on 200 interphase nuclei confirmed cytogenetic data. There was no evidence of growth hormone deficiency. Laboratory tests showed low insulin-like growth factor-1 (IGF-I). Bone radiographic imaging of the left carpal demonstrated a difference between bone age and chronological age of 2 years. Blood routine test, liver and kidney function, blood glucose and insulin, thyroid function were normal. The ultrasound examination of heart, uterus, ovary and urinary system, as well as cranial magnetic resonance imaging (MRI) were normal. A possible explanation might be the aploinsufficiency of the short stature-homeobox-containing gene (SHOX gene) that is present in the pseudoautosomal region of sex chromosomes. This study performed in our patient is yet ongoing.

J11.03
De Novo 4p deletion and 4q duplication in a female dysmorphic child

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Wolf-Hirschorn Syndrome (WHS) is a well recognized chromosome 4p16.3 microdeletion syndrome with characteristic craniofacial features, pre and postnatal growth retardation, hypotonia, intellectual disability, EEG abnormalities, congenital heart defects and corpus callosum agenesia. Direct duplications on the chromosome 4q represent an infrequent chromosomal finding. Here, we report the cytogenetic and molecular cytogenetic findings and clinical manifestations observed in a 19 months old female infant with terminal del4p and dup4q. The infant was delivered by Cesarean section at the 36th week of the mother's twin pregnancy. Her twin was normal. The birth weight of the case was 1750 gr (<-1 sd) and heigth was 42 cm (<-2 sd). She had significant features specific to WHS. Additionally she had bitemporal narrowing, square forehead, mild brachycephaly, large antihelix, bilaterally hand and foot fifth finger clinodactyly. Our patient has increased renal parenchyma echogenity, large antihelix and umblical hernia which are similar to chromosome 4qdup anomalies. Conventional cytogenetic analysis revealed 46,XX,dup(4)(4qter-4p16?::4q31-4qter) and molecular cytogenetic analysis was 46,XX,ishder(4)dup(4)(q31qter)(wcp4+)del(4)(p16.3pter). The karyotypes of the healthy, non-consanguineous parents were normal.
The de novo abnormal karyotype seen in the case have not been described previously. Although phenotypic features of the case are similar to WHS, the patient also has features, including ear and renal anomalies, which may be attributed to the 4q duplication. The patient's phenotype may represent the combined effect of both chromosome aberrations. In order to identify regions of the relevant genes, further advanced molecular analyses are planned.

J11.04
Deletion of 4q28.3-31.23 in the background of multiple malformations

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We would like to address a case regarding a patient who suffered from multiple malformations. The symptoms include small kidneys, epilepsy, dystrophy, hyponatraemia, immunodeficiency with decreased lymphocyte number, most of which are well known in medical literature, however some of them are unique like hypocalcaemia and pulmonary hypertension. Despite preliminary tests, which include GIEMSA banding and FISH, we were not able to detect the genetic alterations behind these symptoms, which is why we expanded the routine method to array CGH. We used Agilent Human Genome G3 Sureprint 8x60K Microarray to detect the cytogenetic deletions in

the patient. We found 14.56MB interstitial deletion on the long arm of chromosome 4, more specifically in the region of 4q28.3-31.23, where a total of 47 genes were affected. Eight out of these genes affected could play and important role in the manifestation of these symptoms. These genes include NR3C2, IL15, PCDH18, SETD7, ELMOD2, GAB1, HHIP, and SMAD1. We also examined the patient's parents with the same type of microarray. As no deletions were detected in either parent we concluded that the deletion found in the patient was a de novo alteration, which leads us to believe that the loss of these genes could give us a possible explanation for the clinical features.
This research was supported by TÁMOP-4.2.3-12/1/KONV-2012-0028.

J11.05
It is all phenotype caused by 6qter deletion?

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We report ok a 5 years old boy with a prenatal ultrasound diagnosis of biventricular hydrocephalus. He was born with a birth weight of 2580 g, a length of 46 cm and a head circumference of 33 cm. He did not show muscular hypotonia but his psychomotor development was delayed: he sat at the age of 11 months and he walked independently at the age of 17 months. He spoke single words at the age of 12 months but he could not make complete sentences until 3 years of age. Brain MRI showed colpocephaly, thinned corpus callosum and pons, hypertrophy of massa intermedia. His EEG was normal. Neurological evaluation showed muscular atrophy and hypotonia, joint laxity and mild intellectual disability. On the clinical examination at 5 years of age, the patient had a weight of 15 kg (3th centile), a height of 105 cm (10th centile) and a OFC of 47 cm (<<3th centile). The examination showed failure to thrive, microcephaly, triangular face, downslanting palpebral fissures, sparse eyebrows, large and posteriorly rotated ears, muscular atrophy and hypotonia, ligamentous laxity, scapular winging and bilateral flat foot . Array-CGH showed a 2.5 Mb microduplication of 2q37 and a 5.3 Mb microdeletion of 6q27 generating a de novo derivative 6 chromosome: this rearrangement is likely to be the result of a translocation involving the distal region of long arm of chromosome 2 and the distal region of long arm of chromosome 6. Array-CGH showed also a maternal 714 Kb microduplication of Xq26.2.

J11.06
A case report with de novo interstitial deletion in 7q21

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In this case we report a male patient with a de novo interstitial deletion in 7q21 which is determined by G-banding. Patient was referred our clinic for undescended testes and mild intellectual disability. He was five years old and one of the triplet pregnancies. Two other siblings were girls. The pregnancy resulted from in vitro fertilization. The siblings and parents karyotype analyzes were normal. The clinical features of the patient were cerebral cerebellar hypoplasia, partial androgen resistance, undescended testis and mild intellectual disability. We are now planning to make further molecular investigations to find a relationship between the candidate genes of these features.

J11.07
New case of 7q11.23 duplication syndrome in a polymalformed Saudi child

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7q11.23 duplication syndrome referred to as "duplication of the Williams syndrome region;" is a recently-documented genetic disorder caused by a heterozygous duplication of contiguous genes at 7q11.23 mediated by nonallelic homologous recombination (NAHR) between large flanking Low Copy Repeats, leading to duplication or to deletion like observed in williams beuren syndrome.
Less than 100 cases have been described and the phenotype is not yet well defined. Most prominent phenotypic characteristics, mentioned in literature review are severe speech delay, language delay, a characteristic facies, hypotonia, developmental delay, and social anxiety. Congenital anomalies as heart defects, diaphragmatic hernia, cryptorchidism and non-specific brain abnormalities are sporadically reported.
We report here an 1 year-old male with 7q11.23 microduplication detected by array-CGH analysis performed because of speech delay, growth retardation, hypotonia and polymalformative syndrome associating congenital heart diseases, hydronephrosis, cleft lip, macrocephaly and polydactyly. Fluorescence in situ hybridization, using ELN gene probe, confirmed the diagnosis, revealing a tandem duplication of the Williams-Beuren critical region detectable only on interphase nuclei and karyotype study was normal.
This case is one of the most severe reported in the literature and it allows us to expand the phenotypic spectrum of this syndrome.

J11.08
A new familial case with 8q24.11-q24.3 complementary rearrangements: phenotypes associated with pure deletion and mosaic duplication

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Genomic rearrangements of 8q distal bands have been associated with several clinical entities, such as Langer-Giedion Syndrome. We report a unique familial case with complementary chromosomal aberrations involving the same distal 8q region, detected on fetus (deletion) and mother (mosaic duplication). Chromosome analysis on chorionic villi at 13 weeks of gestation for increased Down Syndrome risk after maternal serum screening revealed 46,XX,del(8)(q?23q?24.2) karyotype. The 8q deletion was defined as interstitial by subtelomeric and painting specific FISH probes. The deletion size was investigated by microarray and parental karyotype was performed. Microarray result was arr[hg19] 8q24.11q24.3(119,142,459-144,825,972) x1 with a 25 Mb deletion. The involved region included 74 OMIM genes and no recurrent microdeletion syndromes. Maternal karyotype was mos 46,XX,dup(8)(q?24.1q?24.3)[22]/46,XX[28]; the mosaic interstitial duplication was confirmed also by FISH and microarray, which disclosed the same fetal breakpoints. Only two cases have been described with pure 8q24 duplication characterized by microarray (Concolino et al., 2012;Wheeler, 2010) and none in mosaic condition. Mother had hydrocephalus at birth, short stature, facial dismorphisms, psychomotor and mild cognitive delay. Fetal ultrasound investigation was normal. Similar deletions have never been reported. We speculated about possible clinical consequences evaluating the role of genes mapped in the deleted/duplicated region, such as KCNK9, associated with Birk-Barel mental retardation dysmorphism sindrome (#612292); in brain tissues, it is expressed only from maternal allele and this might have a possible role in maternal and newborn phenotype. We have also suggested a possible mechanism of formation of this unique familial case

J11.09
Application of array CGH technique for postnatal identification of constitutional abnormalities

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The identification of cytogenetic imbalance is an important component of clinical genetics. Genetic abnormalities have been associated with 6-13 % of stillbirth, but the true prevalence may be higher. Chromosomal abnormalities often cause specific and complex phenotypes resulting from an imbalance in the normal dosage of genes located in a particular chromosomal segment. Furthermore, many multiple malformation syndromes are caused by deletion or duplication of genomic region. In the interest of diagnosis, conventional cytogenetic analysis such as chromosomal banding technique is applied as the first choice, which allows for the unambiguous identification of each human chromosome with the detection of aneuploidy and many large structural rearrangements, including translocations, large deletions and duplications. The molecular cytogenetic platform on which our study is based on is array comparative genomic hybridization (aCGH), which is a useful diagnostic method to detect a complex cytogenetic syndromes and diseases. The aim of our study is to extensively apply, further applied and validate sensitive high throughput array CGH to efficiently investigate the cytogenetic abnormalities in all regions of Hungary. We introduced the NimbleGene array platform and we started the molecular cytogenetics analyses in 7 cases. Out of the 7 samples, in which the causative chromosomal effect was confirmed, a gain was identified at four patients (range of the gain was from 39 Mb to 1.3 Mb) and in three cases loss of different regions was detected (from 67.4 Mb to 0.17 Mb). The underlying genes were selected and the results are currently discussed with the clinicians.

J11.10

Sub-microscopic chromosomal imbalances in a patient with mild dysmorphic features, developmental delay, excessive skin grooves and hypoplasia of the corpus callosum

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Chromosomal imbalances are the major cause of developmental delay combined with dysmorphic facial features. Many of these imbalances are caused by submicroscopic deletions or duplications that are undetectable at the level of traditional cytogenetic analysis. Array-based comparative genomic hybridization (array CGH) is a powerful and high-resolution approach for detection of DNA copy number variants (CNVs).
We report a 7 month old boy with mild dysmorphic features, developmental delay, excessive skin grooves of limbs and hypoplasia of the corpus callosum. We have used genomic array CytoChip Oligo (BlueGnome, Cambridge, UK), format 2x105K, version 1.1. and BlueFuse Multi software, version 2.2. The 2x105K array detects 100 Kb imbalances on the backbone and has tiling of 20 probes over 137 OMIM disease loci. Array CGH- analysis revealed cryptic deletion of 15q11.2 region spanning 4,68 Mb and an amplification spanning 19,31 Mb of 13q12.11-13q13.3. The CytoChip results were confirmed with MLPA. The influence of the known genes in the imbalanced regions and their correlation to the phenotype will be discussed.

J11.11

The BBS12 gene mutation is cause of Bardet Biedl syndrome in two individuals from south west Iran

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Bardet Biedl Syndrome (BBS) is a muli-organic disorder with variation in symptoms in affected individuals. Nevertheless, the main signs of the BBS disease are: vision less, Obesity, Polydactyly in hand and foot, intellectual disability, and abnormalities in the genitalia. To date, 14 genes are identified involving in the pathogenesis of the BBS. Mutations in these genes cause difficulties in the cell movement and other chemical signaling pathways.The BBS1, BBS2 and BBS10 count for more than 50% of all detected mutations in BBS affected individuals, worldwide. In contrast, the BBS12 gene mutation is causative for less than 1%. However, two BBS patients with above mentioned clinical symptoms referred to us for genetic counseling and molecular genetic testing. We screened firstly the BBS1, BBS2, and BBS10 genes for both individuals with negative results. But to our surprising, we found a novel nonsense mutation in the one and a novel missense mutation in the other affected individual within the BBS12 gene. In both cases, parents were heterozygous for detected changes. To validate the pathogenic consequence of the missense mutation, 65 healthy individuals were checked. Recently, a BBS patient has been reported from north Iran with a disease causing mutation in the BBS12 gene. We also suggest the BBS12 gene as a strong candidate for mutation hunting in the BBS affected individuals in the Middle East, at least in Iran.

J11.12

Case report of patient with Charlie M syndrome - cytogenetic and molecular analysis

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Charlie M syndrome, oromandibular-limb hypogenesis syndrome, is a rare facio-neuro-skeletal disorder characterized by limb defects amputation-like, cleft palate, hypoplastic incisors, midfacehypoplasia, dysmorphic face and facial nerve paralysis. Authors in this paper present a case of a boy, age 6, who was referred for genetic consultation because of clinical appearance suggestive of Charlie M syndrome.
Cytogenetic analysis of the proband's peripheral blood using GTG banding showed unbalanced karyotype with derivate chromosome 4: 46,XY,add(4)(p15?). Molecular analysis using multiplex ligation-dependent probe amplification (MLPA) (commercial kit for microdeletion syndrome detection P096, MRC Holland) detected LOH for WHSC1 critical region in 4p16.3. Further karyotype analysis of both parents revealed that mother was a carrier of reciprocal translocation 46,XX,t(1;4)(q42;p15). Final conclusion was that patient had unbalanced rearrangement, 46,XY,der(4)t(1;4)(q42;p15) mat including derivative chromosome 4 with partial deletion of 4p15-4pter and partial trisomy of 1q42-1qter, which has resulted in fusion of genes on two different chromosomes.
In this work authors will discuss details of genetic analysis and possible combined effect of identified unbalanced chromosomal alterations on phenotype of the proband.

J11.13

A rare chromosomal rearrangement of 46,XX, der (7) in dysmorphic female with mental retardation

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Chromosomal rearrangements are rare structural rearrangements with three or more breakpoints and exchange of genetic material between two or more chromosomes. The rearrangements may be balanced or unbalanced, and are classified into type I with three to four breaks and familial origin; and type II with five or more breaks, which generally arise de novo. Unbalanced chromosomal rearrangements may lead to significant clinical consequences such as multiple congenital anomalies, dysmorphic features, and mental subnormality in the progeny. Balanced chromosomal rearrangements are often not associated with any phenotypic abnormalities and may remain undetected in family members through multiple generations. Case presentation: The patient was a 5.5 years old girl referred to genetic center with delayed development. She was born from second cousin familial marriage by normal vaginal delivery, walked and spoken from 2 years old. Congenital right knee dislocation was treated. Delayed speech was limited in about 3 words and was suffering from speech apraxia. The signs were hypertelorism, strabismus, epicanthal fold, narrow palpebral fissure, sparse eyebrows, strabismus, decayed teeth, clinodactyly of fifth fingers, increased distance between toes 2 and 3, semisyndactyly of the toes 2 and 3, and shortened fourth and fifth metatarses. There were two mental retardation girls in common cousins of parents. Also, parents had three other pregnancy resulting two spontaneous abortions and one male neonatal death due to multiple congenital heart disease. Chromosomal study of proband on the basis of GTG-banding was revealed 46,XX, der(7), t(7;10)(p22; q24).

J11.14

Focus on chromosome 22 - Rare Chromosome Disorders

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Many genetic conditions are related to changes in particular genes on chromosome 22. We present three unrelated cases diagnosed in our Genetics Department with rare chromosomal abnormalities involving the structure of this chromosome.
Cat-eye syndrome is characterized by a recognizable clinical picture, although the variability of the physical features is enormous. A female infant, one month old, was referred with following major malformations: hypertelorism, narrow palpebral fissures, cleft palate, bilateral preauricular pits, bilateral coloboma of the iris, anal atresia with a fistula from the rectum to the vagina, complex heart malformation. Cytogenetic evaluation identified an additional marker and by FISH examination has been confirmed the involvement of chromosome 22.
Phelan-McDermid syndrome/22q13 Deletion Syndrome is a genetic syndrome caused by disruption of the SHANK3/ProSAP2 gene on the terminal end of chromosome 22. A newborn male with neonatal hypotonia, mild dysmorphic features and large, fleshy hands was suspected with chromosomal abnormality. G-banded chromosome analysis identified terminal deletion involving 22q13.3. Parental karyotypes were normal. FISH analysis confirmed deletion of SHANK3 gene.
Microdeletion 22q11.2 syndrome associated with XXY karyotype (Klinefelter syndrome). This double chromosomal abnormality is a rare finding in clinical practice. A case with DiGeorge phenotype was confirmed by conventional cytogenetic testing and FISH analysis, but the results revealed also XXY karyotype.
There is a great variability of clinical phenotypes in chromosomal disorders: some of clinical findings are distinct and others are very common. Clinical skills combined with standard and molecular technologies could offer a definite diagnosis and proper genetic counseling.

J11.15
Microstructural genomic imbalances in patients with congenital malformations

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In the current study 52 patients were selected for array CGH analysis after implementing stringent criteria (a combination of congenital malformations, dysmorphic features and behavioral disorders, and patients with multiple abnormalities and the presence of at least one major anomaly). A combination of methods was applied - cytogenetic analysis, FISH method, oligo DNA microarrays.
The results of analysis of microarrays revealed definite etiology in 9 out of 52 patients tested. Fifteen pathological aberrations were found in them. All pathological findings were validated by FISH analysis. Genotype/phenotypic correlations between different patients were confirmed. In addition, the majority of the patients tested (41 patients) showed 124 normal variations in the number of copies and 108 variations of unknown clinical significance (34 patients). Analyses of the type and distribution of the different variations was performed and the clinical significance of variants of unknown nature was discussed.
Our results show the advantages of high resolution microarrays for clinical diagnosis of patients with congenital malformations associated intellectual disability. The results also highlight the need for extensive population studies revealing the molecular nature and clinical significance of different CNVs and the creation of detailed maps of variations in the Bulgarian population. This would facilitate greatly the precise interpretation of specific genomic imbalances in clinical aspect and would ease the widespread introduction of microarrays in diagnostic practice not only for postnatal diagnosis of individuals with developmental delay and dysmorphism, but also for prenatal genetic diagnosis.
Acknowledgements: Grant 02/76-21.12.2009, National Science Fund, Bulgaria.

J11.16
Duplication on chromosome 17 and deletion on chromosome 20 in a patient with craniosynostosis

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We present molecular cytogenetic analysis of a boy with syndromic craniosynostosis. He was born to healthy parents after an uncomplicated pregnancy. At birth he presented with saggital craniosynostosis and seizures. Further examination revealed complications including a cardiac malformation. At eight years and two months the patient showed neuropsychomotor and behavioral anomalies. G-banding showed an apparently normal male karyotype. A DNA sample was taken for chromosome microarray analysis (CMA) using the Affymetrix 750k CytoScan array. The results showed terminal alterations of chromosomes 17 and 20. Specifically, there was a duplication on the long arm of chromosome 17 (chr17:78,952,204-81-,060,886), together with a deletion of 1.4 Mb of long arm of chromosome 20 (chr20:61,643,144-63,003,805). The deletion on chromosome 17 includes more than 80 genes and miRNAs, while the deletion on chromosome 20 includes more than 50 genes and miRNAs. The data are indicative of an unbalanced translocation of part of chromosome 17 to chromosome 20. Partial trisomy of chromosome 17q has been previously reported in the literature, and the patient reported here shows phenotypic overlap with the previously reported spectrum, including psychomotor delay, craniofacial assymetry and other dysmorphisms. Previous reports also suggest that severity of the phenotype associated with 17q partial trisomy may be associated with partial monosomies of other chromosomes, and this also appears to be the case with the current patient.

J11.17
Atypical presentation of 17q21.31 microdeletion syndrome

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The combination of developmental delay and dysmorphic features is frequently caused by different chromosomal imbalances. Many of these imbalances are result of submicroscopic deletions or duplications that are impossible to detect by conventional karyotyping. One of the best analysis which can be used in such cases is the array-based comparative genomic hybridization (array CGH) as it is a high-resolution approach for detection of DNA copy number variants (CNVs).
We report a 15-month-old boy with dysmorphic features, mild developmental delay, congenital glaucoma and partial coronal craniostenosis. We have used genomic array CytoChip Oligo (BlueGnome, Cambridge, UK), format 2x105K, version 1.1. and BlueFuse Multi software, version 2.2. The 2x105K array detects a loss of 231 Kb that overlaps 3 HGNC and 1 OMIM gene. OMIM disease: Chromosome 17q21.31 deletion syndrome (610443). The maximum overlap between an ISCA pathogenic region of type loss and this region is 30%. 56% of the region is covered by significant polymorphisms of type loss (DGV: 56%, ISCA: 0%). The CytoChip results were confirmed with MLPA. The influence of the known genes in the imbalanced regions and their correlation to the phenotype will be discussed. The above mentioned features do not correlate with the typical presentation of Koolen-De Vries syndrome as aspected from the aCGH results.

J11.18
5p13.3p13.2 duplication associated with severe intellectual disability, congenital malformations and chromosome instability manifested as aneuploidy

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Chromosome 5p13 duplication syndrome is usually attributed to increased copy number of NIPBL and FLJ13231 genes. Here, we report on a duplication producing phenotypic outcome similar to chromosome 5p13 duplication syndrome, spanning 5p13.3p13.2. Using SNP/oligonucleotide CGH, a duplication within 5p13.3p13.2 affecting a genomic locus of 994 kb spanning TARS, ADAMTS12, RXFP3, SLC45A2, AMACR and C1QTNF3 in a one-year-old boy presented with severe intellectual disability, developmental delay, corpus callosum hypoplasia, microcephaly, congenital cataract, congenital optic atrophy and large ears. It is noteworthy that the present case exhibits more severe phenotype than previously described in chromosome 5p13 duplication syndrome. Therefore, duplication of these disease-associated genes causes a more severe subtype of this duplication syndrome. Additionally, chromosome instability manifesting as aneuploidy has been observed in the index case. Bioinformatic pathway analysis has shown that chromosome insatiability is likely to result from alteration of aneuploidization and cell cycle regulation pathways through interaction of RXFP3 product with APP and PSEN1/2, alteration to which disrupts the mitotic spindle and directly inhibits mitotic microtubule motors. Furthermore, the duplication has the potential to cause chromosome instability through altered interactions between AMACR product and ubiquitins. Thus, high-resolution analysis of copy number variations in this case provided for efficient correlation between genotype and phenotype data, which can be used to elucidate pathogenic processes in a given patient. In addition, this case representing a severer variant of chromosome 5p13 duplication syndrome has also allowed defining previously unknown pathway to chromosome instability. Supported by the Russian Federation President Grant (MD-4401.2013.7).

J11.19
A patient with partial 12q dublication and 10q deletion

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We report a female patient with facial dysmorphic features, prenatal and postnatal growth retardation and diarrhea. This patient was 18 months old and referred from pediatric service. She was born at 8 months, weighing 2000 g (<3 centile). Her mother and father were healty. On physical examination, her weight was 4500 g (<3 centile) her height was 64 cm (<3 centile) and her head circumference was 39 cm (<3centile). The dysmorphic features were frontal bossing, arched eyebrows, hyperrtelorism, and wide nose bridge. Secundum ASD and PDA were also detected with echocardiography. After cytogenetics analyses, partial 12q duplication (q24.11-q24.33) and partial 10q deletion (q26.3) were detected with array CGH. We observed that the patient features and array CGH results were compatible. As a result, array CGH can be used to define a better karyotype phenotype correlation in patients with unbalanced chromosome abnormalities.

J11.20
Identification of microdeletion 8q23.3q24.11 by MLPA in patient with multiple hereditary exostoses

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Multiple hereditary exostoses (EXT) is an autosomal dominant disorder characterized by multiple projections of bone capped by cartilage, most numerous in the metaphses of long bones, but also occuring on the diaphyses of long bones. EXT type I is caused by mutation in the gene encoding exostosin-1 (EXT1) which maps to chromosome 8q24. Here, we report on the second case microdeletion of 8q23.3q24.11. The patient is a 15-year and 6 month-old boy with dysmorphic features, dyslaly, multiple exostoses, delayed bone maturation and short stature. Dysmorphic features included expressed thick eyebrows, sinofris, large and prominent ears, protruding philtrum, microcephaly. Initial conventional karyotyping was normal. The MLPA (multiplex ligation-dependent probe amplification) screening with SALSA P245 kit showed deletion of EXT1 gene. The deletion was than confirmed by SALSA MLPA P228 indicating 1.46 Mb distal deletion in 8q23.3q24.11 region including EIF3H gene (exon 8) and EXT1 gene (exon 2-11). Because the parents were not available for study, we were not able to determine if this deletion was de novo or inhereted. There is only one case which included 1.46 Mb 8q23.3q24.11 deletion reported so far. This case highlights the importance of using MLPA technique for accurate characterization of rare chromosomal rearrangements in order to make possible genotype-phenotype correlations and to understand the genetic mechanisms involved. Finally, we recommend to perform MLPA in a patients with multiple hereditary exostoses phenotype in whom no mutation is identified in the EXT1 gene.

J11.21
The clinical phenotype in a familial deletion 18p syndrome

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Deletion 18p syndrome is characterized by dysmorphic features, growth deficiencies, and mental retardation with a poorer verbal performance. About 1 in 50,000 babies is born with a deletion of 18p. Most reports suggest that 18p deletions affect girls more often than boys. Until now, few families have been described with limited clinical description. Women with del(18p) are fertile and seem to have a normal miscarriage rate. We report transmission of deletion 18p from a mother to his son. The proband is 8 years old and has short stature, dysmorphic features, polymorphous dyslalia and moderate mental retardation. The mother also presents dysmorphic features, mild mental retardation and has better verbal abilities than his son. Our report presents a mother with del(18p) syndrome having two miscarriage and no analysis was performed on the fetus. Chromosome analysis from the proband and their mother revealed the same chromosomal deletion: 46, XX, del(18)(p11.2).
This report sheds new lights on the familial del(18p) syndrome. Cognitive performance may be more variable than previously suggested within the same family. Management needs to be handled by a multidisciplinary team and includes speech therapy, hormonal (if necessary) and psychological care. Patients have an essentially normal life expectancy but will need to attend regular medical visits. Genetic counselling for these patients should take into account these new data, especially in regard of a wider variability of intellectual outcomes and better verbal performance.

J11.22
A rare case with De Novo Isochromosome 18p Syndrome

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Tetrasomy 18p is a very rare chromosomal disorder which affects males and females equally. The small extra metacentric marker chromosome results from a spontaneous mutation early in embryonic development in most of the cases. Here we report a de novo supernumerary i(18p) in a 8 months old female dysmorphic child. The case was delivered by Cesarean section at the 38th weeks of gestation with anthropometric parameters <-2 sd. Other significant features of the case were growth retardation, neonatal feeding problems, microcephaly, seizures, hearing loss, strabismus, refractive errors, high arched palate, and constipation. An additional metacentric marker chromosome was revealed by conventional cytogenetic analysis. The chromosome constitutions of the parents were normal. Based on physical features of the case, FISH analysis specific to chromosome 18 were performed and tetrasomy 18p was diagnosed. The clinics of the patient was compared with the previously published cases.

J11.23
Clinical and genetic examination of syndromic limb defects

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Limb developmental defects are known to be rare conditions that occur in about 10 / 10 000 live births, but their significance cannot be negligible. Our aim was to work out a genetic examination protocol that can help to find the most effective way to reveal the background of limb developmental defects. Following detailed clinical inspection, genetic examinations were performed in selected cases. Firstly, we usually indicated chromosomal analysis that was followed by FISH in negative cases and in the other cases specific we performed molecular diagnosis. In this paper we would like to feature some of our cases - with compound syndrome including limb developmental defects - where genetic examinations were available: 4q deletion syndrome, Holt-Oram syndrome, CHARGE syndrome, Down syndrome.
Dysmorphic features, connected symptoms may help to find exact diagnosis in compound syndromes and give chance for genetic examinations in chromosomal or monogenic syndromes. Interdisciplinary collaboration is recommended for proper diagnosis, genetic counseling and understanding of the pathogenesis. Despite the latest techniques, genetic background of limb development and its defects still belongs to an unrevealed part of science.

J11.24
A de novo marker chromosome derived from 15q in a patient with growth retardation: genotype-phenotype correlation

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We report a female patient with growth retardation who was referred to our clinic from pediatric service. She was the first child of 41 year old mother and was born at 31 weeks with 1640 gr birth weight. Her mother and father was healthy and have normal karyotypes. On physical examination, shorth neck, shorth philtrum, high arched palate, hypertelorism, simple ear, sacral diple, macrocephaly and hypothenia were detected. Cardiological evaluation was normal. In the cytogenetic analysis 47,XX,+mar was detected. We performed chromosomal microarray using an Affymetrix 750K SNP array platform. The application of array offered a precise characterization of the marker chromosome plus additional findings. The most significant finding confirming the conventional analysis was a 10,145 kbp duplication on chromosomal location15q11.2-13.3. Additional unconfirmed findings above the cut off values involvingthe sites containing OMIM genes were: A 837 kbp gain within 4p15.32, and a 1066 kbp loss on the X chromosome's short arm, p22.33. We observed that the patient features and array CGH results were compatible. As a result, array CGH can be used to define a better karyotype phenotype correlation in patients with dysmorphic featues which can not be expained by known syndromes.

J11.25
Recurrent pregnancy loss and familial marker chromosome: case report

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19-year-old female patient with recurrent pregnancy loss referred to Department of Medical Genetics from Department Obstetric and Gynecology of Afyon Kocatepe University. Her physical evaluation was normal. Biochemical and hormon test results were normal. They had second cousin marriage. There was an ectopic pregnancy and a 10 week intrauterine dead history in their 6 years marriage. The karyotype of the abort fetus was 46,XY. The family history was not specific, but postnatal exitus was reported in two of her sisters. The karyotype of the propand was detected as 47,XX+mar[108]. The marker chromosome was regular and had same structure in every metaphase plate. Centromere was not detected by C-banding. Physical examination of the Proband's husband was normal and his karyotype was 46,XY. The mother of the proband had 46,XX[71]/47,XX,+mar[29] karyotype and father had 46,XY karyotype. The proband has 7 sisters and two of them had regularly the same marker chromosome in their karyotype, the rest had normal karyotype. Sisters who had marker chromosome have postnatal exitus history in their pregnancies. Fluorescence in situ hybridization-FISH report is expected in order to detect the origin of extra chromosomal structure. In our case report, we aimed to evaluate the effect of marker chromosome in recurrent pregnancy loss ethiology.

J11.26
The Case Of Michel Aplasia In Russian Family With Congenital Sensorineural Deafness: Results Of Temporal Bone Ct-Images Analysis

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In this study we present data on the temporal bone computed tomography of rare case of inner ear malformation - Michel aplasia in patient with congenital bilateral sensorineural deafness from Russia (Sakha Republic, Yakutia, Eastern Siberia). The CT-images (1 mm) of temporal bone has been performed. This case of Michel aplasia was characterized by symmetrical agenesia of the cochlea and semicircular canals, bilateral abnormality of the facial nerve canal, and abnormality of the internal auditory canal on both sides. Results of this study confirm high diagnostic importance of the temporal bone computed tomography for detailed characterization of developmental abnormalities of inner ear.

Study was supported by RFBR (#12-04-00342_a, #12-04-98520_r_vostok_a, #12-04-97004_r_povolzhye_a, #14-04-01741_A), SB RAS Integration project #92 «Ethnogeny of indigenous peoples in Siberia and North Asia: comparative, historical, ethno-social and genomic analysis», the Sakha Republic President grant for Young Researchers for 2014 (RP#80), RAS Program «Fundamental Sciences for Medicine» (#30 for 2013-2015), and «Scientific and Educational Foundation for Young Scientists of Republic of Sakha».

J11.27
Interstitial del(3)(p26.3p26.1) in a patient with deletion 3p syndrome

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Partial deletion of chromosome 3p is a rare disorder with variable chromosomal breakpoints and consequently phenotypes. Although most of these deletions involve the 3p terminus, interstitial deletions may also give rise to features of the syndrome.

We report the case of male patient with mental retardation and minor dysmorphic features and a 7.4 Mb deletion of 3p26.3p26.1 [arr 3p26.3p26.1 (61,891 7,527,372) ×1], encompassing the genes CHL1, CNTN6, CNTN4, CNTN4-AS2; IL5RA, TRNT1, CRBN, LRRN1, SETMAR, SUMF1, ITPR1, EGOT, LOC100507582, BHLHE40, ARL8B, EDEM1, MIR4790 AND GRM7. We compare the clinical phenotype of this patient to previously reported cases of 3p syndrome.

Microarray technologies are increasingly becoming the tool of choice to accurately determine the underlying genetic cause and resulting phenotype in patients with mental retardation and multiple anomalies. aCGH allows to precisely determine the length and breakpoints in order to better understand a child's future development and needs.

In the present case molecular karyotyping has characterized a 3p deleted region with haploinsufficiency of neurodevelopmental genes associated with cognitive deficit and mental retardation, which may help to identify genes important to growth and development that contribute to the deletion 3p syndrome phenotype and aid in better understanding the molecular basis of the 3q syndrome.

J11.28
16p13.11 microduplication: a case report

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The short arm of chromosome 16 is very rich in segmental duplications, predisposing this region of the genome to a number of recurrent rearrangements, namely deletions and duplications. Although it is already known that there is a strong association between 16p13.11 deletion and neuropsychiatric disorders, the clinical significance of its reciprocal duplication is not clearly defined yet. 16p13.11 microduplication that results of non-allelic homologous recombination is a very rare genetic alteration which can be associated with variable clinical features including behavioural abnormalities, developmental delay, congenital heart defects and skeletal anomalies. We report a 7-years-old boy with global developmental delay, speech absence, microcephaly, dysmorphic facial features and inexpressive *facies*. Microarray analysis revealed a 3.3Mb duplication comprising the 16p13.11-p12.3 region, which was confirmed by fluorescence *in situ* hybridization with a BAC clone for 16p13.11. Eight annotated genes are present in this region including *NDE1*, the candidate gene for neurological and behavioural phenotype. Although this microduplication has been found in the normal population, is significantly enriched in patients with autism, schizophrenia and cognitive impairment. Several case reports until now suggest that this genomic abnormality has incomplete penetrance and variable expressivity and can constitute a new syndrome. With this case we intend to contribute to expand the spectrum of clinical findings associated to this genomic abnormality and provide further knowledge of the pathogenic involvement of this duplication.

J11.29
Immun deficiency in monosomy 18p

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Immune deficiency may be a rare feature in chromosomal disorders. A 31-year-old man with immunodeficiency, intellectual disability and facial anomalies was admitted to the genetics department. The patient was born to nonconsanguineous parents. He had congenital hypothyroidism and intellectual disability. During childhood, he had recurrent oral aphthous lesions, ear infections and pneumonia. Investigations revealed IgA, IgG and IgM deficiencies with a normal lymphocyte count. Magnetic resonance imaging of brain showed hyperintense lesions in peripheral white matter. On physical examination, he had short stature, and dysmorphic facial characteristics including flat nasal bridge, low-set ears, epicanthic folds, and short philtrum. Facial dysmorphic features, intellectual disability and immunodeficiency may suggest some dysmorphic syndromes including 22q11.2 deletion, ICF and others. Karyotype analysis revealed a partial chromosome 18p deletion: 46,XY,del(18)(p11.1).

Chromosome 18p partial deletion is one of the most common deletion syndromes. The estimated frequency is 1 in 50,000 live-born infants. The cause of this disorder is deletion of short arm of chromosome 18 or sometimes deficiency in a ring 18 chromosome. IgA deficiency, central nervous system abnormalities such as holoprosencephaly, and mild to severe intellectual disability usually accompany.

J11.30
A report of partial monosomy of distal 5p and partial trisomy of distal 19q in a family with Charcot-Marie-Tooth disease

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We present two sisters with mild developmental delay/ intellectual disability and additional clinical features. The younger girl, 21 months of age, had hypotonia, short stature, microcephaly, hip dysplasia, delayed teeth eruption, bilateral epicanthus, wide nasal bridge, short philtrum, short neck, clinodactyly of fifth finger, low serum calcium and parathormone. Clinical features in her sister, 11 years of age, were similar, but in contrast, she had macrocephaly, and additional skeletal anomalies: kyphosis, pectus carinatum, contractures of elbows and knees, right clubfoot. Positive family history of Charcot-Marie-Tooth disease was revealed (patient`s mother had duplication of CMT1A region, patient`s grandfather had clinical symptoms of CMT). Testing for PMP22 duplication was performed, and it was positive for the older sister. Karyotype of both sibs established by GTG banding was 46,XX, der(5)t(5;19)(p15.3;q13.2). Subsequent chromosome analysis in parents revealed maternal balanced translocation t(5;19)(p15.3;q13.2), which was later confirmed by FISH. The same translocation was detected in grandmother.

Both del5p and dup19q are described in literature as pathogenic imbalances. 5p terminal deletion causes cri-du-chat syndrome (mewing cry, microcephaly, epicanthus, depressed nasal bridge, fifth finger clinodactyly, hypotonia). 19q13.2àqter duplication is associated with wide range of congenital anomalies and dysmorphic facial features, including growth retardation, microcephaly, heart defects, hypoplasia of the gallbladder, renal anomalies, hypertelorism/flat nasal bridge, dysplastic ears, downturned mouth corners, clinodactyly. Synthesis of del5p15.3 and dup19q13.2 symptoms determines the clinical phenotype of our patients. Additional skeletal malformations in older sister are caused by CMT. This report provides clinical characterization of previously unreported chromosomal rearrangement.

J11.31

Clinical characterization of a patient with mosaic microdeletion 7q36.1-qter

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Individuals with 7q36.1-qter microdeletion may have a wide range of clinical manifestations including developmental delay, craniofacial abnormalities (agenesis/hypoplasia of corpus callosum, microphthalmia, choanal atresia/stenosis), skeletal anomalies (absent sacrum), heart defects (atrial septum defect, patent ductus arteriosus, pulmonary segmentation defects), obesity, urogenital system anomalies (ectopic/supernumerary kidneys, hydronephrosis, micropenis), seizures and behavioral changes. We present the clinical features of a 3-year-old boy with mosaic7q36.1-qter microdeletion. Our patient presented with unspecified developmental delay and cognitive impairment with speech delay. Mild facial dysmorphism was noticed, such as upslanted palpebral fissures, prominent nasal bridge and narrow palate. Another phenotypic finding was tapered fingers. Additional features included unilateral cryptorchidism, low testicular volume and childhood obesity. He had numerous motor mannerisms including body rocking, facial posturing, self-touching. Cytogenetic and molecular cytogenetic FISH analysis revealed a mosaic male karyotype with a terminal deletion of the long arm of chromosome 7 in 90% analyzed blood cells. The karyotype is described as 46,XY,del(7)(q36.1q36.3)/46,XY. Both parents have normal karyotypes. No family history of congenital anomalies or mental retardation was referred. Our findings, therefore, suggest that the phenotypic consequences are very variable. We came to a conclusion, that a post zygotic terminal deletion occurred in this boy, after performing FISH analysis, which is a powerful tool in low quantity mosaic cell line detection. Further investigation by array-CGH is needed to identify chromosomal breakpoints and the exact deleted region.

J11.32

Mowat-Wilson phenotype in two patients with normal ZEB2 gene study: an example of somatic mosaicism?

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Mowat-Wilson syndrome (MWS) is a multiple congenital anomaly syndrome characterized by typical facial dysmorphism, moderate to severe intellectual disabilities, epilepsy and variable congenital malformations. MWS is caused by eterozygous mutations or deletions in the Zinc finger E-box-binding 2 gene, ZEB2: sequence analysis detects mutations in approximately 81% of individuals; FISH detects large deletions encompassing all or part of ZEB2 in approximately 15% of persons; chromosomal rearrangements that disrupt ZEB2 cause MWS in approximately 2% of individuals; an additional 2% have intermediate-sized deletions that can be detected by techniques such as quantitative PCR, MLPA, or gene-specific array-CGH. We describe two patients with a clinical diagnosis of MWS not confirmed by genetic tests. The first patient is a 5 years old female with typical facial dysmorphisms, aganglionic megacolon, hypoplasia of the corpus callosum, growth retardation with microcephaly, psychomotor retardation with speech delay and epilepsy. Sequence analysis and FISH study of ZEB2 gene detected no alterations; arrayCGH was normal too. The second patient is a 4 years old female with typical facial features, pulmonary stenosis and bicuspid aortic valve, partial agenesia of the corpus callosum, ambiguous genitalia with scrotal labia, growth retardation with microcephaly, psychomotor retardation with speech delay, epilepsy, chronic constipation, RGE and dyschromic cutaneous areas. Sequence analysis of ZEB2 gene and arrayCGH did not find any alteration.
The two patients could be a first example of somatic mosaicism for ZEB2 gene anomaly. Study are in progress in order to demonstrate this hypothesis.

J11.33

Multiple pterygium syndrome: a case report

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Multiple pterygium syndromes include a group of multiple congenital anomaly disorders characterized by webbing (pterygia) of the neck, elbows or knees and joint contractures . Males had small penis and scrotum or cryptorchidism; females had aplasia of the labia majora and small clitoris. This syndrome included fusion of cervical vertebrae, scoliosis, flexion contraction of fingers, and rocker-bottom feet with vertical talus and facial dysmorphism with long face, high-arched palate, small mouth, and retrognathism. The multiple pterygium syndrome is phenotypically and genetically heterogeneous. It is also called as Pterygium Colli syndrome, Escobar syndrome or Pterygium syndrome. Multiple pterygium syndrome is a rare syndrome. This condition may behave sometimes as a dominant, but there clearly appears to be a recessive pterygium syndrome. İn this case, described a 9 year old female patient with pterygia of the neck, axilla and popliteal, flexion contraction of fingers, camptodactyly and rocker-bottom feet, short neck, kyphoscoliosis, downslanting palpepral fissüres, low-set ears, high-arched palate, low-set hairline, aplasia of the labia majora. To provide molecular verification of Multiple pterygium syndrome, direct sequencing of CHRNG gene is planned.

J11.34

Novel mutation in exon 13 of the TCOF1 gene in the patient with Treacher-Collins syndrome

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Treacher-Collins Syndrome (TCS) is a rare craniofacial disorder, associated with an abnormal differentiation of the first and the second pharyngeal arches during fetal development. The major features of the disease include: midface hypoplasia, micrognathia, microtia, conductive hearing loss and cleft palate. The estimated incidence is 1/50000 live births, with 60% of the cases resulting from *de novo* mutations. The syndrome is mostly caused by mutations in the *TCOF1* gene, which encodes the serine/alanine-rich protein named Treacle. TCS can be also caused by mutations in the *POLR1C* and *POLR1D* genes. Over a hundred mutations of the *TCOF1* gene in TCS patients have been described. About 70% of recognized mutations are deletions, which lead to a frame shift, formation of a termination codon and shortening of the protein.
We report a novel mutation of *TCOF1* gene in male patient with typical facial symptoms of TCS. Patient presented: prominent forehead, absent of auricular canals, third degree microtia, conductive deafness, sparse eyebrows, cleft of right eyelid, palpebral fissures slant down, broad base to nose, underdevelopment of zygomatic region, malar flattening, micro- and retrognathia. No internal defects were diagnosed.
A multitemperature single-stranded conformation polymorphism (MSSCP) analysis and direct sequencing were performed. A novel, heterozygous deletion c.1978delC was detected. Patient's parents were tested and no mutation was observed. The c.1978delC deletion causes a reading-frame shift and premature termination of translation at 677aa. We believe, that these findings will facilitate a precise diagnosis of the patient and extend our knowledge on the pathogenesis of TCS.

J11.35

Novel PTPN11 gene mutation in Iranian patient with Noonan syndrome

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Introduction Noonan Syndrome (NS) is an autosomal dominant, variably expressed, multisystem disorder with an estimated prevalence of 1 in 1000-2500. After trisomy 21, NS is the second most common syndromic cause of congenital heart disease. In recent years study on the molecular mechanisms of this disorder have been improved and expressed some pathophysiological mechanisms that cause the complications up to this abnormality such as the great range of medical and developmental features. **Methods** Genomic DNA samples were extracted from peripheral whole blood of 25 NS patients using the standard procedure. For each patient, exons 1-15 of the PTPN11 gene individually amplify by polymerase chain reaction (PCR) using 15 sets of designed primers. The amplified fragments of PTPN11 gene were purified and directly sequenced. **Result** Three known PTPN11 mutation hotspots (exons 3, 8, 13) were checked first, and a previously identified mutation (Asn308Asp) was found in only two patients. We also found a novel non-synonymous substitution (Asp155Asn) in exon 4 of an eight years old patient. **Conclusion** This study is the first report of PTPN11 mutations in Iranian patients with NS. A non-synonymous mutation was found in exon 4 that is novel in NS but has registered before as somatic mutation in cancer patients with large intestine tumor. The affected probands who are negative for PTPN11 mutations will be screened for other 10 genes mutations involved in NS using next-generation sequencing.

J11.36

Oromandibular-limb Hypogenesis Syndrome Type IIIC? A case in Russia

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Oromandibular limb hypogenesis sindromes (OLHS, OMIM %103300) are extremely rare hereditary syndromes with overlap of phenotypes, most of them occur sporadically. OLHS constitutes combinations of congenital malformations of mandible, tongue, upper and lower limbs and normal mental development. The pathogenesis of OLHS is still unknown, however, it is considered to have several possible causes: heat-induced vascular disruption; teratogenic etiology and genetic origin of these syndromes. According to Hall's classification OLHS is divided into five categories; with the only necessary criteria for inclusion - hypoglossia. However, in some cases, there is a considerable overlap of symptoms, making it difficult to define the phenotypic boundaries between OLHS. We report the case of a 3-year-old girl. She is the second child of healthy, nonconsanguineous parents, born after an uneventful pregnancy, by normal delivery. She was born in 39gw with low birth weight(1700g) and length(46cm). She has a sister, who is normal. There is no family history of congenital abnormalities. Currently she has low weight(9kg), height(80cm) and microcephaly(44cm). She has a mental retardation and absence of speech. Her face has signs of hypoglossia, ankyloglossia, cleft palate, malocclusion with normal teeth, epicanthal folds, low-set and rotated ears. She also has oligodactyly hands and feet. Chromosomal study of peripheral blood lymphocytes confirmed the 46,XX karyotype. By Hall's classification our case conforms to OLHS type IIIC: Glossopalatine ankylosis with hypoglossia - hypodactilia. However, as we know, combination OLHS type IIIC with mental retardation, microcephaly, low weight and height has not been described before.

J11.37

A case with frontonasal dysplasia and 2q36.1q31.2 deletion

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A 7 years old girl was referred to our genetics lab. The first and only child of non- consanguinuous parents, she was delivered at 9 months by C-section. Her birth weight was 2690 grams. She was diagnosed with renal stones at 6 months. She had bilateral deafness and underwent surgery. At examination she had normal intelligence and development. Her facial features include epicanthal folds, hypertelorism, blue eyes, arched eyebrows, high nasal bridge and short philtrum. She had bilateral simian crease, camptodactyly of fourth finger of right hand and syndactyly of second and third toes.
Her karyotype was normal. Whole genome Oligo Array Comparative Genomic Hybridization was performed using CYTOCHIP ISCA 4X44K whole genome oligo array version 1.1 and was analysed using BlueFuse Multi software. A 3.09 Mb deletion of 2q36.1q36.2 was detected.
The deletion spans nucleotides 222 Mb to 225.1 Mb covering 10 OMIM genes and 13 refseq genes. We compare phenotypic and genotypic findings of other overlapping cases. The deleted region in our patient includes the EPHA4 and PAX3 genes that have been formerly implicated in frontonasal dysplasia.

J11.38

Six patients from four unrelated Tunisian families with Peters plus syndrome harboring the same splice site mutation in the B3GALTL gene

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Peters plus syndrome is an autosomal recessive rare disorder Peters' plus syndrome is an infrequently described entity that combines anomalies in the anterior chamber of the eye with short stature, distinctive facial features often associated with other major/minor additional defects and a developmental delay. Peters plus syndrome is related to mutations in the B3GALTL gene, localized on chromosome 13 (q12.3q13.1), leading to the inactivation of the B1-3glucosyltransferase.
In this study, we screened the B3GALTL gene in six patients, from four unrelated families, with typical Peters plus syndrome. We reveled inter and intra familial phenotype variation. However the novel homozygous c.597-2-A>G mutation was identified in all patients suggesting an effect founder of this mutation. Functional study using an ex-vivo approach showed that this mutation causes complete skipping of exon 8 in the B3GALTL cDNA, which altered the open reading frame of the mutant transcript and generated a PTC within exon 9. This finding potentially elicits the nonsense mRNA to degradation by NMD.
All these data confirm an important role of the B3GALTL gene test that provides diagnosis confirmation and improves genetic counseling for the families.

J11.39

PITT-HOPKINS Syndrome: A new case of intragenic deletion detected by array-CGH

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Pitt-Hopkins syndrome is a rare syndromic mental disorder, mainly characterized by severe intellectual disability with stereotypic movements, typical facial gestalt (deep-set eyes, broad beaked nose, wide mouth with bow-shaped upper lip and widely spaced teeth), childhood-onset hyperventilation and seizures.While PTHS appears to be a recognizable clinical entity,it seems to remain underdiagnosed principally when characteristic dysmorphic features are less typical due to similarities with other known genetic syndromes (Rett, Angelman).PTHS is an autosomal dominant condition caused by haploinsufficiency of the TCF4 gene on 18q21.2. The molecular abnormalities identified in more than 100 patients include 40% of point mutations, 30% small deletion or insertion and 30% of partial or total gene deletions. We report on a 25-year old boy with severe intellectual disability,absent language, ataxic gait, anxiety and agitation, dysmorphic features and smiling appearance.Due to the lack of the hyperventilation and the presence of atypical dysmorphisms, we decided to start genetic investigations by array-CGH (Bluegnome LTD, 44K-feature whole genome). The result was a loss of 103Kb on 18q21.2 spanning from 53,045,402 bp to 53,149,037 bp (hg19) within the TCF4 gene. This gene has 20 exons (the first and the last non coding), with several isoforms reported. Our deletion includes exons 4-6(NM_001083962) and is predicted to result in a frameshift. At our knowledge, this is the second one described in literature involving these 3 exons. Reporting our case we want to contribute to the phenotype-genotype correlation in Pitt-Hopkins syndrome, mainly in those cases with a small intragenic deletion and a less typical phenotype.

J11.40

Genotype-phenotype correlation with ring chromosome 11

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Ring chromosomes usually result from distal breakage of both chromosomal arms and carriers often exhibit a general overlap in phenotype. Jacobsen syndrome (JBS) is a rare contiguous gene disorder caused by terminal 11q deletion, characterized by intellectual disability, various physical anomalies and a distinctive facies. In this study we report a 12 yo boy with a ring chromosome presenting with global developmental delay, characterized by hyperactivity and repetitive behavior, hypertension, obesity, dyslipidemia and food compulsion. Clinical findings include short stature, microcephaly, bitemporal narrowing and occipital flattening, short nose with long filter, small carp mouth, low-set ears, short neck and systolic murmur. Cytogenetic analysis by GTG banding revealed karyotype 45,XY,-11[18]/46,XY,dicr(11;11)[4]/46,XY,r(11)[78]. Array-CGH using 2X400kb platform (Agilent) showed a large 8.6Mb terminal deletion: 11q24.2q25(126,368,150-135,006,516)x1, which includes 40 genes. There was no deletion in the 11p region suggesting that the ring was formed by fusion at 11q24.2 with 11ptel region. This interpretation was validated by FISH using 11q25, 11ptel and 11qtel probes. Our genotypic results could explain the short stature, compulsive behavior and ADHD, as well the early-onset hypertension (associated with KCNJ5 gene) compatible with JBS. However, his phenotype does not include thrombocytopenia (related to FLI-1, ETS-1 and NFRK3 genes) or kidney abnormalities (KCNJ1 and ADAMTS15 genes). We suggest that some characteristics described in JBS cannot be explained by monosomy of single genes, but rather the combination of contiguous genes, or gene-gene interactions.

J11.41

Partial SHOX duplication in a daughter and her father associated with short stature.

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We report a case of a familial partial duplication of the SHOX gen detected in a 14 year old girl and her father. The girl was referred to us for short stature and development delay. She has a Dandy Walker abnormality of the brain and

no other congenital malformations. She was born at term, her developmental milestones were delayed, she has borderline mental retardation and attends special school. Her height is 152.5 cm (3rd percentile), weight 70.3 kg (97th percentile) and head circumference 51.5 cm (3rd percentile). She has mild dysmorphic features. Karyotype is 46,XX. SNP a CGH (HumanCytoSNP-12-v2.1 Illumina) detected 190,9 kb duplication of Xp22.33 (422.642-613.567) encompassing most of the SHOX gene. The finding was confirmed by MLPA (MRC, Holland, Human Telomere -5, kits P036 - E1; P070 - B2; P245-B1; P106-B1 MRX), which detected a duplication in the SHOX gene region. The same duplication was revealed by MLPA in her father, who measures 170 cm (10th centile) and has no congenital malformations. Isolated duplications of SHOX gene are rare and their effect on height is not clear. Seventeen patients with full and sixteen with partial duplication of the SHOX gene were reported in the literature. Some were ascertained through studies of particular conditions associated with short stature (idiopatic short stature, Léri-Weill dyschondrosteosis), in those who were not, the stature varied from normal to tall. The effect of SHOX gene duplication on stature is still not clear.

J11.42
Somatic mosaicism in patients with r(18) and congenital heart disorder

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Syndrome r(18) - ring 18 chromosome cytogenetically is characterized by a complete or mosaic forms a ring chromosome 18 with the absence of the distal portions of the long and short arm of the chromosome. The clinical picture includes multiple dysmorphia, combined with varying degrees of mental retardation. Committal involvement of cardiovascular system and congenital heart defects typically are not described in cases of r(18).
We observed 18-year-old patient with mental retardation, multiple facial dysmorphia, and subaortic stenosis. During standard karyotyping by PHA-stimulated lymphocytes patient revealed 46, XY, r(18) karyotype. Since congenital heart defects are not common feature of r(18) we hypothesized mosaic chromosomal aberrations and performed additional FISH analysis on blood smears using Aneu Vision (CEP 18) and To Tell Mix 11, Mix 12 probes.
The study revealed three signals of chromosome 18 centromere and subtelomeric deletion of chromosome 18 in 15% of the cells, contained two signals from the centromere of chromosome 18 with subtelomeric deletion to form a ring in 80% of cells, 5% of the uncultured cells in blood smear contained two signals from the centromere of chromosome 18 without subtelomeric deletion.
In conclusion we describe clinical case of somatic mosaicism in patients with r(18) in combination with 18 trisomy in 15% of blood cells, leading to combined phenotype of mental retardation and congenital heart defect.

J11.43
Molecular study of the gene TCOF1 in the syndrome of Treacher Collins : about 7 cases

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Treacher Collins Syndrome (TCS) is a rare clinical entity which associates essentially craniofacial dysmorphism and deafness. Symptoms can sometimes be severe being life-threatening when choanal atresia exists. Our objective is to look for the TCOF1 (5q32-q33.1) mutations most frequently reported in the literature in Tunisian patients affected by TCS. We were interested in the study of the mutations in 11 of the 24 exons of the gene TCOF1 by the method of direct sequencing in 7 patients among whom 4 family cases. These exons are reported as hotspots of mutations. The confirmation of TCS diagnosis by molecular biology was possible for 3 of our 7 patients. The sequencing allowed to identify a new mutation not reported in the literature which was found in 2 cases from the same family (a father and his daughter). An already described mutation was found in 1 sporadic case. Eight polymorphisms were found, among which five are known. The mutations found in our patients occur in repetitive sequences which support the hypothesis of polymerase errors. No mutations were detected in exon 24 which account for 20 % of already reported cases. The molecular study of the TCS allows the confirmation of the diagnosis, the orientation of the clinical follow up and the prenatal diagnosis in the severe forms.

J11.44
Co- occurence of tetrasomy 12p and trisomy 12q

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A female neonate was born at a gestational age of 38 weeks by spontaneous vaginal delivery, with a birth weight of 3600 gr (50th-75th centiles). Antenatal ultrasound had shown minimal pelvicalyceal dilatation of left kidney, and no other abnormalities were detected on abdominal ultrasound scan. Postnatally, she was noted to have hypertelorism, prominent and wide forehead, upslanting palpebral fissures, long philtrum, pigmentary mosaicism in both feet, hyperplasia of the gingiva and hypotonia. Cranial magnetic resonance imaging showed an asymmetrical dilatation of right pontocerebellar cistern, with a suspicion of an arachnoid cyst. On follow-up, she had progressive hyperpigmentation over both lower extremities and a skin biopsy was performed with a clinical diagnosis of Pallister-Killian syndrome. Karyotype analysis from peripheral blood lymphocytes revealed 46,XX and karyotype analysis of skin fibroblast sample revealed 47,XX,+i(12)(p10). This finding was confirmed by fluorescence in situ hybridization by whole chromosome paint 12, that revealed 47,XX,i(12)(p10).ish (wcp12+). Further FISH analysis for 12p and 12q terminals with Vysis ToTelVysion 12 probe (12p – 8M16, 12q – VIJRM2002) revealed 47,XX,+i(12)(p10).ish +i(12p)(8M16x4, VIJRM2002x3). Combination of tetrasomy 12p and trisomy 12q is a previously unreported condition.

J11.45
Tetrasomy 18p in a patient with happy demeanor

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An 8 years old female patient was referred to clinical genetics department because of facial dysmorphic features, speech and motor delay. She was the second child of nonconsanguineous parents. Maternal age at delivery was 31 years, and the baby was born by spontaneous vaginal delivery at 39th gestational weeks, with a birth weight of 1200 gr (below 3rd centile). Psychomotor development was delayed; she sat independently at 2 years of age, walked at 6 years of age, and spoke a few words at 6 years of age. On physical examination at the age of 8 her anthropometric measures were as follows: height 119.5 cm (3rd-10th centiles), weight 22 kg (3rd-10th centiles), head circumference 49 cm (below 3rd centile). She presented with microcephaly, prominent nasal bridge, prognathism, malformed ears and long fingers. Her behaviour was characterised by inappropriate and frequent laughter. In echocardiographic evaluation patent foramen ovale and atrial septal defect were detected. Cranial magnetic resonance evaluation was normal. Peripheral blood specimens were collected for chromosomal analysis and 47,XX,+i(18)(p11. 2) was detected. This finding was confirmed by whole chromosome paint fluorescence in situ hybridisation that revealed 47,XX,+i(18)(p11. 2). ish i(p18)(p11.2) (WCP 18++). Tetrasomy of 18p is found one in every 140,000 live births, affecting males and females equally. The syndrome expresses itself with developmental delay, cognitive impairment, happy demeanor, growth retardation, muscle tone abnormalities, microcephaly, malformed ears, prognathism and congenital heart diseases. This condition must be considered in differential diagnosis of syndromes with microcephaly, happy demeanor and intellectual disability.

J11.46
The weight of a translocation

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We report on a 29 years old woman with secondary amenorrhea and short stature. Pelvic ultrasound revealed a normal uterus and little ovaries. There was no intellectual disability. She has two healthy sisters and her mother had a first trimester spontaneous miscarriage. Chromosome analysis revealed 46,X,der(X)t(X;15)(p14;q22.3) of maternal origin. Array-CGH showed a 39.6 Mb Xp22.33→Xp11.4 deletion and a 33 Mb 15q23→15q26.3 duplication. It was also observed a 92 Kb 5q22.2 microdeletion which involves MCC gene. Any genomic imbalances, deletions or duplications of an X chro¬mosome have a much more severe impact on males than fe¬males. In males, most cytogenetically visible de¬letions of the X chromosome involve the terminal portion of Xp (Xp22.2→Xpter), which leads to nullisomy of the deleted region, has been recognized as a cause of variable contiguous gene syn¬dromes. The phenotypes depend on the extent and posi¬tion of the deletion and are limited by the presence of male lethal genes in Xp22.2 at about 10-11 Mb from the telomere so larger Xp deletions extend¬ing beyond the KAL1 locus are very rare. Females with similar Xp22 deletions are often phenotypically normal except for short stature, because of skewed X-chromosome inactivation. Xp deletion women show primary amenorrhea or sometimes secondary amenorrhea. This is a pregnancy planning's dilemma: if our couple

will decide to undergo medically assisted procreation programs transmission of derivative X chromosome to sons would be letal instead transmission to daughters would determine a unpredictable variable phenotype in order to skewed X-chromosome inactivation.

J11.47
Trichorhinophalangeal syndrome type II due to a novel 8q23.3-q24.12 microdeletion detected by oligo-SNP array associated with novel genital findings.

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Trichorhinophalangeal syndrome type II (TRPS2; OMIM 150230, Langer-Giedion syndrome, LGS) is a contiguous gene deletion syndrome located on 8q23.1-q24.1. Depending on the size and position of the heterozygous deleted region, the genetic defect principally involves TRPS1, RAD21 and EXT1 genes. TRPS2 presents characteristic clinical findings with multiple cartilaginous exostoses and frequently, intellectual disability. In the present study we analyzed a female with TRPS2 by Oligo-SNP array and detected a de novo 8q23.3-q24.12 microdeletion of 5.464 Mb on 8q23.3- q24.12 involving seven OMIM genes (CSMD3, TRPS1, EIF3H, RAD21, SLC30A8, MED30 and EXT1). In addition, UTP23, MIR3610 and exon eight of SAMD12 were deleted. The final cytogenetic result was 46,XX, arr [hg19] 8q23.3-q24.12 (113,858,753-119,323,017) x1 dn. Parental testing of all procedures showed normal results. Our patient had genital anomalies with normal intelligence not previously reported. The analysis at molecular level of delete region in TRSP2 is imperative to understand the pathogenesis of the disease

J11.48
Congenital absence of the portal vein in a child with Turner Syndrome

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Congenital absence of the portal vein in a child with Turner Syndrome Introduction: Turner Syndrome is a disease caused by a total or partial loss of an X chromosome in women, showing a frequency of 1 per 2500-4000 live-born females. (1,2) Characteristic features of the disease are short stature, gonadal dysgenesis, webbed neck,cubitus valgus and congental cardiovascular anomalies. Congenital absence of portal vein, however, is rare, and there are no systemic reviews detailing the prevalance of the absence of the portal vein in patients with Turner Syndrome. 13 year old girl was admitted to emergency service for high fever. At the physical examination her respiratory rate was 60/min, she had toxic appearence. She had webbed neck and short stature.She had a shilded chest with no breast tissue and prepubertal nipples. She had splenomegaly. Laboratory tets revealed high transaminases. Chromosomal studies showed monosomy pattern compatible with Turner Syndrome (45XO). Echocardiography was consistent with aortic coarctation and bicuspid aortic valve. Patient underwent dynamic biphasic computerized tomography(CT) imaging to investigate the causes of liver disease. CT revealed the absence of portal vein. She had grade II esophageal varices in the upper gastrointestinal endoscopy. The final diagnosis was a case of Turner's Syndrome with absent portal vein, portal hypertension and hypersplenism.
References:1. Gravholt CH. Epidemiological, endocrine and metabolic features in Turner Syndrome. Eur J Endocrinol 2004; 151:657-87.
2.Bondy CA, Bakalov VK. Investigation of cardiac status and bone mineral density in Turner Syndrome. Growth Horm Res.2006;16:S103-8.

J11.49
An ~1,6 Mb interstitial deletion on Xp22.31 in a patient with psychomotor developmental delay, microcephaly, epilepsy, ichthyosis, and spastic tetraplegia.

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We present a 6-year-old boy, born at 41 weeks of gestation to non-consanguineous, healthy parents. The pregnancy was complicated by respiratory infection in the first trimester of gestation. At birth, body weight and length were normal, and head circumference was 33 cm (3 centile). The boy was evaluated at genetic counselling unit at the age of 3 years because of severe psychomotor retardation, absent speech, epilepsy, spastic tetraplegia and severe microcephaly (- 4,04SD). Generalized ichthyosis was observed. There were no dysmorphic features or structural malformations. Brain MRI showed thin corpus callosum without other abnormalities. Results of oligonucleotide arrayCGH (180 K V8.1) showed an ~1,6 Mb interstitial deletion on chromosome Xp22.31 encompassing a few genes, including VCX (candidate gene for intellectual disability), PNPLA4 , and STS associated with X-linked ichthyosis (XLI). Further analysis of the family revealed that the deletion is maternal in origin. The same Xp22.31 deletion was also found in her two healthy sisters; her grandfather presented only with ichthyosis. Some authors have reported single cases of the Xp22.31 deletion, involving the VCX gene in patients with ichthyosis and normal intelligence. Therefore, it is not clear whether the deletion is the only cause of patient's pathology, especially intellectual disability, epilepsy, and spastic paresis. Incomplete penetration or another gene within Xp22.31 deletion (e.g. PNPLA4) or unknown mechanisms might be responsible for these symptoms in our patient. Comparison with the similar cases from the literature and genotype - phenotype correlation will be discussed.

J11.50
Partial deletion of 9p: familial case of Alfi syndrome

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Monosomy 9p syndrome also known as Alfi syndrome is a rare genetic disease characterized by mental retardation, developmental delay, facial dysmorphism and various type of feminization in male.
We report two cases of Alfi syndrome in siblings.
One patient (9 year old girl) was initially diagnosed with dysgerminoma of the right gonad and gonadoblastoma of the left gonad. Following symptoms were identified: severe mental retardation, general development delay, generalized hypotonia, contractures of all joints including talocrural, knees, and phalanges as well as complete gonadal dysgenesis. Floating movements of eyeballs and epilepsy were also observed. The external female genitalia were normal. Histological analysis of gonads revealed sclerosis and no follicles as well as absence of cilia on the surface of salpinx.
Cytogenetic analysis of peripheral blood cells identified male karyotype with unbalanced translocation 45,XY, der(9)t(9;21)(p22;q11),-21. Deletion of fragment 9p22-pter was confirmed by serial FISH analysis.
Patient's sister (5 year old girl) had identical symptoms except for sex reversal and karyotype 45,XX,der(9)t(9;21)(p22;q11),-21.
Family history was remarkable for unidentified genetic disorder with mental retardation and some chromosome 21 abnormality in mother and severe genetic disease in maternal brother. Most probably abnormal karyotype in this family was inherited through female line.

J11.51
Aspects Of The Early Neurogenetic Diagnostics Of Klinefelter Syndrome

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Background: In the study are analyzed peculiarities of clinical manifestations and cytogenetic features in Klinefelter syndrome, which is a sex chromosome abnormality. The aim of research is to help achieve an early neurological diagnosis and initiation of measures to improve the development of children.
Material and methods: A group of 84 boys with Klinefelter syndrome was investigated during medical genetic counseling in the Center for Reproductive Health and Medical Genetics, presenting phenotype selection criteria such as: developmental anomalies of the external genitalia - peno-scrotal hypospadias, micropenis, small testes, cryptorchidism, cranio-facial dysmorphism, waist high and disproportionate, hypogonadism, gynecomastia, mental retardation, psychosocial problems.
Results: Homogeneous form or trisomy 47, XXY (27 cases - 84.5%) was the most common chromosomal abnormality diagnosed in the 32 patients with Klinefelter syndrome, followed by mosaic form (47 XXY/46, XY: 1 case - 3.1%), polysomy X-Y (48, XXYY: 1 case - 3.1% and pentasomia - 49, XXXXY: 1 case - 3, 1%) and variants of structural abnormalities of autosomal chromosomes (47, XXY, inv (5): 1 case - 3.1%), associated with robertsonian translocation (47, XXXY, Rob (13:14): 1 case - 3.1%). Most patients with KS had been diagnosed in puberty (23 cases - 71.9%), 6 patients (18.7%) were diagnosed at prepubertal, and only 3 patients (9.4%) were diagnosed during early childhood.
Conclusion: Neurogenetic diagnosis during early ontogenetic development and cytogenetic analysis (karyotyping, Barr test) is necessary for better investigation of children with suspection on SK to confirm the clinical diagnosis and timely provide genetic counseling.

J11.52

Prophylaxis of Congenital Malformations in Pregnant Women of a Risk Group

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Background: Efficient stabilization of genetic diseases spreading in population of Moldova means, first of all, to improve prophylaxis of congenital malformations in pregnant women. The role of genetic counseling in the prevention system of genetic diseases is analyzed in the study. The main prophylaxis measures and prenatal diagnosis methods applied to pregnant women of risk group are identified by authors.
Materials and methods: Retro-prospective study of investigation included 8937 pregnant women who have asked for medico-genetic counseling in CRHMG, in 2008-2012. Group I: 4473 pregnant women from medium and high risk group. Group II: 4464 pregnant women from low risk group.
Results: Two researched groups were comparable in age, gestation period, degree of genetic risk. The age of women in genetic risk group was from 17 years to 44 years (average age 26,1 ± 5,3 years). Prenatal diagnosis contributed to the identification of severe fetal pathologies to 478 pregnant women, which constituted 5,4% of total number of investigated cases. Amniocentesis with study of the fetal karyotype has allowed the identification of numerical and structural chromosomal abnormalities to 67 patients (3,0%). Abnormalities of the central nervous system are the most common in the structure of the serious fetal pathologies (1,4%), followed by abnormalities of the cardio-vascular system (0,87%), osteomuscular system anomalies (0,51%), abnormalities of renal system (0,64%) and digestive system (0,6%).
Conclusion: Genetic counseling and prenatal diagnosis methods (fetal ultrasound, biochemical screening, karyotyping) helps to reduce up to 50% of the frequency of chromosomal abnormalities and congenital malformations to newborns.

J11.53

The results of clinical and molecular analysis of data of patients with branchio-oculo-facial syndrome

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Branchio-oculo-facial syndrome (BOFS, OMIM #113620) is a rare autosomal dominant disorder, characterized by branchial cleft sinus defects, ocular anomalies, a dysmorphic facial appearance including lip/palate cleft or pseudocleft and associated with mutations in TAFAP2A gene. Clinical features analysis and DNA-testing are performed in 7 BOFS patients. 3 patients present three main clinical BOFS features: cervical cutaneous aplasia linear loci, ocular anomalies, orofacial cleft, 4 patients are symptomatic with ocular anomalies, orofacial cleft and accessory BOFS signs. 5 patients have lacrimal duct stenosis, one has distopy of lacrimal point in upper lip area. Orofacial clefts are observed in all 7 patients (2 - upper lip pseudocleft, 2 - upper lip cleft, 3 - upper lip/palate cleft). 2 patients have developmental abnormalities of auditory ossicles, 1 - atresia of external acoustic meatus, 4 - anatomically narrow acoustical ducts. Conductive hearing loss is diagnosed in 5 BOFS patients. Sequensing of TFAP2A gene coding region revealed missense- mutations in 4 BOFS patients. One patient has p.Arg251Gly mutation previously described, 3 patients from 2 families have novel mutations: p. Arg213Ser and p.Val280Asp. Novel mutations are not detected in healthy members of the families.

J11.54

Establishment of national birth defect surveillance in Lebanon: preliminary data

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Background: Following the WHO recommendations in 2012, the National Collaborative Perinatal Neonatal Network (NCPNN) in collaboration with the Centers for Disease Control and Prevention and the Ministry of Public Health (MOPH) inaugurated the establishment of a national Birth Defect (BD) surveillance program. A previous study conducted on 19 Lebanese hospitals, members of the NCPNN, between 2003 and 2007 had shown an incidence of 3.25% of BDs in neonates with a significantly higher occurrence in consanguineous mating. This is our preliminary data on BDs in Lebanon, from May 1st to November 30, 2012. **Methods: I**nfants born with major BDs from hospitals across Lebanon were reported. Incidence of BDs was calculated using data provided from the MOPH. **Results:** 464 infants born with a major BD were reported. The overall rate was 18.2/1000 live births. The most common were musculoskeletal defects (4.2%), mainly clubfoot (24.3%), central nervous system defects (4.01%), mainly spina bifida (40.2%) and cardiovascular defects (3.29%), with septal defect accounting for 29.7%. Facial/neck defects represented only 0.63%. Among infants with spina bifida, 8.3% of the mothers were on folic acid before pregnancy while 36.1% started it after conception. Among infants with clefts, 64.1% of the mothers reported smoking exposure during pregnancy. 30.8% of all mothers were overweight/obese before pregnancy. Parental consanguinity was reported in 33.4% of the cases; among those, 53.2% were first cousins. **Conclusion:** Ascertaining the prevalence and determinants of BDs through proper surveillance would lead to preventive measures contributing to a measurable reduction of BDs in our country.

J11.55

Arthrogryposis and perisylvian polymicrogyria: report of an emerging phenotype

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In recent years a few patients with perisylvian polymicrogyria associated with arthrogryposis have been reported. The underlying genetic cause of this condition is not yet known.
We report on a newborn male whose pregnancy was characterized by the detection of arthrogryposis, bilateral club feet, micrognathia, and a small VSD on prenatal sonogram. The patient was born full term: BW 2673 g (5th centile), BL 46 cm (5th centile), OFC 34 cm (20th centile). Physical examination at birth showed minor facial anomalies, proximal and distal contractures of the upper and lower limbs consistent with arthrogryposis multiplex congenita, lack of palmar and plantar creases, and bilateral talipes equinovarus. A brain MRI evidenced bilateral perisylvian polymicrogyria. Family history was not contributory.
The SNP array [Affymetrix Cytoscan HD microarray (average resolution of 50 Kb for deletions and 400 Kb for duplications)] showed a microduplication of 825 Kb in chromosome 14q12 (29.372.798-30.197.863, hg 19) and a microdeletion of 420 Kb in chromosome 7p22.1 (4.743.281-5.163.491, hg19). The duplication contains a control region for the *FOXG1* gene. The deletion contains several genes associated with muscular function, including *FOXK1*. The interpretation of this result is still unclear, and analysis of the parents is pending. We will proceed with exome sequencing if neither of the chromosomal imbalances are considered to be causative.
In conclusion, we propose that the association between perisylvian polymicrogyria and arthrogryposis is a novel discrete phenotype. The results of the genetic analysis will allow better insight into the molecular etiology of this recently described entity.

J11.56

Prader Willi syndrome clinical signs and phenotypic features at Lithuanian health science university hospital

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Backround: Prader Willi syndrome (PWS) - a rare genetic disease that manifestates in 1:10000 or 1:25000 births. The aim of the study was to evaluate when first starting to emerge Prader Willi syndrome symptoms and how they are distributed according to sex.
Methods: In this retrospective analysis data were collected from 29 outpatients, who had clinical symptoms of PWS. The patients weight and height were evaluated at birth and the first visit to the doctor at the time (weight and height procentile evaluated), as well as the face, extremities, breast development and other changes.
Results: All patients were applied for the obesity to the doctor, when average age was 3.23 years. Comparison of average age between boys and girls showed no statistically significant difference .The almond -shaped eyes were 33.3% of girls and 47.4% boys. Thin upper lips 33.3% of girls and 36.6% boys. Short limbs were 50.0% of girls and 57.9% boys. Tapering fingers 22.2% of girls and 26.3% boys. Sandal gap 11.1% of girls and 15.8% boys.
A comparison of the other symptoms (sucking reflex absence, epilepsy, stigma) dependence on sex, a statistically significant relation has not been established. These symptoms occurred in 66.7% girls and 47.4% boys.
Conclusions:
Average ages of patients was 3.23 years, when they first applied for the obesity to the doctor. Weight on first visit to the doctor does not depend on birth

weight.Statistically significant association between all clinical symptoms and sex has not been established

J11.57
First case report of Seckel in Ecuador

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Introduction: Seckel syndrome was described in 1960 and it is also known as bird-headed dwarfism. This syndrome is a rare autosomal recessive disorder. It is characterized by severe intrauterine and postnatal growth retardation, low birth weight, severe microcephaly, craniofacial dysmorphism with characteristic bird headed appearance, prominent beaked triangular nose, micrognathia and variable mental retardation. Other multiple anomalies associated are cleft lip and palate, club foot, scoliosis, gastrointestinal malformations, multiple skeletal malformations, cardiovascular, endocrine, hematopoietic and central nervous systems abnormalities. At the moment 100 cases has been reported and this is the first case reported in Ecuador and the second in Latin America

Case report: Woman of 15 years old. The mother did not have bad habits during the pregnancy. The father is a first cousin of the mother. The patient is 91 cm in length and weights 9.2 Kg., exhibits severe signs of malnutrition. The patient is bird-headed, presents microcephaly, multiple skeletal malformations, ears are deformed and positioned very low, scoliosis, multiple anomalies associated are cleft lip, deformed teeth, clinodactyly, asymmetric pelvis, as well as other characteristics signs. She is aggressive and presents severe mental disability and she suffered convulsions for the first years of her life. Gonadotropin hormones are at a low level. FSH: 1,07mUI/ml, LH: <0,10mUI/L, these suggest a structural problem in the hypothalamus. Karyotype is normal 46,XX. But the patient has no uterus, ovaries or secondary sexual characteristics, but she is not diagnosed as a case of turner syndrome.

J12.001
Polymorphism of P53 gene - exone 4 codon 72 in endometrial carcinoma: Correlation with tumor type

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Endometrial carcinoma is the fourth most common cancer among women in developed countries. Endometrial cancer patients may benefit from systemic chemotherapy, alone or in combination with targeted therapies if the disease is clinically diagnosed prior to expansion and metastasis to other organs. The aim of this study was to evaluate the prognostic role of p53 polymorphism and comparison with tumor type and grade in human uterine endometrial carcinoma. Seventy five patients with endometrial carcinoma and seventy five patients with o malignancy were studied for possible mutations in exon 4 p53 gene using polymerase chain reaction and restriction fragment length polymorphism techniques and sequencing. P53 polymorphism in exon *4* showed no significant difference in the genotype or allele prevalence between case and control groups. We found the Pro allele and genotype frequency to be insignificantly higher in cases than controls (Pro allele 6 and 8/3% respectively; genotypes: Arg/Pro 61 and 84/7%, Pro/Pro 5 and 6/9%, respectively). there was no significant difference in the allele distribution between tumor type and grade because it was need many number of cases.

J12.002
Association between polymorphisms rs1801270(Ser31Arg) ,rs762624,rs3176336 of CDKN1A gene in sporadic colorectal cancer

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Background: Colorectal cancer is the third most commonly diagnosed cancer in both men and women. Progressive loss of cell cycle control is an important feature on the colorectal cancer. p21 (CDKN1A/CIP1/WAF1), one of the cyclin-dependent kinase inhibitors, plays a key role in regulating the cell cycle. The aim of this study was to investigate associations of the CDKN1A gene polymorphisms (rs3176336,rs1801270,rs762624) with risk of colorectal cancer (CRC) in an Iranian population.

Methods: The study subjects were 150 cases of colorectal cancer and 150 controls for any polymorphisms. Genomic DNA was extracted using standard salting out method. Genotypes were determined using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method.

Results: A significant relation was found between rs1801270 in the CDKN1A gene and colorectal cancer. The distribution of AC genotypes among sporadic CRC patients was more frequent than that in the control group (P value = 0.003).We found no significant difference between studied polymorphismS and colorectal cancer .

Conclusion: to our knowledge this is the first study on association of CDKN1A \polymorphisms with CRC risk in Iranian population. Our findings indicated that there is association between rs 1801270 and risk of colorectal cancer

J12.003
The 1303CA mutation of solute carrier family member 2 gene in lung cancer

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As an essential element for human life, iron is widely involved in many important metabolic processes, such as DNA synthesis, electron transport, and oxygen delivery. Numerous studies have found a positive correlation between iron storage and risk of cancers such as hepatic cancer, renal cancer and lung cancer(LC). Solute carrier family-member-2(SLC11A2) is the only transmembrane iron transporter known to be involved in cellular iron uptake. Mutations in SLC11A2 gene are associated with hypochromic microcytic anemia with iron overload. One of SLC11A2 gene variants, 1303C/A, occurs in the coding region of SLC11A2 and results in an amino acid change from leucine to isolecine. Due to the role of SLC11A2 in iron regulation, 1303C/A mutation in SLC11A2 gene (rs144863268) was investigated in 100 subjects (LC patients: n=50; healthy-controls: n=50) by method of PCR-RFLP. The resulting 362-bp amplification products were digested by SfaNI and were analyzed by electrophoresis in a 2% agarose gel. The 1303C/A mutation destroys a SfaNI site. Accordingly mutant alleles showed only the undigested, 362-bp band, whereas wild-type alleles were identified on the basis of a digestion pattern showing a 197- and a 165-bp band. No significant differences in allele (A allele χ^2=0.91, p=0.338; C allele χ^2=1.01, p=0.315) and genotype (χ^2=2.70; p=0.259) frequencies of the gene polymorphism were found between the healthy controls and lung cancer patients. According to our preliminary data SLC11A2 1303C/A mutation is not linked with lung carcinogenesis. We would expect significance with the increasing number of patients. The project was supported by ESOGU (Grant no:201241020)

J12.004
Variant type PML-RARA fusion transcript in AML: Detection and interpretation of results by RT-PCR

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Acute myeloid leukemia (AML) is characterized by a number of features that requires accurate diagnostic and specific treatment approach. The t(15;17)(q24;q21) which generates the PML-RARA fusion transcript presents the diagnostic hallmark of AML. PML-RARA positive patients express one of three hybrid transcripts within PML gene and are denoted bcr1 (long), bcr2 (variant), and bcr3 (short) forms. The importance of specific breakpoint region is its utility as prognostic factor for individual's likelihood of relapse and possibly their response to therapy treatment.

In this study we present detection of the most uncommon breakpoint region, bcr2, by RT-PCR technique. We analyzed 95 pediatric AML patients and examined the incidence of three most frequent fusion transcripts obtained by standardized RT-PCR protocol (European BIOMED-1 Concerted action). In 28 cases, positive for one of the AML rearrangement, 9 patients (32%) had AML1-ETO rearrangement, 3 (11%) had CBFB-MYH11, and 16 (57%) had PML-RARA. Among 16 PML-RARA patients, five (42%) were presented as bcr1 positive (multiplied with PMLA1-RARAB primers) and showed nonspecific extra band multiplied with bcr3 primers (PMLA2-RARAB). These results are interpreted as bcr2 positive, but to ultimately determine whether it is bcr1 or bcr2 transcript, we are now investigating length of these products by sequencing. After these analyses we will determine the rate of bcr2 products among our patients.

Cytogenetic analysis from bone marrow samples showed normal karyotype. Establishment of bcr2 rearrangement is important because of its decreased response to treatment with all-trans retinoic acid (ATRA) depending on where the break occurs within PML exon 6.

J12.005

Novel Additional Chromosomal Abnormalities in patient with Acute Promyelocytic Leukaemia and ATRA resistance

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Additional chromosome aberrations (ACA) have been observed in 23-43% of Acute Promyelocytic Leukaemia (APL) cases, with trisomy 8 being the most frequent (31-46%). Here we describe one case of APL with ACA at presentation occurred in our hematology department in April 2013 and never been reported in literature. A 22 years old male patient who was referred to our institution for mucosal and cutaneous bleeding. Laboratory exams showed anemia, leucopenia and thrombocytopenia, with increasing prothrombin time, activated partial thromboplastin time and fibrin degradation products. A blood smear evidenced atypical agranulate promyelocites with Auer Rods in faggots. Bone marrow aspirate for morphological, immunophenotyping , cytogenetic and biomolecular analyses was performed. The patient showed a 46,XY,t(5;21)(q31;q22)[17]/46,XY,t(5;21)(q31q22),t(15;17)(q24;q21)[3].ish t(15;17)(PML+;RARA+,PMLdim)[8] karyotype. The aquired , non constitutional nature of the translocation t(5;21) has been confirmed by a cytogenetically normal result of phytohemagglutinin-stimulated blood analysis. Molecular analysis performed by RT-PCR disclosed the presence of bcr-1 PML-RARα gene fusion transcript. Follow up laboratory exams showed persistence of a normal karyotype and molecular remission, but in January 2014 bone marrow cytogenetic analysis revealed a complex karyotype:49,XY,t(5;21)(q31;q22),t(15;17)(q24;q21),+mar1,+mar2,+mar3[2]/
46,XY[18].ish t(15;17)(PML+;RARA+,PMLdim)[7/100] with presence of bcr-1 isoform in RT-PCR, normal CBC count and MRI imaging showing initial NCS envolvment. The incidence and prognostic significance of ACA in APL is still a controversial matter and when such abnormalities are found there is no evidence to support the use of alternative therapeutic strategies to ATRA(all-trans retinoic acid) and chemotherapy. Now patient is under treatment with arsenic-trioxide.

J12.006

Increased frequence of the rs2066853 variant of aryl hydrocarbon receptor gene in patients with acromegaly

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Introduction Aryl Hydrocarbon Receptor (AHR) regulates Cytochrome P4501A1 (CYP1A1) expression and other xenobiotic target genes to antagonize environmental contaminants effects. It controls also cellular proliferation, senescence and apoptosis. AHR gene polymorphisms were associated with several tumours' occurrence and deregulation of AHR expression was demonstrated in somatotropinomas. In this study, we analyse the sequence of the AHR gene in patients with sporadic acromegaly. Patients and methods We evaluated 70 patients (M = 27, age 57.5 ± 12.8 aa ± SD) and 157 controls. Exons 1, 2, 3 5 and 10 of AHR was screened by direct sequencing in patients and controls. **Results** Polymorphism rs2066853 (c.1661 G>A) was identified in 18/70 acromegalic patients and in 9/157 healthy subjects (25.7 vs 5.7%, $\chi 2$ 18.98 p <0.0001, OR 5.7, 95% CI: 2.4081 - 13.4558). Moreover, rs4986826 (c.1708 G>A) variant was identified in two patients but not in controls ($\chi 2$ 4.62 p <0.05). Mean IGF-1 ULN (2.93 ± 1.07 vs 2.29 ± 0.86, p <0.05) and the prevalence of cavernous sinus invasion (χ^2 6.08, p <0.05) were higher in patients with rs2066853 polymorphism than in the other ones. Moreover, an increased prevalence of differentiated thyroid cancer (χ^2 7.53, p 0.02), bladder tumour (χ^2 34.66, p 0.0001) and lymphohematopoietic neoplasm (χ^2 6.41, p <0.05) was found in the former group than in the latter. Further studies are needed to better elucidate the functional consequence of this AHR polymorphism, whether it could impact on xenobiotic sensitivity or affect other AHR-mediated cell-cycle deregulation mechanisms.

J12.007

Cytogenetic pattern profilling in Acute Lymphoblastic Leukemia of childhood in North Indian population

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Acute lymphoblastic leukemia(ALL) comprises of 70 - 80 % of childhood leukemias. Its diagnosis is based on hematological parameters like blast population, blast morphology, flow cytometry. ALL arises as a result of genetic damage to the lymphohematopoetic progenitor cell leading to malignant transformation. This study was a cross-sectional study done on paediatric age group patient of North Indian population on confirmed cases of ALL on bone marrow examination. The bone marrowsamples were subjected to cytogenetic study using immediate, 24hr and 48hr protocols. FINDINGS: 1.Hypodiploidy was the commonest finding in our study with an incidence of 78.57% which is similar to that found in studies from East India where it is reported to be 63.6% and in West India it is 38.4%. In World Literature, this is similar to Africa population studies where hypodiploidy showed an incidence of 37.5%.Hyperdiploidy was commonest in North India (27%) in another study and South India(14.8%) and Asia(69.5%) and Europe(63%). Pseudodiploidy was commonest in America(41%). 2.B Cell ALL(95%) and T Cell ALL(5%) in our study is dissimilar from other studies, B Cell (70%-76%) and T Cell(24%-30%) 3. Structural abnormalities seen :43,X,del(4),dic(5)-(6)-(2)-(20)+(14) in 1(7.14%) and 42Y6p del dic 13-(7),-(9),-(16),-(X) in 1(7.14%). RECOMMENDATIONS Cytogenetics should play a key role in risk stratification and treatment protocols considering the heterogeneity of the pediatric ALL

J12.008

New, prognostically relevant chromosomal lesions in AML assessed by array CGH - a preliminary study

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Acute myeloid leukemia (AML) is a biologically heterogeneous disease. Identification of chromosomal aberrations, as well as cryptic genome lesions is important for precise diagnosis and risk assessment. The aim of our study was to determine the most frequent clonal chromosomal aberrations, to assess their prognostic value and to compare the results obtained by classical cytogenetics and by array CGH. Bone marrow aspirates were collected from 21 patients diagnosed with AML and from 8 healthy blood donors. The material was divided into two parts. One part was used for classical cytogenetic analysis (GTG-banding and RHG-banding) and FISH. The second part was used for DNA isolation and array CGH analysis. Chromosomal abnormalities detected by classical cytogenetic techniques most often relate to chromosomes: 5, 17 (24%), 8, 15, 16, 18, 21 (19%), 3, 7, 11, 14, 20 (14%), 4, 12, 13 and 22 (9,5%). The array CGH study indicated regions: 16p13.3, 16p22.3q24.2, 5q14.1q35.2, 8p23.1p11.21, 8q12.1q24.3, 15q11.2, 15q15.1q15.3, 15q22.1q24.1, 15q25.2q25.3, 18p11.32q23, 3p26.3, 3p14.1p12.2, 3q11.2q21.1, 3q27.2q29, 7p12.3q36.3, 17p13.3p13.1 and 17q12. The above aberrations were confirmed by FISH. Array CGH method enabled to determine the regions of the feasible prognostic value during 36 months follow up. Aberrations in regions 5q14.1q35.2, 16p13.3 and 18p11.32q23 had a negative impact on the prognosis. AML patients with these lesions had shorter overall survival. The changes in the region 15q22.2q25.3 were associated with longer survival and favorable prognosis. Larger group of AML patients included in the study will enable the verification of prognostic significance of reported chromosomal changes.

J12.009

Complex translocation t(8;21;17)(q22;q22;p11.2): a masked variant of t(8;21) in a patient with AML-M2

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The t(8;21) translocation occurs in 5-12% of acute myeloid leukemia (AML) cases, and in 40% of AML FAB M2 type, with well defined and specific morphological features. Over 70% of patients show additional chromosome abnormalities: loss of Y or X chromosome in half cases. Variant translocations involving a variable third chromosome account for approximately 3-4% of all AML-M2 with fusion transcripts. Although the t(8;21) is easy to recognize cytogenetically, rearrangements between 8q22 and 21q22 may be masked with complex or cryptic translocations. This translocation leads to the fusion of the AML1 (RUNX1) gene on chromosome 21 and the ETO gene on chromosome 8, and results in a transcriptionally active chimeric gene, AML1-ETO on the derivative 8 [der(8)]. Here, we present a case of a patient, 41 years old male, with AML-M2 with a three-way translocation, involving the chromosomes 8, 21, and 17. The diagnosis, evaluated by morphology and immunophenotype in bone marrow, was indicative of AML M2 without specific features suggesting the presence of t(8;21). Cytogenetic study of

bone marrow cells revealed the karyotype as 45,X,-Y,t(8;17)(q22;p11.2) in 20 metaphases. FISH analysis using a 17p13 probe (Cytocell p53 Deletion) on previously G-banded metaphases showed that one of the LSI p53 signals was on chromosome 21. This led to the discovery of the cryptic translocation t(8;21;17); the presence of rearrangement AML1-ETO was confermed by RT-PCR (using BIOMED-I protocol). Variant t(8;21) translocations should be suspected in AML M2 and specifically looked for whenever 8q22 and 21q22 rearrangements are found.

J12.010

Molecular-genetic investigation of aFAP/MAP syndromes among Russian patients

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Attenuated Familial Adenomatous Polyposis (aFAP) and *MYH*-associated Polyposis (MAP) are the important inherited colorectal cancer syndromes. Germline mutations in *APC* gene provide aFAP, biallelic (or heterozygous in some populations) mutations in *MYH* gene cause MAP. We investigate *APC* and *MYH* genes among 25 patients with adenomatous polyps (4-95). Heterozygous missense mutations in *MYH* gene were analyzed in control group including 106 healthy probands. As a result 2 mutations p.I1307K in *APC* and 4 missense variants in *MYH* (2 p.G169D, p.G382D and p.D382D) genes were found. All 6 patients with mutations had more than 20 polyps (p=0,0026). Only 1 missense variant (p.G169D) in *MYH* gene was found among 106 healthy probands (p=0,016). Frequency of germline mutations in *APC* is 8% (2/25) and 16% (4/25) in *MYH*. The value of the heterozygous mutations for *MYH*-associated Polyposis was demonstrated for the first time among Russian patients.

J12.011

Expression of genes for drug resistance and metabolism in muscle-invasive bladder tumors

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Background:
The primary endpoint of this study was evaluation of the expression of genes related to the resistance to the most common anticancer drugs in muscle invasive bladder carcinomas.
Materials & Methods:
Gene expression analysis of the 168 genes from two panels for Cancer drug resistance and metabolism (PAHS004) and Cancer Drug Targets (PAHS507z) was performed. A total of 47 transitorial cell bladder cancer samples of stage pTa, pT1, pT2, pT2b and lymphoepithelioma-like pT2a were investigated. Gene expression analyses of the individual samples as well as the pool samples (pTa, pT1 and pT2) in comparison to negative and positive (non-malignant lesions) controls were carried out.
Results:
The pool analysis revealed statistically significant difference (p<0,0001) in the expression level between muscle invasive and non-invasive bladder tumors. More than 10 times higher expression is observed for AKT1, AURKA, CSTB, EGFR, ERBB2, ERBB4, HDAC and MDM2 genes which are targets for current and trial anticancer drugs. More than 5 times up-regulation was established for ABCC1, ABCC3, ARNT, CYP1A5, CYP3A5, EPHX1, MVP, PPARG and TXN genes. These are involved in the multi-drug resistance and metabolism of cyclosporine, steroid hormones, PAHs as well as anticancer drugs Vincristine, Thiopurine and Taxol.
In the fourteen tested individual samples we found an up-regulation for AHR, AR, CCNE1, CLPTM1L, CYP1A1, CYP3A5, MVP and TOP2B genes. In the 78,5% from them the expression of the PPARG gene, which activity is influenced by retinoid, was elevated.
Acknowledgements: Contract № ДМУ 03/48, 12.12.2011 of the Ministry of Education and Science, Bulgaria.

J12.012

Matrix Metalloproteinase and Tissue inhibitor of metalloproteinase gene polymorphisms involved in bladder cancer development

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The aim was to investigate MMP1 (rs494379, -519 A>G), MMP9 (rs3918242, -1562C>T; rs17576, 2660A>G), MMP12 (rs2276109, -82 A>G), MMP2 (rs2285053, -735C>T), TIMP3 (rs 9619311, -1296T>C) polymorphisms and bladder cancer susceptibility in population of The Republic Bashkortostan (Russian Federation). A total of 307 bladder cancer patients and 271 controls were genotyped by PCR- RFLP. It was found 2660A> G MMP9 association with bladder cancer development in the dominant model (p=0.044, OR=1.45, 95 % CI (1.01-2.07)) and MMP9 2660G/-1562C haplotype significantly more common in patients (34.52 % vs. 21.17%, OR=1.90, 95 % CI (1.22-2.97)) compared with control. It was defined MMP2 -735C>T significant association with bladder cancer development in the overdominant model (p=0.0009, OR=2.21, 95 % CI(1.38-3.53)). -1296T>C TIMP3 associated with disease in additive model (p=0.016, OR=1.44, 95 % CI(1.07-1.95)). No association with bladder cancer was observed for -519 A>G MMP1 and -82 A>G MMP12. It was found -735C> T MMP2 was significantly associated with the development of bladder cancer as an invasive (OR=1.93) and non-invasive forms (OR = 2.17). Analysis of gene - environment interactions showed a statistically significant interaction between MMP2 and MMP9 polymorphisms with the status and smoking index (p=0.014 and p=0.017, respectively) in dominant model. Thus we can assume MMP9, MMP2, TIMP3 polymorphisms under investigation make a definite contribution to the bladder cancer development.
The investigation was partially supported by RFBR 14-04-97006 r_povolzye_a, 14-06-97003 r_povolzye_a, 13-04-00287 A; RFH 13-06-00101.

J12.013

Bladder cancer risk associated with XPD polymorphism in the Belarusian population

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Bladder cancer (BC) is the fourth most common cancer among men in Europe, and the annual incidence rate is 1000 in Belarus. Among risk factors, cigarette smoking and occupational chemical exposure lead to accumulation of DNA damage contributing to carcinogenesis. In this context, polymorphism of error-free excision repair genes seems to affect the cancer risk. Polymorphism of XPD Asp312Asn, XRCC1 Arg399Gln, OGG1 Ser326Cys, ERCC6 Met1097Val was studied in the group of BC patients (336 individuals) as compared to the control group (370 volunteers). The allelic variants were determined in DNA samples using the PCR-RFLP method. Minor allele frequencies in Belarus were close to those in Caucasians and significantly differed from their frequencies in Asians. Analysis of polymorphisms in a single gene revealed increased BC risk in carriers of heterozygous genotype Asp/Asn of XPD gene that was pronounced in the elderly (OR95%CI=3.34 [1.35-8.28] p=0.009). The combination of this genotype with homozygous genotypes Ser/Ser of OGG1 or Met/Met of ERCC6 also increased cancer risk. Analysis of interaction between four genes showed that carriers of combinations Asp/Asn, Arg/Arg, Ser/Ser, Met/Val and Asn/Asn, Arg/Arg, Cys/Cys, Met/Val possessed more predisposition to cancer (OR95%CI=3.20 [1.47-6.95] p=0.003 and OR95%CI=9.97; p=0.1 respectively). Thus, XPD312 polymorphism was predominantly associated with BC risk in Belarus.

J12.014

Cell free DNA quantification in urine of patients with bladder urothelial carcinoma

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Aim of the study Cell free DNA (cfDNA) represents one of the new bladder cancer markers. Comparision of total urine cfDNA values of the control group and of the patient one was the aim of our study. Patients and methods Naturally voided second morning urine samples were collected from 99 individuals and 3 different groups of individuals were compared: (16 healthy volunteers (HV), 20 control patients with urological diagnoses different from bladder cancer (CP) and 63 patients with bladder cancer (BCP) (pT0 in 5 cases, pTa in 21, pT1 in 17, pT2 in 8, pT3 in 6, pT4 in 6 cases, both LG and HG in each stage group). Results: The comparison showed statistically significant difference between HV and BCP (p value = 0,000049). There were no statistical differences between the CP and BCP (p value = 0,372059) or between CP and HV groups, as well (p value = 0,046749). Patients were then divided into two groups according to their cancer stage. The group 1 (pTa patients, patients with another urological diagnoses and healthy volunteers) and the group 2 (patients with stages pT1 and more). Comparing these two groups by Mann-Whitney test we have shown significant difference with p value < 0.001. Conclusion: pTa patients significantly differ from pT1

ones and more regardless to the cancer grade. Patients with stages pT1 and more have significantly higher ucfDNA levels than other individuals. Acknowledgements: Supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic no. NT12417.

J12.015

BRCA1 Gene Polymorphisms and chromosomal aberrations as a risk of borderline ovarian cancer patients in Tamil Nadu population, South India

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Ovarian cancer is the leading cause of death due to gynaecological malignancies among women. The aim of the study was to analyse the BRCA1 gene polymorphisms and major chromosomal aberration in the borderline ovarian cancer (BOC) patients. A population-based, case control study of ovarian cancer was performed in Tamil Nadu. Inferred consent forms were obtained from 34 BOC patients individually (Case & Control). We have examined for BRCA1 Q356R amino acid changing polymorphisms. Cytogenetic analyses were carried out using 3 mL of blood (100 metaphase). The major chromosomal aberrations were gains from chromosome arms and deletions of 1p, 12q, 14q, 15q, 16p, 17p, 17q, 19p and 19q sites in BOC patients. The correlation between family history, age, infertility, and diet - body weight were selectively evaluated and analysed. The most significant chromosomal abnormalities like deletions were recorded in BOC patients compared to the control subjects. Findings reveal that the patients above 60 years of age were at high risk. Thus the study concludes that the BRCA1 gene polymorphisms and chromosomal aberrations play a key genetic role along with the risk factors for BOC patients.

J12.016

The role of BRAF V600E mutation as a potential marker for prognostic stratification of papillary thyroid carcinoma: A long-term follow-up study

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Papillary carcinoma is the most prevalent malignancy of thyroid gland, and its incidence has been recently increased. The BRAFV600E mutation is the most frequent genetic alteration in papillary thyroid carcinoma (PTC). The role of BRAFV600E mutation as a potential prognostic factor has been controversially reported in different studies, with short-term follow-up. In this study we evaluated the role of BRAFV600E mutation as a potential marker for prognostic stratification of patients with papillary thyroid carcinoma in long-term follow-up. We studied 69 PTC patients with a mean follow-up period of 63.9 months (median: 60 m). The BRAFV600E mutation was analyzed by PCR-single-strand conformational polymorphism and sequencing. The correlation between the presence/absence of the BRAFV600E mutation, clinicopathological features and prognosis of PTC patients were studied. The BRAFV600E mutation was found in 28 of 69 (40.6%) PTC patients, and it was significantly more frequent in older patients (P<0.001), in advanced tumor stages (P=0.006), and in patients with history of radiation exposure (P=0.037). Incomplete response to treatment in PTC patients was significantly correlated with certain clinicopathological characteristics (follow up time, distant metastases, advanced stage, first thyroglobulin (fTg), history of reoperation and external radiotherapy, and delay in iodine therapy) but it wasn't related to the presence of BRAFV600E mutation. Prevalence of BRAFV600E mutation was 40.6% in patients with papillary thyroid cancer in northeast of Iran. The BRAFV600E mutation was associated with older age and advanced tumor stage but was not correlated with incomplete response during follow up.

J12.017

Study of genes BRCA1 and 2 in clinical cases of breast and/or ovarian cancer in the Molecular Genetics Unit of Ferrara

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Germinal mutations in two high susceptibility genes BRCA1 and BRCA2 explain around 25 % of familial breast cancers. Patients with a probability value above 10% in the risk assessment calculated by different prediction tools (CaGene, BOADICEA and Cuzick-Tyrer)) or belonging to the profile 3 of Modena's criteria were selected for the molecular analysis of BRCA 1 and 2 genes by direct sequencing and MLPA. Molecular analysis was completed on 80 patients and for both genes we identified 8 pathogenic mutations (frameshift, missense, nonsense) recurrent in unrelated patients or private. According to the literature, in about 10% of the identified variations the pathogenic significance was not known. The mutations were distributed along the entire gene sequence, therefore the search for specific mutations hotspot it is not adoptable as a procedure for the implementation of genetic testing. To date, the Breast Cancer Information Core Database (BIC) contains more than 3000 different sequence variations in BRCA1 and BRCA2 genes. We have found the same mutation in different patients associated to the occurrence of different cancer at a different age, therefore to date it is not possible a specific genotype-phenotype correlation. The data obtained in our laboratory so far suggest that the genetic risk of breast cancer could be caused by rare variants and being able to distinguish as deleterious or neutral the large number of new variations with unknown pathogenic significance is a difficult task. Early identification of individuals at risk allow to implement protocols and periodic clinical surveillance for certain risk profiles.

J12.018

Analysis of a novel BRCA1 splicing mutation in hereditary breast and ovarian cancer woman

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Hereditary breast and ovarian cancer (HBOC) is the commonest malignancy in women. HBOC is a familial disease accounting for about 10% of all breast cancers, BRCA1 and BRCA2 being the most prevalent genes involved in this pathology (1). They play a role in the maintenance of genome stability, particularly in the homologous recombination (HR) pathway for double-strand DNA repair (2). BRCA1/BRCA2 germline mutations are related to an increased risk of developing HBOCs, therefore the carrier identification is crucial especially before the onset of the disease. Furthermore, testing for BRCA gene mutations allow to improve the clinical management of high-risk patients and of their mutation carriers family members. However, the correct interpretation of variants with uncertain significance or of novel variants still remains a problem for genetic counseling.
Here we describe a novel intronic variant identified in a HBOC patient and involved in the BRCA1 gene splicing regulation. This variant is predicted to be deleterious according to Human Splice Finder and NetGene2, causing the loss of a canonic donor splice site at position +2 in the intron 21 of BRCA1 gene. Variant effects were experimentally verified on patient cDNA by PCR amplifications using different primers pairs *ad hoc* designed. Our results indicate that intron 21 is completely retained in the transcript RNA. Despite other studies are needed to confirm the role of this splice variant, our preliminary results strongly support the pathogenicity of the novel found mutation.

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J12.019

Polymorphisms in the FGFR2 and miR146 genes are associated to increased risk in familial breast cancer in young women.

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Breast cancer is the most common cancer diagnosed in women worldwide and 7% of all breast cancer cases occur in women under 40. Only 5-10% of all breast cancer have genetic predisposition and the number of women in the general population with breast cancer attributable to mutations in highly penetrance genes BRCA1 And BRCA2 is very low. These observations have led to the proposal that breast cancer susceptibility is largely polygenic and recent GWAS studies have identified some regions in the genome which contains SNP that can involved risk of developing breast cancer including FGFR2 (rs2981852) and miR146 (rs2910164). Moreover, several SNPs at the BRCA1 gene such as c.2731C>T (rs7999917), c.3232A>G (rs16941), c.3667A>G (rs16942), could modify the risk. We studied 124 women under 40 with breast cancer divided in two main groups: patients who carried pathogenic mutation in BRCA1/2 genes (BRCA+ n=22) and noncarriers (BRCA- n=102). DNA was extracted from peripheral blood and allelic discrimination was performed using TaqMan SNPGenotyping Assay. We found a significant association between genotype TT in the FGFR2 polymorphism and BRCA+ group (OR=0,133 CI95%=0,026-0,687p=0,024). Furthermore, we found that the G allele in the miR146 polymorphism is related with

BRCA- group (CG OR=6,792 CI95%=1,489-30,978 p=0,013 ; CG+GG OR=4,16 CI95%=1,316-13,152p=0,015) Thus we can conclude that genotype TT of FGFR2 polymorphism might be associated to increased the risk of breast cancer in presence of BRCA1/2 mutation while allele G of mir146 polymorphism could be related to an increase of breast cancer in patient noncarriers of BRCA1/2 mutation.

J12.020

Cytogenetic heterogeneity in breast cancer and its relationship with the morphological types of infiltrating component

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Solid tumors are characterized by considerable interindividual and intratumoral genetic heterogeneity. High genetic variability of breast cancer (BC) probably manifested in the presence of various types of structures of infiltrative component (morphological structures): tubular, trabecular, solid, alveolar and discrete groups of tumor cells. It may be assumed that different types of morphological structures are manifestations of phenotypic diversity, which is based on a set of specific cytogenetic differences. The aim of this study was to investigate intratumoral cytogenetic variation in BC and its relationship with the morphological subtypes of breast cancer. Material for analysis was taken from single patient with breast cancer. We isolated five morphological structures from two regions of breast cancer with laser microdissection (PALM, Carl Zeiss, Germany). Analysis of chromosomal aberrations was performed using Agilent microarrays (SurePrint G3 Cancer CGH + SNP Microarray Kit, 4x180K). The number of identified unbalanced chromosomal aberrations in the various structures ranged from 24 to 270. While the least amount of rearrangements found in tubular structures and the largest - in solid. The cluster analysis results indicate that the samples from different types of histological structures obtained from the same region of tumor clustered together rather than between similar cellular phenotype across the tumor. Furthermore, number of common chromosome aberrations between different tumor regions was not higher in samples with similar morphology than in samples with different morphology. It is suggested that similar histological structures may originate from cell clones with different karyotype.

J12.021

Analysis of BRCA1 and BRCA2 mutations in breast cancer patients from Uzbekistan

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Breast cancer is the most common malignancy in women and affects approximately 1 out of 10 females. Risk of developing breast cancer is strongly influenced by genetic factors. Germ-line mutations in BRCA1 and BRCA2 genes are the primary cause of up to 5-10% of breast cancer incidence. To reduce increased risk of developing cancer or to increase the likelihood of early detection, carries of BRCA1 or BRCA2 mutations are offered surveillance programs and effective preventive medical interventions. This study aimed to investigate the contribution of BRCA mutations to breast cancer cases in Uzbekistan. 67 patients with breast cancer as well as the sex and age matched control group of healthy individuals (n=103) were included in this study. By means of real-time allele-specific PCR we analyzed DNA samples of these groups for the presence of BRCA1 5382insC, BRCA1 4153delA, BRCA1 185delAG, BRCA1 300T>G and BRCA2 6174delT mutations. 3 unrelated samples (4.5%) were found to be positive for the heterozygous 5382insC mutation representing a possible founder mutation in the Uzbek population. In the investigated group of patients we didn't find BRCA1 4153delA, BRCA1 185delAG, BRCA1 300T>G and BRCA2 6174delT mutations. All investigated mutations were not found in any DNA sample of control group. The presented data confirm contribution of BRCA1 5382insC mutation to breast cancer development in Uzbek people and taking into account a high disease penetrance in carriers of BRCA1 mutation, it seems reasonable to suggest inclusion of 5382insC mutation test in screening programs for breast cancer prevention in Uzbekistan.

J12.022

Preliminary study of BRCA1/BRCA2 mutations in Bulgarian women with breast cancer

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The mutations in BRCA1 and BRCA2 genes are the most significant genetic determinants of predisposition to develop breast cancer (BC). There is still insufficient data about BRCA1/2 mutations in Bulgarian BC population. It was a preliminary study aiming to investigate the frequency of five common deleterious point mutations (reported before in Bulgarian patients) and large genomic rearrangements (LGR) in BRCA1/2 genes in a target group (defined by preliminary prepared selective criteria) of Bulgarian women with BC.

The list of patients diagnosed with BC was taken from the Cancer Registry (2009-2013) of University Hospital, Pleven. One hundred and seventy five women with BC were interviewed and pedigree was constructed for each of them. The patients were classified into ten categories, according to personal, disease and family history. Based on the selective criteria we defined a target group of 79 Bulgarian women with BC. They were screened for five deleterious point mutations (5382insC; C61G, in BRCA1 6174delT, 9326insA; 9908delA in BRCA2) by DNA sequencing and for LGR by Multiplex Ligation Probe Amplification. Among the screened women we found: one deleterious mutation (5382insC in BRCA1) with frequency 1.2%; two polymorphic variants in BRCA2 gene - rs4987117 in exon11N and rs9534262 in exon17 with frequency 2.7% and 36% of the patients, respectively. None LGR was detected.

In conclusion, the insufficient results of our study suggest that the strategy for genetic screening in Bulgarian patients requires complete analysis of the BRCA1/2 genes and stringent compliance to the internationally accepted BCLC/NCCN criteria.

J12.023

Mutation Analysis of ESM-1 Gene in Breast Cancer Patient Tissues

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Breast cancer is the second common type of cancer in the world. In developed countries, breast cancer is one of the leading causes of women deaths. In recent years, many genes have been identified related to breast cancer. But underlying molecular mechanisms have not been clarified yet. Endocan, (ESM-1: endothelial cell-specific molecule-1) soluble proteoglycans, is secreted by endothelial cells. Expression of ESM-1 gene is regulated by the cytokines. The expression increases with the stimuli of VEGF, TGNF-α, LPS and IL-1β in endothelial and epithelial cells. The ESM-1 gene function has an important role in tumor angiogenesis and growth. It has been shown to involved in pathogenesis of lung, liver, gastric and breast cancer. ESM-1 gene is reported to have an effective role in cell proliferation, cell migration, invasion, and metastatic spread of cancer cells in recent studies.

In this study, we analyzed ESM-1 gene mutation in breast cancer from paraffin-embeded tissue samples of breast cancer patients. All three exons for ESM-1 gene mutation were sequenced by Sanger Sequencing.

Our results showed a homozygous silent mutation (CAG>CAA) in the second exon at codon 118. Another case displayed the same mutation heterozygously. In nine cases, the polymorphism TTG>CTG was evaluated as being remarkable in the third exon of poly-A region which maybe the miRNAs binding site.

J12.024

Association of estrogen receptor-α A908G (K303R) mutation with breast cancer risk

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Genetic mutations in premalignant breast lesions may have a role in malignancy progression or influence the behavior of subsequent disease. A point mutation in estrogen receptor-α (ER-α) as A908G (Lys303→Arg) was originally involved to hypersensitive to estrogen breast hyperplasia. We detected this mutation among Iranian women with invasive breast cancer. A population-based case-control study was conducted in 150 newly diagnosed invasive breast cancer and 147 healthy control individuals controls to screen for presence of the ER-α A908G mutation by using single-strand conformation polymorphism (SSCP) analysis and 33Pcycle DNA sequencing. We detected the 10.7% ER-α A908G mutation in the form of heterozygote genotype only among cancer patients ($\chi 2=22.752$, $P=0.00$). The allelic frequency of mutant allele AGG in codon 303 was significantly ($\chi 2=29.709$, $P=0.001$) higher in patients with the family history of breast cancer (28.9%) than those without the family history of breast cancer (1.9%). Our data suggest that ER-α

codon 303 mutation is correlated with various aspects of breast cancer in Iran. ER-α genotype might represent a surrogate marker for predicting breast cancer developing later in life.
Keywords: Breast cancer, mutation, estrogen receptor, PCR-SSCP, lymph node metastasis

J12.025
The association between G-2548A polymorphism of leptin gene and breast cancer risk

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Introduction: The Breast cancer is the most common cancer in woman and the second cause of death in women between 35 to 55 years. Risk creation the breast cancer is in time life woman 12/5% (one of the eight cases) and risk of death from breast cancer 3/6% (one case of twenty eight case). Leptin hormone is secreted from adipocytes which is involved in the regulation of body weight and serum levels are correlated with breast cancer risk. Genetic polymorphisms G-2548A promoter region of leptin gene expression and hormone secretion rates of faty tissue will be affected. The purpose of this study is to investigate the relationship between G-2548A polymorphism in the leptin gene and breast cancer risk.
Material and methods: We here carried out a case-control study that included 50 patients and 50 healthy subjects. We examined the genotype distribution of Leptin promoter G-2548A polymorphism, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach,to investigate the possible role of this SNP as a risk factor in breast cancer.
Results: Statistical analysis showed is that the AG genotype frequencies in the patient group 26% and in the control group 48%.AG genotype the region -2548 leptin gene has a protective effect on breast cancer risk and this genotype than other genotypes reduces the breast cancer risk (OR:0.4, 95%CI: 0.16-0.97, P: 0.04). There is a significant association between G-2548A polymorphism of leptin gene and breast cancer risk. This polymorphism can be used as a diagnosis marker for breast cancer.

J12.026
Evaluation of immunostaining by hormone receptors and the receptor HER2 in breast cancer

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Breast cancer is the most common cancer in women worldwide. The evaluation of the expression of estrogen receptors and progesterone and HER- 2 has become essential for the treatment of patients bearing breast cancer since it provides information of prognostic and therapeutic order. The purpose of this study was to evaluate the expression of hormone receptors and Her-2 protein in breast cancer , study the correlations between them and with other prognostic factors, compare our results with those in the literature and evaluate our immunohistochemical technique . This is a prospective study of 130 cases of breast cancer diagnosed at the Laboratory of Pathology of the University Military Hospital and Regional Oran (HMURO) since the year 2006 until 2009. The evaluation of the expression of hormone receptors and HER2 by immunohistochemistry was performed . ERs are expressed in 64% of cases, PR in 71% of cases and HER2 in 31 % of cases. The correlation between the percentage and intensity of marking the RE and RA was statistically significant . On PR , they were correlated with age but not with tumor size or lymph node status with . HER2 was associated with histological type . Our results are generally consistent with those in the literature and our immunohistochemical technique has proved its reliability. The development of molecular study and collaboration with the various departments of our University and Regional Military Hospital of Oran (HMURO) means help understanding some of our patients .

J12.027
Analysis of microRNA expression in breast cancer tumor: identification of cluster co-expressed miRs.

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The investigation of 14 microRNA (miR-10b, miR-21, miR-31, miR-155, miR-29a, miR-195, miR-192, miR-182, miR-125b, miR-145, miR-221, miR-222, miR-34a, miR-335) expression levels in a sample of breast cancer tumors in relation to the normal breast tissue was performed. All patients of the sample were not subject to radiation, neither to chemotherapy. The expression level of miR-182 most often was increased - in 81% of cases. In 59% of cases there was over expression of miR-182 - more than 10 times. The expression of miR-31, potential metastasis suppressor, was decreased in 54% of cases. There was identified cluster co-expression 5 microRNA: with increased expression miR-10b, miR-21, miR-155, miR-34a and miR-335. The cluster includes 33% of studied tumors. Frequency over expression of these miRs among tumors in the cluster was 4 times higher compared to other tumors ($p < 0.00001$). The transcriptional regulation analysis shows that 4 microRNAs of cluster are transcriptional targets of p53 and RelA-NF-kB. All the tumors in the cluster were characterized by infiltrating lobular and mixed variants (infiltrating lobular combined with infiltrating ductal) breast cancer and were positive in the expression of estrogen and progesterone receptors. The cluster includes 60% of all tumors with local metastases in lymph nodes (i.e., metastases occur approximately 3 times more often than in the other tumors of the sample). It allows assuming existence of the association co-expressed in this cluster microRNA with pathomorphological features of the tumors, in particular, with a predominant presence of metastases.

J12.028
Breast carcinomas with negative estrogen and progesterone receptor status and the expression of various potential useful biomarkers

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In primary breast cancer, hormonal receptor status is very useful in predicting response to endocrine therapy and prognosis. Negative ER, PR cases have limited therapeuting options, so it is very helpful to identify biomarkers that can be used as targets for the new targeting therapies, or can be used for prognostic value. We studied 623 tissue samples from operable primary breast adenocarcinomas and 101 of them had negative receptor status. From these 101 cases 52.3% was c-erbB2 positive vs 27.3% of the total amount, and the HER-2 gene was amplified in 36.6% vs 16.8% of the total cases. p53 was expressed in the 59.5% of the negative ER cases vs 26.6% of the total amount. The expression of the stem cell markers was almost identical in positive and negative ER cases, except of cd24 which was expressed in 25%vs80% respectively (p0.02) of the cancer cells of this group. According to our results the positivity of c-erbB2 is higher in negative cases and many of the negative c-erbB2 immunohistochemically cases show amplification of the gene, proposing that herceptin may be a benefit in these cases. The high percentages of the p53 and cd24 expression shows that this group of cases tends to have poorer prognosis, as cd24 is expressed in the more primitive mammary stem cells. A CISH or FISH test is recommended for this group, whatever the c-erbB2 result of the protein is, in order to give the oncologist more detailed information about the biological behaviour of these cancer cells.

J12.029
Study of Her-2/neu and TOP2A Expression in Familial Versus Sporadic Breast Carcinoma

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Genetic predisposition is one of the risk factors that increase the incidence of breast cancer. Her-2/neu gene is considered the most frequently amplified oncogenes and is accompanied with amplification or deletion of TOP2A gene. Our study aims to compare familial breast cancer (FBC) patients with sporadic breast cancer (SBC) and to evaluate the hormonal status, immunostaining for Her-2/neu, and Her-2/neu and TOP2A copy number alterations by fluorescence in-situ hybridization (FISH). Twenty two patients with invasive breast cancer were involved: 12 positive for the criteria of FBC. Sections of formalin-fixed paraffin embedded blocks were subjected to immunostaining for Her-2/neu (Hercep test), estrogen receptors, progesterone receptors and FISH for Her-2/neu and TOP2A genes copy number alterations. Pathological parameters and immunohistochemical markers proved that FBCs were more aggressive than SBC. TOP2A gene amplification was observed in 25% of FBCs compared with 20% of SBC patients. Co amplification of both Her-2/neu and TOP2A genes were observed in 8.3% of FBC patients and in 20%SBCs. In contrast, Her-2/neu amplification was observed in 25% of FBCs compared with 10% of SBCs. Importantly, TOP2A amplification without Her-2/neu gene amplification was observed in 16.7% of FBCs but was

not observed among SBC patients.
In conclusion TOP2A gene amplification occurs both in FBCs and SBCs but more frequently among FBCs independent of Her-2/neu amplification. This study highlights the importance of TOP2A gene amplification in diagnosis of FBC. In addition, a combined approach using immunohistochemical analysis and FISH can optimize Her-2/neu testing for breast cancer patients.

J12.030
Burkitt lymphoma: Unfamiliar presentation of a familiar disease

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Recently, Hematology has been witness to great strides in genetic advancement. Lymphoma is now regarded as heterogeneous genetic disease. The genetic aberrations not only aid diagnosis, but also dictate choice of therapy, therapeutic response and portend prognosis. This is such a case exampling application of cytogenetics. A 4 year frail child presented with abdominal pain and distension of 15 days duration. Ultrasonography and Magnetic Resonance Imaging revealed bilateral renal mass. Provisional clinical diagnosis of Bilateral Nephromatosis / Neuroblastoma was offered. Preliminary guided Fine Needle Aspiration showed monomorphous population of vacuolated lymphoid cells suggestive of malignant lymphoma - Burkitt type. Peripheral smear and bone marrow study on aspirate material revealed 46,XY,t(2;8), add(11)(q24) confirming cytological diagnosis. The patient succumbed to organ failure with short hospital stay of 20 days. We present our case of Burkitt lymphoma with unusual presentation, variant translocation and poor performance. Documentation of such cases can direct compile new prognostic factors, advent of new therapeutic regimens in the future.

J12.031
Expression analysis of four cancer-testis antigens in breast cancer tissues and cell lines

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Introduction: Testis-specific genes which has normal expression in restricted tissues, contains a subgroup demonstrating expression in various malignancies known as Cancer-Testis Antigen (CTAs). Considering testis as an immune privileged organ, aberrant expression in cancers could cause spontaneous humoral and cell-mediated immune responses. Breast cancer is a promising target for vaccination and immunotherapy using CTAs. **Methods and material:** We analyzed the expression of 4 CTAs, PEPP-2, ODF4, ACRBP and SPATA19 in 40 samples of invasive ductal carcinoma (IDC), adjacent normal tissue and 10 fibroadenomas, as well as MCF-7 and MDA-MB-231 cell lines using RT & Real Time RT-PCR. **Results:** ACRBP was expressed in normal tissue. SPATA19 expression was not significantly different in normal and cancer tissues. ODF4 and PEPP-2 were expressed in 62.5% and 22.5% of IDC samples respectively. Real Time results showed increased expression of ODF4 and PEPP-2, 2.96 ($p<0.001$) and 3.31 ($p<0.01$) times in IDCs samples, respectively in comparison with testis tissue. Both genes were up-regulated in MCF-7 and MDA-MB-231 cell lines compared with normal testis sample. Comparing the expression of ODF4 & PEPP-2 in MDA-231 with MCF showed 1.758 & 1.77 ($p<0.001$) times up regulation in MDA-MB-231, respectively. **Conclusion:** ODF4 and PEPP-2 could be potential cancer biomarkers. Therefore, they can be used for active immunotherapeutic interventions. Expression of ODF4 and PEPP-2 in malignant tissues and their absence in benign and normal tissues make them putative cancer biomarkers. We showed ACRBP expression in normal breast tissue, so its application as cancer biomarker or in immunotherapeutic approaches is under question.

J12.032
CAT C262T, GSTM1, GSTT1 and CML risk

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Background: Polymorphisms of oxidative stress related genes enzymes are known to influence the metabolism of different carcinogens and have been associated with incidence of various types of cancer. **Matherial and method:** The aim of this study is to evaluate the influence of catalase (CAT) and glutathione S-transferase (GSTM1/GSTT1) as risk factors for Chronic Myelogenous Leukemia (CML) development. We performed a case-control study which included 75 CML patients and 150 healthy volunteers, with no history of malignancy. The genetic polymorphism of CAT C262T was assessed by RFLP-PCR while for GSTM1 and GSTT1 Multiplex PCR assay was used, both followed by gel electrophoresis. **Results:** For CAT gene polymorphism the molecular analysis identified homozygous mutant allele TT in 13.33% of CML patients and 5.34% in the control group (p=0.3072), while heterozygous CT was found in 21.33% of study population and in 46.66% control cases (p=0.0008). The prevalence of GSTM1 null genotype in the patients group was 60% and in the control group 53.33% (p=0.3939). GSTT1 null genotype frequency in CML patients was 17.33% while in the control population it was 16% (p= 0.8494). We found no correlation between GSTM1 and GSTT1 null genotypes in CML development. Our results show that CAT C262T variant genotype is significantly associated with CML risk (p=0.016, OR=2.042 CI95% 1.151-3.623) **Conclusion:** These preliminary results show that CAT C262T genetic polymorphism might be related with CML development. Acknowledgement: This work was supported by Research Grant number 19/11.12.2013 from University of Medicine and Pharmacy Tîrgu Mureş, România.

J12.033
Expression analysis of CD44 isoforms in Esophageal Squamous Cell Carcinoma patients

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Background: CD44 is a member of the cell adhesion molecules family. In normal cells, CD44S, along with CD44V3 and CD44V6 play roles in cell motility, migration, and adhesion, while in tumor cells they are involved in tumor invasion, progression, and metastasis. The aim of this study was to evaluate the expression of CD44S, V3 and V6 in esophageal squamous cell carcinoma (ESCC) and their correlations with clinicopathological features of the patients.
Methods: The expression of CD44S, V3 and V6 was compared in tumoral and distant tumor-free tissues of the esophagus in fifty ESCC patients using comparative real-time PCR.
Results: A significant overexpression of CD44S, V3 and V6 mRNA was observed in 13, 11 and 9 tumor specimens, respectively. The co-expression of the genes (S&V3) were significantly associated with grade of tumor differentiation, stage of tumor progression, and depth of tumor invasion and (S&V6) with grade of tumor differentiation and (V3&V6) with grade of tumor differentiation, stage of tumor progression and depth of tumor invasion.
Conclusion: Our finding suggested that the reduced expression of CD44 v6 is associated with invasion and stage of tumor and the level of CD44V3 mRNA expression was associated with tumor invasion. In addition, Simultaneous expression of these genes has an important role in tumor prognosis. Investigating the potential role of alternative splicing in cancer progression may therefore lead to the development of novel therapeutic interventions.

J12.034
Is the chromosome Y loss in oncohematological male patients a disease-related marker or an age-associated phenomenon?

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Loss of the Y-chromosome (-Y) is observed in peripheral blood of healthy elderly men, however it may be found also in bone marrow cells of males with different subtypes of hematological malignancies. Therefore it is unclear whether -Y occurs as normal age-related event or it is a marker of neoplastic clone. The aim of our study was to review molecular cytogenetic findings in bone marrow cells of patients with oncohematological diseases and to assess the role of the Y-loss during malignant process.
We found loss of the Y-chromosome in bone marrow samples of 142 males (median age 72 years, range 21-89 years) with myeloid or lymphoid disorders. The most frequent diagnosis was myelodysplastic syndromes and acute myeloid leukemia (54 patients). All samples were analyzed by conventional G-banding and by interphase FISH. Cut-off level for FISH was established at 10% and the extent of cell clones with Y-loss varied from 12 to 98%. Most of the patients (131) showed a clone with -Y at the time of diagnosis. In 114 cases Y-loss was the sole abnormality and in 17 patients it was associated with autosomal structural and/or numerical aberrations. In 11 patients -Y clone was detected during the progression of the disease.
As quoted in the literature (Van Dyk, 2001) the presence of >75% cells with -Y is associated with the malignant disease. In our cohort, this was found in 25 patients (18%) and therefore such findings may be considered as related to malignity.
Supported by MHCR 00023736, RVO-VFN64165, GACR P302/12/G157/1.

J12.035

Polymorphism of *XRCC1*, *XRCC3* and *XPD* genes and risk of chronic myeloid leukemia in a Romanian population

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The genetic polymorphisms of X-ray repair cross-complementing group 1 (*XRCC1*), X-ray repair cross-complementing group 3 (*XRCC3*) and xeroderma pigmentosum complementation group D (*XPD*) repair genes may lead to genetic instability and leukemogenesis. The purpose of the study was to evaluate the association between *XRCC1* gene Arg399Gln, Arg280His and Arg194Trp, *XRCC3* Thr241Met and *XPD* Lys751Gln repair gene polymorphisms and the risk of development of CML in Romanian patients. A total of 156 patients aged 20 to 78 years diagnosed with CML (mean age 51.5 ± 1.1 years) and 180 control individuals (mean age 49.8 ± 2.1 years) were incuded in this study. The *XRCC1, XRCC3* and *XPD* genotypes were determined by the polymerase chain reaction-restriction fragment length polymorphism assay. We found no association between chronic myeloid leukemia and *XRCC1* and *XRCC3* variant in any of investigated cases. A significant differences were observed in the variant genotype frequencies of the *XPD* Lys751Gln polymorphism between the the patients with CML and control group (for variant homozygous genotypes, OR = 2.37; 95% CI = 1.20-4.67; p-value = 0.016 and for combined heterozygous and variant homozygous genotypes, OR = 1.72; 95% CI = 1.10-2.69; p-value = 0.019). This was also observed when analyzing the variant 751Gln allele (OR = 1.54; 95% CI = 1.13-2.11; p-value = 0.008). Our results suggest that the *XPD* Lys751Gln variant genotype increases the risk of chronic myeloid leukemia.

Acknowledgement: This work was supported by Internal Research Grants of the University of Medicine and Pharmacy Tîrgu Mureş, România. Project No. 19/11.12.2013

J12.036

Investigation of the role of VHL and SETD2 gene mutations in human clear cell renal cell carcinoma development in patients from Russia

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Renal cell carcinoma is the most common neoplasm affecting the adult kidney. One of the most frequent events in clear cell renal cell carcinoma (ccRCC) is inactivation of Von Hippel-Lindau gene (VHL). A series of studies recently demonstrated that a number histone modifying and chromatin remodeling genes, including SETD2, have mutation in ccRCC. Furthermore, SETD2 gene is within a 50-Mb region on chromosome 3p that encompasses VHL and is deleted in ~90% of ccRCC.

The goal of the study was to investigate inactivation of VHL and SETD2 genes by mutations. We studied 105 DNA samples of tumor tissues and normal renal parenchyma in ccRCC patients from Bashkortostan Republic of Russia, using PCR, SSCP and direct sequencing. Mutations in VHL gene were found in tumor tissues with the frequency of 21,9% (23/105). We detected 22 mutations in 23 ccRCC patients, including 8 point mutations (35%), 13 deletions (60%) and 1 insertion (5%). Ten somatic mutations hadn't been described in literature previously. VHL inactivation through sequence alterations in tumor DNA didn't differ by histopathologic characteristics or occupational exposure. Analysis of SETD2 gene was performed in 50 ccRCCs DNA samples and matched normal tissues. According to literature, mutations in SETD2 gene are detected in 4-15% cases of ccRCC. In our research SETD2 gene mutations were not found. This may be due to an insufficient number of samples in the investigated cohort. Further studies of chromosome 3 tumor suppressor genes in ccRCC tumorigenesis and validating the clinical impact of these novel mutations are required.

J12.037

Familial inversion accompanying unbalanced karyotype in a case with CML

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%90-95 of patients with CML have t(9;22)(q34;q11) Philadelphia chromosome. Other abnormalities besides the Ph chromosome are observed as %7 in chronic phase and %40-70 in blast crisis. Generally additional abnormalities are translocations and deletions, the inversion rate is just %2. Inversions constitute %10 of all structural chromosome aberrations and generally had no phenotypic effects. In inversion carriers, depending on which chromosome or segment are involved, the risk of reproductive wastage increase.

Here we reported a 43 years old female patient with Ph chromosome and additive chromosomal abnormalities which has been diagnosed in CML. In the analyses performed using BCR-ABL1 FISH probe into the bone marrow interphase cells, %12 of the cells were normal, %20 had classical fusion, in %68 of the cells increased ABL signals were present giving rise to complex rearrangements. In the molecular genetic analysis the BCR-ABL1 was positive (IS:0.75924).

The karyotype of bone marrow cells was 46,XX,?add(16)(q24),inv(18), add(21)(q22),Ph(+).C and G-banded metaphases of peripheral blood had 16qh+ and inv18, however add21(q22) and Ph chromosome which are observed in the bone marrow cultures were not encountered. The patient had 7 pregnancies but she had only 2 healthy children. Her father and 1 child also had 16qh+ and inv18. It was concluded that only Ph+ and add 21(q22) was present in the leukemic clones of the patient and that 16qh+ and inv18 was inherited.

As a result, it was concluded that evaluation of the contribution of inversions identified in haematologic malignancies to unbalanced karyotypes and carcinogenesis should be made wisely.

J12.038

The comparison of cytogenetic & fluorescence in situ hybridization study on bone marrow & peripheral blood cells in 30 chronic myelogenous leukemia patients

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BACKGROUND: Chronic myeloid leukemia (CML) is a myeloproliferative disease. The cytogenetic hallmark of CML is philadelphia chromosome (Ph). The aim of this study was to diagnose assumed CML patients, to monitor CML patients under Imatinib therapy on the bone marrow and peripheral blood samples by cytogenetic and fluorescence in situ hybridization (FISH), and also to compare the results on both specimens. **MATERIALS & METHODS:** Chromosome banding and FISH analysis was performed on 30 suspected CML patients on the bone marrow (BM) and peripheral blood (PB) specimens. **RESULTS:** The comparison of FISH and karyotyping in 30 patients on BM and PB specimens, respectively showed that 9 (30%) and 8 (26.66%) of them were Ph+, and only (18.18%) of Ph positive patients showed atypical patterns. In comparison between BM-cytogenetic and PB I-FISH, BM-cytogenetic was more reliable than PB I-FISH to detect Ph. **CONCLUSION:** Our data demonstrate FISH analysis is a rapid, reliable and sensitive technique. Our study in comparison between BM and PB, showed BM can't replace by PB, even in detecting by FISH.

J12.039

Translocatioon t(7;11)(p15;p15) in a patient with chronic myelogeneous leukemia (CML) Ph-positive

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Translocation t(7;11)(p15;p15) is observed in 2% de novo AML and, much rarely, in Ph-positive CML in blastic phase (BP). The translocation results in a fusion between NUP98 and HOXA9 with a pathogenetic role in leukemogenesis. A 40-yr old caucasian male was diagnosed with CML-BP. He came to our attention for a marked leukocytosis (190 x 10^3/ml) and anemia (Hb 7.6 g/dl), with a normal platelet count and mild splenomegaly. Conventional cytogenetic and fluorescent in situ hybridization (FISH) analyses detected a t(9;22)(q34;q11) combined with a translocation t(7;11)(p15;p15), and BCR-ABL together with NUP98-HOXA9 rearrangements, respectively. The patient was at first treated with hydroxyurea briefly followed by high dose imatinib (400-600 mg daily). Then, based on the absence of complete response (CR), sequential therapy with dasatinib (140mg daily) was administered which resulted in primary resistance. Sequencing analysis of ABL-KD domains revealed mutation E255K, within the P-loop site. Two months later a new induction protocol (AML1310) was administered without response. The patient is alive in relapse six months from diagnosis. To date, only seven CML-BP Ph positive cases have been reported associated with translocation t(7;11). In the present case, TKIs resistance was likely subsequent both to mutation E255K, which shows a moderate resistance to dasatinib, combined with the presence of t(7;11) as an additional chromosomal abnormality. Furthermore, unsuccessful chemotherapy still represent a possible effect of t(7;11)/ NUP98-HOXA9 fusion gene which is known to be associated with

severe prognosis in AML cases inhibiting hematopoietic precursor differentiation and increasing self-renewal of hematopoietic stem or progenitor cells.

J12.040

Study of *C-MYC* amplification and expression in gastric cancer samples using CISH and IHC methods

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Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths). Gastric cancer is the fourth and fifth most common cancer in men and women, respectively. The northern and northwestern regions of Iran are high risk areas for gastric cancer.
Gastric cancer carcinogenesis refers to accumulation of genetic alteration of multiple genes such as oncogenes, tumor suppressor and mismatch repair genes. *C-MYC* proto-oncogene is one of the most frequently activated oncogenes, and is estimated to be involved in 20% of all human cancers.
To evaluate *MYC* copy number and its protein expression, CISH and IHC analyses were performed in 50 gastric adenocarcinomas among Iranian individuals. 29 samples showed low amplification, 9 and 12 samples showed moderately and high amplification, respectively. MYC immunoreactivity was observed in 27 samples. In 31 samples either *MYC* amplification or MYC immunoreactivity were observed. 19 samples showed low amplification and were negative IHC. Among 31 samples, 9 samples showed low amplification, 8 and 14 samples were moderately and high amplification, respectively. Among 31 samples, 9 samples had strong signal, 16 samples moderately signal, 2 samples poor signal and 4 samples negative signal for IHC. The majority of patients with IHC negative had low amplification. Therefore, *C-MYC* amplification was correlated with the C-MYC protein overexpression.
This study showed that there was amplification in early gastric cancer and could be used as a therapeutic target. Therefore, this will contribute to early diagnosis, therapeutic and prognosis of gastric adenocarcinoma.

J12.041

Extended genetic analysis of Swedish patients with suspected hereditary colorectal cancer (The SWEN study)

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Background
The life-time risk for colorectal cancer (CRC) in the Swedish population is approximately 5 % with a mortality second only to that of lung cancer. It is estimated that 5 % of all CRC develop in individuals with a Mendelian predisposition for the disease, for example Lynch syndrome (LS), familial adenomatous polyposis (FAP), *MUTYH*-associated polyposis (MAP), juvenile polyposis syndrome (JPS), Peutz-Jeghers syndrome (PJS) or PTEN hamartomatous tumor syndrome (PHTS). However, diagnose-selective genetic screening fails to detect a disease-causing mutation in the majority of patients with suspected hereditary CRC.
Aim
The SWedish Extended genetic analysis of hereditary colorectal Neoplasia (SWEN) is a prospective national study involving researchers and clinically active staff at the cancer genetics clinics in Sweden with the overall aim to improve genetic testing and the care of patients and families with genetic susceptibility for CRC.
Material and Methods
Six hundred adult patients with clinically suspected Mendelian CRC of any type are offered mutation screening of the 11 genes associated with LS, FAP, MAP, JPS, PJS, PHTS and additional selected candidate CRC susceptibility genes. Genetic and clinical data for each family is registered in a national database followed by genotype-phenotype correlation studies.
Current Status
The study is ongoing since February 2014 and inclusion of patients will continue for at least three years.
Significance
The results from the SWEN study should improve genetic testing, personalized risk estimation and effectiveness of surveillance programs for individuals with hereditary predisposition for CRC.

J12.042

Microsatellite instability analysis in sporadic colon cancer patients in Croatia

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Colorectal cancer is a result of accumulation of genetic and epigenetic alterations associated with the transformation of normal colonic epithelium to colon adenocarcinoma. Two major pathways involved in colorectal carcinogenesis, the suppressor and the mutator pathway have been identified, with different clinical behaviors and response to chemotherapy. Approximately 15% of sporadic colorectal carcinomas (CRC) arise from mutator pathway which is characterized by microsatellite instability (MSI) caused by deficient mismatch repair system (MMR) and defects in MMR genes.
In this study we have examined the incidence of MSI using the NCI Bethesda panel of microsatellite markers (Bat-25a, Bat-26, D2S123, D5S346 D17S250) in 200 sporadic CRC patients. Analysis was performed using ABIPRISM 310 genetic analyzer. The sample was denoted as MSI high (MSI-H) if two or more of the markers demonstrated instability. The sample was denoted as low microsatellite instability (MSI-L) if only in one of the analyzed markers the MSI was detected.
The MSI was detected in 11/200 (5,5 %) analyzed samples and 8 tumors with MSI-H and 3 tumors with MSI-L were identified, while the remaining tumors showed no instability and were classified as microsatellite stable (MSS). MSI was detected in the following markers: Bat25a in 8 patients, Bat26 in 7 patients, D2S123 in 10 patients, D5S346 in 6 patients and D17S250 in 8 patients.
There was no statistically significant correlation between the MSI and clinico-pathological characteristics, although MSI was more frequent in larger, poorly differentiated and advanced stage tumors.

J12.043

Quantitative JAK2^{V617F} mutation and cytogenetic abnormalities in MPNs: Legnano Hospital experience

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The detection of molecular and cytogenetics alterations is important for the diagnosis, prognosis and classification of myeloproliferative neoplasms (MPNs). In our institution bone marrow conventional and molecular cytogenetic and detection of JAK2^{V167F} on isolated granulocytes from blood sample are performed at clinical presentation of suspected MPN. Recently evaluation of JAK2^{V167F}allele burden has been introduced in our diagnostic flow chart. While the V617F acquired mutation in JAK2 gene has been described in a high proportion of MPN patients and allele burden (heterozygosity versus homozygosity) correlates with a higher risk of secondary fibrosis, there is no specific cytogenetic alteration but some recurrent abnormalities in MPNs. Among 34 JAK2 mutated patients since the introduction of quantitative JAK2^{V167F} measurement, 3 patients has also shown an abnormal karyotype. One patient (case 1) presented 2 related clones with trisomy of chromosome 8 and 9, deletion of chromosome 20q has been observed in case 2 and a t(4;20) translocation involving deletion of TET2 gene has been observed in the third case. Prognostic significance of cytogenetics findings at diagnosis and correlation with JAK2^{V167F}allele burden is presented. A specific discussion of trisomy 9 case will be done due to the difficulty of interpretation of the allele burden.

J12.044

Clinical and genetic features of paediatric acute lymphoblastic leukaemia in Down syndrome in the Nordic countries

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To address clinical and genetic differences between acute lymphoblastic leukaemia in Down syndrome (DS-ALL) and non-DS-ALL and to ascer-

tain prognostic factors in DS-ALL, we reviewed all 128 paediatric DS-ALL diagnosed in the Nordic countries between 1981 and 2010. All had B-cell precursor ALL, comprising 2.7% of such cases. Within the DS-ALL group, platelet counts and the incidence of extramedullary disease were higher in girls. 5-year event-free survival (EFS) and overall survival were significantly poorer for DS-ALL patients with white blood cell (WBC) counts ≥50 x 10^9/l. The DS-ALL and the 4,637 non-DS-ALL patients did not differ as regards sex ratio and WBC counts, but the age distributions varied between the DS and non-DS cases, with age peaks at 2 and 3 years, respectively, and the platelet counts were lower in the DS-ALL group. Abnormal karyotypes were more common in non-DS-ALL, and there was a significant difference in the distribution of modal numbers, with only 2% high hyperdiploid DS-ALL cases. There was no significant difference in 5-year EFS between DS-ALL and non-DS-ALL patients in the recent NOPHO ALL-2000 protocol (0.670 and 0.785, respectively; *P*=0.11). The present study adds further support for increased survival of DS-ALL patients during the last few years.

J12.045

Allele-specific real-time PCR detection of EGFR exon 19 and 21 mutations in various clinical non-small cell lung cancer specimens

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The aim of our study was to evaluate the effectiveness of EGFR gene mutation detection in diverse materials from 707 NSCLC patients using the two ultra-sensitive allele-specific real-time PCR assays in the routine diagnostic procedure.
629 samples were analyzed by PNA-LNA PCR clamp, 164 samples by CE-IVD certified allele-specific PCR assay and 86 samples by both methods. PNA-LNA PCR clamp products were analyzed by Sanger sequencing to confirm rare mutations. NSCLC specimens were characterized by tumor cells content and type of fixation [Table].
62/707 (9%) specimens were positive for EGFR mutations: 32 detected in exon 19, including 4 rare deletions and p.R748K substitution (c.2243G>A); 28 p.L858R (c.2573T>G) mutations, 1 rare variant of p.L858R (c.2572_2573delinsAG) and 1 double mutation p.L858M+p.L861Q (c.2572C>A; c.2582T>A) detected in exon 21. There were 71% women in the EGFR+ group. 98% of EGFR+ were adenocarcinoma samples, thus mutation frequency among adenocarcinoma patients was 9.8% (61/617 patients). Ultrasensitive PNA-LNA PCR clamp assay and allele-specific PCR CE-IVD test presented high results conformity with 95.3% overall percent agreement (95%CI=90.9-99.8) and Cohen's Kappa score of 0.9 (95%CI=0.88-0.92).
Both real-time PCR assays provided reliable and robust detection of most common EGFR activating mutations in various clinical NSCLC specimens.

NSCLC specimens

fresh-frozen tissue	FFPE tissue	FFPE biopsy	cytology smear
84	299	198	126

J12.046

An investigation of relationships between hypoxia-inducible factor-1a gene polymorphisms and endometrial cancers

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Background: Endometral carcinoma is the most common malignant tumor of the female genital tract and the fourth most common cancer in women after breast, colorectal and lung cancers. Hypoxia-inducible factor -1 (HIF-1) is a key transcription factor that regulates cellular response to hypoxia. HIF-1 plays important roles in the development and progression of cancer through activation of various genes that are involved in crucial aspects of cancer biology, including angiogenesis ,energy metabolism, vasomotor function, erythropoiesis, and cell survival. The aim of the present study was to investigate the association between *HIF-1* 1772 C/T polymorphisms and endometrial cancer. **Patients and methods**: 75 patients with endometrial carcinoma and 75 patients who underwent hysterectomy for non tumoral reasons selected for evaluation of *HIF-1* 1772 C/T polymorphisms by PCR-RFLP and sequencing. **Results: Our findings** showed that the T allele and genotype TT in *HIF-1* 1772 C/T was significantly associated with endometrial cancer risk in comparision with control group. **Conclusions:** Our results suggest that the C1772T polymorphism of the HIF-1a may be involved in development and progression of endometrial carcinoma.

J12.047

The EMT gene expression signature and somatic mutations in colorectal cancer with peritoneal carcinomatosis

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Colorectal cancer (CRC) often metastasizes even at early stages that is the main cause of death. Epithelial-mesenchymal transition (EMT) is a complex process that is required for dissemination of tumor cells. EMT leads to the transformation of epithelial cells phenotype to mesenchymal phenotype. Tumors with mesenchymal subtype is associated with poor prognosis.
EMT, somatic mutations in *KRAS* and *BRAF*, microsatellite instability were investigated in 17 tumor samples and 20 carcinomatosis nodes from 20 CRC patients with peritoneal carcinomatosis. To determine EMT program gene expression profiling *ZEB1, ZEB2, VIM, SNAIL1, CDH1* by real-time PCR was analyzed. EMT was detected in 5 of 17 tumor samples (29%) and 17 of 20 carcinomatosis nodes (85%). These CRC samples were characterized by high frequency of somatic mutations (11 *KRAS* and 2 *BRAF* mutation, 65%), microsatellite stability (17 MSS and 3 MSI-L tumors) and low grade. We observed a high concordance between tumor and carcinomatosis node for mutations and MSI status, but not for EMT. We suppose the tumors eventually may lose mesenchymal phenotype (mesenchymal-epithelial transition) or intratumoral heterogeneity exists. Our data shows that the most of CRC with peritoneal carcinomatosis undergo EMT process.

J12.048

Role of Dido1 in the progression and development of esophageal squamous cell carcinoma

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Background: Bone morphogenetic proteins (BMPs) are implicated in several processes during embryonic development and adult tissues homeostasis. Many cancers are linked to either the BMPs or the molecules functioning in this signaling pathway. Dido1 is a novel BMP-specific Smad-regulated target gene, which is studied in few cancers. However, its expression level has not been yet elucidated in esophageal squamous cell carcinoma (ESCC). To determine this level and its probable clinicopathological consequences, expression of the gene was analyzed.
Methods: Dido1 expression in fresh tumoral and distant tumor-free tissues from 50 esophageal squamous cell carcinoma samples was compared by real-time polymerase chain reaction (PCR).
Results: Dido1 mRNA expression level was overexpressed in 26% of tumors, while its underexpression was detected in 18% of ESCC samples. There was a significant correlation between level of Dido1 mRNA expression and increased depth of tumor invasion (P= 0.043). Furthermore, Dido1 mRNA expression was correlated with the progressed stage of tumor cells in ESCC samples (P = 0.04). Dido1 mRNA expression was inversely correlated with the age of patients (P< 0.05).
Conclusions: These results indicate a relationship between Dido1 expression and depth of tumor invasion and staging. Along with the promising evidences of its role in regulation of BMP signaling pathway which is one of the main involved pathways in tumorigenesis, Dido1 may have a role in progression and invasiveness of ESCC.
Keywords:Esophageal Squamous Cell Carcinoma (ESCC); Dido1 gene; Expressional analysis; Real-time PCR; BMP signaling pathway

J12.049

Impact of HES1 on depth of tumor invasion in esophageal squamous cell carcinoma

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Notch signaling is one of the main involved pathways in cell differentiation and organogenesis and its deregulation may lead to tumorigenesis. In this pathway, targeted to the CSL (CBF1, Suppressor of Hairlesss or Lag-1) complex, notch intracellular domain (NICD) releases corepressors and recruits MAML1 as coactivator triggering the activation of notch signaling transcription complex. HES1 (Hairy enhance of split-1) is one of the notch signaling target genes which is a bHLH transcription factor acting as a proliferation stimulator through the suppression of cell cycle inhibitors such as p27 and p21. HES1 mRNA expression in fresh tumoral tissues from 50 esophageal squamous cell carcinoma (ESCC) samples was compared to their margin normal by real-time polymerase chain reaction (RT-PCR). Thirteen out of 50 cases (26%) had HES1 underexpression while HES1 overexpression was observed only in 4 (8%) samples. HES1 underexpression was significantly

correlated with tumor depth of invasion (P = 0.02). Although we have not observed any significant correlation between the HES1 expression and notch activation in ESCC, this study is the first report that elucidated the HES1 underexpression in ESCC and revealed its correlation with indices of poor prognosis.

J12.050

Clonal genomic changes in progression of multiple myeloma to extramedullary relapse and plasmocellular leukemia - case report

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Extramedullary relapse (EM) of multiple myeloma (MM) is defined as infiltration of plasma cells (PC) outside of the bone marrow. EM is an aggressive form of the disease with a adverse outcome.

We present a case study of a 52-year-old female diagnosed with MM in 2008. Initial cytogenetic analysis (G-banding) proved hypotriploid karyotype (63-64 chromosomes) with structural and numerical abnormalities. Using cIg-FISH, we detected disruption of IgH gene, t(4;14), gain 1q21 and trisomy of chromosomes 9 and 15. In 2011, patient relapsed twice and progressed in 2011. Peripheral blood was infiltrated by abnormal PC and an extramedullary lesion in the head was formed. At the time of progression, the same chromosomal abnormalities were present in the bone marrow, peripheral blood and the EM lesion: deletion of RB1 and TP53, IgH rearangement, t(4;14), gain(1q21) and non-hyperdiploidy. Array-CGH showed that genome profiles before and after progression were different - during disease progression, hyperdiploid karyotype turned into nonhyperdiploid karyotype together with loss of trisomies of chromosomes 2,3,7,8,9,11,17,18,19,20 and gain of new abnormalities - deletion of 1p, 2p, 4q, 11p, 12p, 13, 14q, 17p and 22p. Similarly, sequencing of TP53 showed different mutations before and after progression.

We suppose that the extramedullary lesion originated by an expansion of one clone of tumor plasma cells from the bone marrow. This is confirmed by identical genome profile of both tested samples.

Work was supported by grants NT13492 and OP VK CZ.1.07/2.3.00/20.0183 project

J12.051

Germline mutations of apc and myh genes in patients with familial adenomatous polyposis in populations from central western Spain

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Germline mutations in APC are asociated with the development of familial adenomatous polyposis (FAP). This syndrome is characterized by the presence of hundreds to thousands of adenomatous polyps in the colon and rectum. An attenuated form of FAP, called attenuated familial adenomatous polyposis (AFAP), is related to mutations in the 5' end, in exon 9 and in the 3' end of the APC. In addition, biallelic mutations in MUTYH gene have also been identified in patients with colorectal adenomas and in APC-negative patients with FAP or AFAP.

Molecular analysis in APC performed to 50 Spanish families diagnosed with FAP (n=38) and AFAP (n=22) has allowed the identification of pathogenic mutations in 85% of FAP families and 21% of AFAP. In total, we found 22 pathogenic mutations: 7 were nonsense and 13 were frameshift (7 deletions, 3 duplications, 2 insertions and a deletion together with an insertion). Ten of these mutations are reported in this work for the first time. We have also detected two novel splicing mutations. In this study, we detected 6 variants of unknow significance and we performed population-based studies, segregation studies and In Silico studies to further define these mutations. In patients who showed no point mutations in the APC gene were analyzed for large rearrangements by multiplex ligation-dependent probe amplification (MLPA).

Analysis of MUTYH revealed 6 pathogenic mutations in 8 unrelated families: 7 biallelic and 1 monoalellic variants.

Thus we have characterized 12 novel mutations in the APC gene involved in Familial Adenomatous Polyposis.

J12.052

FGFR2 K367E mutation is frequently found with low burden in patients with early onset aggressive colorectal cancer

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We conducted next-generation sequencing using Ion AmpliSeq™ Cancer panel (Life technologies) which covers relevant regions across 46 oncogenes and tumor suppressor genes on 5 primary colorectal tumors. The analyzed patients presented similar clinical features: early onset (39-50yrs), a highly aggressive disease, distal localization, microsatellite stable tumors and a lack of a family history of malignant diseases. The mutations found (Table 1) generally correlate with the most abundant alterations in colorectal cancer. A K367E mutation in exon 9 of the FGFR2 gene was found with low burden (8-11%) in the tumors of 3 patients. This is a novel mutation that results in AA substitution in the extracellular juxtamembrane region and might initiate spontaneous dimerisation of the receptor, leading to factor-independent growth and hyperresponsiveness to ligand, as suggested for similar defects in endometrial cancer and patients
with craniosynostosis syndromes. Having in mind the low burden of the variant, we believe that K367E mutation is a late event alteration in a subclone which may be responsible for the highly aggressive pattern of the disease. This should be clarified by mutational burden analysis of the primary and secondary tumors of these and other patients.

Table 1. Mutations spectrum in the analyzed patients *

patient	PIK3CA	BRAF	TP53	FGFR2	KRAS	APC	FBXW7
CC330			R248F (67%)	K367E (8%)	G13D (55%)		R465C (70%)
CC553			R273C (63%)	K367E (11%)		Q1349X (50%)	
CC562					G13D (10%)		
CC567	G1049R (34%)	V600E (28%)	G245D (50%)	K367E (13%)			
CC571	R88Q (29%)						R465C (26%)

*The mutation burden is presented in parentheses.

J12.053

FGFR3 and TP53 gene mutations in bladder cancer in the Belarusian population

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Bladder cancer represents the second most common genitourinary tumor, with approximately 390,000 new cases every year worldwide. Mutations in the FGFR3 and TP53 genes have been shown to define two divergent pathways in the pathogenesis of bladder urothelial carcinomas.

The purpose of this study was to perform molecular screening for FGFR3 and TP53 mutations in 243 bladder tumors and to assess the relation of clinicopathological variables with mutation status of these two genes. Mutations in the FGFR3 and TP53 genes were detected by SNaPshot method and PCR-single-strand conformational polymorphism analysis followed by DNA sequencing, respectively.

FGFR3 mutations were identified in 115 (47.3%) specimens and TP53 alterations were found in 46 (18.9%) tumors. In 12 cases (4.9%), FGFR3 and TP53 mutations coexisted; in 94 cases (38.7%), neither mutation was found. Analyses of nonmuscle-invasive and muscle-invasive tumors alone revealed that FGFR3 and TP53 mutations were independent events (p=0.76 and p=0.11, respectivelly). An inverse correlation between mutated FGFR3 and mutated TP53 was observed: FGFR3 mutations were strongly associated with low-stage (p<0.001) low-grade (p<0.001) tumors, whereas the frequency of TP53 mutations was significantly higher in tumors of higher stage (p<0.001) and grade (p<0.001). Moreover, the presence of metastasis was strongly associated with TP53 mutations and the lack of FGFR3 alterations. Our data are consistent with the idea that FGFR3 and TP53 mutations represent two distinct mechanisms of bladder cancer development. In our study, FGFR3 mutations were associated with favorable conventional prognostic factors, whereas TP53 alterations were related to adverse disease parameters.

J12.054

Association of rs2294008 polymorphism in PSCA gene with gastric cancer susceptibility in Uzbekistan

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Gastric carcinogenesis is a complex, multistep and multifactorial process, in which many factors are implicated. Genetic factors play an important role in the development of gastric cancer(GC). Recent studies have shown that single-nucleotide polymorphisms (SNPs) in several genes are associated with increased GC risk, indicating that genetic variation contributes to gastric carcinogenesis.

Located on chromosome 8q24.2, the prostate stem cell antigen (PSCA) gene encodes a 123-amino acid glycoprotein related to the cell-proliferation inhibition and cell-death induction activity. SNPs in PSCA gene have been found associated with gastric cancer risk in a genome-wide association study. This association has been replicated in several populations, but also there are some conflicting results.

This study aimed to investigate the association between polymorphic variant of PSCA gene(rs2294008) and susceptibility to gastric cancer in Uzbekistan.

105 patients with gastric cancer and the sex and age matched control group of healthy individuals (n=116) were included in this study. DNA samples isolated from these groups were genotyped by means of PCR and RFLP method. Comparative analysis of resulting genotypes showed a statistically significant association between gastric cancer and C/T genotype(p=0.0009, Pearson's χ2 test). The odds ratio (OR) of increased relative risk of developing gastric cancer for CT genotype carriers was 4.75 (CI95% 2.68 - 8.41).

These findings supported that PSCA rs2294008 C>T polymorphism may contribute to the susceptibility to gastric cancer. Genotyping of rs2294008 PSCA gene polymorphism can be recommended as a criterion for identification of high risk groups concerning developing of gastric cancer in Uzbekistan.

J12.055

Polymorphism of the thymidylate synthase gene and sensitivity of gastric cancer to 5-FU chemotherapy.

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Thymidylate synthase (TS) is a key enzyme in nucleotide biosynthesis and is the main intracellular target of 5-FU. There are TYMS polymorphisms in the 5' and 3' untranslated region associated with expression of TS.

We investigated the clinical and prognostic importance of TYMS polymorphisms for gastric cancer patients. We examined of 5' (TSR2/TSR3 and G>C) and 3' (del 6) untranslated region polymorphisms in 80 gastric cancer patients after surgical treatment and chemotherapy with 5-fluorouracil.

We have shown, that the genotype 2R/2R is significantly more often found in patients with regional lymph node metastases (N1-3) (p = 0.0475), genotype -6/-6 is significantly more common in patients with intestinal gastric cancer (p = 0.0375), as well as in patients, who have high/moderately differentiated adenocarcinoma (p = 0.033).

In the study of the effectiveness of chemotherapy we have shown, that genotype 3G/3G was associated with the poor prognosis, development of local recurrence, carcinomatosis, the appearance of distant metastases during the first 3 years after surgery and 5-FU treatment (p = 0.024). Genotype 2G /3C was significantly frequent (p = 0.023) in patients without progression of the disease within the first 3 years after surgery and 5-FU treatment. In the study of the TYMS gene 3'-untranslated region (del 6) polymorphism in the patients after surgical treatment and chemotherapy, we found no significant differences.

We suggest that TYMS 5'UTR and 3'UTR polymorphisms can serve as individual prognostic factors and as predictors of adjuvant treatment effectiveness in patients with locally advanced gastric cancer.

J12.056

Relationship between genetic and protein expression of antifole chemotherapies metabolic enzymes and sensitivity in head and neck cancer cell lines

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Introduction: Antifolate chemotherapies disrupt cellular proliferation by blocking folate dependent enzymes. Data show variability in outcomes with antifolate chemotherapies and it can be associated to genetic factors. **Objectives:** To investigate relationship between genetic and protein expression of *MTHFR, DHFR, TYMS* and *SLC19A1* folate genes for 5-fluorouracil (5-FU) and methotrexate (MTX) antifolate chemotherapies response in two head and neck cancer cell lines. **Methods:** HEP-2 (laryngeal cancer) and HN13 (oral cavity cancer) cell lines were treated with 0.25, 25.0, and 75 μM of MTX and 10 ng/ml, 50 ng/ml, and 100 ng/ml of 5-FU, separately, for 24 hours/37°C. Real-time PCR and Western blotting techniques were performed. ANOVA and Bonferroni's post hoc tests were utilized for statistical analysis. P<0.05 was considered significant. **Results:** Regarding to 5-FU treatment, there was increased expression for *TYMS* mRNA in HEP-2 cell line with 100 ng/ml 5-FU, and increased expression for *DHFR* and *TYMS* mRNAs in HN13 cell line with 100 ng/ml (p<0.05). Regarding to MTX treatment, there was increased expression for all mRNAs evaluated in HEP-2 cell line treated with 75 μM MTX. *SLC19A1* gene presented lower expression in HEP-2 cells treated with 0.25μmol of MTX. For HN13 cell line and MTX chemotherapy, there was increased expression for *DHFR* and *SLC19A1* mRNAs in cells with 75 μM MTX (p<0.05). There was not association of protein expression and chemotherapies evaluated. **Conclusion:** Folate genes expression alterations in cell culture model may predict mechanisms of resistance and sensibility for antifolate chemotherapies and give support for studies in clinical practice.

J12.057

Double knockdown of apoptotic genes and their relationship with other mechanisms involved in tumor cell survival

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The simultaneous inhibition of two important genes - p53 and TNF - involved in tumor cell development and evolution, with the siRNA technology, will hopefully bring us new significant and interesting data in triple negative breast cancer (TNBC) cell signaling. TP53 is involved in the intrinsic apoptosis pathway, and the tumor necrosis factor (TNF) is the main inducer of the extrinsic apoptosis signaling mechanisms. In order to evaluate the impact of TNBC dual gene knockdown, we used PCR array technology (qRT-PCR) to analyze the most relevant genes involved in apoptosis along with other staining assays that will unravel important and new data in the tumor cell pathways cross-talk. Our experiments were performed on a triple negative breast cancer cell line (Hs578T) and in relation with untreated cells that serve as control. After analyzing the transcript quantification data we obtained statistical relevant results for 16 genes, of which 12 were up-regulated and 4 down-regulated. By simultaneously inhibiting p53 and TNF genes on Hs578T cells, we observed that they affect the angiogenesis mechanisms by inhibiting the tumor cell network formation and self-degradation of cellular components through autophagy. By integrating the expression profiles of triple negative breast cancer untreated cells with the ones obtained from knocking down p53 and TNF, simultaneously, we obtained a network-based platform, that is important in finding a functional interaction between the networks of intrinsic and extrinsic apoptosis cell signaling pathways and other mechanisms involved in tumor cell angiogenesis, autophagy and cell death in breast cancer.

J12.058

Genetic profiles' comparative Analysis: Adult glioblastomas vs young adult

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Background : Glioblastomas (GB) are malignant astrocytic tumors, which often occur in patients aged over 55 years. However, younger age (<40 years) was also described in several GB cases. Therefore, GB are newly divided into 2 subtypes according to patient's age: young adult GB (Y.GB) (40 years).

Basically, clinical presentation and histopathological descriptions are similar between the 2 GB subtypes. Nonetheless, the assumption of different tumorogenic pathways was suggested. This hypothesis is supported by molecular alterations differences between Y.GB and A.GB.

Purpose: This study aims to compare the genetic profiles between Y.GB and A.GB in Tunisian glioblastomas.

Methods: 43 samples composed by 40 A.GB and 13 Y.GB were collected during the period of 5 years from 2009 to 2013. In order to investigate discriminative genetic alterations between the 2 GB groups, tumoral DNA was analysed by MLPA (Multiplex Ligation Probe Amplification). We used the 4 SALA MLPA probemix designed for Gliomas analysis: P370, P088, P105 and ME011.

Results: Similarities were observed between the 2 GB groups, concerning tumor size and locations as well as some chromosomal alterations: 10q, 17q and 19q. Interestingly, 1p 19q co-deletion, 1p deletion and CDKN2A (p14)

were only found in A.GB group. While IDH1 mutation was more frequently detected in Y.GB.
Conclusion: GB genetic profile variability noticed in our sample cohort supports the hypothesis of different tumorogenic pathways between A.GB and Y.GB. Despite of the crucial role of clinical and histopathological data in tumor diagnosis, molecular investigations seems of a great importance as well.

J12.059

Association analysis of rs7903146 (C>T) variant of TCF7L2 Gene with Glioblastoma in a Turkish Population

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Glioblastoma (GBM) is the most common malignant brain tumor in the adult population defined as grade IV astrocytoma according to severity of necrosis and vascular proliferation. It can be seen at any age, but commonly between the ages of 45-75. GBM is difficult to treat and presents high morbidity and mortality risks. There is no curative treatment. Patient's genetic structure is a factor as important as the choice of chemotherapeutic agents, dose, time of administration that directly affect the process of chemotherapy. Transcription factor 7-like 2 (TCF7L2) gene, located on chromosome 10q25.3, encodes a transcription factor which is involved in the Wnt/β-catenin signaling pathway. Active nuclear complex which TCF7L2 forms with β-catenin induces the expression of target genes involved in cellular proliferation, evasion of apoptosis and tissue invasion and metastasis. It is demonstrated in association with many cancer types, including breast, prostate, lung and colon cancers. We performed a study to investigate the association between TCF7L2 rs7903146 variant and another cancer type glioblastoma. Forty-three patients who primarily diagnosed as GBM were recruited to this study from Department of Neurosurgery of Faculty of Medicine of Ankara University in Turkey. None of the patients received any chemotherapeutic agents or radiation therapy before surgical resection of the tumor. DNA was isolated using isolation kit from patients' cancerous and their corresponding adjacent non-cancerous tissues. rs7903146 were genotyped by PCR-RFLP. No association was determined between rs7903146C>T and GBM after analyzing carried out using dominant, additive, and recessive models (P>0.05).

J12.060

Analysis of autophagy polymorfisms in glioblastomas

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BACKGROUND
Autophagy is an adaptive and highly regulated response in unfavorable conditions such as starvation. During this process, long-lived proteins and organelles are self-eaten by double membranes vesicles, called autophagosomes, which transport them through the cytosol to the lysosome, where they will be degraded. The resulting macromolecules can be recycled to the cytosol for reuse. Autophagy plays an important role in cellular development and differentiation and it has been reported that its malfunction is implicated in several diseases including cancer. Glioblastoma is a very infiltrating and aggressive heterozygous glioma. Its behavior is directly related to their genetic and chromosomal alterations. Here, we have analysed common polymorphisms in autophagy genes in order to evaluate the role of these variants in modulating glioblastoma risk.
PATIENTS AND METHODS
112 newly diagnosed patients with primary glioblastoma (grade IV) and 189 controls without cancer were included in the study. Genomic DNA was extracted from peripheral blood using phenol/chloroform procedure and genotyped using TaqMan 5'-exonuclease allelic discrimination assays (Applied Biosystems) (table 1). Statistical analysis was performed using SPSS software.
RESULTS
No significant differences were found in genotype distribution for ATG16L1 RS2241880 ATG2B RS3759601 and ATG5 RS2245214 between patients with glioblastoma and control subjects. However, statistical differences in genotype distribution for ATG10 RS1864183 were found. The A allele was associated with increased risk of developing glioblastoma (OR = 2,788, 95% CI 1,40-5,55; p= 0,004).
CONCLUSIONS
ATG10 RS1864183 POLYMORPHISM IS ASSOCIATED WITH SUSCEPTIBILITY TO SUFFER GLIOBLASTOMA, SUPPORTING THE IMPORTANCE OF AUTOPHAGY IN CANCER DEVELOPMENT.

J12.061

Genetic polymorphisms in GSTM1, GSTT1, GSTP1 and the susceptibility to basal cell carcinoma in Iranian population

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The glutathione S-transferase (GSTs) are polymorphic supergene family of detoxification enzymes that are involved in the metabolism of numerous potential carcinogens. Several allelic variants of polymorphic GSTs show impaired enzyme activity and are suspected to increase the susceptibility to various cancers. To evaluate the relationship between polymorphisms in the GSTs (GSTT1, GSTM1, GSTP1) and basal cell carcinoma (BCC), 50 BCC patients and 50 healthy controls were studied. Following tissue biopsy and DNA extraction genotypes were analyzed by GAP-PCR and ARMS-PCR. The frequencies of GSTM1 null genotypes in patients and controls were 34% and 6%, respectively. The overall frequency of GSTT1 null was higher in cases as compared with controls (p=0.01, Odds ratio (OR) = 3.78, 95% confidence interval (CI), 1.34-10.63). The GSTP1, Ile/Val genotype and the Val allele were higher in cases than controls (p=0.0001, OR = 12.67% CI = 4.90-32.73; p=0.001, OR = 5.52, CI = 2.56-11.89), respectively. The results obtained demonstrated that presence of two genotypes of GSTM1 null and GSTT1 null and allele GSTP1 Val lead to increase for BCC.

J12.062

BRIP1 Screening in Spanish Breast and Ovarian Cancer Families

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Up to 75% of Hereditary ovarian carcinomas (HOC) are attributed to mutations in *BRCA1* and *BRCA2* genes, whereas DNA mismatch repair genes would cause about 10% of them. Genes involved in FA-BRCA pathway will account for an additional percentage of HOC.
Several studies identify BRIP1 (a FA-BRCA pathway gene) as a moderate-penetrance breast cancer susceptibility gene in BRCA1/2 negative familiar cases. Truncating BRIP1 mutations have been described as cancer susceptibility alleles in families with a great ovarian cancer burden. Thus, we aimed to assess whether BRIP1 alterations may contribute to breast and ovarian cancer (BOC) susceptibility as c.1702_1703delTT mutation has been associated with BOC in Spanish population.
Forty-nine out of 140 families In North West-Central of Spain with family history of breast and ovarian cancer carried a pathogenic mutation in BRCA1 or BRCA2. We performed BRIP1 mutational analysis in 72 BRCA negative families. Mutation screening was performed with High Resolution Melting Curve Analysis (HRMA). Cases displaying abnormal HRM patterns were evaluated by direct Sanger Sequencing.
There is no evidence of pathogenicity in the identified variants. Mutation c.508-31C>G was found in ovarian cases. Mutation c.2755T>C was carried by BOC patients. Around 60% of the samples harbors c.2637A>G and c.3411T>C changes. Variant c.577G>A has been previously reported in a UK study with similar frequency of occurrence.
Although clearly pathogenic mutations have not been identified in the samples screened, BRIP1 polymorphisms may increase ovarian cancer risk, so these findings can be used as a potential tool for improving cancer diagnosis and treatment planning.

J12.063

Analysis of polymorphisms in the EGFR pathway involved in the response and toxicity of treatments in head and neck squamous carcinoma

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Head and neck squamous carcinoma (HNSC) is one of the most prevalent cancers in Spain. The classic risk factors strongly associated with developing HNSC are tobacco smoking and alcohol consumption.
The common therapies in HNSC are platinum-based chemotherapy, radiotherapy and/or monoclonal antibodies against EGFR, although the response rates are very variable. We have evaluated the influence of some polymor-

phisms in different genes of the EGFR pathway and antibody dependent cellular cytotoxicity receptors (ADCC) associated with differences in toxicity and responses of these treatments.
Genomic DNA was extracted from peripheral blood samples of 91 Spanish HNSC patients treated with monoclonal antibodies against EGFR. The selected polymorphisms realized by QPCR with TaqMan® probes were: FCGR2A rs1801274, FCGR3A rs396991, EGFR rs28384375, rs2227983, rs17336639 and KRAS lc6 rs61764370.
Statistical analysis was performed comparing the different distribution of the polymorphisms and patients were stratified according to treatment and response.
We found differences in the distribution of FCGR2A A519C (rs1801274) polymorphism (p=0.012), conferring an increased risk of developing dry skin in carriers of two copies of the C allele; OR=4.018 (1.257-12.850). Moreover, we found that carriers of the heterozygous genotype in K-RAS lcs6 polymorphism (rs61764370) exhibit a decrease in global toxicity of anti-EGFR antibodies therapy; p=0.014, OR=0.294 (0.107-0.805).
Our results support the correlation between some germline polymorphisms in some genes in the EGFR pathway and ADCC receptors, and the differences in the toxicity of anti-EGFR antibodies therapy.

J12.064
Conventional cytogenetics and FISH analysis in hematological disorders

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Non-random chromosomal aberrations are a common feature of many hematological disorders, making cytogenetic analyzes essential for the most common abnormalities in myelodysplastic / myeloproliferative diseases, leukemias and lymphomas.
Between March 2013 and January 2014, 77 bone marrow samples were analyzed cytogenetically from adults aged between 23 and 82 years. Where possible three independent cultures (direct method, 24 and 48 hours) were established and a minimum of 20 metaphases were analyzed.
The karyotype of 70 patients was analyzed successfully. For 7 patients with insufficient number of metaphases FISH analysis was performed using BCR/ABL1plus probes (MetaSystems). Complementary specific FISH probes (MetaSystems) were used according to clinical hematological diagnosis for 5 patients with normal karyotype.
Among the successfully karyotyped samples, 35 cases of CML, and 3 cases of ALL showed various homogeneous or mosaic chromosome abnormalities. The most common abnormality was the presence of the Philadelphia chromosome (Ph), found in varying percentages (between 9 and 100%) with 2 cases having an additional cell line with double PH chromosome. One case had a complex translocation involving chromosomes 9, 10 and 22. Other common abnormalities were trisomy 8, isochromosome 17, autosomal hyper and hypodiploidy.
Identification of cytogenetic abnormalities by conventional karyotype is important to confirm the diagnosis and provide useful information for classification, staging and prognostic information, for choosing therapy conduct and for signs of remission or relapse. Because some cases show a normal karyotype by conventional banding, FISH and aCGH analysis are valuable techniques recommended for identifying the presence of possible submicroscopic abnormalities.

J12.065
Her-2neu gene in neck squamous cell carcinomas (SCC) a potential target for therapy

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Background: Over-expression of the proto-oncogene cerbB-2 (HER-2/neu) has been shown to be a prognostic marker and also a target for therapy in many cancers but conflicting data exist about the prevalence of HER-2/neu over expression in SCC of the head and neck.
Design: The status of Her-2/neu was evaluated in a series of 36 SCC of the larynx and oro-pharynx to verify the frequency of over-expression of HER-2/neu and evaluate the correlation with traditional diagnostic parameters of this neoplasm. A Hercep test kit was used to detect HER-2 expression, and a Path Vysion kit was used for gene amplification.
Results: On immunohistochemical (IHC) staining HER-2/neu was positive only in 8 cases (8/36) and these were further tested by fluorescent in situ hybridization (FISH) with positive gene amplification in 3 cases (3/8), cep17/Her-2/neu = 4.22 - 4.84. One case with IHC staining 3+ do not have gene amplification and 4 cases with 2+ and 1+ at the IHC evaluation have no amplification (cep17/HER-2/neu = 1.64); tumors with positive gene amplification were grade 3 (4/8) or had basaloid features.
Conclusion: Even there are a small percentage of these tumors that have gene amplification for HER-2/neu, at present, patients who may potentially benefit from molecular targeted therapy targeting HER-2/neu for SCC of head and neck should be identified by gene amplification analysis using FISH in IHC 3+ patients.

J12.066
Colorectal cancer risk in relation to Hypoxia Inducible Factor-1α (HIF-1α) and Von Hippel-Lindau (VHL) gene polymorphisms

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Colorectal cancer (CRC) is a multifactorial disease involving environmental and genetic factors. Unhealthy diet habit, smoking, and environmental carcinogenic agents are risk factors of CRC, such as alcohol, low methionine, low folate diets and exposure to white soil stands.
Hypoxia plays a critical role in activating hypoxia-inducible factor-1α (HIF-1α) and leads to numerous metabolic changes such as angiogenesis, anaerobic glycolysis and erythropoiesis. HIF-1α protein level is known to be regulated by von Hippel-Lindau (VHL) ubiquitin-proteasome system but in hypoxic conditions HIF-1α protein level increases. Mutations in VHL ve HIF-1α genes can affect the connection of VHL protein to HIF-1α and cause cancer and cardiovascular diseases.
The aim of this study is to investigate the relation of the C1772T (rs11549465) and G1790A (rs11549467) polymorphisms of HIF-1α gene and the functional rs779805 polymorphism of 5'UTR region of the VHL gene, regulating the oxygen-dependent degradation of HIF-1α, with the risk of colorectal cancer. In the study, 92 patients who have been diagnosed to have colorectal cancer and 101 healthy controls were included. PCR-RFLP ve ARMS-PCR molecular diagnostic methods were used for genotyping.
For statistical analysis student t, chi-square (χ^2), Fisher's exact tests and SNPStats were used. According to genetic models, CT/TT genotypes of HIF-1α C1772T polymorphism were found to increase the risk of colorectal cancer in patients (p=0,049; OR 1,96; 95% CI 1,02-3,77; AOR 4,79 (1,07-21,48). For G1790A ve rs779805 polymorphisms, no significant deviation was observed between patients and controls (p>0,05).

J12.067
Delineation of high-risk Human papillomavirus genotypes in Iranian breast cancer patients

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Contribution of Human papillomavirus (HPV) in tumor-genesis has been proved for a while, but it`s function in the pathogenesis of breast cancer is still not clear. In this investigation we checked the prevalence of main genotypes of HPV and their association with breast cancer in pre/post-operative Iranian patients. 77 formalin fixed paraffin embedded (FFPE) breast invasive ductal carcinoma and their normal adjacent tissues (NAT) were included in the study. L1-born primer pairs applied for amplification and detection of HPVs via nested-multiplex polymerase chain reaction. The HPV positive samples were further genotyped using DNA sequencing. Pretty High percentage of HPV (38%) was detected in breast (core) ductal carcinoma compared to 4% in the NAT. High-risk HPV genotypes 16, 18, 31, 33, 35 and 45 were highly associated with the advanced stages of tumor, while low-risk types 6 and 11 were present in NAT. In malignant tissues, HPV-16 was the most abundant genotype followed by type 18 and 33.The high risk HPV genotypes in breast cancerous tissue may represent its contribution in breast carcinogenesis. Using HPV vaccination program is highly recommended to reduce this type of cancer.

J12.068
Expression of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2) gene in negative and positive human Cytomegalovirus Glioblastoma multoforme tissues

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Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor. The median survival of glioblastoma patients is 12 months. The (possible) relationship between Human Cytomegalovirus (HCMV) infection and cancer has been investigated for decades. Detection of viral DNA, mRNA,

and antigens in tumor tissues suggests that HCMV infection has a role to play in the etiology of several human malignancies. HCMV gene products can promote the various signalling pathways critical to tumor growth, including PDGFR, PI3K/AKT, STAT3 and GSK-3b, that are involved in apoptosis, angiogenesis, invasion and immune evasion. IGFBP2 is a biomarker of the PI3K/AKT pathway so we decided to evaluate the expression of this gene in 3 groups: HCMV-negative glioblastoma multiforme tissues, HCMV-positive glioblastoma multiforme tissues and non tumor tissues. The presence of Human Cytomegalovirus was assessed by cytomegalovirus detection kit. Human Cytomegalovirus was present in %75 of glioblastoma tissues. Then RNA was extracted, cDNA was synthesized and Real-time PCR was performed. Then the rate of increased expression was calculated using the Livac or 2-ΔΔCt. ΔCt of samples in the three groups were compared using ANOVA (Analysis of Variance). The expression of IGFBP2 gene relative to GAPDH gene in HCMV-negative glioblastoma tissues and HCMV-positive glioblastoma tissues, respectively was increased 5.486 and 15.032 times compared to non-neoplastic brain tissues. ANOVA tests showed that the difference of mean ΔCt for IGFBP2 gene between healthy subjects and patients with HCMV positive and HCMV negative glioblastoma tumors statistically significant.

J12.069
2 Novel Somatic Mutations In Exon 15 Of The Apc Gene In Iranian Familial Adeno Polyposis Coli Patient

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Abstract: The adenomatous polyposis coli (APC) gene is considered to be a gatekeeper in colorectal tumorgenesis. 60% of somatic mutations in the APC gene are concentrated in a region called the mutation cluster region (MCR). In this study, our goal was to perform the genetic analysis of 2 patients (index patients) who had been selected by colorectal cancer features, and to identify the genetic changes in the MCR region at the APC gene. **Methods:** Mutation analysis of the MCR, which spans codons 1286-1513, was performed on the paraffin-embedded cancerous tissue samples using macrodissection, nested PCR and direct sequencing of purified PCR fragments. **Results:** In our study 2 new somatic mutations detected in these patients .In one patient we have detected a CGA to TGA as a Nonsense mutation that lead to Arg to premature Stop codon at the 4507 nucleotide position (Codon 1503) and in another patient , we describe a G→A Transition (ACG to ACA) at nucleotide position 4638 in exon 15 (MCR region) which causes a silent mutation since both normal and mutated alleles encode a Thr residue at codon 1546. Thess mutations have not been described previously. **Conclusion:** this observation could suggest differences in the frequency of pathological mutations in APC among different populations; however epidemiological studies must be performed to confirm this theory which it is not the aim of our present work. **Keywords:** APC, Iranian Familial Adenomatousis Polyposis (Iranian FAP), somatic mutations

J12.070
Detection of K-RAS gene activating mutations, an important factor in therapeutic management of CRC Romanian patients

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Activating mutations in the K-RAS gene, a known oncogene involved in the EGFR signaling pathway, were found in a variety of human tumors, including colorectal carcinoma. These mutations, detected mostly in codons 12 (82%) and 13 (17%), represent an early event in the development and progression of colorectal cancer (CRC), being associated with poor response or resistance to anti-EGFR therapy. K-RAS mutation status is used to predict the response to anti-EGFR therapy.
The aim of our study was to detect the K-RAS activating mutations (in codons 12 and 13) in order to contribute to the selection of CRC patients for anti-EGFR therapy.
DNA was extracted from formalin-fixed, paraffin-embedded samples (tumor cellularity between 10-90%) from 102 Caucasians CRC patients. The patient aged between 21-81 years, and sex distribution was 1:1. The K-RAS mutational status (codons 12 and 13) was identified by PCR-RFLP.
About 35-45% of colorectal tumours have been reported having mutation in the K-RAS gene, that activates cell signalling downstream of EGFR. In our study, such mutations were detected in 43 (42,15%) patients, of which 35 (81,40%) patients with mutations in codon 12 and 8 (18,60%) patients with mutations in codon 13. None of the patients presented mutations in both codons.
Detection of activating mutations in the K-RAS gene is required in all CRC patients, being an important factor for the therapeutic management. Our results were in accordance with international data, contributing to an effective selection of CRC patients for the anti-EGFR- therapy.

J12.071
Association of IREB2 and FAM13A variants with lung cancer and chronic obstructive pulmonary disease in Poland

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Several genome-wide association studies (GWAS) have identified loci located at 15q25 (*IREB2*) and 4q22 (*FAM13A*) associated with chronic obstructive pulmonary disease (COPD) and lung cancer (LC). Dysfunctions of *IREB2* gene, as a regulator of iron homeostasis, may lead to oxidative failures, relevant in pathogenesis of lung diseases. *FAM13A* gene is involved in modulation of RhoA molecule activity, suggesting both anti-inflammatory and tumor suppressor function. The aim of our research was to determine the association between *IREB2/FAM13A* polymorphisms and Polish COPD and LC patients. We have examined four variants in *IREB2* (rs2568494, rs2656069, rs10851906, rs13180) and three in *FAM13A* (rs1903003, rs7671167, rs2869967) among 149 COPD, 468 LC patients and 524 controls, using TaqMan® genotyping assays. The frequency of AA genotype/A allele of *IREB2* rs2568494, were significantly higher in LC cases compared with controls (P=0.0081, P=0.0043). The *FAM13A* rs2869967 was significantly associated with COPD and combined COPD with LC+COPD group (CC genotype: P=0.0007, <0.0001; C allele: P=0.0009, 0.0001, respectively). The rs1903003, rs7671167 *FAM13A* variants confers a protective effect on COPD (both P<0.002). Haplotype-based tests identified significant association between the *IREB2* AAAT haplotype and LC cases (P = 0.0021). The *FAM13A* TTC haplotype was associated with an increased risk of COPD and COPD with LC+COPD group (P=0.0013 and P=0.0003, respectively). Results were independent of age, sex, lung function and smoking history. These studies have confirmed that the *IREB2* variants contribute to increased risk of LC, whereas the *FAM13A* predispose to increased susceptibility to COPD.
Supported by the National Science Centre, Poland (2011/01/D/NZ5/02841).

J12.072
Genetic Polymorphism of NOS2 -954G/C in Nasal Polyposis. A case Control Study in a Population Group of Northern Romania

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Background Polymorphisms for genes encoding chemosensitive signalling proteins like NOS2, might contribute to the variability in individual susceptibility to nasal polyposis. NO produced by the inducible NO synthase enzyme NOS2A is generated at high levels in certain types of inflammation, so that the role of NOS2 might be important in nasal polyposis etiopathogeny. **Study Design** This is a cross-sectional, randomized, case control study for the evaluation of the frequency of -954G/C NOS2 polymorphism alleles among patients with nasal polyposis. **Subjects** The study included 92 cases of nasal polyposis diagnosed patients (nasal endoscopy and CT scan examination), and 107 healthy unrelated controls. **Methods** -954G/C NOS2 genotyping was carried out using PCR amplification of relevant gene fragment was followed by restriction enzyme digestion. Detection of the variant alleles was determined through analysis of resulting restriction fragment length polymorphism (RFLP) followed by gel electrophoresis. **Results** Molecular analysis revealed an increased frequency of -954G/CNOS2 variant allele in the study group compared to the control group (p=0.043; OR= 1.77; CI=1.02-3.09). **Conclusions** The main finding of our study is that mutant genotype of -954G/C NOS2 is considered to be a risk factor for nasal polyposis development.

J12.073
Clinical features of sebaceous skin lesions and diagnosis of Lynch syndrome: a case series from Genetic Health Queensland

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Muir-Torre syndrome is a clinical sub-type of Lynch syndrome which is associated with sebaceous skin lesions and keratoacanthomas in addition to internal malignancies. As established in other Lynch syndrome-associated cancers, these skin lesions demonstrate loss of mismatch repair (MMR) protein expression on immunohistochemical (IHC) staining, allowing targeted germline mutation analysis of the MMR genes associated with Lynch syndrome (*MLH1, MSH2, MSH6, PMS2)*. However, not all lesions with loss of MMR protein expression will result in a molecular or clinical diagnosis of Muir-Torre or Lynch syndrome, as this protein loss may be due to a sporadic mechanism. There is emerging literature that the specific type of sebaceous lesion, along with its location on the body, can provide an indication as to which lesions are more likely to have loss of MMR protein expression and subsequent association with Muir-Torre/Lynch syndrome. In order to assess this theory within the experience of Genetic Health Queensland (GHQ), an internal audit was performed. All patients seen at GHQ between 2005 and 2013 with a primary reason for referral of a sebaceous skin lesion were included in a retrospective analysis. The information reviewed included subtype and location of lesion, age of diagnosis, personal and family history of cancer, IHC results, MMR gene testing results and outcome of assessment. A detailed analysis of this information will be presented, including the proportion of individuals with sebaceous lesions in which a clinical or molecular diagnosis of Muir-Torre/Lynch syndrome was made.

J12.074
RET proto-oncogene main exons' mutations in Iranian patients with medullary thyroid carcinoma

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Introduction: Thyroid cancer is the most common endocrine malignancy. Medullary thyroid carcinoma (MTC) account for 5-10% of all thyroid cancer types. It occurs in both hereditary (25%,hMTC) and sporadic (75%,sMTC) forms which are associated with gain of function mutations in the RET proto-oncogene.
Material and methods: We have started MTC genetic screening since 2001. Our study included 360 individuals, including 161sMTC, 39FMTC, 8MEN2A, 3MEN2B, 4pheochromocitoma (215 index cases), and 145 relatives. Genomic DNA was extracted from peripheral blood leukocytes, and the six exons of the RET gene (10, 11, 13, 14, 15, 16) were amplified by PCR and examined by DNA sequence analysis to detect mutations.
Results: A total of 20 different types of missense RET mutations were identified in 78 patients. All of the MEN2A patients had mutations in codon 634. Mutations outside of codon 634 occurred only in FMTCs and sMTCs. A mutation at codon 918 was found in all MEN2B patients. p.C634Y was the most common mutation in our study followed by mutations p.C630Y, p.C634R, p.634S. The G691S/S904S haplotype was identified in 120 patients and 70 relatives. A novel C>T intronic variant (intron 11, chr position 10:43612012) was also detected.
Conclusion: Exon 11 and after that exon 10 were the most frequently mutated exons of RET proto oncogene in MTC patients in our population. As about half of patients with the hot spot mutations had the G691S/S904S haplotype simultaneously, further analysis needs for clarifying the effect of multiple risk alleles in MTC development.

J12.075
Molecular cytogenetic diagnosis of melanocytic lesions

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Although histopathological analysis is the "gold standard" for melanoma diagnosis, distinguishing potentially lethal melanomas from benign melanocytic nevi with atypical histopathologic features needs further analysis. Most melanomas have chromosome copy number abnormalities. Fluorescent In Situ Hybridization (FISH) is an applicable technique in the diagnosis of these chromosome abnormalities and so its usability is emerging in melanoma diagnosis. The aim of this study was to evaluate the diagnostic role of the FISH in the assessment of malign melanomas and melanocytic nevi. Histologically evaluated most suitable formalin-fixed, paraffin-embedded (FFPE) tissue sections of 25 malign melanoma tumors and 25 melanocytic nevi samples were analysed for copy number abnormalities of CCND1, MYB, RREB1 and centromere of chromosome 6 but no FISH result could be obtained from a tumor tissue. Copy number abnormalities were detected in 20 malign tumors (83.8%) and four melanocytic nevi (16.0%) and statistically significant differences were seen between the tissues for each gene (MYB deletion and RREB1/CEP6 p<0,05 , RREB1 amplification p<0,01 and CCND1 amplification p<0,001). Histopathologic features of the melanocytic nevi tissues with gene copy number abnormalities were similar to the characteristics of malign melanomas and they had had premalignant diagnosis. The CCND1 amplification was the predominantly seen abnormality and its importance as a marker should be evaluated in detail. The study demonstrated that copy number analysis of FFPE tumor samples by FISH is a reliable method in the diagnosis of melanoma and the technique is also informative to understand molecular mechanism of the melanocytic tumors.

J12.076
Prevalence of *P16* methylation and prognostic factors in plasma cell myeloma of single institution in Korea

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Background: The primary purpose of this study was to investigate the prevalence and characteristics of *p16* methylation and determine the prognostic implications of the clinical data, hematologic data and *p16* methylation changes in plasma cell myeloma (PCM). **Methods:** We reviewed clinical characteristics, laboratory tests and investigated the response to combination chemotherapy, and survival time. DNA methylation of the *p16* gene was tested by methylation specific PCR and evaluated the clinical significance. **Results:** A total of 103 patients were enrolled in this study. The patient median age was 59.0 years at diagnosis and male to female ratio was 1.15:1. According to the International Staging System (ISS), patients were diagnosed as stage: I (n=17, 16.5%); II (n=41, 39.8%); III, (n=39, 37.9%); (not classified 6). Forty five (43.7%) patients and thirty six (35.0%) patients showed abnormal karyotype and complex karyotype on chromosome study, respectively. The*p16*methylation was found in 39 of 103 (37.9%) patients, but there was no significant association of *p16* methylation status with other clinical, laboratory factors and survival outcome. The male gender, albumin and complex karyotype were independent prognostic factors for overall survival by multivariate analysis (P<0.05). **Conclusions:** The male gender, albumin and complex karyotype were independent prognostic factors in PCM. And *p16* methylation was relatively common in PCM, but didn't influence a response to survival outcome.

J12.077
Analysis of oncogenic mutations in 52 MGUS patients - two case reports of KRAS mutation

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Monoclonal gammopathy of undetermined significance (MGUS) is a premalignant condition permanently associated with a risk of progression into malignant disease, especially to multiple myeloma (MM). While in MM, oncogenic mutations have been described in several studies, no mutation study was done in abnormal plasma cells (aPCs) in MGUS. In our study, we performed genetic analysis in 52 samples of aPCs (CD138+CD19-CD56+/-) of MGUS patients by aCGH (SurePrint G3 CGH+SNP, 4×180K, Agilent) together with High Resolution Melting (HRM) followed by Sanger sequencing in HRM positive cases. By HRM, we focused on DNA single nucleotide mutations in 11 hot spots of MYC, NRAS, KRAS, DIS3, BRAF, FAM46C and TP53 genes. We found two cases of KRAS mutations p.Q61L and p.A146T with damaging prediction effect. Both MGUS cases showed positive IGH disruption (in 56% and 85% aPCs) analysed by I-FISH, but only the first case with p.Q61L mutation showed unbalanced chromosomal changes in genome-wide profile: hyperdiploidy (+3, +5, +9, +11, +15, +19), losses of chromosomes 8, 13 and Y, 7 segmental losses (1p34.2-p13.1, 6p23, 6q12-q27, 7q36.3, 12p12.1-p11.23, 12q12, 12q21.2-q23.3) and 4 segmental gains (6p25.3-p23, 6p23-p11.1, 6q11.1-q12, Xq21.33-q28). Interestingly, only this patient has progressed after 6 months to MM. Genome-wide profiling of the second patient with p.A146T mutation did not show any unbalanced chromosomal changes and the patient is still stable for more than 16 months. In our study, we showed that KRAS p.Q61L mutation combinated with chromosomal changes could

be potential markers of MGUS progression. Support: IGA NT13492,OPVK CZ.1.07/2.3.00/20.0183, MH CZ-DRO (FNBr, 65269705)

J12.078

Gene polymorphisms of microRNAs in Helicobacter pylori- induced high risk atrophic gastritis and gastric cancer

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Aims: The purpose of this study was to evaluate potential associations between miRNA-related gene polymorphisms (miR-27a, miR-146a, miR-196-a-2, miR-492 and miR-608) and the presence of gastric cancer (GC) or high risk atrophic gastritis (HRAG) in European population.
Methods: Gene polymorphisms were analysed in 995 subjects (controls: n = 351; GC: n = 363; HRAG: n = 281) of European descent. MiR-27a T.C (rs895819), miR-146a G.C (rs2910164), miR-196a-2 C.T (rs11614913), miR-492 G.C (rs2289030) and miR-608 C.G (rs4919510) single nucleotide polymorphisms (SNPs) were genotyped by RT-PCR.
Results: SNPs of miRNAs were not associated with the presence of GC or HRAG. We observed a tendency for miR-196a-2 CT genotype to be associated with higher risk of GC when compared to CC genotype, however, the difference did not reach the adjusted P -value (odds ratio (OR) - 1.46, P=0.032). MiR-608GG genotype was more frequent in GC when compared to controls (OR 2 2.34), but significance remained marginal (P=0.029). A similar tendency was observed in a recessive model for miR-608, where CC + CG vs GG genotype comparison showed a tendency for increased risk of GC with OR of 2.44 (P=0.021). The genotypes and alleles of miR-27a, miR-146a, miR-196a-2, miR-492and miR-608SNPs had similar distribution between histological subtypes of GC and were not linked with the presence of diffuse or intestinal-type GC.
Conclusions: Gene polymorphisms of miR-27a, miR-146a , miR-196a-2, miR-492, miR-492a and miR-608were not associated with the presence of HRAG, GC or different histological subtypes of GC in European subjects.

J12.079

Association of a SNP in miR-576-5p binding site of NHLH2 gene with neuroectodermal tumours in paediatrics

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Backround: Neuroectodermal tumours represent a large group of tumours of central as well as peripheral nervous system that arise from the cells of neuroectoderm. NHLH2 (nescient helix-loop-helix 2, also known as HEN2 or NSCL2) is a member of basic helix-loop-helix (bHLH) transcription factor family, expressed in neuroectoderm and also involved in tumorogenesis. Hereto, we focus on rs12405792, a single nucleotide polymorphism in the miR-576-5p biding site of the NHLH2 gene.The aim of the study was to investigate the rs12405792 polymorphism in paediatric patients with neuroectodermal tumors and other malignancies.
Material and Methods: The study involved the total of 40 of paediatric patients with neuroectodermal tumors (neuroblastoma, ganglioneuroblastoma, medulloblastoma, pineoblastoma, retinoblastoma, astrocytoma, ganglioneuroma, ependymoma, paraganglioma, glioblastoma, melanoma, dysembryoplastic neuroepithelial tumors), the total of 108 patients with non-ectodermal tumorsand 20 healthy controls.
Results: There were significant differences in genotypes frequencies between the investigated cohorts; the genotype distributions were different in neuroectodermal tumors compared to other groups (p = 0.03), the relative frequency of GG genotype (55 %) being increased in neuroectodermal tumors compared to healthy controls and non-neuroectodermal malignancies.
Discussion: In this study, we observed significant differences in genotype frequencies of rs12405792 between the neuroectodermal and non-neuroectodermal tumors and healthy controls. The functional effect of this SNP in miR-576-5p binding region to NHLH2 expression has to be further elucidated in order to explain possible effects of the SNP in neuroectodermal tumors.

J12.080

Alterations of miRNA expressions in plasma of AML patients at the time of diagnosis

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We studied miRNA expression profiles in patients with acute myeloid leukaemia at diagnosis and in the first remission and we compared them with profiles found in healthy controls. Plasma samples were collected from 8 patients at the first diagnosis of AML and at the first complete remission and from 10 healthy volunteers. Isolated miRNA were transcribed to cDNA and applied on TaqMan Human MicroRNA Array A to find the expression of 381 genes. Relative quantification and statistical analyses were performed with Expression Suite v1.0.3 and with qbase+ v2.4. Relative quantification was calculated using the reference gene miR-16 or the global mean. Both values of miRNA expressions were compared between the patients and controls using Mann-Whitney tests with multiple testing corrections. The differences of various statistical significance were found. Among them there were 12 miRNAs (miR-125a-5p, miR-145, miR-221, miR-652, miR-744, miR-146a, miR-181a, miR-191, miR- 27a, miR-340, miR-134, let-7d) giving the p values < 0.01 with both applied normaliazation methods and another 4 miRNAs (miR-199a-3p, miR-331-3p, miR-324-5p, miR-28-3p) giving p values < 0.01 using reference gene miR-16 and p value between 0.01 - 0.02 using global mean. All these miRNAs were overexpressed in patients at the time of diagnosis only, but not at the first complete remission. With regards to miRNA expression profiles of healthy subjects, we compared the profiles in the morning and the afternoon samples of healthy volunteers and we detected no significant differences refering to circadian rhytmicity. Supported by the Ministry of Health of the Czech Republic, RVO VFN64165.

J12.081

Investigation of DNA mutations in Mitochondrial D-Loop region at thyroid nodules in Turkish Population-Preliminary findings

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Mitochondrial defects have been associated with various human conditions including cancers. However mitochondrial DNA (mtDNA) alterations within the highly variable D-loop control region have been reported as a frequent event in cervical cancer, breast cancer, gastric carcinoma, colorectal cancer, hepatocellular cancer, lung cancer and renal cell carcinoma. Specifically, it was suggested that the D310 region is more susceptible to oxidative damage and electrophilic attack.
Aim of this study to investigate if the accumulation of mtDNA mutations plays a role in tumorigenesis in thyroid nodules in Turkish population. Clinically and pathologically examined 178 tissue samples belonging to the 77 patients; including 58 surrounding healthy tissues, 51 hot thyroid nodules (HTN) and 69 cold thyroid nodules (CTN), were enrolled to the study. DNA extraction from tissue samples was done by using phenol-chloroform methodology. D-Loop region between 15-484. nt and 15971-16411. nt were amplified by PCR. DNA sequencing reaction of D-Loop region between 15-484. nt was performed on Bekcmann Coulter Genomelab Automated DNA Sequencer.
The same polymorphisms were detected in both healthy and nodular tissue samples belonging to the same patients. But two different polymorphisms (A73G; T89G) were detected as heteroplasmically in HTN tissue of 31th patient different from healthy tissue. Beside this in HTN tissue of 33th and CTN tissues of 13th patient D310 polymorphisms were also detected as heteroplasmically different from healthy tissues.
As a result, the obtained data indicates that mtDNA alterations in the D-loop region could happen before tumorigenesis in thyroid, and they might also accumulate during tumorigenesis.

J12.082

The occurrence of *EZH2* mutations in patients with *BCR-ABL*-negative myeloproliferative neoplasms

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Recent studies have revealed a number of epigenetic alterations that contribute to myeloproliferative neoplasms (MPNs) pathogenesis and determine the clinical outcome. According to the published data, mutations involving *EZH2*, which encodes a histone methyltransferase, are founded in 6% cases of primary myelofibrosis (PMF), 1% of polycythemia vera and 1-3% of essential thrombocythemia regardless of the presence of mutations in *JAK2* or *MPL*. *EZH2* mutations may be of prognostic value in MPN's at the time of

transformation to the blastic phase. The goal of our research was to determine the frequency of mutations in *EZH2* in two groups of patients with different chromosomal aberrations. We examined 47 patients with *BCR-ABL*-negative MPNs. The first group included 20 patients with normal karyotype, and 16 patients with the isolated chromosomal aberrations del(13)(q22), del(20)(q12),-Y associated with favorable prognosis, and add(22)(q13), del(1)(p32), del(6)(q15), t(10;12)(q22;p13) that are referred to as intermediate risk. The second group included 11 patients with complex abnormalities and the chromosomal aberrations of unfavorable prognosis +8, +7, inv(7)(p11;q21). Mutations in 8,10,17,18,19 exons of *EZH2* were defined by sequence analysis. The Ile713Thr mutation in *EZH2* gene was detected in 2.1% (1/47 cases). The patient with mutation had a del(6)(q15) karyotype which is associated with an intermediate risk, and he subsequently underwent transformation from PMF to myelodisplastic syndrome in 9 months after the disease onset. Conclusions: mutations in the *EZH2* gene could be preliminarily assessed as additional prognostic markers of unfavorable prognosis in patients with *BCR-ABL*-negative MPNs and with different chromosomal aberrations. Further studies are needed.

J12.083

Presence of RET Proto-oncogene mutations and polymorphisms in Iranian families with multiple endocrine neoplasia type 2A

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Introduction Multiple Endocrine Neoplasia type 2A (MEN 2A) is a complex autosomal dominant inherited syndrome characterized by medullary thyroid carcinoma (MTC), pheochromocytoma and primary parathyroid hyperplasia. In patients with only one or two clinical features, identification of a germ line RET (**RE**arranged in **T**ransfection) mutation on chromosome 10 or the identification of the clinical features of MEN 2A in other first degree relatives is required to make the diagnosis. **Methods** We analyzed blood DNA from 4 Iranian families with three generations of MEN2A including 22 affected persons with MTC and 10 with pheochromocytoma. RET hotspots were amplified in probands and sequenced for mutation detection. RFLP test was used for identification of mutations in family members. For fidelity analyzing of used RFLP test, all members sequenced. **Result** The causative mutation in all families was found to be Cys634Tyr missense substitution. The simultaneous presence of a functional SNP resulting in Gly691Ser was also detected in exon 11 of all the affected cases. Two known silent substitutions and one new silent mutation was also detected in exons 10 and 13. RsaI restriction endonuclease can detect all carriers of Cys632Tyr mutations. **Conclusion** Simultaneous presence of Cys634Tyr mutation in cysteine rich region and Gly691Ser polymorphism in trans-membrane domain of RET tyrosine kinase have been detected in all patients. This study shows that Cys634Tyr mutation is recurrent in Iranian patient with MEN2A and suggest the RFLP method for detection of this mutation. All asymptomatic carriers of mentioned high risk activating mutation were recommended for prophylactic thyroidectomy.

J12.084

Coordination of expression of cell cycle related genes in Multiple Myeloma and Plasma Cell Leukemia

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In multiple myeloma (MM), malignant cells retain the self-renewing potential. Majority of myeloma cells stay in the G1 phase, however after leukemic transformation in plasma cell leukemia (PCL) myeloma cells become highly proliferative. We anticipate that complex "re-setting" of cell cycle gene expression coordination during leukemic transformation creates required background for proliferation restoration. The aim of this study was to define and describe complex "re-setting" of cell cycle gene expression coordination in MM and PCL. Gene expression profiling was performed in 7 healthy donors, 6 MM and 7 PCL patients utilizing Affymetrix GeneChip Human Exon 1.0 ST Arrays. Genesets, connected with cell cycle regulation (GO:0045786; GO:0045787) and regulation of apoptotic process (GO:0043065; GO:0043066) together with all descended direct connected genesets were taken for the GSEA analysis and Gene Set Differential Coordination Analysis. Comparison of PCL, MM and healthy donors revealed coordinating expression changes between regulation of mitosis, apoptosis and cell cycle arrest for both positive and negative regulation genes. In MM, co-expression changes were associated with early phase of cell cycle, whereas in PCL with both - early and late phase of cell cycle. We anticipate that expression of cell cycle positive regulators is in dynamic equilibrium with cell cycle negative regulators. We suppose that this equilibrium serves as a compensatory mechanism to oncogenic events. Despite compensation mechanisms activation, whole regulatory complex seems to be imbalanced by growing "oncogenic stress" during MM to PCL progression. Supported by grants NT12130, NT13190 and OPVK CZ.1.07/2.3.00/20.0183.

J12.085

MYCN oncogene amplification in advanced neuroblastoma patients from Republic of Macedonia

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Neuroblastoma is characterized by striking clinical heterogeneity, including subsets that show spontaneous tumor regression. Several acquired genetic alterations such as amplification of the MYCN oncogene, deletions of chromosome bands 1p36 and 11q23 and unbalanced gains of 17q regions have been well-characterized and shown to be correlated with tumor behavior, including response to treatment. Amplification of the MYCN oncogene identifies a group of patients who have a poor prognosis. The aim of our study was to evaluate the prognostic role of MYCN oncogene amplification in our cohort of children with neuroblastoma. Ten children (age 9 months to 4 years) with previously untreated neuroblastoma, were evaluated at diagnosis for MYCN gene amplification. Fresh surgical specimens were obtained during excision of the tumor or during bone marrow fine needle aspiration and analyzed using MLPA P252 kit. MYCN amplification was observed in 8 patients (4 with stage IV and 4 with stage III disease), and it was not present in the two patients with stage 2A disease. The disease was lethal in six patients with MYCN amplification with overall median survival of 10 months. The other two patients with MYCN amplification are alive, but with a recurrent disease. In conclusion, MLPA analysis is simple, fast and cost-effective method that enabled accurate detection of MYCN amplification in our patients with neuroblastoma. Our study also shows that MYCN amplication is frequent event in patients with an advanced neuroblastoma and confirms it as a poor prognostic factor

J12.086

Elucidation of genetic changes associated with a rare form of familial myelodysplastic syndrome

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BACKGROUND Myelodysplastic syndrome (MDS) is a clonal proliferative disorder of the bone marrow characterized by ineffective hematopoiesis, peripheral blood cytopenias, and increased risk of developing acute myeloid leukemia (AML). Identifying predisposing gene mutations is critical for effective diagnosis, assessment of prognosis, and therapeutic counselling. A Caucasian family with an autosomal dominant inheritance pattern of early onset MDS was identified. No common exposures to potential carcinogens were present and clinical genetic testing indicated that regions 5q31 and 7q22, frequently associated with sporadic MDS, were not causal, suggesting an inherited predisposition. **METHODS/RESULTS** In-depth genetic analysis was undertaken. Six samples were acquired, including four clinically affected males across 2 generations (age of onset 25-69; one RAEB MDS, two unclassified MDS, one unknown MDS), a suspected unaffected female family member (aged 40), and an unaffected spouse (aged 72). Single-nucleotide polymorphism genotyping was performed on the suspected unaffected and three affected individuals. Data was analyzed using a haplotype mapping technique for autosomal dominant disorders. Using a cut-off of 3cM, affected individuals were found to share 89 genomic regions. Whole-exome sequencing on two affected individuals, combined with the mapping data, revealed 1904 shared variants in those regions. **FUTURE DIRECTIONS** Whole-exome sequencing on a third affected individual is underway to reduce the list of potential causative variants. After analysis, variants will be prioritized based on the genes known function and mutation severity. Top candidate genes will be sequenced in all available family members to determine which variant(s) track with the disease.

J12.087

NAT2 acetylation polymorphisms in bladder cancer risk

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Bladder cancer is known to be modulated by environmental carcinogens. Polymorphisms in the genes coding for the enzymes involved in the metabolism of carcinogens are considered as susceptibility factors for bladder cancer. N-acetyltransferase 2 (NAT2) is a Phase II enzyme of the xenobiotic metabolism pathway, which is involved in detoxification of arylamines and heterocyclic amines. Therefore, polymorphisms of the NAT2 gene and its related acetylation status may confer a risk factor for the development and progression of bladder cancer. In this study, we have investigated NAT2 481C>T, 590G>A and 857G>A polymorphisms, corresponding to NAT2*11A, NAT2*6B and NAT2*7A alleles, in terms of bladder cancer risk. For this purpose, genomic DNA samples of 129 bladder cancer and 148 healthy subjects were genotyped by PCR-RFLP upon their informed consent. We have shown that NAT2*11A allele have a trend for protection against bladder cancer (p=0.059; OR=0,761), and significance of protection increases (p=0,005; OR=0,539) when non-smokers are excluded. We also categorized the individuals according to their acetylation status as slow, intermediate and fast acetylators, however, we could not find a statistically significant asscociation between bladder cancer and acetylation status.

J12.088

Evaluation of genotoxic risk of Tunisian hospital workers exposed to low levels of ionizing radiation (IR)

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Over the years, (IR) has become a universal diagnostic and therapeutic tool, making the largest man-made contribution to the population dose. Thus, medical personnel represent the group most consistently exposed to low doses of IR.
Many cytogenetic studies have been conducted among hospital workers exposed to IR.
The cytokinesis-blocked micronucleus (CBMN) assay is widely used, since it represents a reliable test to assess radiation-induced chromosme damage and it's a valuable biomarker in many biomonitoring studies on human populations exposed to IR.
The aim of our study is to assess chromosomal damage in Tunisian hospital workers occupationnally exposed to low levels of IR.
The CBMN in peripheral lymphocytes of 67 exposed workers compared to 43 controls.
The clastogenic/aneugenic effect of IR was evaluated using the CBMN assay in combination with flurescence in situ hybridization (FISH) with pan-centromeric DNA in all the exposed subjects and controls.
The centromere analysis performed in our study showed that MNs in hospital staff were predominantly centromere negative and the mean negative labeled micronucleus (C-MN) frequency was significantly higher in the exposed subjects than in the controls (9.04±4.57‰ vs. 1.17± 0.77‰). The multivariate regression analysis showed that only the time of exposure ti IR had a significant effect on the level of MNs and C-MN.
The results of the study confirm the well-known clastogenic properties of IR.

J12.089

The detection of mutations in gene PTEN in patients with ovarian cancer from Bashkortostan Republic of Russia

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PTEN (phosphatase and tensin homolog) localized on chromosome 10q23.3 is tumor suppressor, which mutations cause both hereditary and sporadic forms of malignancies, including ovarian cancer (OC). The first germinal mutations in this gene have been identified in the study of hereditary syndromes: Cowden, Bannayan-Riley-Ruvalcaba and Lhermitte-Duclos. PTEN is negative regulator of the PI3K/AKT/mTOR pathway by dephosphorylating phosphatidylinositol 3,4,5-triphosphate to phosphatidylinositol -4,5-bisphosphate thus counteracting PI3K function. The operation of PTEN is necessary to control apoptosis, migration and proliferation of cells in the organism.
We carried out an analysis of structural changes in the PTEN in 250 patients with ovarian cancer from different ethnic groups of the Republic of Bashkortostan. The detection of mutations was performed by high resolution melting curve analysis (HRM) and confirmed by direct sequencing. The study of nucleotide sequence of the PTEN in patients with OC were detected changes: c.904A>C (Ser302Arg), c.217G>A (Glu73Lys), previously described polymorphism c.132T>C (Gly44Gly), and changes in introns: c.209+9G>C, c.209+10T>C.
Using the program Polyphen-2, we have found that the change c.904A>C, located in exon 8 of PTEN, is not pathogenic, and therefore does not contribute to the development of ovarian cancer. Mutation c.217G>A (Glu73Lys), located in exon 4 of PTEN, is found in phosphatase domain of protein PTEN that may possibly affect the impaired function of the protein and subsequently lead to tumor formation.
This study was supported by RFBR-Povolzhye 14-04-97088

J12.090

microRNA expression in circulating samples of pancreatic ductal adenocarcinoma patients

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Pancreatic ductal adenocarcinoma (PDAC) is a rare disease, whose incidence rates are very close to mortality rates, due to its difficult and late diagnosis. Noninvasive diagnostic biomarkers emerges as a possibility to perform a safe diagnosis, preferably early in the disease course. Recently, microRNAs (involved in the initiation and maintenance of tumors, and able to discriminate the presence or absence of certain diseases) were discovered as stable molecules in circulating samples. We evaluated the expression levels of six miRNAs (miR-21, -34a, -155, -196a, -200b and -376a) in serum (24 PDAC and 9 healthy patients) and saliva (10 PDAC and 10 healthy patients) samples. The aim of our study was to investigate the presence of diagnostic and/or prognostic biomarkers. Expression levels were measured by relative quantification using qRT-PCR. In serum, miR-21 and miR-34a were significantly more expressed in samples from patients with PDAC (P<0.001 and P=0.001) and both were able to distinguish patients with and without disease with clinically acceptable sensitivity and specificity (AUC for miR-21 and miR-34a of 0.894 and 0.865). We also found a strong and positive correlation between the expression levels of these two miRNAs in serum (r_s=0.681; P<0.001; Kappa-tests: 0.477; P=0.022). However, the mechanism related to this correlation remains unknown. In salivary samples, generally, miRNA levels were very low. No difference in miRNA expression levels was seen between cases and controls. These findings suggest that the chosen miRNAs are not adequate as diagnostic biomarkers in saliva, although these findings require confirmation in a larger series of cases.

J12.091

Association of Platelet Derived Growth Factor-B (PDGF-B) and Human Epidermal Growth Factor Receptor -2 (HER-2/neu) Single Nucleotide Polymorphisms (SNP's) with Gallbladder Cancer

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Purpose: Gall bladder cancer (GBC), a highly malignant gastrointestinal tumour, is very common in north India. Platelet derived growth factor-B (PDGF-B) at chromosome 22q12.3-q13.1 and Her-2/neu at 17q12-q21 play an important role in tumour angiogenesis. PDGF-B and Her-2/neu overexpression has been found in many cancers. We studied PDGF-B and Her-2/neu single nucleotide polymorphisms (SNPs) in GBC and gall stone associated benign diseases viz. chronic cholecystitis (CC) and xantho-granulomatous cholecystitis (XGC).
Methods: DNA was extracted from blood in patients with GBC (n=195), CC (n=140), XGC (n=47) and normal controls (n=300). PDGF-B polymorphisms were investigated using ARMS PCR for +286A>G and +1135A>C, and for Her-2/Neu Ile^{655}Val by PCR-RFLP method.
Results: +286A>G polymorphism homozygous GG genotype, and +286G allele was found to be risk associated for GBC (OR=5.25 P=<0.0001 and OR=2.02 P=<0.0001). Recessive model (GG vs. AA+GA) of +286A>G polymorphism was risk associated (OR=4.78, P=<0.0001) whereas dominant model (AA vs. GG+GA) was risk protective (OR=0.56, P=0.003) with GBC. +1135A>C polymorphism CC genotype and +1135C allele was risk associated (OR=3.19, P=<0.0001 and OR=1.81, P=<0.0001) with GBC. Recessive model (CC vs. AA+AC) was risk associated (OR=2.75, P=0.0003) whereas dominant model (AA vs. CC+AC) showed protective association (OR=0.56, P=0.0024) with GBC. In HER-2 Ile^{655}Val polymorphism, dominant model and Val allele of this polymorphism was risk protective for XGC. In haplotype

analysis, haplotypes ACIle (OR= 1.48), GAVal (OR=1.70) and GAIle (OR=2.00) were risk associated with GBC.
Conclusion: PDGF-B polymorphisms may play a role in carcinogenesis of GBC and need further evaluation.

J12.092
'3-Phosphoglycerate Dehydrogenase Polymorphism in male patients with thyroid gland cancer

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Introduction: Thyroid gland cancer (TC) is considered a rare malignancy, accounting up to 2% in males. Recent studies suggest important role of both environmental and genetic factors in cancerogenesis. 3-Phosphoglycerate dehydrogenase (3-PHGDH) gene overexpression is assosiated with patogenesis of human cancer and contributes to cell proliferation.
Aim: The objective of our study was to assess the association of PHGDH gene polimorphism in group of males with thyroid gland cancer and control group of healthy men.
Methods: The survey was carried out in the Department of Human Genetics-Medical School, University of Belgrade. The study has encompassed 80 man diagnosed with thyroid gland cancer in Center for Endocrine Surgery, Clinical Center of Serbia Serbia and 100 health males volonteers. The DNA was isolated from the periferal blood with solting out method. The genotypes 3-PHGDH polimorphism were determined by Polimerase Chain Reaction and Restriction Fragment Lenght Polimorphism. Gel-electrophoresis was used to separate DNA fragments.
Results: There was a significant difference in frequency of TT, CT and CC between
experimental and control groups of rs541503 polymorfism (Hi2= 38.924; p=0.001). There were
significantly more TT in experimental group, while in control group there were more CT (Hi2=33.186; p=0.001) and CC (Hi2=21.734; p=0.001). Conclusion: In our study we found TT genopype as the most frequent in patients with thyroid gland cancer. The results of our study also suggest that C allele might be factor of risk associated with thyroid gland cancer. It is necessary to undergo further testing with more adequate test groups.

J12.093
Incidence of PIK3CA mutations in breast cancer correlated with histopathological characteristics of the tumor

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Gain-of-function mutations in PIK3CA have been found in breast cancer, but prognostic value of PIK3CA mutation status is controversial. The goal of the study is the analysis of PIK3CA hotspot mutations and their correlation with clinicopathological parameters. Paraffin-embedded tissue sections were obtained from biopsy specimens of 95 breast cancer patients characterized histologically and immunohistochemically including histological grade, regional lymph node status (pN), estrogen receptor, progesterone receptor and HER2 status. We investigated exon 9 and exon 20 of PIK3CA gene in 95 breast tumor samples by DNA sequencing. We observed mutations in 27.3% (26/95), all with exception of 2 cases were mutually exclusive. Mutations in exon 9 represent 16.8% (16/95); mutations in exon 20 represent 12.6% (12/95). We observed associations between mutations and the histopathological characteristics of the disease. In exon 9, we detected E542K (5/16), E545K (9/16), Q546K (2/16) missense changes. These mutations showed significant correlation with lower grade (p=0.0074) and pN status without metastases (p=0.0415). Mutations in exon 20 H1047R, H1047Y and G1049R were associated with higher age of patients (p=0.0249). The E545K mutation correlated with lower grade (p=0.0013) and with pN status (p=0.0232) particularly; the H1047R mutation was significantly more frequent in lobular type of breast cancer (p=0.0354). We have shown that the mutations in exon 9 of PIK3CA were associated with favorable prognostic factors. The PI3K signaling pathway plays a critical oncogenic role in the development of human breast cancer and the prevalence of its dysregulation advocates its potential as a feasible therapeutic target.

J12.094
Molecular subtyping of three aggressive PCa patients from Bulgaria in correlation with clinic-histological data

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Prostate cancer (PCa) is among the most prevalent neoplasms worldwide, whereas metastatic PCa is one of the leading causes of death in men. Here we report three Bulgarian patients with strongly aggressive, hormone-refractory PCa. The application of the following molecular markers: DD3 overexpression, GSTP1 promoter hypermethylation, TMPRSS2-ERG gene fusions and mutations in androgen receptor (AR) gene for diagnostic purposes was assessed. We attempt to correlate the molecular data to both histological and clinical data. The obtained molecular profile in the three Bulgarian patients coincides with the clinical and histological data of aggressive, hormone-independent PCa. There was no association between the tumor stage (assessed by TNM as T2) and the detected molecular profile of aggressive cancer behaviour. None of our cases was with positive family history and no somatic mutations were detected in the AR gene. The rest of the markers: DD3 overexpression, GSTP1 gene promoter hypermethylation and TMRSS2-ERG fusion were positive in fresh prostatic tissues and biopsies from all three patients, whereas only one blood sample showed triple positive result. The appearance of PCa specific molecular markers in blood was considered as a predictor for a pronounced migration and dissemination of prostatic tumor cells in circulation. The GSTP1 promoter hypermethylation is the earliest epigenetic fluctuation, which indicates cancerous changes in the gland the first and long-lasting marker, in the blood circulation. The molecular profile during cancer treatment could be used to predict the individual response
Acknowledgements: the study was supported by the grants №4-D/2011 and 26-D/2012, Medical University Sofia, Bulgaria.

J12.095
Analysis of the *PCA3*, *TMPRSS2-ERG*, *TERT* genes expression in biopsy samples and urine sediments as potential markers for diagnostics of prostate cancer

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Prostate-specific antigen (PSA) concentration in the blood can be increased in hyperplasia or adenoma, analysis of biopsies due to the heterogeneity of prostate cancer (PCa) requires highly skilled pathologist. Search of new molecular markers for noninvasive diagnostics of PCa is an actual oncourology problem. The aim of this study is analysis of *PCA3*, *TMPRSS2-ERG*, *TERT* expression in biopsies and urine sediments as potential markers for the diagnostics of PCa. We have analyzed 72 biopsies, which included 21 adenocarcinoma, 29 prostate intraepithelial neoplasia (PIN) and benign prostatic hyperplasia (BPH), 22 cases of inflammatory lesions; 10 urine sediments obtained after prostatic massage. Genes expression was analyzed by real-time PCR with endogenous *GAPDH* and tissue-specific *KLK3* controls. There was a correlation between the grade in normal/low- /high PIN/adenocarcinoma and expression of *PCA3* (r = 0,691). The threshold ΔCt(PCA3-KLK3) 3,9 provided optimal sensitivity and specificity in biopsy: 86% and 96%, respectively. *TMPRSS2-ERG* expression was detected in 48% of adenocarcinomas and 11% of high PIN, *TERT* - 71% and 22%, respectively. Combination of *PCA3*, *TMPRSS2-ERG*, *TERT* has detected PCa in biopsies with 95% sensitivity and 97% specificity. Analysis of 10 urine sediments has classified correctly 9 cases. The high PIN is a precancerous stage, more than 50% of it proceeds in adenocarcinoma. This fact explains the difficulties in diagnosis of high PIN versus PCa using markers of carcinogenesis. Thus, analysis of *PCA3*, *TMPRSS2-ERG*, *TERT* expression could be useful for PCa detection in the biopsies and urinary sediments of patients with elevated PSA.

J12.096
Vitamin D receptor gene BsmI, FokI, ApaI and TaqI polymorphisms and the risk of prostate cancer among men of Russian, Tatar and Bashkir ethnic origin.

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Prostate cancer (PCa) is one of common tumors among men in Russia. Low levels of vitamin D are implicated as a potential risk factor for PCa development, and vitamin D receptor (VDR) gene may be important in the on-

set and disease progression. In this study, SNP variants rs2228570 (FokI), rs1544410 (BsmI), rs7975232 (ApaI), rs731236 (TaqI) in the VDR gene were investigated in PCa patients and controls of Russian (N=122 and N=95, respectively), Tatar (N=70 and N=170), and Bashkir ethnic origin (N=42 and N=82) to determine whether they are associated with PC risk. We evaluated the association between these SNPs in the VDR gene and PCa risk as well as clinical characteristics (prostate-specific antigen level, clinical stage, pathological stage) in men (237 PCa patients who underwent a prostatectomy and orchectomy) using logistic regression.

We did not observe significant differences for either the VDR BsmI, ApaI and TaqI genotype and allele frequencies in PCa patients and healthy individuals. However, the frequency of the VDR *T/*T genotype of rs2228570 (FokI) was statistically different between PCa patients and controls of Tatar ethnic origin (OR = 1.88, 95%CI=1.22-2.58, p=0.0023). There wasn't association of the studied VDR BsmI, ApaI and TaqI polymorphisms with clinical characteristics of PCa patients. We found linkage disequilibrium between the BsmI *A and ApaI *C alleles (D'=84%). Our study indicates that VDR FokI variant might increase PCa risk in Tatars. However, current limitation for small cohorts might have false positive effects; therefore it should be overcome via further large-scale validating studies.

J12.097

Expression of Zn^{2+} metabolism genes in prostate cancer

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During prostate carcinogenesis prostate tumor cells lose the ability to accumulate Zn^{2+} ions in high levels. The aim of this study was to investigate the expression of four genes ZIP1, ZIP7, MT1-F and MT2 involved in the maintenance of homeostasis of zinc cations in prostate cells in tumor tissue and in benign prostate hyperplasia (BPH) by RT-PCR and determine whether there is an correlation between the mRNA expression of four genes and the TNM classification, Gleason score, PSA level and age and thus evaluate its diagnostic and prognostic potential.

Isolation of mRNA from prostate cancer in 65 patients has been performed in period 2011 - 2013. As a control group, 27 patients with BPH were used. Statistically significant lower relative expression of MT1-F and ZIP1 genes was detected in prostate cancer tissue than in BPH ($p<0.00048$, $p<0.0082$, resp.). The PSA level correlated positively with the ZIP7 expression ($p<0.0099$). Decreased expression of all four genes correlated with higher age. No correlation of the gene expression with the TNM classification and Gleason score was observed.

According to our results, it is possible to consider that the genes MT1-F and ZIP1 may be candidate tumor suppressor genes for prostate cancer. **Supported by Diana Lucina, the Ministry of Health project for conceptual development of research organization 00064203** and by grant MSM 0021620808.

J12.098

Association between the RAD50 rs17166050 polymorphism and risk of childhood leukemia and adult cancers

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The RAD50 gene encode one of the protein of the MRE11-RAD50-NBN complex involved in cellular response to DNA damage and the maintenance of genome stability. The aim of this study was to answer the question whether the RAD50_rs171660505 polymorphism may be associated with childhood leukemia or adult cancers. We estimated the frequency of RAD50_rs171660505 polymorphism in the group of 220 children diagnosed with leukemias and in the 280 non-selected breast cancer (BC) patients, 175 with a single laryngeal cancer (LC) and 115 with multiple primary tumors but one malignancy (primary or second primary) localized in the larynx (MPT-LC) and 68 multiple primary tumors localized in the head or neck (MPT) and controls (n=504). The analysis was performed by multi-temperature single-strand conformation polymorphism technique. We performed two molecular tests to examine any potential function of the detected the c.551+19G>A SNP in RAD50 gene. The frequency of either the AA genotype or A allele of RAD50_rs17166050 were significantly different in controls compared to childhood leukemia group (ALL+AML) ($P<0.0019$ and $P<0.0019$, respectively). In MPT group, heterozygous genotype GA was significantly more frequent, even after multiple testing correction, than in controls ($P=0.0205$). The cDNA analysis of AA or GA genotypes carriers has not revealed evidence of splicing abnormality of RAD50 pre-mRNA. We measured the allelic-specific expression of G and A alleles at c.551+19G>A and the statistically significant overexpression of the G allele has been observed. This data demonstrate that some specific alternations of the RAD50 gene may be associated with childhood ALL.

J12.099

Research of association between the SNP309 of MDM2 gene and the occurence retinoblastoma in Algerian population

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Retinoblastoma is an intraocular malignant tumor that usually affects the child in the first months of life. The gene responsible for this disease is an anti concogene called RB1. Retinoblastoma is due to the biallelic inactivation of RB1 gene. The loss of this gene does not confer a growth advantage due to the activation of p53 pathway. Events that would block or attenuate this pathway seem necessary for the development of retinoblastoma as the proto-oncogene MDM2 that negatively regulates p53. Indeed, the close relationship between MDM2, P53 and RB1 in retinoblastoma tumorigenesis makes the SNP 309 T > G of MDM2 gene as a good candidate for the development of this cancer.

This study is based on the search for a possible association between the polymorphism 309 T>G MDM2 gene and the occurrence of retinoblastoma in the Algerian population. We performed a case control study including 73 patients and 100 controls. The 309 T > G polymorphism was determined by PCR-RFLP method.

Our results show that there is a significant increase of the G allele in cases compared to controls ($p = 0.0002$). Therefore, this allele seems to decrease susceptibility to retinoblastoma in our population.

J12.100

Atypical *RUNX1* rearrangements in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS)

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The *RUNX1* (*AML1*) gene is a key regulator of hematopoiesis which is frequently rearranged in acute leukemias and myeloid malignancies. More than 55 different translocations of *RUNX1* have been described. However, majority has been reported rather sporadically and as complex *RUNX1* rearrangements they remain undefined at the molecular level. A series of 21 adult patients with uncommon structural abnormality of 21q21-q22 detected by conventional cytogenetic/multicolor FISH methods and diagnosis of AML or MDS was collected. Three patients with no further available material were excluded from the study. All the others were tested for involvement of the *RUNX1* using FISH analysis with Vysis LSI TEL/AML1 or LSI AML1/ETO probe (Abbott). The split signal of *RUNX1* gene was confirmed in four patients with: 1) t(8;21;7)(q22;q22;p13); 2) t(3;21)(q12;q22); 3) der(21)ins(21;10)(q22;q?)t(14;21)(q11;q22); 4) der(12)t(12;22)(p11.2;?)t(12;21)(q13;?),der(21)t(5;21)(?;q21)t(5;12)(?;q13). Further FISH analyses have been focused on the reciprocal translocation t(3;21)(q11;q22) and have been performed with BAC probes located at 3q11-12. The breakpoint has been specified between clones RP11-138C11 and RP11-153N1. In conclusion, four until now unreported *RUNX1* alterations with the new potential fusion partners are described. One of them was specified into 293 kbp region at 3q12. Two protein and three RNA coding genes have been mapped at this interval. The description and identification of atypical *RUNX1* fusions on the larger cohort of patients is an important tool for understanding and characterization of the pathogenic mechanisms of *RUNX1* rearrangements. Supported by MHCR for conceptual development of research organization 00023736, RVO-VFN64165/2012, GACR-P302/12/G157, RVOUK-/27/LF1/1.

J12.101

SALL4 as a new biomarker for early colorectal cancers

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Colorectal cancer (CRC) is one of the most commonly diagnosed cancers in the world. SALL4, newly identified oncogene, is a member of family of zinc finger transcription factors. Our aim in this study was evaluation of SALL4 mRNA absolute copy number in the peripheral blood of CRC patients, to in-

troduce a probable new prognostic or diagnostic molecular marker for CRC. Method: Peripheral mononuclear cells from 51 blood samples of CRC patients and 60 healthy controls, as well as 21 serum samples from the same patients were examined using absolute quantitative real-time RT-PCR to evaluate the exact copy number of SALL4 mRNA. Results: The blood copy number of SALL4 in recruited CRC patients was significantly higher in comparison with the healthy controls (p = 0.0001). This high copy number was not only inversely associated with the depth of tumor invasion (p=0.045), but also was significantly correlated with the higher grade of tumor differentiation (p=0.029). Furthermore, the copy number of SALL4 was also found to be elevated in all serum samples of CRC patients where high copy number of SALL4 was significantly associated with the higher grade of tumor differentiation (p = 0.026). Discussion: Our results emphasize the potential of SALL4 as a biomarker for detection of early stages (I/II) of CRC which are not invaded to the adventitia. Since early detection of CRC is correlated to improved outcomes, SALL4 may be introduced as a critical biomarker for efficient screening of patients, who are in early stages of CRC tumorigenesis.

J12.102

CDH1 intronic mutation c.2440-6C>G misclassification: not confirming earlier described splicing aberration.

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E-cadherin, encoded by the gene named CDH1, is a protein important for cell-to-cell adhesions. Germinal mutations in the gene trigger hereditary diffuse gastric cancer (HDGC). In this work, we have investigated c.2440-6C>G transversion found in our patient with a family history of gastric carcinomas. The mutation that is located in acceptor splice site of the last exon was previously described as pathogenic, affecting splicing of the mutated intron (1). However, the identity of aberrant splicing variant (detected by RT-PCR from patient's blood) and even the segregation of mutation in family was not so obvious. Recently, other research group found no aberrant mRNA in their patient carrying the same mutation (2). In our patient, we did not detect any splicing aberration in the blood-derived mRNA, nor we did see any difference in splicing when using our hybrid minigene system specialized for the analysis of the last introns/exons splicing. Furthermore, the mutation does not clearly co-segregate with the disease in our patient's family, being detected in the unaffected father's side of family whereas the mother's affected side could not have been examined. In conclusion, we propose that this mutation should be regarded rather as benign or with uncertain effect on gene expression and HDGC development. Nonetheless, further investigations of mRNA from the affected tissue might be helpful.

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J12.103

Correlation between amplification and expression status of ERBB1, MYC, Her2/neu, and TOP2A oncogenes in Iranian women with sporadic breast cancer and their relationship with Clinical and Immunohistochemistry prarameters

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ERBB1, *MYC*, *Her2/neu*, and *Top2A* oncogenes are known to be involved in the development, prognosis and response to the therapy in breast cancer. We extracted DNA and RNA of 81 primary sporadic breast cancer tumor tissues and 30 normal breast tissues. IHC, MLPA, RT-PCR and FISH techniques were used. The *Her2/neu* gene showed the most significant correlation between amplification and overexpression in 23 patients. The concurrent increase in amplification and overexpression for *Top2A* gene was statistically significant. *ERBB1* and *MYC,* showed no correlation between their amplification and overexpression. Significant concordance between co-amplification of *Her2/neu* and *Top2A* genes was detected. The amplification of *Her2/neu* gene was associated with negative progesterone receptor state, higher grade of the tumor, and increase in MVD; the overexpression of the gene was concordant with negative hormone receptor state and higher grade and stage of the tumor. The amplification of *MYC* was seen more in younger patients (<50yr) and was correlated with higher cell proliferation factor (KI-67). The amplification of *TOP2A* was detected more in stage 3 tumors. Co-amplification of *MYC-HER2* genes and co-overexpression of *TOP2A-HER2* genes were detected more in higher grade and stage tumors; co-overexpression of *TOP2A-HER2* genes were related to higher MVD. The rate of co-amplification of *MYC-ERBB1* genes was higher in tumors greater than 5 cm. In conclusion, besides *HER2* gene, the amplified *TOP2A* and *MYC* genes have crucial role in predicting the prognosis of the patients; the co-amplification and co-overexpression of these genes with *HER2* gene have significant prognostic roles.

J12.104

Evaluation of HER2 status in sporadic breast cancer amongst Iranian women using IHC, FISH, MLPA, and Real time RT-PCR techniques

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Objectives: Human epidermal growth factor receptor (HER) status is an important prognostic factor in breast cancer. There is no globally accepted method for determining HER2 status, and which method is most precise is still a matter of debate.
Methods: A total of 93 Iranian women with sporadic invasive breast cancer were studied. We analyzed *HER2* mRNA expression by quantitative reverse transcription-PCR (qRT-PCR) and *HER2* DNA amplification using multiplex ligation-dependent probe amplification (MLPA). To assess the accuracy of the RT-PCR and MLPA techniques, a combination of IHC and FISH was used, substituting FISH when the results of IHC were ambiguous (2+) and for those IHC results that disagreed with MLPA and qRT-PCR and named it IHC-FISH.
Results: The correlations between IHC-FISH and qRT-PCR or MLPA were 0.945 and 0.973, respectively. The ASCO/CAP guideline IHC/FISH correlation with MLPA was (0.827) and with RT-PCR was (0.854).
The correlations between the IHC results (0, 1+ as negative, and 3+ as positive) and qRT-PCR and MLPA techniques were 0.743 and 0.831, respectively.
Conclusion: Given the shortcomings of IHC analysis and greater correlations between MLPA, qRT-PCR, and FISH methods than IHC analysis alone with each of these three methods, we propose that MLPA and real-time PCR are good alternatives to IHC. However a suitable cut-off point for qRT-PCR is a prerequisite for determining the exact status of HER2.

J12.105

Clinical Utility of Measuring Expression Levels of Stanniocalcin 2 in Patients with Colorectal Cancer

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Stanniocalcins (STC) are glycoprotein hormones which were originally found in the endocrine gland of bony fish. Recently, Microarray expression data revealed that the expression level of Stanniocalcin was higher in tumors. Regarding the need for novel prognostic biomarkers, in place of the existing routine histopathological methods in early screening and the choice of therapy options in Colorectal Cancer (CRC), we examined Stanniocalcin 2 expression levels in patients with CRC and assessed their association with clinicopathogical data. We examined the mRNA expression levels of Stanniocalcin 2 in CRC in 48 tumor tissues and 48 marginal tissue samples using real-time reverse transcription-polymerase chain reaction (RT PCR). Clinicopathological data of patients were collected after fulfilling criteria and the pathological evaluation of their tissue samples. STC2 mRNA expression levels were higher in tumor tissues than the control marginal groups. (r=0.36, p=0.02). The median expression level in tumor groups was 31829 and the minimum and maximum levels were 2366 and 25183989, respectively, with the median expression level in marginal groups, 7303.5 and the minimum and maximum levels, 681 and 1296806, respectively. The mRNA expression level of STC2 was significantly associated with tumor size (p=0.04) and the histology of tumors (p=0.03). Taking into account the results of this study, high levels of STC2 expression were seen to be associated with larger tumour sizes with poor histological characteristic. We also showed that STC2

can be as a marker (indicator) to differentiate between tumor borders and margins, potentially improving accuracy during surgery.

J12.106

Characteristics of hematologic malignancies with coexistence of t(9;22) and inv(16)

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Background : The coexistence of the t(9;22)(q34;q11.2) and inv(16)(p13q22) is one that has been described in chronic myeloid leukemia (CML) mainly in myeloid blast phase (BP), de novo acute myeloid leukemia (AML) and a few therapy-related AML (t-AML). In this study, we evaluated 7 cases of hematologic malignancies with coexistence of t(9;22) and inv(16) and tried to characterize their laboratory and clinical findings.

Materials and Methods : We found 7cases with coexistence of t(9;22) and inv(16) in data of the Catholic Blood and Marrow Transplantation Center. We reviewed available data including clinical informations and peripheral blood smears, bone marrow (BM) aspirates, cytochemical stains, core biopsy specimens and chromosomal analyses. And we analyze the mutations of genes including IKZF1, NPM1, FLT3, N-RAS,K-RAS, c-KIT and TP53.

Results : 4 CML (1 chronic phase, 2 accelerated phase, 1 blast phase) and 3 AML (1 de novo AML, 2 AML) were identified. The percentage of circulating blasts and BM eosinophils were higher in AML than in CML (53% vs. 5%, 30% vs. 5.5%, respectively). The proportion of each chromosomal abnormality and follow-up karyotype were informative to identify which was a secondary change. The BCR-ABL1 a p210 fusion transcript was associated with CML and a p190 fusion was associated with AMLs. One patient with AML revealed two mutations; c-KIT D816V and TP53 E11Q.

Conclusion : Our experience demonstrated that BM morphology, initial and follow-up cytogenetics and type of BCR-ABL1 and CBFB-MYH11gave us valuable information to characterize CML and AML with coexistence of t(9;22) and inv(16).

J12.107

Extended RET proto-oncogene screening in medullary thyroid cancer

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Introduction: Medullary thyroid carcinoma (MTC) is accounts for 5-10% of all thyroid cancers. The molecular pathology of MTC is constitutive of *RET* proto-oncogene. *RET* mutations have an important role for diagnosis and confirming MTC. The most common mutation follow in the exons 10, 11,and 14-16. In this study some others exons examined in MTC patients. **Material and methods:** 298 participants were included, 196 patients (111 female, 85 male) and 112 relatives (58 female, 54 male). Genomic DNA was extracted from peripheral blood leucocytes and nucleotide change detection of exons 2, 3, 5, 8, 12, 17, 18 of the *RET* gene was performed by PCR and direct DNA sequencing methods. **Results:** A total of 10 different nucleotide substitutions were identified. There was not found any SNP in exons 5 and 8. One missense mutation (R982C) in exon18 and three synonymous mutations (A45A, V125V, G733G) were found in exons 2, 3, and 12 respectively. A novel C>T intronic variant (intron17, chr position 10:43620286) was also found. **Conclusion:** This study was focused on some *RET* gene exons that did not consider comprehensively before. It seems, molecular screening of the *RET* gene in MTC patients should not be limited to hotspot exons. Probable deleterious, protective, and/or additive effects of these SNPs with or without *RET* hotspot mutations in MTC development remained to be clarified.

ID	Consequence type	Base change	AA change	Allele frequency (%)	Phenotype (n)		
					sMTC	hMTC	relatives
rs1800858	Synonymous variant	A/G	A45A	23.15	32	12	29
rs1800859	Synonymous variant	C/A	V125V	0	1	1	-
COSM918120	Synonymous variant	C/T	G733G	0	1	-	-
rs17158558	Missense variant	C/T	R982C	4.34	8	5	7
rs2435351	Intron variant	G/A	-	33.42	40	17	45
rs2505530	Intron variant	G/T	-	41.05	50	20	52
rs2472739	Intron variant	A/G	-	25.16	52	23	48
rs72781242	Intron variant	A/G	-	66.66	80	26	91
-	Intron variant	C/T	-	0.85	2	-	2
rs2742236	Intron variant	G/A	-	29.86	56	24	69

J12.108

TP53 Arg72Pro and MDM2 SNP309 polymorphisms and colorectal cancer risk: a West Algerian population study

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The tumor suppressor gene *TP53* and its regulator *MDM2* are both key players involved in multiple pathways including apoptosis, cellular transcriptional control, and cell cycle regulation. Common germline polymorphisms in these genes may affect colorectal cancer susceptibility. An arginine-to-proline substitution at codon 72 in the p53 gene is reported to decrease apoptotic potential, while a thymine-to-guanine polymorphism at nucleotide 309 (named SNP309) of murine double minute 2 *MDM2* gene increases its transcription. These two polymorphisms therefore may be of importance in colorectal carcinogenesis. The relation of these polymorphisms to colorectal cancer in the Algerian population was addressed in this study.

DNA samples from 121 controls and 116 cases were genotyped for these two polymorphisms by PCR/RFLP then confirmed by sequencing.

Unexpectedly no significant association was found between this potential marker *TP53* Arg72Pro and CRC (p>0.05). However, our findings reveal that individuals with the *MDM2* SNP309 GG genotype have a low risk of CRC (OR=0.49; 95% CI, 0.24- 0.98, p=0.04) relative to the TT genotype and with more significance in females (OR= 0.16; 95% CI,0.06-0.41, p<0.05). Moreover, no significant association was observed between the combined *TP53* and *MDM2* genotypes and colorectal cancer.

Contrary to initial expectations that the GG genotype with high *MDM2* levels will increase cancer risk, our results demonstrate that the *MDM2* SNP309 GG genotype is associated with decreased risk of colorectal cancer. This is suggesting that other mechanisms independent of increased *MDM2* levels can influence cancer susceptibility.

J12.109

Acquired uniparental disomy (UPD) involving the short arm of chromosome 17 in patients with myelodysplastic syndromes (MDS) and complex karyotype

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Acquired uniparental disomy (UPD), which can be an important step in cancer development and progression, are found in various subtypes of haematological malignancies including myelodysplastic syndromes (MDS). The aim of the study was to evaluate the frequency of 17p UPD in bone marrow cells of patients with newly diagnosed MDS and complex karyotypes and to assess correlation of 17p UPD with mutations of tumour suppressor gene TP53 located at 17p13.1.

Bone marrow samples from 49 patients were analyzed by aCGH/SNP (BlueGnome) and UPD of 17p was found in 7 of them (14%). All cases had deletion of 5q and additional recurrent chromosomal aberrations: 7q deletion (4x), monosomy 7 (1x), or 12p deletion involving ETV6 gene (5x). Average extent of 17p UPD was 14-20 Mb involving TP53 gene. In all 7 cases with 17p UPD, two copies of TP53 gene were detected by FISH (Abbott). DNA isolated from bone marrow cells of 5/7 patients was NGS-sequenced (Roche) and in all of them homozygous TP53 mutations were found.

Our study confirmed that acquired UPD 17p is a recurrent cytogenetic defect in patients with MDS with complex karyotypes and is strongly associated with homozygous mutations of TP53 gene. This lesion is cryptic unless analyzed by SNP array. UPD might have a fundamental role in tumorigenesis, therefore further characterization of 17p UPD will lead to better understanding of the initiation and progression of MDS.

Supported by RVO-VFN64165, GACR P302/12/G157/1, PRVOUK-P27/LF1/1.

J12.110

Association between polymorphism of glutathione S-transferase genes (GSTM1) and uterine leiomyoma in Iranian population

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Introduction: Uterine leiomyoma is one of the most common benign smooth muscle tumors, occurring in 20-40% of women in their reproductive years. Although, the initiator/initiators of uterine leiomyoma are unknown, several predisposing factors such as age, nulliparity, obesity and genetic factors have been identified to be contributed to the pathogenesis of the disease. In regards to genetics factors contributing to uterine leiomyoma, a few studies have investigated the role of GSTM1 gene polymorphisms and incidence of uterine Leiomyoma. However, further studies are needed to clarify the effects of genetic variations in uterine leiomyoma. Therefore, this study was carried out to investigate the associations between GSTM1 null genotype and uterine leiomyoma in Iranian population.
Methods: In this case-control study, blood samples were collected from 50 women with uterine leiomyoma and 50 healthy controls. Genomic DNA was extracted and GSTM1 gene polymorphisms were detected using Gap-PCR. Allelic and genotypic association was evaluated by Chi-square and Fisher's exact tests.
Results: The frequency of GSTM1 null genotype was significantly different (p = 0.01) in cases (42%) compared to controls (18%). In addition, the results indicated that the presence of GSTM10/0 genotype increased risk of uterine leiomyoma in case group compared to control group (OR: 3.56; CI 95%: 1.35-9.37; p = 0.01).
Conclusion:
This study would be important to report the first data on the relationship between GSTM1 gene polymorphisms and risk of uterine leiomyoma in Iranian population.The findings revealed the association between the GSTM1 null genotype and uterine leiomyoma among Iranian population.

J12.111

Study of thrombosis factors related polymorphisms including PT (rs1799963), FGB (rs1800790) and PAI-1(rs1799889) in Iranian affected women with uterine myoma

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Introduction: Myomas are benign, solid, monoclonal tumors of the smooth muscle cells of the endometrium. Myomas occur in 20%-40% of women in their reproductive years. Venous thromboembolism (VTE) is a common complication cancers. Although several thrombosis genetic risk factors related to cancer are known; however, their contributions to thrombotic tendency in cancer patients have conflicting results. However, the association of venous thrombosis with large uterine myoma has been reported previously, the influence of thrombosis related factors polymorphism on incidence risk of uterine myoma is unclear.
Material and methods: In the present study, three selected thrombotic factors gene polymorphisms have been evaluated by ARMS-PCR method. We have focused on the prevalence of PT G20210A, FGB -455G/A and PAI-1 4G/5G polymorphisms in 70 women with clinically diagnosed uterine myoma and 70 healthy controls. The data were analyzed by SPSS19 software and using χ 2 test.
Results: The results of this study demonstrated that the PT (rs1799963), FGB (rs1800790) and PAI-1 (rs1799889) polymorphisms were not correlated with an increased risk of uterine myoma in the study population (p>0.05).

J12.112

Next generation sequencing in sporadic retinoblastoma reveals somatic mosaicism

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In about 50 % of „sporadic „ cases of Retinoblastoma no constitutive *RB1* mutations can be identified. Recent research suggests that, at least in some of these cases, somatic mosaicism of *RB1* can be found. The increased availability of Next Generation Sequencing (NGS) technology improves our ability to detect the exact percentage of patients with *RB1* mosaicism. Using NGS we re-tested a series of 42 patients with sporadic Retinoblastoma. Twelve patients had constitutive mutations, in apparent heterozygosis, that were identified according to traditional techniques, whereas 30 patients had no mutations. Among the 30 patients with no mutations, NGS identified the mutation in a „mosaic state", varying between 8 and 27% in 3 cases. Among the cases with apparent heterozygosis according to traditional methods, mutations in a mosaic state -varying from 3 to 28%- were found in 100 % of cases when, in addition to blood samples, it was possible to test more than 3 tissues ,i.e. ocular tissue, urine and/or oral mucosa. Present results confirm that 10 % of patients with sporadic retinoblastoma with no apparent mutations in RB are actually cases with low-rate mosaicism. In addition, our results show for the first time that many sporadic cases with 50% mutations in blood -that have been interpreted up to now as de novo mutations occured at gametogenesis- could actually be postzygotic events.

J12.113

XPD Lys751Gln and Arg156Arg Polymorphisms and Acute Myeloid Leukemia risk

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Defects in repair pathways are involved in cancer pathogenesis. Therefore, DNA repair genes might be involved in acute myeloid leukemia (AML) susceptibility. Some studies have shown an associations between risk of de novo acute myeloid leukemia and XPD (XPD Lys751Gln) gene polymorphisms. In the present study, we investigated the possible association between single nucleotide polymorphisme in two key regions of XPD, codons 156 and 751 and LAM in a Romanian population. The study included a total of 102 patients with AML and 153 heathy control subjects without any malignancy. The findings of our study suggest that XPD Arg156Arg polymorphism is not significantly associated with AML risk. In our study the XPD 751Lys/Gln variant heterozygous genotype was significantly associated with a increased risk of developing AML (p - value = 0.005). In the case of XPD 751, the heterozygous and homozygous variant genotypes were significantly enriched in AML cases compared to controls (OR = 2.08; 95% CI = 1.237-3.498; p-value = 0.006). There was a significantly better overall survival among AML patients with wild-type homozygous compared to those with at least one variant allele, in the case of XPD Lys751Gln polymorphism. Our preliminary results show that XPD Lys751Gln genetic polymorphism may be a genetic risk factor for acute myeloid leukemia.
Acknowledgement: This work was financially supported by Internal Research Grants of the University of Medicine and Pharmacy Tîrgu Mureș, România (grant number 1/30.01.2013).

J12.114

Clinical, hematological and molecular-genetic variability of acute myeloid leukemia

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The aim of the study was to determine the heterogeneity of AML patients with FLT3-ITD mutation and CD7 aberrant expression.
Mutation FLT3/ITD was detected by PCR in 21/101 (20.8%) patients. The cytogenetic analysis revealed 13 pts with normal karyotype (NK) and 8 pts with different chromosomal aberrations. A high WBC count was found to be significantly associated with FLT3/ITD mutation (p=0.049). CD34 expression in control group and group with FLT3-ITD was 83.3% against 90.0% (p=0.635). We found the significant association of FLT3/ITD mutation with aberrant expression of HLA-DR (50.0% cases with FLT3/ITD against 5.6% without FLT3/ITD) and CD7 (100% samples with FLT3/ITD mutation and 55.6% of samples without FLT3/ITD) markers.
The expression of CD7 in the group of 31 patients is varied over the range from 20 to 97.7% (average 70.4%). We formed 2 comparative groups: 13 pts with CD7 expression below the average and 18 – above. Patients with high level of CD7 expression were older than patients with lower expression:

57.5 vs 44 years, respectively (p=0.011). There was no significant difference between these two groups in respect of FAB and karyotype variants, the presence of FLT3/ITD mutation and CD34 expression.
Summary. Leukocytosis, aberrant expression of CD7, and/or hyperexpression of HLA-DR on blast cells are probable markers for the detection of FLT3-ITD mutations in AML patients with NK. Patients with AML and CD7 aberrant expression are heterogeneous in morphological, cytogenetic and molecular characteristics. CD7 expression in AML blasts is not an independent prognostic factor.

J12.115

Tailored Targeting of Inflamed, Regenerative, and Mutated K-RasG12D Upregulated cDNA Microarray Gene Signatures in Pancreatic Ductal Cells

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Pancreatic ductal adenocarcinoma (PDAC) harbors a universal K-RasG12D mutation that incites extracellular matrix (ECM) invasion and metastasis. Importantly, human Pancreatic Ductal Cells (PDCs) recapitulate ERK2 phosphorylation and gene upregulation only within a 3D ECM. By differentiating PDCs with stepwise p53, Rb/p16INK4a, and K-RasG12D mutations, we present a model that readily permits high-throughput cDNA microarray analysis of invasive gene signatures for personalized targeting. Specifically, populations of human PDCs in different chronologic and phenotypic stages of neoplastic formation and murine PDCs isolated ex vivo from inflamed, regenerative, or neoplastic Pdx1-Cre;LSLKrasG12D/+ pancreata were acquired. RNA was extracted and its cDNA was analyzed on genotypically-focused, invasion-specific microarray plates. As expected, K-RasG12D was the overwhelming driver of upregulated, invasive genes within the phenotypic and histologic neoplastic populations. Indeed, genes associated with cancer stem cells and necessary for PDCs to escape the ECM (metalloproteinases, tenascin-c, vitronectin and CD44) were induced 2.5 to 35-fold based distinctly on unrestricted K-Ras signaling. Uniquely however, when PDCs were stratified to pathologic stage (inflamed, regenerating, neoplastic) or mutational iteration (p53 ± Rb/p16INK4a ± K-RasG12D), there was substantial variation in the gene signatures regulating invasive potential. Indeed RNAi, shRNA, and pharmacologic inhibition of invasive genes and proteins successfully inhibited invasion or ceased neoplastic formation only if tailored to unique genes uncovered in that PDC population's gene signature. As the majority of patient's with PDAC ultimately die of metastasis, 'personalizing' the targeting of gene products is necessary to effectively attenuate each PDC population's invasive potential.

J12.116

Rare complex chromosomal aberrations in CML: A report on 300 cases

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CML is characterized by *BCR-ABL* mutation in over 90%, which indicates a favorable prognosis. Involvement of third or more chromosome(s) has been reported in 5-10% of CML. The presence of additional abnormalities is rarely reported and thus merely been discussed for prognostication. However, management of leukemia varies considerably with the involvement of additional chromosome(s). We present data on 300 CML patients of which 30 (10%) appeared with additional rearrangements in addition to *BCR-ABL* mutation. Conventional G-banding analysis was the first step followed for chromosomal characterization of leukemic condition. In cases with cryptic rearrangements, FISH was employed for confirmation of CML. PHA-stimulated lymphocyte culture was performed in all cases carrying additional translocation for differentiation of acquired or constitutive pattern. Complex and rare abnormalities observed in 10% CML are presented in bold in the table below. Cases with only trisomy 8, i(17q) and extra Ph chromosome have been excluded in this classification. Of these, constitutive aberrations were of important concern for family members of blood-lineage. Half yearly follow-up study of these cases showed secondary clones and no response to imatinib. Presence of complex aberrations might have played a different mechanism of imatinib action. The study highlights the pattern of rare rearrangements in CML and its importance for molecular investigation of such complex situation and pharmaceutical alteration of TKI for understanding prognosis of *BCR-ABL* in a complex situation.

Complex chromosomal rearrangements in CML [no.]	
46,XX,**del(3p)**,t(9;22) [75%]/49,XX, **del(3p)**,t(9;22),**+8,+19** [10%]/50XX, **del(3p)**,t(9;22),**+8x2,+19** [15%] [1]	46,XX,**t(7;22)**,t(9;22) [100%][2]
45,XY,**-7**,t(9;22) [57%]/45,XY,**-7**,t(9;22), **extra Ph** [43%] [7]	47,XY,**t(9;22;15;17),+1(p35q44),del(20 q13)** [100%] [1]
46,XX,**t(9q34;22q11;12q13;14q32;16q 23)** [100%][1]	46,XY,t(9;22),**del(17q21)** [100%] [1]
46,XY,**t(8;17)**(q13;q25),t(9;22) [100%]/47, XY,**t(8;17)** (q13;q25),t(9;22),**extra Ph** [55%]/47, XY,**t(8;17)**(q13;q25),t(9;22), **extra Ph,+8** [10%] [1]	46,XY,t(9;22) [30%]/ 47,XY,t(9;22),**+19** [30%]/48,XY,t(9;22),**+8,+19** [20%]/49,XY, t(9;22),**+8,+19,extra Ph** [20%] [2]
45,XX,t(9;22),**t(13;15)** [100%] [4]	46,XY,t(9;22) , **+ complex rearrangements** [100%] [5]
46,XY,**der(1)t(1q:?),**t(9;22) [100%] [3]	46,XY,t(9;22),**del(5q)** [100%] [2]

J12.117

Common genetic variants in NEFL influence gene expression and neuroblastoma risk

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The genetic etiology of sporadic neuroblastoma is still largely unknown. Using genome-wide association study, we identified single nucleotide polymorphisms (SNPs) associated with neuroblastoma at the LINC00340, BARD1, LMO1, DUSP12, HSD17B12, HACE1 and LIN28B gene loci, but these explain only a small fraction of neuroblastoma heritability. Other neuroblastoma susceptibility genes are likely hidden among signals discarded by the multiple testing corrections.
Eight genes were selected based on their proven involvement in neuroblastoma differentiation. Here, we tested SNPs at the eight candidate genes for association with disease susceptibility in 2101 cases and 4202 controls. We replicated the associations of the identified gene in an independent cohort of 459 cases and 809 controls. Replicated associations were studied for cis-effect using gene expression, transient overexpression and cellular differentiation assays.
NEFL showed three SNPs associated with neuroblastoma (rs11994014; Pcombined=0.0050; OR=0.88, rs2979704; Pcombined=0.0072; OR=0.87, rs105911; Pcombined=0.0049; OR=0.86). The protective allele of rs1059111 correlated with increased level of NEFL expression and we observed significant growth inhibition upon over-expression of NEFL, specifically in neuroblastoma cells carrying the protective allele. We also demonstrated that NEFL expression enhanced differentiation and impaired proliferation and colony growth in soft agar of cells with protective allele and basal NEFL expression while impairing invasiveness and proliferation of cells homozygous for risk genotype. Finally, high NEFL expression in diagnostic primary neuroblastomas was associated with better overall survival (P=0.03; HR=0.68). Our study shows that common variants of NEFL influence neuroblastoma susceptibility and indicates that NEFL likely has a role in disease initiation and progression.

J12.118

Characterization of the rs2802292 SNP identifies FOXO3A as a modifier locus predicting cancer risk in patients with PJS and PHTS hamartomatous polyposis syndromes

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Hamartomatous polyposis syndromes (HPS) are inherited conditions associated with high cancer risk and frequently carrying mutations in the LKB1 or PTEN genes. Estimation of cancer risk is crucial in order to optimize surveillance, but no prognostic markers are currently available for these conditions. Our study is based on a 'signal transduction' hypothesis relying on the crosstalk between LKB1/AMPK and PI3K/PTEN/Akt signals at the level of the tumor suppressor protein FoxO3A. Of note, the FOXO3A rs2802292 G-allele was shown to be associated with longevity, improved insulin sensitivity and increased expression of FoxO3A mRNA.
Thus, we typed this polymorphism in 150 HPS unrelated patients. We found a significantly higher risk for malignancies in female patients and TT genotype carriers compared to those having at least one G-allele. Indeed, subgroup analysis for each HPS syndrome revealed a G-allele-associated beneficial effect on cancer risk occurring mainly in males.
Our results suggest an inverse correlation between the copy number of the protective allele (G) and the risk of cancer and might be useful to optimize

surveillance in HPS patients. Further investigations will be needed to confirm our hypothesis and to ascertain whether differences exist in terms of therapeutic response across genotypes

J12.119
Gene expression of MMP9 and its prognostic role in patients with gliomas

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Background: Brain tumours, especially glioblastoma multiforme is an aggressive cancer characterized by extensive glioma invasiveness. Matrix metalloproteinase (MMP)-9 have been implicated to play a critical role in this process. **Methods**: In the present study we analyzed the expression of MMP9 mRNA in 59 gliomas (astrocytomas and oligodendrogliomas) and 14 nonneoplastic brain tissues. MMP9 gene expression was detected by real-time quantitative RT-PCR assay. **Results:** The expression level of MMP9 mRNA in glioma tissues was significantly higher than that in corresponding nonneoplastic brain tissues. MMP9 was observed with high expression level in 36 out of the 59 (61%) glioma tumours. Overall survival rates of patients with high MMP9 mRNA expression were obviously lower (12 months) than the patients in the other two groups - the median survival was 48.7 and 22.9 months for the down-regulated and normal MMP9 mRNA expression, respectively (p< 0.001). The increased expression of MMP9 mRNA was also significantly correlated with low Karnofsky performance score (p= 0.001). Multivariate analysis showed that high MMP9 mRNA expression was an independent prognostic factor for glioma patients (p= 0.002). **Conclusion:** Our results indicate that the overexpression of MMP9 mRNA is closely associated with poor clinical outcome. It may be used as specific prognostic biomarkers and for the future could be molecular targets in the treatment of malignant gliomas. Acknowledgements: This work was supported by: Infrastructural Grant DUNK01/2/2009 and Grant No.49/2009 funded by National Science Fund, Ministry of Education and Science, Bulgaria; Grant No.25-D/2012 by the Science Fund, MU-Sofia, Bulgaria;

J12.120
Individual variability in escape from nonsense mediated decay may influence the clinical severity of patients with nonsense mutations in the upstream region of the hMLH1 gene

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We previously reported the MLH1 S131X as founder mutation in patients with the Lynch syndrome from Macedonia. The mutation has a high penetrance with different time of disease onset ranging from 32 to 55 years. We speculated that this variability might in part result from a leaky nonsense mediated decay (NMD) of mutant transcripts, which results in a truncated protein and dominant-negative effect in patients with a more severe disease. Herein, we present in vivo expression data of the S131X mutation in PBMCs and 3 tumors of 2 patients with the time of onset at the age of 33 and 49. Since both patients were also heterozygotes for the common I219V polymorphism, the output from each allele was analyzed by measuring the relative amounts of transcripts containing either I219 or V219 by direct sequencing of PCR amplified cDNA. The mutant transcripts in the patient with the later onset of the disease were completely absent in the 2 synchronous tumors (stomach and colon), whereas in the patient with an early onset of the disease they were present in a significant amount (50%) both in the tumor and in PBMCs, indicating the presence of leaky NMD. Having in mind that we previously detected a variable amount of normal transcripts from alleles with splicing mutations in patients with β+-thalassemia, we suggest that inter-individual variability in the capacity for posttranscriptional processing of mutant transcripts is a general mechanism which influences the clinical severity of inherited diseases resulting from truncating mutations.

J13.01
Genetic alterations in breast cancer patients from Saudi Arabia by FISH

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Breast cancer remains a worldwide public health concern. The incidence and mortality of breast cancer varies significantly in ethnically and geographically distinct populations. In the Kingdom of Saudi Arabia, it ranks number one in terms of incidence as well as cancer related mortality in females. Although the age-standardized incidence rate for breast cancers in Saudi Arabia is 3.4 fold lower compared to United States, the median age of onset is 47 years, significantly lower than 62 years observed in patients from United States. Amplification of the two oncogenes: Her-2/neu and c-myc and deletion of the tumor suppressor gene p53 are frequently encountered in breast carcinomas. Our objective was to evaluate the association between Her-2/neu, c-myc, p53, and clinicopathologic variables in breast cancer patients using fluorescence in situ hybridization (FISH). FISH analysis for Her-2/neu, c-myc, and p53 was performed on 60 samples with breast carcinomas and 22 samples with benign breast lesions. Amplification of HER-2/neu was seen in 7/60 (11.7%) cases and amplification of c-myc was seen in 11 of 60 (18.3%) cases; neither was associated with adverse clinicopathologic variables or survival. Deletion of p53 was seen in 29/60 (48.3%) cases and was associated with poor histologic grade, compared to the benign group. There was impact of genetic alterations on overall survival and disease-free interval. The results indicate that the p53 gene plays a significant role in breast carcinogenesis and the early onset of the disease among Saudi female individuals.

J13.02
Hemolysis to Red Blood Cell

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Hemolytic due to hemolysis, the abnormal breakdown of red blood cell (RBCs), either in the blood vessels (intravascular hemolysis) or elsewhere in the human body (extravascular). It has numerous possible causes, ranging from relatively harmless to life-threatening. The general classification of hemolytic is either inherited or acquired. Treatment depends on the cause and nature of the breakdown. Symptoms of hemolytic anemia are similar to other forms of anemia (fatigue and shortness of breath), but in addition, the breakdown of red cells leads to jaundice and increases the risk of particular long-term complications, such as gallstones and pulmonary hypertension. In the present study, the *V. cholerae* were tested for their ability to cause hemolyis. The WT caused an excessive destruction of RBCs. However, several *V. cholerae* mutants did not hemolyse the chicken RBCs as the WT did. The genes that caused the reduction of hemolytic activity could not be determined as the mutant strains carried multiple gene mutation. In the previous study, hly gene was reported to cause hemolyis. Hly gene is repressed by hap, which is expressed late in infection.

J13.03
Postreplicative semihistonal form of the chromatin is cause of education and disappearance of chromosomes during cell cycle

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It is suggest a hypothesis, that replication creates a new form of chromatin, which exists from G2 period of interphase to telophase. The replication doubles of DNA, but the amount of nucleosomic histones in the nucleus remain the same as before replication in G1 period. Therefore daughter nucleofilaments of G2 period have twice less histones than before replication. This is a new post-replicative semihistonal form of chromatin, „semichromatin". Helix formation and condensation are important structural properties of semichromatin. They are responsible for the formation and development of the chromosomes. It is known that helix formation and condensation latst in the chromosomes during of prophase - metaphase.
Consequently, the chromosomal material is represented semichromatin of the nucleus all this time. The hypothesis shows that the nucleus doesn't contain a stock of free histones, and a nuclear pores can't pass quickly all histones of cytoplasm in a nucleus. The cytoplasmal histones reach of semihromatin of chromosomes only after the destruction of the nuclear membrane in prometaphase. They gradually double the number of histones of semichromatine of chromosomes in a anaphase - telophase and return it into full for histones threadlike chromatin, ie nucleofilamenty. Thus, replication creates a semihistonal form of chromatin and it is the reason of formation of chromosomes. Synthesis of new histones in cytoplasm fills histones semichromatin and return in full histones chromatin, i.e. synthesis of new histones is the reason of disappearance semichromatin and chromosomes from view a light microscope.

J13.04

Alkyl Mercury chloride compounds induced genotoxicity in human blood cultures and corrective role of Ascorbic acid (Vitamin C)

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Methyl mercury chloride is a xenobiotic metal that is a highly deleterious environmental pollutant. The biotransformation of mercuric chloride (HgCl2) into methyl mercury chloride (CH3HgCl) in aquatic environments is well-known and humans are exposed by consumption of contaminated fish, shellfish and algae. The genotoxicity induced by mercury compounds remains controversial. Therefore we have investigated the genotoxic effect of methyl mercury chloride (MMC; CH3HgCl) at two concentrations (100, and 1000 µg/L) and the role of ascorbic acid (Vitamin C) at a single concentration of (9.734 mm) on MMC-treated short-term human lymphocyte cultures. We assessed the chromosomal aberrations (CAS), sister chromatid exchange (SCE) and COMET assay in control and MMC-treated lymphocyte cultures with and without Vitamin C supplementation. The results showed that MMC has increased the frequency of CAS and SCE/cell in a dose-dependent manner than control values. CH3HgCl also, induced DNA damage in determined by COMET assay. These effects were prevented by the addition of Vitamin C to MMC-treated lymphocyte cultures. Data revealed that, mutagenic activity of MMC and the protective role of Vitamin C on mercury compounds-induced genotoxicity in human lymphocyte cultures is probably due to its strong antioxidant and nucleophilic nature

J13.05

Application of cytogenetic in Biodosimetry

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The evolution of the world demand will make that Algeria will develop, in a near future; nuclear energy .This will require a competence in this field, and particular in the evaluation effect of the radiations emitted in the event of radiobiology accidents. The importance of the effects of ionizing radiation is to increase the risk of cancer or genetic defects. The most reliable method of biological dosimetry, is analysis of chromosomal modifications radiation-induced, particularly, the dicentric chromosomes, in lymphocytes of peripheral blood. For this purpose, we established two calibration curves Dose/effect, by the methods of cytogenetic in uniform staining. The selected experimental conditions are the same as those used by the team of Pr J,F. BARQUINERO (University Autonomy, Barcelona, SPAIN). The first was established like witness, from the blood of a Spanish person. As for the second, it was conceived for the Algerian laboratory from a blood sample of an Algerian person, without history to exposure to radiations. The frequency of dicentric chromosomes increases as dose and the statistical analysis shows that the values obtained follow a Poisson distribution revealing thus that the irradiations were correct and homogeneous. The two curves obtained from the dicentric analyses, obey the quadratic linear model and the relation Dose/effect is expressed by the equation: $Y = C + \alpha D + \beta D2$.
This study has allowed us, in Algeria to establish a dose effect curve for biodosimetry laboratory, which could in the future be part of an international biodosimetry network

J13.06

Dysgonosomies at University Hospital Hassan II Fes: Cytogenetic and molecular aspects

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Abstract. The dysgonosomies are represented by all the anomalies of the X and Y sexual chromosomes.
The main dysgonosomies are represented by Klinfelter syndrome which includes the presence of at least one extra X chromosome in a masculine karyotype 46, XY and Turner syndrome, which is linked to the complete or part absence of X chromosome. Monosomy X is not the only form of this syndrome. In half cases we find mosaic and / or structural abnormality of the X chromosome and sometimes the Y. Other less frequent dysgonosomies are represented by Double Y, Triple XXX , Men XX and Women XY. We report our first series collated between October 2009 and Marsh 2014 at the unit of medical genetics and Oncogenetics CHU Hassan II Fes. Many abnormalities found: Klinfelter syndrome (n: 9), Turner syndrome (n: 21), 12 cases with chromosomal formula (45, X), 3 cases of mosaic, 4 cases of isochromosome of the X long arm and 2 cases of Turner syndrome with the Y. Finally a patient with double Y (n: 1). The cytogenetic investigations used: metaphase karyotype; FISH (Fluorecent In Situ Hybridization) used „probe SRY Region (LSI SRY (Yp11.3) Spectrum Orange / CEP X Spectrum Green)", and multiplex PCR SRY / DXS1684.Through this communication we will display our different dysgonosomies (Klinfelter syndrome, Turner syndrome, double Y) in all their aspects: circumstances of discovery, clinical aspect, management and genetic counseling. The classical and molecular cytogenetic diagnostic of dysgonosomies allows better medical and surgical care and proper genetic counseling.

J13.07

Genetic heterogeneity of Fanconi anemia (FA)-A in Egyptian patients

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Fanconi anemia (FA)-A is the most frequent complementation group and is detected in approximately two-thirds of studied FA patients in most countries. The aim of the study was to screen the common mutations previously reported in the international literature within exons 27, 34, 38, and 43 of the FANCA gene among Egyptian FA patients. **Patients and methods** The study included 24 Egyptian FA patients of unrelated consanguineous pedigrees and diagnosed by positive chromosomal breakage studies using diepoxybutane. Ten healthy unrelated individuals matching age and sex were included as the control group. Genomic DNA amplification, sequencing of exons 27, 34, and 43 of the FANCA gene, and restriction enzyme analysis for the exon 38 3788-3790del mutation were performed for patients and controls. **Results** No mutations were detected within the studied FANCA gene exons. **Conclusion** This is the first molecular study of FA in Egyptian patients that proved absence of the common mutations previously reported in other countries this may denote molecular heterogeneity in Egyptian patients. Further studies are recommended to establish the underlying mutations responsible for Egyptian FA cases as an important step in disease control.

J13.08

Over expression of the glycoprotein P human in chemoresistance

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The "Multidrug resistance" (MDR) is an important obstacle to the success of the chemotherapy of many human cancers. The cellular multi chemoresistance is due to the over expression of the glycoprotein P, which confers the phenotype MDR to the cells. The aim of these researches if the localization of the fixation site of the steroid chemosensitizing in order to prepare efficacious modulating molecules of the MDR "phenotype". The first object has consisted in increasing the expression level of the Pgp in the R7 cells born of (descended from) a patient attacked by an erthroleuchaemis expressing concentrations of doxorubicin. The characterization of the Pgp presence has been realized by affinity photomarking with azidopine tritiée. An augmentation of the Pgp concentration is observed in the membranous fractions of the R7 cells treated by the doxorubicin. The second aim has consisted in preparing different radio active chemomarkers by peptidic coupling of bromo acetic [14C] the terminal amine of the progesterone derivatives substituted on the carbon 11 but the introduction of different hydrophobic chains, and in testing these chemomarkers with the membranous fractions of the R7 cells treated with the doxorubicin.

J13.09

The diversity of MED12 gene mutations in uterine fibroid cells

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Uterine myoma (UM) is a benign and most common tumor that affects 20-45% of women of fertile age. Previously it was shown that somatic mutations in the MED12 gene occur in most women with uterine myoma. We analyzed exon 2 nucleotide sequence of MED12 gene from 94 DNA samples extracted from fibroids, endometrium, myometrium cells and peripheral blood leukocytes of 17 women with uterine leiomyoma. Twenty-four samples exhibited various MED12 gene mutations. No mutations were found in DNA from myometrium cells and peripheral blood leukocytes. Two mutations have been identified in codon 44 from endometrial tissue sample. In 22 of the 47 samples (47%) isolated from fibroids we found different mutations of the MED12 gene. Each fibroid is characterized by its MED12 mutation. Up to five different mutations per individual patient could be identified depending on the amount of fibroids. Altogether we identified 18 mutations in codon 44 and 5 deletions of varying lengths. A single nucleotide substitution c.92T>A was also found within the area of alternative splicing. MED12 gene mutations were identified in 12 of the 17 women (70%). Mutations of MED12 gene could make substantial contribution in UM progression by modifying the activity of other genes that encode proteins involved in cell

proliferation and blast transformation.

J13.10

Serotonin effects on NDUFS2 gene expression, a schizophrenia animal model research

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Schizophrenia is a chronic and disabling psychiatric disorder with unknown cause that affects about 1% of the population worldwide. In last researches we have seen a significant higher expression of ndufs2 in schizophrenic mouse models in compare with normal controls. we have studied expression changes of 200 schizophrenic mouse models with and without serotonin treatment. Based on sex and treatments program, Mouse models have separated to four groups. Group A(male) and B(female) have been treated with serotonin(40 microg/kg body weight/day) for 6 weeks , and group C (male) and D(female) did not treated with serotonin. All mouse models were in the same age (15-16 weeks old) and all situations including air, light, feeding and etc were completely similar to four groups. After this period, mouse models killed and RNA isolated from prefrontal and hypo camp of their brains. We have investigated ndufs2 expression by using qRTPCR. Results shown significant lower expression of ndufs1 in models of group A and B in compare with models of group C and D. Also normal control group results confirmed our last researches output about increasing expression of ndufs2 in schizophrenic mouse models. Control group gene expression rate was lower than all other four groups. This research shown a significant relation between serotonin treatment and ndufs2 expression in brain but Since the neurotransmitter does not easily cross the blood-brain barrier the mechanisms of serotonin effect to genes expression in prefrontal and hypo camp are not clear and needs more studies.

J13.11

Extended expression of promyelocytic leukemia (PML) during in vitro neural differentiation process of mouse embryonic stem cells (mESCs) purposes the importance of PML in cellular pluripotency and nervous system development

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Pro-myelotic leukemia (*PML*) is one of the major proteins in promyelotic leukemia nuclear bodies (*PML*-NBs). *Pml* gene has located on 9B mouse chromosome. Retinoic acid (RA) exerts its tumor growth suppressor activity and terminal myeloid differentiation of granulocyte\monocyte progenitor (GMP) cells via *PML*-NBs in RA pathway. In addition, RA as a natural morphogen guides posterior patterning in embryo neural development. Based on these two scenarios the aim of this study was to define if there was any revenue for PML-NBs in RA dependent neural development. For this reason, RA was used as a neural inducer for *in vitro* neural differentiation of mouse embryonic stem cells (mESCs). In mESCs, neural precursor cells (NPCs) and neural cells (NCs) obtained from this differentiation process *Pml* mRNA and protein levels were assessed by quantitative real time RT-PCR (q-RT-PCR) and western blotting. qRT-PCR results showed that *Pml* had a maximum expression in mESCs and this expression clearly had decreased in NPCs and NCs, although no significant differences existed between two latter groups. Interestingly, three un-foresight protein bands about 170, 130 and 70 kDa similarly were detected in these cell types on western blots. Based on qRT-PCR results, PML expression may have an important role in cellular pluripotency. However, the appearance of three similar bands in western blots from ESCs, NPCs, and NCs led us to assumption that PML might be necessary in cellular pluripotency and nervous system development. Although till now these protein bands were not reported.

J13.12

The effect of carvacrol on prostatic cancer cells line PC3, Using comet assay technique

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Background and aims: Nowadays, cancers are one of the biggest concerns of human societies. Polyphenolic compounds and antioxidants are a key factor in the prevention or treatment of various cancers. In this study, the effects of carvacrol on prostatic cancer cells line PC3 were investigated using comet assay technique.
Methods: 130, 230, and 360 µM concentrations of carvacrol were selected according to IC_{50} using MTT assay on cells line PC3. Then, alkaline electrophoresis was done and 100 comet pictures were analyzed with CASP software and all results were analyzed by SPSS statistical software.
Results: The IC_{50} for carvacrol was determined at 360µM by MTT test. Rate of tail to head in alkaline electrophoresis at 130, 230, and 360 µM of carvacrol concentrations were 15.9±2.1, 38.7±4.2, and 65.3±2.0 percent, respectively.
Conclusion: Carvacrol is one of the effective polyphenolic compounds in treatment the cancers and has destructive effects in prostatic cancer cells line PC3. The genomic destruction effects of carvacrol on cells line PC3 is more effective in near the IC_{50} concentration.

J13.13

Random Aneuploidy in Amniocytes from Aneuploidic Pregnancies

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Aneuploidy may represent genetic instability. Amniocytes of fetuses with aneuploidy carry characteristics of genetic instability. Individuals with chromosomal aneuploidies may develop various types of malignancies. In this study, looking for random aneuploidy, we applied FISH technique, using different probes, to amniocytes from pregnancies with trisomies 21 and 18, and 47,XXY (study group) and compared them to amniocytes from pregnancies with normal karyotypes (control group). A significantly higher rate of random trisomy and more triploidies were observed in trisomy 21 and 18 cases. However, in the 4 cases of 47, XXY higher rates of random trisomies, but not triploidy were observed. Monosomies appeared in both study and control groups, which could be the result of technical problems. The observed differences in the random aneuploidy rates between the somatic and sex chromosome aneuploidies might reflect different mechanisms of random aneuploidy between the two types. Triploidy was significantly higher in the somatic aneuploidies compared to the sex aneuploidy and control groups. As previously shown in CML patients, triploidy, which occurred more often in the study group, probably reflects an increased predisposition to develop malignancy compared to random aneuploidy. In most aneuploidies, malignancy will develop as a result of an oncogenic event that occurs in addition to the existing genetic instability. Occurrence of triploidy may reflect such an event.

J13.14

Small Supernumerary Marker Chromosome Originating From Chromosome 10 Associated With an Apparently Normal Phenotype

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A normal 40-years old Italian woman was referred for cytogenetic evaluation before having undergone controlled ovarian hyperstimulation, in vitro fertilization and embryo transfer. Family history was noncontributory except for her long-term infertility. QFQ-banding revealed a karyotype 47,XX,+mar[49]/46,XX[51] with one sSMC. Studies by aCGH and FISH confirmed that the marker originated from chromosome 10, with duplication of 10p11.21p11.1 segment. Molecular cytogenetic characterization in her parents, sister and brother defined a normal karyotype 46,XY for father and brother while the mother was 47,XX,+mar[61]/46,XX[39] and sister was 47,XX,+mar[77]/46,XX[23] with sSMC derived from chromosome 10. Chromosome 10 is rarely involved in the formation of marker chromosomes. Eight cases have been reported in literature and in six out of these, sSMCs were associated with an increased risk of abnormal phenotype. In our case, there was an apparently normal phenotype, probably associated with minimal involved material and gene content. Some sSMCs derived from the same chromosome, but in spite of this, a great variation in phenotype was observed, probably due to the degree of mosaicism that may vary in different patients as well as in different tissues in the same patient. Furthermore, most sSMCs were ascertained in phenotypically abnormal subjects, but it isn't always possible to correlate the phenotype with sSMC; so it is difficult to predict the precise phenotype-karyotype correlation and phenotypic outcome. This report shows the importance of cytogenetic characterization in patients with comparable chromosome defects, giving the possibility of identifying similarities in the clinical picture that will benefit the counseling of future cases.

J13.15

AvaII-TaqI-HindIII represents a novel informative haplotype at the β-globin gene cluster: Application in carrier detection and prenatal

diagnosis of beta-thalassemia in the Iranian population

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Thalassemia is one of the common monogenic disorders, with a high demand for carrier detection and prenatal diagnosis in the Iranian population. In view of the presence of a large number of mutations associated with the disease, the polymorphic markers present in the β-globin cluster region were commonly used in linkage analysis of the disease. Markers usually show a population dependent base haplotype frequency. Among the polymorphic markers, five markers including AvaII, RsaI, HinfI, TaqI and Hind III were genotyped in 150 unrelated healthy individuals from the Iranian population. The haplotype frequency was estimated using PHASE program and linkage disequilibrium (LD) was analyzed by MIDAS program. Among the 31 possible haplotypes, seven haplotypes showed relatively high frequencies ≥ 5%. The haplotype AvaII-TaqI-HindIII could be suggested as an informative haplotype for possible carrier detection and prenatal diagnosis of beta thalassemia in the Iranian population. Moreover, RsaI and HinfI (located in the hotspot region) were not associated with the 5' sub-haplotypes or 3' sub-haplotypes. The data suggested that RsaI and Hinfl markers might be excluded as strong molecular diagnostic markers in beta-thalassemia carriership and prenatal diagnosis in the Iranian population.

J13.16

Study of cytogenetic stability of induced pluripotent stem cells (iPSCs) using karyotyping and comet assay techniques

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Induced pluripotent stem cells (iPSCs) have capability to undergo unlimited self-renewal and differentiation into all cell types in the body. In order to use iPS cells and apply studies in therapeutic applications, the stability of these cells is essential step. In present experimental study HDF cells were isolated from human foreskin samples and cytogenetic stability of these cells were evaluated in early passages (1-3) using karyotype test and alkaline comet assay. The HDF cells treated with hydrogen peroxide were used as a positive control for alkaline comet assay. The iPS cells with low passage (4-7) derived from reprogrammed HDFs were cultured on MEF feeder layer and cytogenetic stability of these cells were evaluated by karyotype test and alkaline comet assay technique. After karyotype test and comet assay, the results showed that the iPS cells in early passages (4-7) had normal karyotype (46, XY) and DNA damage and comet in these cells were not observed. In addition, HDF cells showed normal karyotype in early passages (1-3) but using comet assay, abnormality and DNA damages in positive control (HDF cells treated with H2O2) was observed. The parameters of alkaline comet assay of iPS cells and HDFs compared with positive control group were statistically significant ($p<0.05$). These findings indicated that comet assay is a sensitive technique and should before performing the functional experiments on iPS cells, cytogenetic stability of these cells must be study. Therefore, for precise evaluation of DNA damage and cytogenetic stability of these cells, both techniques could complete each other.

J13.17

The assessment of lentiviral vectors appliction for gene transformation in human dermal fibroblasts (HDFs)

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Induced pluripotent stem cells (iPSC) are primary undifferentiated cells that are able to create almost any type of cells in the body. The purpose of the present study was production and transmission of TetO-FUW-OSKM lentiviral vector to human dermal fibroblast cells (HDFs) and investigation the application of this vector. In this experimental study after isolation and culture of HDF cells, TetO-FUW-OSKM lentiviral vector containing the reprogramming genes (as transfer vector) and psPAX2 and pMD2.G vectors (as packaging plasmids) were transfected to HEK-293T cell line for virus production. The supernatants of packaging cells were harvested in 48h and 72h after transfection. Then, these viruses were transducted to HDF for reprogramming these cells. The results of this study demonstrated the successful production of TetO-FUW-OSKM lentiviral vector and suitable expression of the transcription factors in HDF cells after transduction. According to these findings lentiviral vectors could be used for gene transmission and reprogramming of adult cells such HDF to generate iPS cells in future studies.

J13.18

A rare case of a 12-year-old boy with a 45,X karyotype: cytogenetic and molecular genetic

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We report the case of a 12 years-old boy of non consanguineous parents. He was born by uterine-incision delivery, weighting 2480kg and measuring 47 cm.. The present height was of 139 cm (height SD score -2.2)< to parental target of 176 cm and weight of 26.3 kg, BMI of 13.61 (SD score-2.98 DS) and head circumference of 52 cm (10°percentile). At physical examination he presented long face, sharp chin, prominent ears, malar hypoplasia, pectus excavatum, joint laxity, cubitus valgus and severe scoliosis. The genitalia were normal for male phenotype but the left testicle were not yet in scrotum He presented mild mental retardation, motor and speech delay The ultrasound examination of heart was normal .
Standard cytogenetic analysys showed a 45,X karyotype. Y microdeletion analysis showed the presence of an intact SRY homeobox region and the ZFY region, instead the regions AZF a,b,c are delete.
Fluorescence in situ hybridation (FISH) with chromosome paints for chromosome Y and with subtelomeric probe for 10q, showed the presence of a rare aberration with an unbalanced karyotype constituted by 45 chromosomes including a der(10) deriving from the Y/10 traslocation. Finally it results a monosomy of the region 10q26.3-qter and a deletion of Yp11.2-qter. The breakpoints were, later, by molecular genetic analysis (Agilent 8x60k array-CGH), ascertained.
The karyotype is:
45,X,der(10)t(Y;10)(q26.3;p11.2).arr Yp11.2 (9,394,173-28,548,485) x0,10q26.3 (131,561,314-135,404,523)x1

J13.19

Developmental role of NF-Y as an epigenetic element on regulatory region of *SALL4* gene in human embryonic carcinoma cells

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Introduction
During development epigenetic mechanisms have key roles in molecular regulation of gene transcription. Through various epigenetic mechanisms, NF-Y act as a histone substitute protein, which precisely binds to the CCAAT box of promoter and has a significant role in chromatin remodelling of their target genes. *SALL4*, spalt-like transcription factor 4, is a coding gene with CCAAT box, play a crucial role in maintaining the properties of embryonic stem (ES) cells and governing the cellular fate. Embryonal carcinoma (EC) cells derived from testicular tumors are worthwhile models for elucidating molecular and cellular genetic and epigenetic functions involved in developmental process due to its similarities with embryonic stem (ES) cells.
Material and Method
In this study, the chromatin immunoprecipitation (ChIP) coupled with real-time PCR was performed using anti-NF-Y antibody, on chromatin extract from a human embryonal carcinoma cell line, named NT2/NTera2, to evaluate incorporation levels of NF-Y on the regulatory region of *SALL4* gene.
Results
The results of ChIP real-time PCR analysis clearly showed a considerable incorporation of NF-Y complex on the regulatory region of *SALL4* in NTera2 cells.
Conclusion
According to the presence of NF-Y protein on *SALL4* regulatory region, it can be concluded that NF-Y has a dynamic epigenetic role in regulation of *SALL4*, a master gene in controlling the molecular pathway of pluripotency during development.

J14.01

An automatic algorithm to extract mid-sagittal plan of fetus in first trimester from 3D volumes

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Background:
In first trimester, there are many important ultrasound examinations during

prenatal care. In evaluation the fetal structure in utero, the three-dimensional ultrasound is the most convenient and powerful tool. However, accuracy image (e.g. mid-sagittal plan) is extremely important. Thus, we proposed an automatic algorithm to extract mid-sagittal plan of fetus in first trimester from 3D volumes.
Materials and Methods:
3D volumes with gestational ages of 11 to 13 weeks were used in the study. We plan a registration method based on the selected feature points. After finishing the registration of image and model with affine transform, we adjusted the scale of model to obtain the optimal size, which is similar to the object in the image. The process to put the statistic shape to the testing volume image is similar to the adoption of all training shapes in the statistic model construction. The following step was to make the geometric information of the statistic shape model was used to avoid the excessive distortion caused
Result:
The measurement results were compared with those manually obtained by an expert. The experimental results show that the proposed method overcomes the difficulties and achieves good consistency between the automatic method and manual measurements.
Discussion:
Due to the difficulties in treating highly-noise US images, the fully automatic image segmentation tool for the fetal ultrasound is lack. The proposed system precisely detected mid-sagittal plan of fetus using 3D US in the first trimester, making it useful for clinical service.

J14.02
Genetic diagnosis of Bardet-Biedl syndrome by MiSeq exome sequencing

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Bardet-Biedl syndrome (BBS) is a rare autosomal-recessive ciliopathy characterized by obesity, postaxial polydactily, retinitis pygmentosa, mental retardation and kidney abnormalities. At least 18 genes have been shown to be associated with BBS, therefore genetic testing for this disease is highly complicated. We consulted a family with strong clinical features of BBS in 2 children. While considering the labor and consumables costs for analysis of at least 7 "common" BBS genes (BBS1, BBS10, BBS2, BBS6, BBS9, BBS12 and BBS13), we opted for exome sequencing as a viable alternative to multiple single-gene tests. Using Illumina MiSeq platform, we identified homozygous BBS7 L656fsX673 (c.1967_1968delTAinsC) mutation in 2 affected sibs; both parents and their healthy son were heterozygous for this allele. Presence of an identical gene defects in non-consanguineous parents is uncommon for BBS, however Russian population was repeatedly shown to have an unusually pronounced founder effect. We are currently examining whether the above mutation is indeed recurrent in Russia: at the time of abstract submission, we genotyped 1817 healthy donors and identified 1 (0.06%) additional carrier of BBS7 c.1967_1968delTAinsC.
We conclude that even medium-throughput next generation sequencing platforms may facilitate clinical diagnosis of genetically heterogeneous disease.

J14.03
Detection of Borna Disease Virus in peripheral blood cells of a number of obese patients in Iran via Nested RT-PCR

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Worldwide prevalence of obesity trends attentions toward the multiple origins of it. The most overlooked one is infectious factor, and among them viruses are the most noticeable. Borna Disease Virus is a nonsegmented, single-stranded RNA virus, usually causes sporadic neurological disease in horses and sheep as its natural host. Although BDV is considered as a non-human virus, recently Serological and molecular evidences investigated the possible association of BDV with specific psychiatric diseases in humans. But the point that we choose it as an etiology of human obesity is that BDV is identified as a cause of rapid increase of body weight with development of an obesity syndrome without obvious neurological signs in Experimental intracerebrally infected Lewis rats. In this study which was done for the first time in Iran, by using nested RT-PCR technique, we demonstrated that BDV RNA was present in peripheral Blood Mononuclear cells of a number of obese patients. 43 subjects took part in this study. The BMI in kg/m2 of the obese subjects were over 30.the detection rate is about 16/2%.sequenceing of positive samples confirmed our finding. These results illustrated the adiposity-promoting effect of BDV occurs in Human being.

J14.04
Reliable and cost effective screening of 16 breast cancer gene panel using sequence capture method coupled with next-generation sequencing in clinical settings

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BRCA1 and BRCA2 are two well-known genes in the background of hereditary cancer syndromes. There is also evidence that several other genes play an important role in the pathogenesis of these type of diseases. Latest population-scaled studies showed that certain mutations in different genes could cause as high risk elevation as BRCA2 mutations. In this study we present a reliable and cost-effective method to analyse the risk assessment of different types of cancer. Using Haloplex, a novel sequence capture method combined with benchtop non-optical next-generation sequencing we were able to achive short turn around time with the screening of 16 genes that could be associated with an increased risk of breast, ovarian and other types of cancer. The analysis of this 16-gene set can explain the inherited background of almost 30% of hereditary and familiar cases of breast and ovarian cancers. Thus, it opens up a high-throughput approach with fast turnaround time to the genetic diagnostics of these disorders and may be helpful to investigate other familial genetic disorders as well.

J14.05
The clinical utility of cell-free DNA analysis for non-small cell lung cancer diagnostics and radical therapy effectiveness monitoring

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Objective: The presence of cell-free DNA (cfDNA) in plasma/serum of non-small cell lung cancer (NSCLC) patients demonstrates promising clinical implications as the minimally-invasive liquid biopsy for diagnostic, prognostic and predictive applications. To date, we performed quantitative evaluation of cfDNA in plasma of NSCLC patients and non-malignant controls. **Methods:** Plasma cfDNA concentration and integrity index (DII) were measured by real-time PCR in 60 NSCLC patients (stage I-IIIA), 100 patients with chronic respiratory inflammation (COPD, sarcoidosis, asthma), 15 patients with non-malignant lung nodules (hamartoma, fibrosis, tuberculoma) and 40 healthy volunteers. **Results:** NSCLC patients presented significantly higher plasma DNA levels (8.0 ng/ml) than patients with chronic respiratory inflammation (3.4 ng/ml) and healthy controls (2.3 ng/ml; p<0.0000), but not than patients with non-malignant lung nodules. The ROC analysis provided 90% sensitivity and 80.5% specificity in discriminating NSCLC from healthy individuals, while 56% specificity and 90% sensitivity in distinguishing NSCLC from any non-NSCLC subjects (p<0.0001). Similarly, the mean DII in NSCLC (4.0) significantly differed from healthy control values (1.0; p=0.000), but not from DII in chronic respiratory inflammation (3.7) and non-malignant nodule group (4.0). During 3-6 month follow-up plasma DNA level were significantly reduced in relapse-free NSCLC patients (2.8 ng/ml), while in relapsed subjects were higher than at baseline. Conclusions: The plasma DNA quantification, though insufficient for routine NSCLC detection, is still superior to diagnostic accuracy of conventional serological markers. Epi-/genetic alteration analysis might improve the diagnostic power of cfDNA. Long-term post-operative plasma DNA level follow-up might prove promising in monitoring of radical NSCLC therapy.

J14.06
Chitotriosidase as a biomarker for the diagnostic approach of lysosomal storage disorders in Colombia

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Lysosomal storage disorders (LSDs) are a group of disorders that enclose the damage of an enzyme and its pathological consequences due to the accumulation of substrate in lysosomes. In Colombia, LSDs are misdiagnosed because many of them are not widely known. A biomarker is defined as a molecule that works as an indicator of disease, being useful for a diagnostic approach. Chitotriosidase (ChT) is a chitin hydrolyzing enzyme that cleaves the 1,4-β linkage of chitin. In human its function has not been entirely elucidated, but its increase has been associated with inflammation, fungal/parasitic infection or LSDs. We propose ChT as a reliable biomarker for the diagnostic approach to LSDs in Colombia. We took DBS samples from 240 patients (112 females and 128 males) with Pompe, Fabry, Metachromatic Leucodystrophy, Mucopolysaccharidosis, Mucolipidosis, Gaucher, Niemann Pick (NP), Gangliosidosis GM1 or Sialidosis, who were previously dia-

gnosed by enzymatic assays in leukocytes. Also 48 patients with symptoms suggestive of LSD and 87 control subjects were analyzed by a fluorometric assay using 4MU-triacetylchitotrioside. While most patients with LSDs showed an increase in ChT (range: 1,1-3118,0 nmol/ml/h), Morquio A patients showed no increase (n=30, range: 5,6-78,1 nmol/ml/h) in relation to the reference value (<96 nmol/ml/h). Gaucher and NP patients showed the highest values (3118 and 1649,6 nmol/ml/h, respectively). Although ChT is not an specific biomarker, it can be used for an LSD diagnosis approach as long as the clinical symptoms strongly suggest it.

J14.07
Chromosomal microarray analysis as first-tier clinical diagnostic test: Estonian five years experience

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Chromosomal microarray analysis (CMA) is now established as the first-tier cytogenetic diagnostic test for fast and accurate detection of chromosomal abnormalities in patients with developmental delay/intellectual disability (DD/ID), multiple congenital anomalies (MCA), and autism spectrum disorders (ASD). We present our experience with using CMA for postnatal and prenatal diagnosis in Estonian patients during 2009-2013. Since 2011 CMA is on the official service list of the Estonian Health Insurance Fund and is performed as the first-tier cytogenetic test for patients with DD/ID, MCA or ASD. A total of 1902 patients were analyzed, including postnatal [1692 (89%) patients and 106 (6%) family members] and prenatal referrals [104 (5%) fetuses]. Abnormal results were reported in 500 (26%) patients, with a total of 585 findings (1-5 per individual): 217 (37%) deletions, 174 (30%) duplications, 176 (30%) long contiguous stretches of homozygosity (LCSH) events (>5 Mb), and 18 (3%) aneuploidies. Of all findings, 230 (39%) were defined as (likely) pathogenic; for 271 findings (46%), most of which were LCSH (159), the clinical significance remained uncertain; 80 (14%) reported findings were classified as likely benign. Clinically relevant findings were detected in 201 (11%) patients. However, the proportion of variants of uncertain clinical significance was high (46% of all findings) demonstrating that the interpretation of CMA finding remains a rather difficult task. Close cooperation between clinicians and cytogeneticists, as well as further data sharing with colleagues are the cornerstones of successful CMA application in clinical practice.

J14.08
Clonal evolution in prospective analysis of chronic lymphocytic leukemia patients detected by FISH and conventional cytogenetics after stimulation with CpG oligonucleotides and interleukin-2

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Introduction: Chromosomal abnormalities are important prognostic factors in chronic lymphocytic leukemia (CLL). During the course of the disease clonal evolution (CE) may occur. CE, defined as acquisition of new cytogenetic aberration, is associated with shorter overall survival. In the majority of published analyses to date, CE has been monitored using FISH. Therefore, the aim of our study was to prospectively assess CE frequency using a combination of FISH and conventional chromosome banding (CBA) after stimulation with CpG oligonucleotides and interleukin-2 (IL-2). The role of other prognostic factors and therapy in development of poor-prognosis CE (new deletion 17p, new deletion 11q or new complex karyotype) was also evaluated. Methods: Betweeen 2008 and 2012, 140 patients with previously untreated CLL were evaluated by FISH (deletion 11q, 13q, 17p, trisomy 12, rearrangement 14q32) and CBA after stimulation. Peripheral blood samples of each patient were provided for baseline and follow-up testing. Mutation status of IGVH gene and expression of CD38 and ZAP70 were also analysed. Results: CE was detected in 15.7 % (22/140) of patients using FISH, in 28.6 % (40/140) using CBA, and in 34.3 % (48/140) of patients by combining both methods. Poor-prognosis CE was detected in 15 % (21/140) of patients and was significantly associated with previous CLL treatment (p = 0.013). Conclusions: CBA after stimulation with CpG oligonucleotides and IL-2 provides more complex information about cytogenetic abnormalities in CLL than FISH. Many patients can acquire new abnormalities during the course of their disease in a relatively short time period.

J14.09
Submitting biomedical data to the European Genome-phenome Archive (EGA)

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The European Genome-phenome Archive (EGA), a service of the European Bioinformatics Institute (EBI), is a permanent archive for all types of potentially identifiable genetic and phenotypic data that has been consented for use in biomedical research, but not for open public distribution. The EGA includes major reference data collections for rare and common diseases, including data derived from the UK10K project, Wellcome Trust Case Control Consortium (WTCCC) and International Cancer Genome Consortium (ICGC), as well as control sets that can be used in addition to the public reference panels such as the 1000 Genomes project. Accepted submissions include manufacturer raw data from genome sequence, transcriptome, epigenome or proteomics experiments. The EGA also stores called variants, genotypes, study summary statistics and associated sample phenotypes. Submission tools designed to facilitate the secure upload of data files and associated metadata are accessible for each submitter using a single log-in and may be run as graphical interface or from the command line. The functionality of the submission tools may be incorporated into submission pipelines for large-scale submitters. The EGA follows strict protocols for information management, data storage, security and dissemination. Authorized access to the data is managed in partnership with the data providing organizations. Future plans include expanding the EGA into a distributed network of data archive and distribution services. A pilot project has already started in collaboration with the Centre for Genome Regulation (CRG) in Barcelona, Spain. The EGA is currently available at www.ebi.ac.uk/ega/.

J14.10
Detection of EGFR gene amplification in head and neck squamous cell carcinoma with paralogue ratio test

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Introduction: EGFR signaling pathway has an important role in the development of head and neck squamous cell carcinoma (HNSCC). Therefore any mutation that affects its components, may influence the course and management of the disease. The amplification of the EGFR gene is such significant event that is present in HNSCC. We compared the efficacy of a targeted detection of the EGFR gene amplification with the paralogue ratio test (PRT) to results obtained with the array comparative genomic hybridization (aCGH).
Methods: Samples of genomic DNA extracted from tumors of 50 patients with various stages of HNSCC were first analyzed with the aCGH method. An oligonucleotide pair that maps inside the amplified EGFR region was selected from a collection at www.prtprimer.org and used for PRT analysis.
Results: The aCGH analysis identified EGFR amplification in 7 samples, with EGFR gene copy number ranging from 3 to 6 copies. Subsequent PRT analysis also identified all 7 EGFR amplifications and it produced concordant results in all remaining samples which were without EGFR gene amplification.
Conclusion: Paralogue ratio test can be adopted for a rapid, targeted detection of gene amplification, such as EGFR amplification present in HNSCC, The method may be applicable as a screening tool and for conformation of aCGH results.

J14.11
Molecular evaluation of carbapenemases and Metallo beta lactamases production among Gram-negative bacteria with carbapenem resistance

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Emerging multidrug resistant (MDR) microorganisms among hospital isolations have been limited the effective drugs to treat or prevent bacterial infections. This study was performed to determine the rates of antibiotic resistance in Gram-negative isolates from clinical samples and to identify carbapenemases and Metallo beta lactamases genes variation in the strain. Identification and assessment of gram negative bacteria with Carbapenem resistant sensitivity to 11 antibiotic was done through biochemistry and disk diffusion methods, respectively. 11 isolates were checked by PCR for identification of blaVIMI,II blaIMP blaSPM blaGES blaNDM blaKPC blaOXA-48 genes MHT and DDST tests were used assessment for Carbapenemases and Metallo beta lactamases production in these strains. The iden-

tified genes were confirmed by sequencing technique.
In this study, a total of 134 isolates were studied, including 57 Escherichia coli (E.Coli) strain, 26 Klebsiella strain, 21 Acenitobacter strain, 17 Pseudomonas strain, 8 Citrobacter strain, 3 Proteus strain, 2 Enterobacter strain. Confirmatory tests showed that 44 strains were EDTA positive and 17 strains were H-Test positive. A PCR based screening revealed the presence of the blaIMP-1 gene in 4 isolates. E.coli showed the highest level of resistance to imipenem. Most of positive H-test (41.17%) and positive DDST (39.13%) strains were Acenitobacter.
Owing to the presentation of blaIMP-1 gene in Klebsiella, Citrobacter, Acenitobacter and E.coli and feasibility of horizontal gene transfer among bacteria, but also due to the importance of Metallo beta lactamase production strains in hospital, change in antibiotic prescription policies are required.

J14.12
Improved methods for preparing high quality genomic libraries from challenging FFPE tumour samples for use on Illumina® sequencing systems

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The research community needs robust and rapid sample preparation workflows for high-throughput sequencing to unlock challenging samples and generate high quality sequence data. These include samples with low input amounts of genomic DNA and highly degraded tumour samples extracted from formalin-fixed paraffin embedded (FFPE) material.
The quality of the libraries and ultimately the sequencing data depends on the integrity of the genomic DNA extracted from FFPE samples. The formalin fixation and paraffin embedding of tissues affects this through fragmenting, cross-linking, and otherwise damaging DNA through various chemical modifications. It is essential to assess the extent of the damage, adjust the DNA extraction procedure to compensate for the damage as much as possible, and adapt the sample preparation workflows to the quality of these samples.
We have developed flexible FFPE-specific sample preparation workflows analysis solutions for tumour/normal low coverage copy number analysis and deep whole-genome sequencing. The end-to-end workflow from sample preparation to answer includes an upfront sample QC step enabling sample triage into three different quality bins (high, medium and low). Depending on the sample quality bin, different input DNA and size selection conditions are recommended for the sample preparation. We have also developed software solutions in BaseSpace and tested third party tools for the detection of somatic variants from tumour/normal analysis, including single nucleotide variants (SNV), structural variants (SV) and copy number aberrations (CNA) data analysis.
We present results obtained with FFPE samples from different qualities sequenced on several Illumina® sequencing systems.

J14.13
Importance Of Poliaspectual Prenatal Diagnostics Of Congenital Malformation

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Background: Congenital pathology is the leading cause in Republic of Moldova of infant morbidity and mortality. Invasive techniques of prenatal diagnosis (amniocentesis) are very important in efficient medico-genetic counseling for determining caryotipe, used in diagnosis of many chromosomal abnormalities (Down syndrome, Patau and Edwards syndromes etc.).
Object of study. Efficient diagnosis and prevention of chromosomal abnormalities and congenital malformation can be assured only by medico-genetic, pedagogical, psychological theory and practice as wide interdisciplinary knowledge on genetic diseases and hereditary pathologies.
Methods. In this research were used a number of practical methods such as gathering of anamnestic data, conversation, questioning, observation, psychological and psycho-pedagogical testing as well as a complex of cytogenetic and molecular-genetic methods. Clinical-genealogical examination of pregnant women from genetic risk group was provided at the beginning of the study.
Results obtained. The diagnosis of chromosomal abnormalities and congenital malformation was provided during medico-genetic counseling and during sessions of qualified psychological assistance, to 74 pregnant women of risk group. Results of the study can be used in family planning and counseling for prophylaxis of congenital pathology as well as to increase the level of awareness of people of risk group on prevention of genetic diseases.
Conclusions. This study stated the necessity of implementation in Moldova of a diversity of medico-genetic strategies during counseling of pregnant women of risk group, for improve the efficiency of diagnosis of chromosomal abnormalities and congenital malformation, which can be achieved by close cooperation between specialists of many fields as genetic physicians, psychologists, pedagogues.

J14.14
Seroprevalence Of Helicobacter Pylori In Children In A Rural Area

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BACKGROUND Helicobacter pylori (Hp) infection has been recognized as a cause of chronic gastritis, peptic ulcer, atrophic gastritis and gastric cancer. **AIM** To analyzed the seroprevalence of Helicobacter pylori in pediatric age in rural area and to evaluate some epidemiologic characteristics. **PATIENTS AND METHODS** the study included 100 patients (80 males; age range 5-13 years) suffering from different gastrointestinal complaints. Blood serology and stool antigen testing were used for the diagnosis of infection due to H. pylori. We interviewed the children with questionnaire about socioeconomics factors, hygiene, living conditions and their dietary habits. **RESULTS** 20 (20%) of the 100 patients were positive for Helicobacter pylori and this positivity had a significantly increasing correlation with age ($p<0.001$). A lower frequency of fermented dairy food, fruits and vegetable consumption was registred among infected children. Among infected patients were noted low socio-economic markers such as croweded living conditions and unclean water. **CONCLUSIONS** Might decrease the risk of Hp infection the use of vitamin C and antioxidants contained in fruit and vegetables. Risk factors for Hp infection are low socioeconomics factors, hygiene and living conditions.

J14.15
Comparison of clinical performances among Roche Cobas HPV, RFMP HPV Papillo Typer and Hybrid Capture 2 assays for detection of high-risk types of human papillomavirus

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High-risk types of human papillomavirus (HR-HPV) is an important cause of cervical cancers. Current cervical cancer screening guidelines suggest that early detection of HPV-16 and HPV-18 may prevent the progression of cervical cancer. We evaluated and compared three HPV DNA tests, Roche Cobas HPV, RFMP HPV Papillo Typer and Hybrid Capture 2 (HC2). Roche Cobas HPV specifically identifies HPV-16 and HPV-18 with concurrently detecting other 12 HR-HPV types and RFMP identifies 74 HPV genotypes. A total of 861 cervical swab specimens from women over 30 years of age were classified into groups of high grade squamous intraepithelial lesion (HSIL) and non-HSIL according to cervical cytology results and analyzed by three assays. The results of direct sequencing or Linear array HPV genotyping test were considered true when three assays presented discrepancies. Concordance rates between Roche Cobas HPV *vs.* RFMP, RFMP *vs.* HC2, and HC2 *vs.* Roche Cobas HPV were 94.5% (814/861), 94.2% (811/861), and 95.8% (825/861), respectively. In 71 specimens with discrepant results, concordance rates between each assay and direct sequencing or Linear array were as follows: Roche Cobas HPV, 35.2%; RFMP, 93.0%; HC2, 25.4%. Clinical sensitivities and specificities for detecting HSIL were 80.3% and 95.8% with Roche Cobas HPV, 83.6% and 95.1% with RFMP and 90.2% and 94.8% with HC2. In conclusion, Roche Cobas HPV, RFMP and HC2 showed high agreement rates each other. Although Roche Cobas HPV and RFMP showed lower clinical sensitivity in detecting HSIL compared to HC2, they would be clinically useful since both provide HPV genotypes.

J14.16
Absolute quantification of transcripts in single leukaemic cells

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We and others have shown that the BCR-ABL mRNA expression is higher in the CD34+ cellular population, representing an enrichment of BCR-ABL+ cells in the progenitor fraction or a higher expression of the fusion gene. Primitive progenitor BCR-ABL+ cells isolated from CML patients will maintain their functionality after Imatinib treatment. Therefore, CML progenitor cells appear to be more insensitive to Imatinib than more mature cells suggesting that TKIs are unable to inhibit the function of the primitive leukaemic stem cells. During the assessment of minimal residual disease (MRD) by RT-qPCR, some of the early leukaemic progenitors of malignant cells with significantly higher levels of BCR-ABL transcript cannot be differentiated from other groups of homogeneous cells. This fact may cause a failure to perform personalised monitoring of MRD; while the sensitivity and reliability of the

assay are directly affected by bulk measurements where the number of normal cells that do not express the target genes. In this study, K562 cells were grown in suspension and a small fraction of these cells was noted to have adhered to the plastic dish after the removal of the media. These adherent cells (K562/Adh) were passaged serially. The FACS separated individual K562 cells into PCR wells with a fixed amount of qPCR buffer. Optimized amplification RT-qPCR was performed on single cells to reveal the nature and significance of cellular heterogeneity with the measurement of BCR-ABL levels in K562 cells was investigated.

J14.17
Novel methods for phenotyping mice pups during the first few hours after birth

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Mice are the preferred species to study the biological function of mammalian genes through mutation but there are still specific challenges to solve in order to characterize their phenotype. As early lethality is common in genetically engineered mice, an important proportion of homozygous mutant mice cannot be phenotyped. When the corresponding heterozygous mice have no phenotype, the effects of this mutation remain unknown. Unfortunately, studies in newborn mice face major difficulties due to the small size of the pups and their sensory and motor immaturity. In order to gain access to the early post-natal phenotype, we developed three dedicated non-invasive devices, suitable for high-throughput screening: - PhysioPups (patented device) is used to assess vital functions: breathing patterns, electrocardiogram (ECG), body temperature, gross body mov ements, and ultrasonic vocalizations. Four animals can be tested simultaneously under controlled conditions of temperature and gas composition. - NeoGAIT is used to assess gait ontogeny. It uses infrared detection of the animal's contact patterns with the floor and custom image analysis software. - MemoryPups is used to assess memory and associative learning abilities. The tests are based on olfaction and thermotactile sensitivity, the only sensory functions operating at birth. This platform has no equivalent worldwide. It allows phenotyping mice pups from the first few hours after birth until weaning. It is currently used to characterize genetically engineered mice, including models of genetic diseases (autism, Prader-Willi, Ondine's syndrome) and to study the toxicity of anti-infectious drugs used in neonates (eg. European TINN project).

J14.18
A comparative study of MPCR analysis for deletion detection within DMD gene using different sets of primers

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Background: Duchenne/Becker muscular dystrophy is X-linked recessive, progressive muscle-wasting disease affecting 1 in 3500 boys, caused by mutations within DMD gene. The patients exhibit pathological deletions in approximately 60% of cases. Since 1988 existing reactions for the multiplex PCR amplification of exons in the dystrophin gene have been modified. We aimed to verify the reliability of the multiplex PCR amplification using two different primers sets in order to define the borders of the deletions. Methods: The patients were diagnosed using standard clinical diagnostic criteria including: EMG and serum creatine kinase (CK) level. Three MPCR assays were performed on 80 DNA samples of patients to amplify 24 DMD gene exons using combined sets of primers designed by Chamberlain and Ashton. Results: All the patients showed a raised serum CK level than normal. MPCR analysis in 4 patients showed deletions of 45 and 48 exons using Chamberlain's primers set. Deletions weren't confirmed by Ashton analysis. The length of 45 and 48 exons amplified using Chamberlain's primers are more extended than Ashton's primers and overlap them. Conclusions: A comparative study of 4 patients showed discrepancies between the results obtained by using primers designed by Chamberlain and Ashton for 45 and 48 exons. MPCR analysis is a reliable method for deletion detection but a noncontiguous and single-exon deletions within DMD gene should be interpreted with caution and confirmed with another technique or using alternative sets of primers to exactly mapping the end point of deletions.

J14.19
NGS targeted resequencing of multiple genes for rare diseases in Bulgaria

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During last years the next generation sequencing (NGS) technologies have significantly enhanced the discovery of causative genetic alterations and identification of mutations in rare diseases. The diseases with locus heterogeneity are still a challenge in clinical practice and sometimes patients remain for years with unknown genetic status.
In order to accelerate the establishment of proper genetic status of patients with rare diseases we analyzed exons of 552 genes underlying rare monogenic recessive disorders using TruSight Inherited Disease Sequencing Panel (Illumina). As model we used diseases with clear unequivocal phenotype (CF, DMD) and included in the study patients in whom routine DNA testing was insufficient to obtain genetic diagnosis. The study included 4 patients: 2 with CF with identified only one mutation, 1 with DMD without deletions/duplications in DMD gene and 1 with polymalformative syndrome including Dandy-Walker anomaly, AVSD, hypoplastic genitalia, MR and facial dysmorphism. NGS revealed CFTR: p.Gly1349Asp as second pathogenic mutation in one patient with CF and DMD: p.His2921Arg in patient with DMD. Both mutations are reported for first time in patients of Bulgarian descent. In one patient with CF NGS revealed only one causative mutation and MLPA showed a large deletion encompassing exons 18-20 of CFTR gene. A homozygous in-frame deletion p.Ser372del in MKS1 gene was found in the patient with polymalformative syndrome.
The NGS technology has the potential quickly and cost-effectively to point out disease causing mutations in patients with rare genetic diseases and gives us the opportunity for better genetic counseling and prophylaxis in affected families.

J14.20
Non-invasive prenatal testing of chromosomal aneuploidies by massively parallel sequencing of circulating free DNA

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Background: cell free fetal DNA (cffDNA) circulates in low concentration in maternal plasma and is a source of genetic material for safe non-invasive prenatal testing (NIPT) by parallel massive sequencing techniques. Aims: validation of a protocol for NIPT and optimization of a method for fetal fraction detection. Methods: low coverage whole genome sequencing was performed on plasma DNA samples from 175 pregnant women (8 - 10 weeks of gestation) for the detection of aneuplodies. Digital PCR assay was used for fetal fraction detection by quantification of RASSF1A, TERT and beta-actin genes. Sequenced reads were mapped against the human reference genome hg19 by BWA. Duplicated reads were removed and unique mapped reads were counted (mapping quality > 20 was required). Read count for each chromosome was normalized based on library size and GC content. Chromosomal aneuploidies were detected by using a z-score statistics. Reference dataset was built by including euploid samples selected on the basis of the correlation between z-scores and coverage in 5 aneuploid cases. This allowed us to estimate the best trade-off between coverage (> 0.4) and sample size (= 140). In the male fetuses, fetal fraction was detected by measuring normalized read count on chromosome Y. Results: sequencing of 175 euploid samples allowed us to set up the experimental protocol and to create the reference dataset. The performance of the dataset was assessed by blinded analysis of twenty pregnancies. Five trisomy 21 and fifteen euploid fetuses were correctly identified by our analysis.

J14.21
FISH and methylation studies in Egyptian children with Prader-Willi syndrome

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Prader -Willi syndrome (PWS) is a complex, multisystem disorders of genetic origin due to lack of paternally active genes on critical area on chromosome (15) (q11-13) . PWS is almost always sporadic and is the most common syndromal cause of human obesity with an estimated incidence of 1 in 25,000 Material and methods:Conventional G-banding, FISH using an SNRPN Prader-Willi /Angelman chromosome region probe by Appligene-Oncor and methylation specific-PCR studies were conducted on 23 patients (13 males and 10 females age ranged from 6.5 months to 20 years selected according to suggested criteria proposed by Gunay- Aygun et al, 2001. Aim: This study aims at confirming/ excluding PWS in clinically suspected patients through a sensible strategy of investigations on the cytogenetic level, FISH technique and molecular level using DNA methylation tests for phenotype/ genotype correlation, early diagnosis and proper counseling. Results: On the cytogenetic level, 10 out of 23 cases showed deletion of 15q (11-

13). FISH confirmed the deletion in in 6 cases with mosaicism in one case (50%); detected deletion in 4 cases with normal cytogenetic findings, and excluded deletion in 13 cases. Molecular methylation studies conducted on 17 patients (6 cases spared upon request) confirmed diagnosis in 10 cases detected 1 new case and excluded PWS in 6 cases. In conclusion: we reinforce the necessity of analysis of DNA methylation within the chromosome 15q 11-13 region, which is an important tool for the correct diagnosis and for detecting etiology among children presenting with neonatal hypotonia, mental deficiency and obesity.

J14.22
Procalcitonin Gene Expression is a Real Biomarker of Aging Process

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Objective: Inflammation is the single greatest precipitator of aging and age-related diseases such as, diabetes, heart disease, alzheimer, arthritis. Inflammation, which takes place on a cellular level, is triggered by a wide variety of factors such as the oxidative stress, hormonal changes and eating a pro-inflammatory diet (Hyperglycemic diet). The procalcitonin gene has promoter sites for NF-kappaβ and AP-1, factors induced under inflammatory conditions. Hyperglycemia is associated with oxidative stress and elevation of advanced glycation end products (AGEs). AGE's interacts the receptor for advanced glycation end products (RAGE) and, RAGE activation is caused by elevation of transcriptional factors NF- kappaβ and AP-1. These factors induce procalcitonin gene expression. The aim of this study was to determine whether or not procalcitonin is a specific marker of aging process. **Research Design and Methods:** 14 type II Diabetes Mellitus (DM) patients within hyperglycemia were studied along with age and sex matched with normal normoglycemic subjects(NNS). Blood samples were taken for measurements of procalcitoninin and analized with kryptor analizator. Procalcitonin levels were elevated in DM patients within hyperglycemia when compared with NNS. There was a statistically significant difference in serum procalcitonin of DM patients within hyperglycemia versus NNS($P<0.01$). **Conclusion:** Our study revealed a raise in serum procalcitonin in DM patients within hyperglycemia. Hyperglycemia associated with increased circulating concentrations of procalcitonin may be a mechanism which explains many of the clinical and biochemical features of aging process. Procalcitonin is a new aging biomarker which can help for discover new drugs to combat aging.

J14.23
Targeted DNA sequencing analysis reveals heterogeneity of single-cell somatic mutations

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Research linking genetic variants to disease has been challenging in the past, because traditional approaches are commonly limited to detecting high frequency variants within a bulk sample. This approach assumes that the sample is comprised of identical cells with the same variants. However, in the case of cancer genomics, somatic mutations accumulate over time and a single tumor can give rise to multiple sub-clones. Employing a multi-cell based DNA sequencing strategy to heterogeneous samples can mask low abundant variants and make it difficult to distinguish them from sequencing errors. Sequencing the genome of individual cells, however, circumvents the problem and provides deeper insight into the molecular heterogeneity within the cell population. The existing single cell whole genome amplification methods and library preparation are costly, laborious, and subject to handling errors. We have developed a method for whole genome amplification (WGA) of single cells using an integrated microfluidic system to automate the capture, lysis, and DNA amplification from up to 96 individual cells in a single workflow. Amplified products harvested from the system are ready for downstream library preparation and targeted, whole exome or whole genome sequencing. Whole genome sequencing results of a few cells indicate improvement in genome coverage, uniformity and GC bias over state-of-the-art WGA methods. We also demonstrate targeted sequencing performance of over 100 single cells compared to bulk unamplified genomic DNA, and show single nucleotide variant distributions in normal/disease-paired breast cancer cells.

J14.24
Interpretation of sex chromosome abnormalities by SNP-array analysis

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Sex chromosome abnormalities represent a special challenge in the analysis of SNP-array data. Data analysis is complicated by the complex structure of the sex chromosomes (PAR regions, interfering homologous regions and only one of each sex chromosome in males). At present, no guidelines can help interpretate the analysis of sex chromosome abnormalities identified by SNP array.
In this study we analysed SNP-array data from 25 samples with known sex chromosome abnormalities.
We present the results from this study and present a general guideline for SNP-array interpretation of sex chromosomes. Hopefully this will aid others in the interpretation of sex chromosome abnormalities identified by SNP-array.

J14.26
NextGen Sequencing: a tool for deciphering the BRCA1/2 and TNBC relationship

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Although during recent years science and technology have generated a great deal of progress in medicine, breast cancer still is the most common malignancy and the leading cause of death by cancer in women. Triple negative breast cancer (TNBC), characterized by the absence of Estrogen and Progesterone receptors and Her2/neu, affects 15-20% of breast cancer patients, has a more aggressive phenotype and occurs at younger ages. Because of its therapeutic limitations, TNBC needs to be investigated in more detail with the help of new technologies. This is why our study focuses on exploring the mutation status of BRCA1/2 genes involved in hereditary breast cancer, and with known mutations in TNBC. This retrospective study on 30 TNBC patients who underwent surgery at The Oncology Institute "Prof. Dr. I. Chiricuta" Cluj-Napoca was conducted with the help of the next generation sequencing platform Ion Torrent PGM. After sequencing the DNA extracted from FFPE tissue, we observed that 20 of the 30 patients presented germline BRCA1/2 mutations, of which seven in BRCA2, 10 in BRCA1, and three in both genes. We identified two mutations that are frequent in patients of European descent with hereditary breast cancers, two that are similar to mutations identified in families of Swedish origin, and several other new mutations. This is the first study that investigates BRCA1/2 mutations in TNBC patients in Romania, and proves that NextGen Sequencing is a competitive and cost-effective BRCA screening method especially in low-income countries where patients cannot afford early breast cancer diagnosis.

J14.27
Comparison of variant calling from whole exome and transcriptome sequencing using CLC Cancer Research Workbench

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Some of the major reasons for high throughput sequencing being more and more frequently applied in cancer research and cancer diagnostics today, are the significant improvements in accuracy, throughput, and speed. In the past, sequencing was clearly the bottleneck but the most time consuming step currently is the data analysis - more precisely the identification and characterization of variants in tumor samples. Whole exome and genome sequencing protocols are commonly used and allow for the identification of SNPs and InDels in protein coding regions. However, both methods fail to show which allele variants are actually expressed in the tumor tissue. Contrariwise transcriptome sequencing (RNA-seq) reveals the expressed alleles and can in addition provide insight into the transcript expression levels, expressed isoforms, and instances of fusion transcripts. Here, we illustrate the benefits of combining the analysis of whole exome sequencing and RNA-seq using CLC Cancer Research Workbench. This software suite includes algorithms for quality control, read mapping, variant calling, and RNA-seq analysis, as well as numerous tools for the comparison and annotation of variants. The analysis is carried out with ready-to-use workflows and the results are visualized in a track based genome browser. In the present work differences found between the variants identified in exome and in RNA-seq data from uveal melanoma samples will be highlighted.

J14.28
Molecular-genetic analysis of predisposition to ovarian cancer among women of Uzbekistan

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Ovarian cancer is the 9th most common cancer, with an estimated 22240 new

cases in 2013. Most women aren't diagnosed until the cancer has spread, leading to a poor five-year survival rate of 43%. Only 10-15% of malignant epithelial ovarian tumors are genetically determined.
The most significant genes associated with ovarian cancer include BRCA1, BRCA2, CHEK2. These genes produce tumor suppressor proteins which help repair damaged DNA and play a role in ensuring the stability of the cell's genetic material. When either of these genes is mutated, DNA damage may not be repaired properly. As a result, cells are more likely to develop additional genetic alterations that can lead to cancer.
This study aimed to investigate the contribution mutations of BRCA1, BRCA2 and CHEK2 genes to ovarian cancer cases in Uzbekistan.
66 patients with ovarian cancer were included in this study. By means of real-time allele-specific PCR we analyzed DNA samples of above mentioned group for the presence of 5382insC mutation in BRCA1, 6174delT - in BRCA2 and IVS2+1G>A - in CHEK2. 6 unrelated samples (9.1%) were found to be positive for the heterozygous 5382insC mutation representing a possible founder mutation in the Uzbek population. We didn't find 6174delT and IVS2+1G>A mutations. The presented data confirm contribution of BRCA1 5382insC mutation for ovarian cancer development in Uzbek people and taking into account a high disease penetrance in carriers of BRCA1 mutation, it seems reasonable to suggest inclusion of 5382insC mutation test in screening programs for breast cancer prevention in Uzbekistan.

J14.29
Multiple genes of small effect and their interactions with environmental factors explain variation in personality traits

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Personality traits are thought to be endophenotypes (high Harm Avoidance (HA), low Self-directedness (SD)) for most psychiatric disorders and predictors of life outcomes. Genetic influences on personality traits are attributable to many genes of small effect and are modulated by environmental factors.
We aimed to examine gene-environment (GxE) and gene-gene (GxG) interaction models based on neurotrophic factor (NGF, BDNF, NTRK2, NTRK3), serotoninergic (SLC6A4, TPH1) and dopaminergic system (DRD2, SLC6A3) gene polymorphisms contributing into personality traits variation in healthy individuals.
In total, 1018 healthy individuals (68% women) from Russia (mean age±SD: 19.81±2.65 years) without any history of psychopathologies were subjected to personality traits assessment via TCI-125 (Cloninger et al., 1993). Involved individuals are Caucasians from Russian (N=409), Tatar (N=290), Bashkir (N=130) and Udmurt populations (N=189). Socio-demographic data including gender, ethnicity, order and season of birth (SOB), place of residence, level of income, childhood maltreatment were obtained. Genotyping of 70 SNPs was performed with SNPlexTM platform (Applied Biosystems). Statistical analysis was conducted with PLINK v.1.07 corrected via FDR-procedure for multiple comparisons.
The present study revealed GxE models demonstrated BDNF Val66Met*SOB (PFDR=0.036), BDNF rs1519479*ethnicity (PFDR=0.042) and 5-HTTLPR*SOB (PFDR=0.05) interactions affected HA. Moreover, variations in SD were caused by interactions between BDNF Val66Met (PFDR=0.048), BDNF rs2030323 (PFDR=0.035) and ethnicity. Accordingly, genetic testing for BDNF and 5-HTT gene polymorphisms assuming gender, ethnicity and SOB confounding is necessary for psychopathologies prevention at early stages.
This work was supported by Russian foundation for humanities grant 13-06-00583a.

J14.30
Validation of a CE marked tool (SEQPRO LIPO RS) based on Next Generation Sequencing for diagnosis of Familial Hypercholesterolemia

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Familial Hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations in the LDLR, APOB and PCSK9 genes. A recessive form is caused by mutations in the LDLRAP1 gene. More than 1500 mutations have been described worldwide, with a mutation spectrum varying depending on the countries. In order to screen any population, we decided to develop a kit (SEQPRO LIPO RS) based on Next Generation Sequencing (NGS). In 2013 we launched a NGS Roche 454 based product with CE mark. We describe here part of the validation of this product in order to obtain the CE mark.
Around 300 patients previously sequenced by Sanger sequencing were used to determine the analytical specificity and sensitivity of the method for point mutation (substitutions and indels), while more than 500 patients previously screened by MLPA were used to verify the ability of the method to detect Copy Number Variations (CNVs) in the LDLR gene.
Reliable variant detection for up to 20 FH patients per run was achieved, with accuracy, specificity and sensitivity similar to Sanger sequencing and MLPA. This algorithm has been fully integrated in software that names the variants according to the HGVS nomenclature, and performs a search in our mutation database, while providing the variants' pathogenicity status.
The SEQPRO LIPO RS tool is feasible for FH detection. Moreover, it serves as a proof of principle for the development of genetic diagnostic tests for any other disease caused by both point mutations and CNVs, cardiovascular or not.

J14.31
DNA microchips for the diagnostics of monogenic hereditary disease in Yakuts

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Yakuts belongs to the monogenic populations with ethnicity specific forms of pathology, with the frequency significantly more higher than in other populations in the world.The genetics research on high frequency diseases in the Republic of Sakha showed the presence of major mutations in the genes specific and often found among people of Yakut origin.One of the major problems for now is to do a heterozygosity screening for found mutations and to organize preventive care program. Until now there are 20 hereditary diseases can be diagnosed in the Republic of Sakha by routine methods such as PCR and restriction.Unfortunately these methods are very time consuming. Alternative method that has been emerging rapidly is a DNA microarray.This main advantage of this method is a possibility to screen thousands of mutations at once making the diagnostic simple and fast. In collaboration with our colleagues from Busan National University(South Korea) there was established a testing system based on DNA microarray for the diagnostic of five high frequency diseases: Enzymopathic methemoglobinemia (OMIM 250800, Pro269Leu mutation in DIA1 gene), 3 M syndrome(OMIM 273750,4582insT in Cul7 gene), SOPH syndrome (OMIM 614800, G5741 A in NAG gene),Nonsyndromic hearing loss 1A type (OMIM220290, IVS1+1G>A in GJB2 gene), Tyrosinemia type1 (OMIM 276700 1090G>C mutation in FAH gene). Since the microarray is well known and rapidly developing, the heterozygocity screening itself using the microarray rather than then microarray establishment have the significance. It will allow reducing the frequency of these diseases in the Republic and the risk of diseased child birth.

J14.32
Pitfalls in molecular genetic testing of m.8344 A>G (MERRF) mutation

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For identification the common mitochondrial 8344 A>G mutation resulting in MERRF (myoclonus epilepsy with ragged red fibers) syndrome a worldwide used molecular diagnostic method is a PCR-RFLP method, using the BanII restriction enzyme. So far it was not a matter of common knowledge, that it is not a trusty technique.
Methods. Mitochondrial 8344 A>G mutation was investigated by PCR-RFLP using BanII restriction enzyme on DNA samples (n=1073). In cases with abnormal restriction pattern mitochondrial tRNA lysin gene region (nt 8105-8536) was sequenced bidirectionally (n=14).
Results. PCR and BanII RFLP analysis detected abnormal restriction pattern indicating m.8344 A>G mutation in 14 cases. Sequence analysis of tRNA lysin gene verified the presence of the m.8344 A>G substitution only in 6 cases. Unexpectedly the m.8347 A>C substitution was present in 8 cases with 9-bp triplication (8272-8280) in 3 samples. The gelphoto showed nearly the same pattern in cases with m.8347 A>C mutation or 9-bp triplication. The BanII cleaves samples with m.8347 A>C and m.8344 A>G substitutions on the same way. The 8347 A>C mutation was prevoiusly not described as pathogenic alteration, however it is a highly conserved nucelotid and has similar effect on the tRNALys as the 8344 A>G mutation. The 9-bp triplication is a polymorphism, its anthropological interest is controversial.
Conclusion. The PCR-RFLP method using BanII restriction enzyme is not a solid method for detecting m.8344 A>G mutation. It may result in pseudo positivity. We suggest to use it only as a screening method, which must be validated always by Sanger sequencing.

J15.01

Brain-derived neurotrophic factor Val66Met polymorphism and cardiac stress reactivity

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Twin studies indicated that heart rate variability (HRV) and particularly its high frequency (HF) spectral component show significant heritability, and their genetic variance is amplified by mental stress. This genetic association study investigated the influence of the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and the triallelic serotonin transporter gene promoter (5-HTTLPR) polymorphism on state anxiety and time and frequency measures of HRV reactivity to mental stress. In comparison to BDNF Val homozygotes, the Met allele carriers showed larger cardiovagal withdrawal during stress. The triallelic 5-HTTLPR did not significantly influence any of the psychophysiological measures. Although BDNF Val66Met only explained a small part of the variance of cardiovagal withdrawal during stress, this polymorphism may be part of a complex genetic pathway underlying the comorbidity of autonomic dysfunctions and emotional vulnerability.

J15.02

Heterogeneity of Tumors - comprehensive biomarker-panels to decipher cancer

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Personalized cancer treatment increasingly enters into clinical routine. Due to the fact that each tumor is individual regarding its genomic structure, the screening for these changes on DNA and RNA level in tumor tissue is becoming increasingly crucial for an individually effective therapy. Drugs like EGFR-Inhibitors in particular and tyrosine kinase inhibitors in general have proven to benefit greatly from companion diagnostics. Despite this fact, however, expectations of clinical impact on the well being of patients were bigger than what was truly observed. Vast improvements were merely seen in a fraction of treated individuals, making a general assumption less than relevant. Even in patients exhibiting great initial response subsequent relapse was quick to follow. Hence a broader approach of analyzing comprehensive biomarker-panels by various techniques should be taken into consideration to account also for heterogeneity and predict maybe the next phase of tumor expansion. After a short overview of high throughput techniques like next generation sequencing and its combination with routine methods like pathological examination, micro-dissection and purification of DNA/RNA, mutation analyses via high resolution melting (HRM), gene expression profiling and epigenetic analysis, the combination of these multiple approaches for personalized cancer therapy are highlighted. The clinical use of this novel and comprehensive approach for an improved diagnostics and thus more effective treatment is demonstrated on the example of prostate and colorectal cancer.

J15.03

The investigation of *BRCA1* and *BRCA2* expression in colorectal cancer

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BRCA1 and *BRCA2* genes are suppressor of malignant tumor and predictive markers sensitivity to platinum-drugs. Malignant transformation and carcinogenesis accompanied by alters the expression of tumor-suppressor genes. Loss of any of these genes expression is associated with positive response on treatment by platinum-drugs. Tumor and normal tissue samples of colon among 30 patients with colorectal cancer were investigated. High level (3 and more time) of *BRCA1* and *BRCA2* expression was found in 22/30 (70%) tumors and 24/30 (80%), respectively. As well high expression of both genes was detected in 19 tumors. The expression of *BRCA1* was not changed in 7 of 30 (23%) tumors and in one tumor was decreased in 7 times. The expression of *BRCA2* was not changed in 4 of 30 (13,3%) tumors and was decreased (in 7 times) in two tumors. The expressions of both the genes were not altered in two cases. However *BRCA1* and *BRCA2* expression was not a coordinately decreasing in neither case. We obtained information about change *BRCA1* and *BRCA2* expression in tumor for Russian patients with colorectal cancer. These data can improve the therapy for colorectal cancer patients by platinum-drugs.

J15.04

The effects of hypericin on p53 and ADAMTS gene expression in MCF-7 breast cancer cell line

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Our objective was to determine the effects of hypericin on MCF-7 breast cancer cells, as it is known to have an anti-tumor effect on the expression and regulation of ADAMTS1, 3, 10 and the P53 gene in breast cancer cells. MFC-7 cells were cultivated and subjected separately to various doses of 1, 5 and 7.5 µg/mL hypericin. After 24 h, RNA was isolated and transcribed into cDNA. Expression analysis was performed by real-time RT-PCR and cell survival was determined by XTT assay. While expression of ADAMTS1 in MFC-7 cells decreased to 0.04 fold after exposure to 1 µg /mL hypericin, expression increased by 5.6- and 36-fold at 5 mg/mL and 7.5µg/mL, respectively. Furthermore, ADAMTS3 expression in MCF7 cells were increased 3.9 fold with the use of 5 µg /mL of hypericin. These concentrations of hypericin did not lead to significant changes in the expression of ADAMTS10 and the P53 gene. XTT tests have shown that hypericin concentration of 7.5 µg /mL lead to significant death of cancer cells. The increase in ADAMTS1 expression may prevent metastasis or facilitate development of an adjuvant factor with tumor-suppressive effects. Hypericin may therefore exert its anti-tumoral and apoptotic effects in MFC-7 cells via ADAMTS1 and ADAMTS3.

J15.05

Effect of Hypericin on the ADAMTS-8 and ADAMTS-9 gene expression in MCF7 breast cancer cells

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Our aim was to investigate the effects of hypericin which is obtained from the plant Hypericum perforatum on the expression and the regulation of ADAMTS8 and ADAMTS9 genes in MCF7 breast cancer cells and on the viability of these cells. MCF7 cells were cultured and were separately exposed to 2, 10 and 50 µl/mL of hypericin. After 24 hours, RNA was isolated from these cells and converted to cDNA. The expression levels of ADAMTS8 and ADAMTS9 genes were evaluated using the real-time RT-PCR. XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, disodium salt) cell viability assay was used to determine cytotoxicity. ADAMTS9 expression in MCF7 cells were increased 1.8 and 3.6 fold with the use of 2 and 10 µl/mL of hypericin, respectively; and decreased 0.7 fold with the use of 50 µl/mL of hypericin. There was no significant change in the ADAMTS8 expression. Rapid cell death was observed in the cancer cells when hypericin was used at a dose of ≥ 50 µl/mL. The increase in ADAMTS9 expression can be a useful factor in the prevention of possible metastasis in breast cancer and for the occurrence of a tumor suppressive effect. Hypericin increases the expression of ADAMTS9, therefore, it may show its antitumoral and antiapoptotic effects by means of ADAMTS9.

J15.06

Effects of tomatine in breast cancer

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Metastasis in breast cancer is still a complicated issue and matrix metalloproteinase family has a significant role in metastasis showing the link between MMP members and tumor development. Tomatine is a secondary metabolite synthesized by tomatoes and has a role in natural defenses against plant fungi, viruses and bacteria. Besides, it is known to disrupt cell membranes and be a strong inhibitor of human cancer cells. In this study, it was aimed to evaluate the effect of tomatine on cytotoxicity and apoptosis on MCF-7 cell line. Firstly, IC50 dose of tomatine was measured as 7.07 µM by using xCELLigence system. Further, it was shown that tomatine induced apoptosis is 3 times greater than control cells with by Annexin V labeling on Flow Cytometry . Additionally, miRNA expression profiles of breast cancer cells were determined by qRT-PCR and 22 downregulated and 15 upregulated miRNAs were found. Active and zymogen forms of MMP-2 and MMP-9 were detected by zymography method and the activations of MMP2 and

MMP9 were decreased significantly. In conclusion, these results show the suppression of matrix metalloproteinase activity on metastasis.

J15.07

Aberrant miRNA Expression by Ellagic Acid Alters Apoptosis and Cell Cycle Regulation in Breast Cancer Cells and Breast Cancer Stem Cells

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Breast cancer has still been the most important subject with other cancer types. This cancer is the most common and second leading cause of death among women. It is known that various plant extracts, including ellagic acid, have anti-proliferative and pro-apoptotic effect on breast cancer cells. However, miRNAs which are associated with tumor initiation for estrogen dependent breast cancer are suppressed by ellagic acid treatment. In this study, it was aimed that ellagic acid could induce apoptosis related mechanism in breast cancer cells and this induction could be trigged by altered expression profiles of miRNAs. Cytotoxic effects of ellagic acid on MCF-7 breast cancer cells and breast cancer stem cells were examined by WST-1 reagent test. Apoptosis and cell cycle analysis were detected by flow cytometry analysis. After ellagic acid treatment, miRNA expression profiles of breast cancer stem cells were determined by RT-PCR. While ellagic acid had no any cytotoxic effect on MCF-7 breast cancer cells, cytotoxic value of ellagic acid for breast cancer stem cells was detected as 24.8 µM dose. Ellagic acid did not induce apoptosis in both cell groups and increased living cells. In the cell cycle, S arrest was observed in MCF-7 breast cancer cells as well as breast cancer stem cells. When analyzed in terms of miRNAs profiles, ellagic acid generally suppressed expression of miRNAs and expression profiles were associated to oncogenic effect. It was suggested that ellagic acid may not be chemo-preventive agent because of miRNA profiles, have oncogene effect.

J15.08

Development of targets for colorectal cancer treatment on a base of small interfering RNA and functional genomics

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The significance of genes for tumor cell viability and understanding of the cell genetic features under which high apoptotic effect of gene silencing will observed - both are important in development of targets. We were searching of genes, silencing of that will increase sensitivity of colorectal cancer cells to oxaliplatin. The panel of genes was formed using of expression data bases and publications analysis. The expression gene profile was determined in dependence on the dose and the time of oxaliplatin action for two colorectal cancer cell lines: HT-29 and HCT-116 $p53^{-/-}$. The over expressed and demonstrating dose dependence under different period of incubation genes have been identified. It was interesting that the identified genes - *c-IAP2* and *Livin* - were the same for both cell lines, although the lines are different genetically. The silencing of genes was performed by small interfering RNA (siRNA). Joint silencing of the gene found has led to a substantial (70-80%) suppression of the cell viability and increase of 5 -10 times the sensitivity of cancer cells to small oxaliplatin doses (5 - 10 µM). Common for HT-29 and HCT-116 is mutation in *TP53* gene. Possibly, this feature is responsible for the similarity of response to oxaliplatin genetically different cancer cells. In conclusion, there were found the genes silencing of that by siRNA gives the possibility to reduce approximately of 10 times the standard dose of chemotherapeutic drug with reaching a high apoptotic effect on genetically different colorectal cancer cells.

J15.09

Analysis of CYP2E1 gene using multiplex HRMA. A study in the Polish patients under sevoflurane anaesthesia

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The CYP2E1 gene encodes a member of the cytochrome P450 superfamily enzymes, which play a key role in the metabolism of inhaled anaesthetics as worldwide used sevoflurane. Potentially nontoxic anaesthetics are converted into simple organic compounds, inorganic fluoride and also vinyl ether, a product of sevoflurane breakdown, which may cause nephro- and hepatotoxicity. Investigations on genetic variability of CYP2E1 gene and the possibility to associate the results with clinical effect of metabolized sevoflurane provide the basis for the development of personalized anaesthesia.

The aim of this study was to establish a simple and economical method for analysis of whole coding sequence of the CYP2E1 and to determine the prevalence of sequence variations in this gene among the Polish patients under sevoflurane anaesthesia. Here we present the molecular test based on multiplex HRMA. The entire coding sequence of the CYP2E1 gene was successful amplified using 14 pairs of primers in the 7 multiplex reactions using the Rotor-GeneQ equipment [Qiagen].

We have analyzed DNA from 41 individuals. After HRMA screening, selected samples with different melting profiles, were sequenced. Among the tested samples we observed relatively high diversity of low-frequency changes. We found 5 different sequence variations, where two are novel changes: p.Gln75Leu (1.2%) and p.His226Tyr (1.2%). The frequencies of known polymorphisms (p.Gly173Ser, rs60452492 - 1.2%; p.Val179Ile, rs6413419 - 1.2%; p.Phe421Phe, rs2515641 - 9.8%) are similar to the other Caucasian populations.

J15.10

Epigallocatechin gallate as inducer of cell death and apoptosis in human oral squamous carcinoma cell line

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The antitumor effects of the green tea compound epigallocatechin-3-gallate (EGCG) have not been studied in detail previously in oral squamous cell carcinoma cells. Expression of the proapoptotic protein (p53) occurs frequently in oral squamous carcinoma as being overexpressed as mutant or oncogene or knockdown as suppressor gene, which is an adverse prognostic factor. Therefore, we examined in detail the molecular effects of EGCG on focusing on the apoptosis signaling pathway, in SSC-4 cell line. Human oral squamous cell carcinoma is a form of cancer that presents a poor prognosis, therefore is an urgent need to determine the molecular mechanisms involved in tumor cell survival and invasion. EGCG is a natural phytochemical previously indicated as chemopreventive and chemotherapeutic agent in multiple cancer types. In the case of treatment with low doses of EGCG, we observed increased apoptosis as and reduced proliferation and invasion as displays the xCELLigence data on SSC-4 oral squamous carcinoma cells. This may be due to the inhibition of pro-survival genes and the activation of cell death mechanisms as results from the qRT-PCR evaluation. EGCG has been shown to exhibit antitumor activities in the case of SSC-4 cell line, and this finding supports the therapeutic implication in oral squamous carcinoma.

J15.11

Epigallocathechin-3-gallate and zoledronic acid induce apoptosis via down-regulation of miR-17-5p and miR-20a-5p in chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is a clonal disorder of hematopoietic stem cells. Epigallocathecin-3-gallate (EGCG) is a major flavonoid of green tea. Zoledronic acid (ZA) is a nitrogen-containing bisphosphonate. MicroRNAs (miRNAs) are small, single strand, non-coding RNA molecules that are responsible for post-translational gene regulation. The aim of this study is determine expression changes of miRNAs related with leukemogenesis with the treatment of EGCG and also define cytotoxic and apoptotic effects of EGCG in K562 human CML cell line. The cytotoxic effects of EGCG on K562 cells were determined in time and dose dependent manner. Total RNA, including miRNAs, was isolated from K562 cells treated with EGCG and ZA 24h and untreated cells as control group. Reverse transcription procedure was performed for cDNA synthesis. Apoptosis assays were performed by using ApoDIRECT Assay. miRNA expressions were showed by RT-qPCR. Expression results were analyzed by using miScript miRNA PCR Array Data Analysis. IC50 value of EGCG and ZA were determined as 50, 60µM, respectively. EGCG

and ZA induced apoptosis 10.9, 2.3 fold and down-regulated miR-17-5p and miR-20a-5p 2.5758, 20.3332 fold according to the control cells, respectively. Up-regulation of miR17-92 cluster, the best defined oncomir group, was associated with a lot of cancer types. Our findings showed that treatment of EGCG and ZA triggered down-regulation of miR-17-5p and miR-20a-5p expression levels which are member of miR17-92 oncomir cluster in K562 CML cells. These novel findings can be valued by exploring the targets of these aberrated oncomirs in leukemia progression.

J15.12

The Effect of Fluoride Agents on the Expression Levels of Pro and Anti-apoptotic Genes in Human Gingival Fibroblasts

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Fluoride is widely used to prevent dental caries in dentistry. It is cytotoxic and produces inflammatory responses in human. The aim of this study was to investigate the effect of fluoride usage on expression levels of BCL-2, BAX, BAD, FASL, P53, CASPASE 3, CASPASE 8, CASPASE 9. Five different fluoride agents [1.23% Acidulated Phosphate Fluoride (APF) and 2% Sodium Fluoride (NaF) gels, 1% Titanium Tetrafluoride (TiF4) and 38% Silver Diamine Fluoride (SDF) solutions and Duraphat Fluoride Varnish containing %5 NaF] were used in human gingival fibroblast cell lines which were obtained from healthy individuals. Gene expressions were analyzed by using real time RT-PCR. No significant change was detected in the expression levels of those genes in NaF group. The expression levels of BAD did not significantly change in all study groups as well. The ratio of pro-apoptotic BAX to anti-apoptotic BCL-2 was greater than 1 which demonstrates that those fluoride agents cause induction of apoptosis. In conclusion we suggest to protect the soft tissues during the application of the fluoride agents, to be careful for patients not to swallow the fluoride agents and to avoid unnecessary usage of fluoride in clinics..

J15.13

Implication of HLA B27 and ITPA polymorphisms in treatment response of HCV infected patients

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Evolution of Hepatitis C virus (HCV) infection is influenced by interactions between viral and host factors. Inosine-Triphosphate-Pyrophosphates (ITPA) gene polymorphisms 21A>C and 94C>A may have a protective role in treatment-induced anemia. Human Leukocyte Antigen B27 (HLA-B27) alleles were associated with spontaneous viral clearance in HCV infection. The aim of our study was to identify HLA-B27 and ITPA genotypes in order to assess the implication of these two human genetic factors in treatment response of HCV patients from Romania. DNA extracted from blood samples of 100 HCV-infected patients was used for genotyping both host factors using PCR-based methods
The study led to the following results:

Patients with HLA-B27 present	ITPA 21 A>C polymorphisms			ITPA 94C>A polymorphisms		
	Homozygous 'wild type' AA	Heterozygous AC	Mutant homozygous CC	Homozygous 'wild type' CC	Heterozygous CA	Mutant homozygous AA
6	76	23	1	88	12	0
	A allele frequency	87.50% (0.875)		C allele frequency	94% (0.94)	
	C allele frequency	12.50% (0.125)		A allele frequency	6% (0.06)	

The HLA-B27 allele frequency (6%) obtained in our study is similar to corresponding data reported in literature for Caucasian populations (8%). Up to now, we found neither association between the ITPA markers and Pegylated Interferon-Ribavirin treatment induced anemia, nor between the presence of HLA-B27 allele and spontaneous viral clearance. To find any correlation between these two genetics human factors and response to Pegylated Interferon-Ribavirin therapy, more HCV-infected patients will be included in our ongoing study.
Acknowledgement: This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number 88/2012, PN-II-PT-PCCA-2011-3.2 (GO).

J15.14

Cytogenetic monitoring of response and karyotype evolution in low-risk MDS patients treated in the leMon5-study

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Besides a more reliable and frequent measurement of cytogenetic response, it was our aim to find out whether lenalidomide treatment in patients with IPSS low- or intermediate I-risk MDS can foster karyotype evolution (KE) and thus increase the risk of leukemic transformation. In this study, only lower risk MDS patients with an isolated del(5q) are included.
We performed a initial screening of bone marrow aspirates by chromosome banding as well as FISH-analysis to ensure an isolated del(5q). For initial screening and frequent cytogenetic follow-up every two to three months (FISH analysis of CD34+ peripheral blood cells), we used panels of 8 to 13 FISH probes. From the initially examined 145 MDS patients, 84 could be included in the study according to the study inclusion criteria. Currently, follow-up data are available for 53 patients. A complete cytogenetic response was observed in 35 patients (66%) after a median follow-up of 15.5 months. In eleven patients (20%) the size of the del (5q) clone did not change. A cytogenetic response was observed after a median of 6 months after initiation of therapy for a median duration of 10 months. In 8 of 53 patients a true karyotype evolution occurred after a median time of 12 months. Conclusion: Our previous cytogenetic results demonstrate the rapid effect of lenalidomide on clones with del(5q) in 66% of patients. The rate of karyotype evolution was 15% and thus does not appear to be significantly increased. Gain of additional abnormalities does not always result in leukemic transformation.

J15.15

The effect of Centaurea lydia on MDM2 gene expression in human acute lymphocytic leukemia cells via transfection of miR-150 by MATRA

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Acute lymphocytic leukemia (ALL) arises from the clonal proliferation of lymphoid progenitors in the bone marrow. It accounts for nearly a quarter of all childhood cancers. *Centaurea lydia* has shown anticancer activity in vitro. miRNAs are single strand, non-protein-coding small RNA molecules that have role in the regulation of gene expression and cell growth, development, apoptosis and hematopoiesis. In recent years, Magnet assisted transfection (MATRA) is one of the most effective non-viral transfection methods. We aimed to evaluate the effect of methanol extract of *C.lydia* (CLM) on apoptosis and MDM2 gene expression levels in CCRF-CEM cells (ALL) via transfection of miR-150 by MATRA.
Cytotoxic effects of CLM in CCRF-CEM cell line was detected in time and dose dependent manner with XTT assay. We determined effects of CLM, miR-150 and the combination of miR-150 with CLM (miR150-CLM) on apoptosis by using ApoDIRECT with FACS and MDM2 gene expression levels with real-time qRT-PCR (TaqMan) in the course of 48 hours. GAPDH was used as "housekeeping" gene.
IC_{50} dose of CLM is detected as 14.95 µM in CCRF-CEM. miR-150 and miR150-CLM were no apoptotic effects on CCRF-CEM cells while CLM induced 10 fold compared to control cells. MDM2 gene expression in CCRF-CEM were found as up-regulated (CLM) 2,44 fold and down-regulated (miR-150) 224,41 (miR150-CLM) 1,34 fold, respectively, according to the control cells. Down regulation of MDM2 gene following the treatment with combination and CLM apoptosis effects, provide evidence that these compounds may serve as potentially effective in leukemia cells via transfection of miR-150.

J15.16

Testing of EGFR mutations, ALK and ROS1 rearrangements, and KRAS-LCS6 polymorphism in Non-Small Cell Lung Carcinoma (NSCLC) in Czech patients

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Introduction: Activating EGFR mutations are associated with good response for tyrosin kinase inhibitors (TKIs) therapy (gefitinib, erlotinib). Also ALK and ROS1 rearrangements are connected with therapeutic options (crizotinib). KRAS is one of the very frequently mutated genes in many various

cancers, including NSCLC. A germline single nucleotide polymorphism (SNP; rs61764370), located in the let - 7 complementary site 6 (LCS6) within the 3`UTR of the KRAS gene, affects the binding affinity of microRNA let-7 to the KRAS mRNA and thus gene expression. It was published that KRAS-LCS6 SNP is associated with the higher risk of NSCLC development, especially in moderate smokers. **Material and methods:** DNA was isolated from biopsy and cytology specimens with verified histological diagnosis. Mutation detection was done by real-time PCR. EGFR patients with no detected mutation were tested for ALK and ROS1 gene rearrangements using the FISH method. SNP (rs61764370) analysis was performed using PCR and RFLP. **Results:** Numbers of positive/tested samples were: Activating EGFR mutations: 50/658 (7,5 %); ALK gene rearrangement: 39/405 (9,6 %); ROS1 gene rearrangement: 1/159 (0,6 %). KRAS-LCS6 NSCLC patients (G-allele): 23/160 (14,3 %) and KRAS-LCS6 healthy controls (G-allele): 61/387 (15,7 %), respectively. **Discussion and conclusions:** Activating mutations of the EGFR gene, ALK and ROS1 genes rearrangements predict possible therapeutic response to TKIs or other small molecules. Our results correlate with generally known findings. No statistically significant difference between frequency of KRAS-LCS6 G-alleles in NSCLC patients and healthy controls was found.

J15.17

Particularities of ATRA therapy in pediatric patients with acute promyelocytic leukemia

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Acute promyelocytic leukemia (APL) is defined by an error in myelopoiesis. Translocation (15;17) giving PML/RARA fusion gene represents the classic molecular disorder found in 92% of patients with APL. Addition of ATRA induces apoptosis and differentiation of promyelocytes acting at multiple molecular levels. We present three pediatric patients (P1, P2, P3) which were diagnosed with APL in our hospital's Oncopediatric department between 2010 and 2013. At admission, they all presented severe bleeding disorders, gingival hypertrophy, thrombocytopenia, anemia, inflammatory syndrome. Bone marrow smear and immunophenotyping established diagnose, later supported by FISH and PCR analyses that detected PML/RARA fusion gene. They received treatment according to the AML BFM 2004 protocol. P2 was allergic to Etoposide, which was withdrawn later from therapy. In later evolution, he was diagnosed with pulmonary aspergillosis presenting massive pleurisy and exudative pericarditis needing surgical drainage. Both P2 and P1 developed ATRA syndrome with complete remission under dexamethasone. Immunophenotype profiles were significantly different for the 3 patients. PCR analysis and FISH were repeatedly performed showing the absence of the fusion gene at the end of the induction therapy for P2 and P1, but not for P3 which needed a supplementary reinduction chemotherapy course. Currently, all three patients are in hematological and molecular remission, being closely monitored. The addition of ATRA to chemotherapy was efficient to our patients, who were early treated and received proper supportive care. ATRA syndrome was manageable, but it still represents a potentially serious complication that has to be confronted.

J15.18

Resveratrol regulates apoptosis by targeting mir-181 family in chronic myeloid leukemia cells

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Chronic myeloid leukemia (CML) is a malignant disorder of the haematopoietic stem cell arisen from the reciprocal translocation between the breakpoint cluster region (BCR) gene on chromosome 22, and the Abelson (ABL) murine leukemia virus gene on chromosome 9, t(9;22)(q34;q11), resulting in the formation of Philadelphia chromosome which has constitutive tyrosine kinase activity, leading to leukemogenesis. Resveratrol is a naturally occurring phytoalexin with apoptotic and growth inhibitory effects in leukemic cells. MicroRNAs play a pivotal role in normal hematopoiesis. In this study we aimed to evaluate the cytotoxic and apoptotic effect of resveratrol in CML cells by questioning miRNA expression levels associated with CML progression. K562 cells were treated with the IC50 dose (100 µM) of resveratrol for 72 hours. Cytotoxicity analyses were conducted by WST-1 analysis. Apoptosis was evaluated by AnnexinV-enhanced green fluorescent protein (EGFP) and by ApoDIRECT In Situ DNA Fragmentation Assay Kit. The RT-qPCR is used for miRNA expressions analysis. miRNA expression levels were evaluated by using miScript miRNA PCR Array. miRNA expression levels was analyzed by using miScript miRNA PCR Array Data Analysis. Log2 transformation was applied. Significant increase in miR-181 family was observed in K562 cells treated with resveratrol according to control. Resveratrol up-regulated miR-181a, miR-181b, miR-181c and miR-181d expressions as 8.96, 6.42, 10.15, 17.19 fold according to the control cells, respectively. Resveratrol upregulated tumor suppressor miR-181 family in K562 cells through induction of apoptosis and this effect can be used for alternative detection of chronic myeloid leukemia progression.

J15.19

Zoledronic acid inhibits glioma cell growth and induces apoptosis by up-regulating miR-22 expression

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Glioblastoma multiforme (GBM) is the most extensive and malignant type tumors of central nervous system. These lethal brain tumors seen in adults and have no-response to standard treatment. Zoledronic acid (ZA) demonstrates anti-tumor activity in various cancers. MicroRNAs are small (19-24 nucleotides) and non-coding RNAs that regulate post-transcriptional gene expression. miRNAs serve as oncogenes and tumor suppressors through unlighted mechanisms in human.
The aim of the study was to evaluate the effect of zoledronic acid on the expression of 84 miRNAs. In our experiments, U87-MG cell line (Human glioblastoma-astrocytoma) is used as an in vitro model of human glioblastoma cells to investigate the cytotoxic and apoptotic effect of ZA towards glioma cells. U87-MG cells were treated with 25 µM (IC50) ZA during 72 hours. Apoptosis assays were performed by using ApoDIRECT In Situ DNA Fragmentation Assay. The RT-qPCR is used for miRNA expressions analysis. miRNA expression levels were evaluated by using miScript miRNA PCR Array.
Results showed that IC50 dose of ZA induced apoptosis 4.25 fold when compared to control cells that untreated with ZA. Also IC50 dose of ZA of miRNA expression results showed that; mir-22 expression level was upregulated 3,08 fold according to control group .
In conclusion, these novel findings showed that ZA can be important in prognosis of glioma and it is necessary to question whether it can be used as a drug candidate in glioma treatment with further research on miR-22 and its target gene expression.

J15.20

Nicotine and Cotinine Dependent Regulation of P-glycoprotein Expression in Caco-2 Cells

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P-glycoprotein (P-gp), encoded by the multidrug resistance gene 1 (*MDR1*), acts as an efflux pump that exports a wide spectrum of drugs across the membrane out of the cell. P-gp is expressed in a number of barrier tissues such as apical membranes of the lower GI tract, blood-brain barrier, liver, kidney, placenta and testis. Therefore, the expression of P-gp is one of the most important factors regulating bioavailability of a wide spectrum of orally administrated drugs.
Previously it was shown that tobacco smoking changes the bioavailability of several P-gp substrates. It's also known that nicotine is metabolized mostly by CYP2A6 and transformed into a cotinine which has an extremely long half-life in comparison to nicotine (about 20 hours versus 2 hours). In the present study we have shown dose-dependent up/down-regulation of P-gp expression in Caco-2 cells by nicotine and cotinine exposure. Cells were treated with three different concentrations of nicotine (5ng/ml, 15ng/ml, 50ng/ml) and cotinine (50ng/ml, 250ng/ml, 750ng/ml) for 96 hours.
P-gp expression was estimated by real-time RT-PCR. We recognized that P-gp expression was increasing in a nonlinear manner. It was shown that the intermediate concentrations of nicotine and cotinine had a lower effect on P-gp mRNA level compared to other concentrations including the lowest one which matched passive smoker's plasma concentrations. Low concentrations lead to 1,5-fold increase of P-gp expression in comparison with untreated cells. It was also shown that cotinine has a role in regulation of P-gp mRNA level in the case of prolonged smoking abuse.

J15.21

The impact of Oncotype DX testing on chemotherapy prescribing patterns in a tertiary referral centre

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Introduction: The use of chemotherapy in node-negative, ER-positive breast cancer has changed dramatically since the introduction of OncotypeDX to determine systemic recurrence risk based on tumour genomic signature. **Aims**: This study aims to 1. Document longitudinal changes in chemotherapy use 2. Assess the impact of new evidence on local protocol **Methods**:

A cohort study was undertaken, including consecutive patients with early node-negative, ER-positive breast cancer diagnosed between 2006 and May 2013. Data was collected regarding clinico-pathological features, OncotypeDX use, recurrence score, and chemotherapy use. All therapeutic decisions followed multidisciplinary discussion, with adherence to guidelines and consideration of trial protocol and OncotypeDX recurrence scores. **Results**: The study group included 476 consecutive patients, of whom 240 (50%) underwent OncotypeDX testing, 96 as part of TAILORx trial. OncotypeDX has been used in 84%(n=125) patients since being made available for use in the public sector (see table).

Time Period	2006-2007	2007-May 2010	June 2010-October 2011	October 2011-May 2013
OncotypeDX test availability	Oncotype Not Available	Oncotype Available on Trial only	Oncotype not publicly reimbursed	Oncotype in Clinical use
Number of patients treated	55	199	73	149
OncotypeDX use (n(%))	0 (0)	96 (48)	19 (26)	125 (84)
Chemotherapy Use (n(%))	34 (62)	101 (51)	33 (45)	48 (32)

Chemotherapy use changed in inverse proportion to the availability of the genomic assay. Of patients undergoing genomic profiling, 138(58%) were spared chemotherapy. A multivariate analysis found factors influencing chemotherapy use to include recurrence score (OR 1.33 per unit increase, p<0.001), and time period of treatment (p=0.04). Traditional factors such as size and grade did not influence chemotherapy use in patients undergoing genomic assessment (p=0.59 and 0.24). **Conclusion**: This study validates the use of molecular testing in the rationalisation of systemic therapy.

J15.22
F8 gene mutations and inhibitor development in hemophiliac patients from West Algeria

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Neutralizing inhibitors development toward factor VIII is one of the most challenging complications in the treatment of hemophilia A. Several studies have suggested that genetic factors influence the development of factor VIII inhibitors such as mutations in the factor 8 gene. The aim of the present study was to analyze the relationship between inhibitor development and F8 gene mutations in a sample of hemophiliac patients from West Algeria.
To study the genetic predisposition for inhibitor development, we genotyped 24 hemophiliac patients with and without inhibitors. Inhibitor detection for all patients was performed using the Bethesda assay once every 3 months. A conventional Fisher's exact test was used for statistical analysis; p-value <0.05 indicate statistical significance.
A total of seven patients had developed inhibitors. Six patients with inhibitors were classified as low responder; whereas, one patient was categorized as high responder. In the present study, we showed that there was any association of the F8 gene mutations and the inhibitor development in our study-group. However, these findings should be confirmed in a larger group of patients.

J16.01
Association of DNA methylation of tumor suppressor genes and histopathological features of breast cancers

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Breast cancer (BC) represents a heterogeneous disease in which many different genetic, epigenetic and environmental factors are involved. The aim of our study was to investigate the association of DNA methylation of tumor suppressor genes and histopathological profiles of BCs. The methylation status of 24 tumor suppressor genes was determined in 124 BCs using MS MLPA001 kit. Methylation of more than 15% was regarded indicative for gene hypermethylation. SPSS statistics was used for statistical analysis. A total of 103 BCs (87.5%) showed a methylation of at least one tumor suppressor gene. Seventeen of the 24 analyzed genes showed methylation. The most frequently methylated genes were RASSF1 (66.1%), APC2 (41.9%), CDH13 (35.5%), GSTP1 (25%), DAPK1 (15.3%), and CASP8 (8.1%). Triple negative BCs showed positive association with ESR1 (p=0.006), BRCA1 (p=0.006) and TIMP3 methylation (p=5x10-5) and negative association with RASSF1 (p=0.003) and GSTP1 methylation (p=0.02). Similar associations showed the BCs with BRCA1ness profiles, determined by MLPA analysis. Estrogen (ER) and progesterone (PR) positive BCs were positively associated with RASSF1 (p=0.001 and p=0.003, respectively) and DAPK1 methylation (p=0.018 and p=0.027, respectively). ESR1, CDH13, BRCA1 and TIMP3 were less frequently methylated in ER+ tumors. RARB1 and CD44 showed positive association with tumor size (p=0.008 and p=0.006, respectively). Herceptine, p53 and Ki-67 positive BCs were more frequently non-methylated at any of the analyzed genes (p=0.05, p=0.022 and p=0.008 respectively). In conclusion, our study shows that DNA methylation is a frequent event in BC and that different genes are methylated in BCs with different histopathological features.

J16.02
Investigation of the promoter hypermethylation in ILC and IDC of the breast

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Backround: IDC and ILC are the most common invasive breast cancer tumor types. About 80% and 10% of invasive breast cancers are infiltrating ductal and lobular carcinomas respectively.
Method: In this study the promoter methylation levels of TWIST, RARβ2, ESR1, GSTP1 and CDH1 genes which are associated with breast cancer were investigated by Quantitative Methylation Sensitive High Resolution Melting Analysis (QMS-HRM). We analysed primary tumor core biopsies from 80 high-risk primary breast cancer patients (tumors ≥2 cm and/or lenfatic metastase and/or distant metastases and/or under 40 years) and their histopathologic types were associated with the methylation levels.
Results: In our study the promoter hypermethylation status were observed at different rates; TWIST, RARβ2, ESR1, GSTP1 and CDH1 methylation frequencies were 25%, 88,75% 72,5%, 82% and 95% respectively. When comparing the promoter hypermethylation of tumor types of the breast, RARβ2, ESR1, GSTP1 and CDH1 genes found to be significant with invasive ductal carcinomas (IDC). RARβ2 and CDH1 genes promoter hypermethylation is found to be significant with invasive lobular carcinomas (ILC). Also the promoter hypermethylation levels of the genes found to be significant with lymph node positivity, ER positivity and HER2/neu negativity.
Conclusions: Our study is important as being the first study that analyzes the association between IDC and ILC tumor types of breast cancer and TWIST, RARβ2, ESR1, GSTP1 and CDH1 genes promotor methylation status in Turkish population.

J16.03
Presenting the genomic cipher adenine = 0, guanine =1, cytosine = 2, thymine = 3 derived directly from the DNA to convert nucleotide names/letters to numbers to study patterns of unique identifiers to detect command genes in the human genome

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Presented is a cipher to convert each DNA nucleotide to a specific number to reveal patterns of unique gene identifiers in the human genome. The cipher was developed by converting tRNA anticodons to 5'-3', then reverse transcribing 5'-3' RNA anticodons to 5'-3' DNA anticodons to facilitate construct of a 4x4x4 prime genomic cube; where the first letter of the anticodon is positioned along the 'x' axis of the cube, second letter positioned along the 'y' axis and third letter positioned along the 'z' axis. Assigning adenine the value of '0', guanine value of '1', cytosine value of '2' and thymine value of '3' places methionine, considered the START anticodon, on one end of the cube and the three STOP anticodons on the opposite end of the cube. The three dimensional image of this arrangement of DNA anticodons demonstrates an orderly stepwise progression of the triplicate DNA anticodons AAA, GGG, CCC, and TTT through the cube. A secondary pattern is demonstrated if the triplicate anticodons nullify anticodons in their rows and anticodons numbering three or more elements are neutral, then there are zero free anticodons in the 'A' 4x4 panel, one free anticodon in the 'G' 4x4 panel, two free anticodons in the 'C' 4x4 panel, three free anticodons in the 'T' 4x4 panel. Analysis produces the cipher Adenine=0, Guanine=1, Cytosine=2, and Thymine=3. Utilizing this cipher to convert nucleotide elements to numbers facilitates study of the human genome to detect command genes that cannot be determined by conventional analysis techniques.

J16.04
Evaluation of New Epi-panel Markers SPG20,ITGA4 and ALX4 in Plasma for Early Detection of Colorectal Cancer

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Colorectal cancer (CRC) is the 3rd most common cause of cancer death in the world.Introducing a suitable method for screening and early detection of CRC will be one of the most important strategies. The presence of cancer-specific DNA methylation biomarkers in the body fluid of CRC patients will provide a simple, non-invasive screening test for colorectal cancer. This study was designed to evaluate methylated SPG20, ALX4, ITGA4 genes as a novel epi-panel for screening and detection of CRC. In the first step 90 blood samples were collected from diagnosed cases as colorectal cancer and 40 samples were collected from healthy volunteers as control from Baqiatalah University of Medical Sciences, Tehran, Iran.DNA from plasma samples was extracted by using Qiagene DNA purification protocol following Bisulfite modified DNA was amplified by using specific primers for Methylation Specific PCR(MSP).Finally the MSP evaluation was performed on the patients and control groups.The results up to now showed that ITGA4, SPG20, ALX4 were hypermethylated in between 30 to 50% of CRC and polyp patients whereas normal samples were rarely methylated. These candidate markers can be considered for further evaluation in screening or diagnostic applications for colorectal cancer.Our findings suggest this epigenetic panel is suitable for screening and early CRC detection. Furthermore, we showed our epi-panel performed better than the individual DNA methylation biomarkers when analyzed in the same plasma samples.

J16.05
Epigenetics, aging and cancer

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Epigenetics is a fast-growing branch of biology. It consists in the study of reversible and transmissible modifications of gene expression without changing DNA sequences. Epigenetic modifications are contributing to the regulation of many physiologic phenomena such us embryonic development, X chromosome inactivation in female and parental imprinting. These modifications are regulated by several mechanisms, the most frequent are: DNA methylation, histones post-translational modifications and non coding RNAs. They are influenced by genetic, environmental as well as stochastic factors.

It has been shown that physiologic aging depends not only on genetic factors but also on „epigenome" modifications, and several age-related diseases such as cardio-vascular diseases, type II diabetes, Alzheimer, auto-immune diseases and cancer have been associated to epigenetic changes. Cancer is, so far, the most studied disease in this context, making „epigenetics" a plausible explanation to the increasing rate of cancer in old. Cancer epigenetic modifications lead to expression alteration of genes regulating neoplastic phenotypes such us cell proliferation and metastasis.

A best knowledge of epigenetic mechanisms gave a possibility to a targeted cancer therapy (epigenetic therapy) and opened many preventive, diagnosis and prognosis perspectives.

J16.06
Gene expression profiles in uranium industry workers of Stepnogorsk city occupationally exposed to ionizing radiation.

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Ionizing radiation (IR) imposes risks to human health and the environment. Taking into account the fact that Kazakhstan is one of the world's leading uranium producer, and considering the magnitude of the damage from Semipalatinsk nuclear test site, the study of radiation effects on genes has always been a priority. It is known that LIG3 and XPA genes may serve as biomarkers for sensitive methods of dosimetry. The purpose of this study is to determine the impact of the IR on gene expression in uranium industry workers of Stepnogorsk city, Kazakhstan.

Peripheral blood samples (n=20, n=19) were collected from workers who were exposed to different radiation doses (50mSv, 20mSv). To examine gene expression profiles of radiation exposed workers quantitative real-time PCR were used. We used B-actin gene as an endogenous control for our study. The expression of LIG3, XPA genes were analyzed and compared between two groups.

Gene expression analysis showed statistically significant difference in expression of DNA repair genes (LIG3, XPA) between two groups exposed to different doses of radiation. The greater the delivered dose of IR, the greater the expression level of DNA repair genes.

Altered expression profiles in this study can be used as biomarkers of dosimetry for uranium industry workers. This strategy on risk assessment should be considered in the construction of industrial safety and health of workers occasionally exposed to IR.

J16.07
Whole genome sequencing of Mycobacterium tuberculosis in Kazakhstan: first sequence results of two clinical isolates

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The project is aimed to create the prerequisites for a personalized approach to the diagnosis and treatment of tuberculosis by identifying and comparing the whole genome sequences of M.tuberculosis strains, isolated in Kazakhstan. Analysis for whole genome sequences obtained using NGS technology will clarify the factors cause of the formation of highly virulent strains of M.tuberculosis, the evolution of local strains, and genetic markers of drug resistance.

Material collection from 50 patients, sputum extraction and determination of drug sensitivity was performed in the reference-laboratory "National Center of Tuberculosis Problems", Almaty, Kazakhstan. DNA libraries for whole genome sequencing were prepared from DNA extracted from the isolates. The sequencing was performed on Roche 454 GS FLX+ NGS platform at the Center for Life Sciences, Nazarbayev University, Astana, Kazakhstan. The sequencing reads from two isolates were assembled into contigs using GS De Novo Assembler. All alignments were done against the M.tuberculosis reference strain H37Rv using GS Reference Mapper.

The sequencing has performed for two M.tuberculosis isolates MTB-476 and MTB-489. 96 M bp with an average read length of 520 bp, approximately 21.8X coverage and 104.2 M bp with an average read length of 589 bp and approximately 23.7X coverage were generated for the MTB-476 and MTB-489, respectively. The genome of MTB-476 consists of 257 contigs, 4204 CDS, 46 tRNAs and 3 rRNAs. MTB-489 has 187 contigs, 4183 CDS, 45 tRNAs and 3rRNAs. The results of genome assembling have been submitted into NCBI GenBank, available for public access under the accession numbers AZBA00000000, AZAZ00000000.

J16.08
Correlation of O6-Methylguanine DNA methyltransferase promoter methylation and clinicopathological parameters in bladder cancer

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Bladder cancer is the one of the most common cancers worldwide and O6-Methylguanine DNA Methyltransferase (MGMT) promoter methylation might be contributing to clinicopathological features of the disease. We analyzed formaline-fixed, paraffin-embedded bladder tissue samples from 101 patients with urothelial carcinoma. All samples were analyzed by real-time methylation-specific PCR. MGMT promoter methylation was detected in 15 (14.9%) of the samples and was not significantly different between any of the groups, although the number of high grade tumors with MGMT promoter methylation was twice the number of low grade tumors. Methylation of MGMT in high grade tumors might play a role in aggressive proliferation of the tumor cells and a poor prognosis.

J16.09
PRKCDBP downstream hypermethylation and its influence on gene expression in squamous intraepithelial lesions and carcinomas

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Protein kinase C delta binding protein (PRKCDBP) is a potential tumor suppressor although molecular basis of its action has been poorly understood. PRKCDBP gene is inactivated in colorectal, lung and breast cancer by genetic or epigenetic alteration which leads to down-regulation of PRKCDBP expression. The purpose of this primary study was to examine PRKCDBP hypermethylation in 7 CpG sites (assays predesigned by QIAGEN) by pyrosequencing and to find out the expression in squamous intraepithelial lesions and carcinomas. A total of 51 cervical specimens were divided into groups with a diagnosis of normal cervix (n=21) and squamous intraepithe-

lial lesion or carcinoma (n=30). Downstream hypermethylation was found significantly hypermethylated in two CpG sites in intron (p=0.0033; 95% CI: -3.26 to -0.69 and p=0.0015; 95% CI: -4.07 to -1.03) and one in exon 2 (p=0.0222; 95% CI -5.06 to -0.41) when compared to group with normal cervix. PRKCDBP gene expression was also reduced in group with cervical lesion or carcinoma. Downstream hypermethylation has been reported as widespread phenomenon with different effect on the gene expression and promoter hypermethylation. These results could indicate the potential impact of downstream hypermethylation on reduced PRKCDBP expression during cervical carcinogenesis.

J16.10
Short runs of homozygosity as an underappreciated mechanism for imprinting disorders

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Runs of homozygosity (ROH) are epigenetic changes encompassing relatively large genomic loci and presenting as stretches of consecutive homozygous genotypes scattered through the genome. Usually, ROH exceeding 5-10 Mb in length are only considered to have an effect on phenotype. On the other hand, heterozygosity losses of a single imprinted gene can produce an epigenetic change leading to imprinting disorders. Therefore, such epigenetic changes have not necessarily to encompass large genomic loci. Studying ROHs in 115 children with intellectual disability and congenital malformations by Affymetrix 2.7M SNP/array, we have found that 1-Mb-long-ROHs are associated with imprinting disorders. In a case of atypical Angelman syndrome a ROH at 15q11.2 (size 1.16Mb) was detected. Furthermore, two cases of atypical Beckwith-Wiedemann associated with ROHS at 11p15.5p15.4 (size: 1.36Mb and 1.15Mb) were identified. In addition, a long ROH at 7p14.2p12.1 (14.95Mb) affecting GRB10 (an imprinted candidate gene for Silver-Russell syndrome) was detected in a child with severe growth retardation, microcephaly and intellectual disability. Three children (2.6%) demonstrated overall ROH burden typical for offspring of a consanguineous relationship. Retrospectively, consanguinity was confirmed by genealogical analysis. Thus, our observations show that 3.5% of intellectual disability cases can be attributed to ROH. Moreover, ROHs can be as short as 1Mb, which are usually overlooked, when SNP/array assays or other methods for epigenetic analysis are applied. We conclude that these epigenetic mutations are a relatively common mechanism for imprinting disorders. Supported by the Russian Federation President Grant (MD-4401.2013.7).

J16.11
The review od epigenetic modification in human thyroid cancer

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Thyroid cancer is the most common endocrine malignancy worldwide. However, there is not a definite treatment for advanced thyroid cancer, which comprises poorly differentiated, anaplastic and metastatic or recurrent differentiated thyroid cancer that do not response to radioiodine; treated patients had not a complete response. Biological therapies have been proposed on the basis of the recognition key oncogenic mutations and these treatments could be effective in stabilizing progressive disease. Epigenetic abnormalities are present in almost all cancers and together with genetic variations led to tumor progression. Epigenetic modification is mostly occur in genes, which play role in the control of cell proliferation and invasion such as RASSF1A, PTEN, DAPK, RAP1GAP, and specific genes of thyroid are mostly epigenetically silenced in thyroid cancer. Rap1, a member of RAS family of small GTPase has been implicated in regulation of mitogenic and oncogenic pathways in thyroid, and its CpG island methylation is reported in some kind of tumors. In addition, miRNAs play important role in cell differentiation, proliferation and survival. They considered as the regulators of gene expression at posttranscriptional level. Deregulation of miRNAs expression is suspected to be an important regulator of tumor development and progressive. Epigenetic modification pattern is different not only between normal and tumors tissues, but also between different stage of malignancy and between primary and metastatic tumor. Therefore these variations may become useful novel biomarkers for diagnostic and treatment tumors.

J16.12
Epigenetics, maternal responsibility and neurological development

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It is believed that epigenetics can partly explain the development of neurological conditions. As epigenetic changes often happen in utero, maternal behavior may affect brain development. This raises questions regarding the responsibility of the pregnant woman. To which extent is she responsible for the neurological status of the future child? Should she, for example, avoid all stress, if this is shown to lead to molecular changes increasing the chance of the child to develop ADHD later in life? Should she ensure that she takes optimum nutrition for development of synaptic plasticity of her fetus? With of this talk I demonstrate first that epigenetics complicates the question of the responsibility of the pregnant woman. To which extent does the increasing knowledge about epigenetics influences in utero further complicate this responsibility, if even behavior of years before the conception may influence a fetus? Is this an individual responsibility or also a collective one? Second, by using the examples of autism and extreme intelligence, I demonstrate that , although discussions about parental responsibility towards future children have centered on duties to prevent suffering or to maximize welfare or 'the chance of a good life', the distinction between prevention and enhancement is difficult to maintain in the context of neurological conditions. By analyzing arguments from the neurodiversity movement, whose members often insist that their neurological condition is not a disease, but an integral part of their identity, I shall demonstrate that there is a need for a revisiting of the concept of maternal responsibility.

J16.13
Anchored Assembly: Comprehensive variant detection using NGS data

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Characterization of large indels, inversions, and multi-nucleotide variants is important for disease studies. These are often undetected by standard pipelines. Spiral Genetics has developed Anchored Assembly, a novel method using direct, *de novo* read overlap assembly to accurately detect variants from next-generation sequence reads. We detect, on average, over 90% of indels and structural variants ≤ 30 kbp in non-repetitive regions. The ability to detect deletions and structural variants is undiminished by variant size, and the ability to accurately detect and assemble insertions continues well into the 30 kbp range.
Anchored Assembly was evaluated against Pindel and BWA + GATK using simulated read data. Datasets were generated by populating chromosome 22 of the human genome reference sequence with a set of SNPs, indels, inversions, and tandem repeats. Anchored Assembly detected, on average, over 90% of SNPs, indels, and structural variants up to 50 kbp with false discovery rates well below 1%. In comparison, Pindel and BWA + GATK had false discovery rates of 10% and 9%, respectively.
Anchored Assembly's range of detection and low false discovery rates may benefit cancer and rare disease studies. The ability to accurately detect structural differences will be useful for characterizing tumor vs. normal samples and analyzing and comparing whole human trio data to identify risk-associated variants.

J16.14
Hidden parts of whole exome sequencing data: non-coding variants and copy number variations

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Sequencing of protein coding regions of human genome (Whole Exome Sequencing; WES) has been wildly used to unravel the mystery behind various inherited human genetic diseases, in the past few years. Despite its great success in the identification of causal mutations for such disorders, a subset of them remained still unsolved. Here, we present the result of our WES studies where analyzing only rare variants in coding regions was not conclusive but further investigation revealed the involvement of non-coding

variants and copy number variations (CNV) in etiology of the diseases. Whole exome sequencing and bioinformatics analyses were performed at Genome Quebec Innovation Center, Montreal, Canada. We successfully identified disease-causing mutations in coding regions of several human rare diseases (e.g. SCARF2: Van den Ende-Gupta syndrome and SNAP29: 22q11.2 deletion syndrome). Additionally, we showed that variants in non-coding regions and CNV have also important value and should not be ignored during bioinformatics analysis of WES data. For instance, in patients with osteogenesis imperfecta type V and in patients with glucocorticoid deficiency, we identified variants in 5'UTR, resulting in the production of longer or truncating non-functional proteins. Furthermore, CNVs were identified as the main cause of the diseases in patients with metaphyseal dysplasia with maxillary hypoplasia and brachydactyly and in patients with osteogenesis imperfecta type VII. Our results highlight that non-coding variants and CNVs can be crucial and they should be considered during WES data analysis, as they can be the only cause of the disease under investigation.

J16.15
An automated benchmarking toolkit for structural variation calling using Next Generation Sequencing data

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Structural variations (SVs), such as insertions, deletions and duplications are found to be one of the major genetic causes of cancer. Identifying and characterizing these events is becoming increasingly important in cancer genomics. Next Generation Sequencing (NGS) technology has made it possible to detect SV events at base pair resolution. Many different computational SV detection tools have been developed, based on information sources such as read depth, discordant paired-end reads, split alignments, and de novo assembly. While some approaches make similar predictions, some approaches disagree in their predictions and deliver rather complementary sets of SV calls. The current challenge for researchers, when being confronted with myriads of tools, is to make a good selection, so as to generate a set of SV calls that is both most reliable and most comprehensive.
To appropriately guide researchers in comparing and selecting most appropriate SV detection tools, we have developed an open source toolkit (available also in a downloadable virtual machine) that allows us to systematically benchmark and evaluate SV detection tools. We have generated realistic, simulated datasets based on comprehensive sets of real SVs from several different genomes. We have put forward the performance statistics of different SV calling methods and created a pipeline for their optimal usage. If desired, more SV detection tools can be added to this pipeline, whose workflow is generic in the choice of tools. Results are summarized in a PDF file where precision, recall and also other useful detection statistics referring to the tools selected are displayed.

J17.01
Angiotensin-I converting enzyme I/D polymorphism gene in Moldavian population

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Background: The angiotensin-converting enzyme (ACE) gene in humans has an insertion-deletion (I/D) polymorphic state in intron 16 on chromosome 17q23. This polymorphism has been widely investigated in different diseases. In this study we aimed to investigate the ACE I/D genotype frequency in Moldavian population with others populations.
Materials and Methods: Have been investigated DNA of 100 healthy children aged up to 17 years, in order to make a comparative analysis of ACE I/D polymorphisms in Moldova with other countries. PCR methods were used for polymorphisms determination of ACE I/D allele. The distribution of genotypes was tested for deviation from Hardy-Weinberg Equilibrium (HWE).
Results: There was no significant difference in the distribution of DD, II and I/D genotypes of ACE polymorphism in Moldavian population (21.3, 28.8, and 48%) and their ethnically matched control from Italian population (36, 16, and 48%), respectively. There two groups also presented with very similar allelic frequencies and were also found to be in Hardy-Weinberg equilibrium, when ($\chi 2=2,05, p<0.15$) indicate statistic confirmation of similarities. The same similarities were found in North Indian, Dutch and Turkish populations and total differences with Kyrgyz population ($\chi 2=13.69, p=0.002$) .
Conclusion: The polymorphism of ACE I/D genotypes frequency in Moldavian population are statistical similar with Italian, Dutch, North Indian and Turkish populations.

J17.02
Establishment of the most common of autosomal dominant spinocerebellar ataxias (ADSCA's) in Bulgarian patients

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Background: The autosomal dominant spinocerebellar ataxia (ADSCA) is large and genetically heterogeneous group of neurological disorder characterized by cerebellar dysfunction. Today are known more than 30 types of SCA's. The most common ADSCA's (SCA1, SCA2, SCA3, & SCA6) are associated with the expansion and instability of CAG trinucleotide repeat (CAG) n encoding polyglutamine proteins.
Aim: The aim of this study was to investigate the most common Spinocerebellar ataxia from SCA1, SCA2, SCA3 and SCA6 in patients from Bulgarian families, and to implement fast and reliable workflow for diagnosis of this disease.
Materials and methods: Our group consists of 63 patients from 50 Bulgarian families with clinical symptoms referred to those of the disease. DNA was extracted from the periferal blood with salting out method. Polymerase chain reaction (PCR) and triplet repeat primed PCR (TP-PCR) were used to confirm the diagnosis.
Results: From 63 analyzed patients SCA was established in 16 patients from 15 out of 50 kindreds (7.5%) . We have determined that SCA1 is found in 5 (2.5%) and SCA2 in 10 (5%) of all families. From all genetically confirmed patients 66% were diagnosed with SCA2 and 33% with SCA1.
CONCLUSIONS: The frequency of SCA2 is substantially higher than that of SCA1, 3, 6 and 7 in patients with SCA from Bulgarian families, unlike the worldwide investigations showing that SCA1 and SCA 3are reported to be most common. TP- PCR proved to be reliable method for routine diagnosis

J17.03
CCR5del32, CCR2-64I та SDF-1 3'A mutations of chemokine receptors genes in West-Ukrainian population

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Ukraine has one of the highest speeds of HIV-1 infection spread in Europe but Western region is the most "trouble-free": 7 compare to 100 - 300 cases of HIV per 100 thousand inhabitants in other regions. A speed of epidemic depends on correlation between susceptible and resistant individuals in single population.
The purpose of study to set up the frequency of allelic variants of genes, which lead to higher resistance to HIV-1 among people of the Western region of Ukraine.
It was used the DNA samples of 200 healthy people (50% men and 50% women). The PCR products were digested with the restriction enzymes and subjected to agarose electrophoresis.
Heterozygote mutations CCR5del32 was detected in 17.0% and homozygote CCR5del32 in 0.5% people with same frequency in both sexes. Heterozygous mutation CCR2-64I was revealed in 14.5% people and homozygotes in 1.5%. This mutation was found in women almost twice often than in men. Heterozygote mutation SDF-1 3'A in studied group was found in 30.5% of people, homozygote - in 3% of all with same frequency in both sexes.
The CCR5del32 was combined with CCR2-64I (1 sample), with SDF-13'A (8), CCR2-64I with SDF-1 3'A (9) and combination of homozygotes mutation CCR2-64I and heterozygotes mutation SDF-1/3'A (1 person). The number of people who do not have any protective genotype is 33%.
Thus, the frequency of mutations in chemokine receptors gene in people of Western region of Ukraine is indicate about the high genetic resistance to HIV-1 infection compared with other ethnic groups.

J17.04
Determination of allele frequencies, duplication and mutation of 17 Y-STR loci in UAE population

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Determining the allelic frequencies and an inconsistency of the transmitted alleles of Y-STR haplotype among different populations is crucial for the correct interpretation of DNA profile matches in paternity and kinship analysis. In this research, STR allele duplications, mutations and the allelic frequencies of the 17 Y-STR loci in the UAE population, will be determined. Cheek swabs samples will be collected randomly from 200 unrelated male individuals. Donors will be UAE citizens, whom ancestors' have long-lived in the country. DNA will be extracted using Genomic DNA Isolation Kit, (NORGEN Biotek CORP, Canada). The quality of the extracted DNA samples will be assessed using agarose gel electrophoresis (AGE). DNA quantification will be carried out using Quantifiler® Duo DNA Quantification Kit followed by

multiplex PCR amplification using AmpFlSTR® Y-filer® PCR Amplification Kit (Applied Biosystems, USA). Capillary electrophoresis (CE) of amplified multiplex PCR will be performed using an ABI 3130 Prism® Genetic Analyzer (Applied Biosystems). The data obtained from capillary electrophoresis will be analyzed and the allelic frequencies of Y-STR loci will be determined using a population-genetics-software called "ARLEQUIN". This study will provide an additional information to the framework of variation involving 17 Y-STR loci in forensic case work samples as well as a further contribution to the Y-STR database for UAE male population. This study will also help to determine allele duplications and mutations plus an inconsistency of the transmitted alleles appeared in the UAE population.

J17.05
Hemoglobin H (HbH) Disease in Khuzestan Province

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Background and Purpose: We studied the α-globin gene genotypes, hematologic values, and transfusion dependency of patients with hemoglobin H (HbH) disease in Khuzestan Province, Southwest of Iran.
Methods: We detected alpha globin gene mutation by Using gap-polymerase chain reaction (gap-PCR) and direct DNA sequencing.
Results: We identified 44 patients with hemoglobin H disease. Of these patients, 15 (34%) had deletional form of HbH disease, 29 (66%) had different form of nondeletional HbH disease. The most frequently observed HbH genotypes were --Med/ -α3.7 in 14 patients (31.8%), αpoly A6α/ αpoly A6α in nine patients (20.5%),and αpoly A4α/ αpoly A4α in five patients (11.3%). Some persons with HbH disease required blood transfusion, whereas some others with the same alpha globin genotype were not transfusion-dependent.
Conclusions: Our data show diversity in the genotype and clinical presentation of deletional and nondeletional HbH disease. This discrepancy between similar genotypes with different phenotypes may be due to other modifying factors. Therefore, we cannot describe a general conclusion regarding the need for blood transfusion in the patients with HbH disease

J17.06
Prevalence of alpha-1-antitrypsin deficiency among Polish patients with lung or liver disorders

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Background: In Poland, the overwhelming majority of individuals with alpha-1-antitrypsin (AAT) deficiency still remains undiagnosed. We estimated the AAT gene frequency and prevalence in a large cohort of Polish chronic lung or liver disease patients eligible for AAT testing.
Methods: Blood samples were collected prospectively from 419 respiratory patients (COPD, emphysema, bronchiectasis, asthma) and 281 patients referred for liver transplantation due to cirrhotic and non-cirrhotic chronic disease. AAT serum concentration was measured by nephelometry and PI-phenotype identified by isoelectrofocusing. The PI*S and PI*Z alleles were confirmed by real-time PCR; rare phenotypes were identified by DNA sequencing.
Results: 53 (12.6%) lung disease patients and 18 (6.4%) liver disease patients demonstrated AAT deficiency phenotypes. Calculated frequencies expressed per 1000 were for PI*Z 46.6 (95% CI: 32.3-60.8), PI*S 20,3 (95% CI: 10.8-29.8) in respiratory patients and PI*Z 19.6 (95% CI: 8.1-31), PI*S 10.7 (95% CI: 2.2-19.2) in liver disease patients. The AAT gene prevalence calculated by Hardy-Weinberg equilibrium were: 1/1.16 for MM, 1/26 for MS, 1/2429 for SS, 1/11 for MZ, 1/530 for SZ and 1/462 for ZZ in respiratory patients and 1/1.07 for MM, 1/48 for MS, 1/8773 for SS, 1/26 for MZ, 1/2393 for SZ and 1/2610 for ZZ in liver disease patients.
Conclusion: Our results show relatively high frequency of AAT deficiency among Polish patients with chronic obstructive respiratory disorders. Estimated frequency for PI*Z and PI*S allele in respiratory group was about two-fold higher than in liver disease patients and four-fold higher than estimated prevalence in healthy Polish population.

J17.07
The epidemiology of anorectal malformations in Russia in 2000-2012

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The aim of this study was to investigate the epidemiological characteristics of anorectal malformations (ARM) in Russia.
Material and methods. We analyzed data from 36 regional birth defects registries of Russia for 2000-2012. The epidemiological analysis included such factors as sex of affected cases, birth outcome, birth weight, maternal age and number of births. Total prevalence per 10,000 births was defined as the total number of cases among live births, stillbirths and induced abortions divided by the total number of all births.
Results. Among participating registries total number of ARM cases was 970 with prevalence of 1.84 per 10,000 births. Most of them (85.26%) are isolated and 14.74% cases are combined with other anomalies. Among isolated cases 47,76% were cases of congenital anomalies of anus (CAA) without fistula (code Q42.3), 33,25% - CAA with fistula (Q42.2), 11,12% - congenital anomalies of rectum (CAR) without fistula (Q42.1) and 7,87% - CAR with fistula (Q42.0). Among the combined cases CAA without fistula were 42%; CAA with fistula - 25,1%; CAR without fistula - 21%; CAR with fistula - 11,9%. The male affected more frequently than female (1,6:1). Among infants with all kinds of ARM are more common the infants with the low birth weight.
Conclusions. On average ARM affected 1 in 5435 births. There are no changes in prevalence of ARM during research period. The regular monitoring of congenital malformations allows the study of the epidemiological characteristics of even the rare birth defects.

J17.08
Status of RANK and MMP9 gene polymorphism in beta-thalassemia patients of Indian origin

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β-thalassemia is an autosomal recessive, commonest hematological disorder found in India. Regular transfusion to sustain life in thalassemia major leads to iron overload. The Cardiac complications and bone deformities are very well documented as outcome of regular transfusion in thalassemics. To evaluate the genetic predisposition for these two important clinical complications, RANK and MMP9 gene prevalence in transfusion dependent cases have been studied. Receptor activator of nuclear factor (RANK) is one of the proteins in regulation of osteoclastogenesis via RANK/RANKL/OPG pathway. In a recent GWAS study RANK was found to be associated with osteoporotic fractures and variations could alter the expression so the two polymorsphisms were studied in beta thalassemia patients. Seventy-five patients of thalassemia major were selected and the genotyping of RANK (G -> A) polymorphism was performed in thalassemia major patients. High prevalence of G allele [86%] was observed compared to 13% prevalence of A allele. Second RANK (insdel C) polymorphism showed 88.6% of insertion cases while remaining were deletions. Matrix metallopeptidase 9 (MMP9) plays a pivotal role in early atherosclerosis, vascular remodeling and development of atherosclerotic lesion. The potentially functional MMP-9 gene polymorphism may contribute to the susceptibility of acute coronary syndrome (ACS). This study includes -1556C>T polymorphism of MMP9 gene in β-thalassemia major patients where 75.3% allele C was found in thalassemia major patients. The present study reports the higher prevalence of wildtype alleles of RANK (+34901G>A, +35966insdelC) & MMP9 (-1556C>T) polymorphism in Thalassemia patients of Indian origin.

J17.09
FTO (rs17817449 and rs9939609) mutations in a Romanian obese children population

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Background: Obesity is a disorder with increasing freaquency in children and adolescents, directly linked with various diseases such as type-2 diabetes, hypertension and atherosclerosis. Variants in the FTO gene have been associated with Body Mass Index in Western European and North American populations.**Aim:** In this research the authors examine the association between the FTO gene variants rs17817449 respectevly rs9939609 and overweight and obesity in a Romanian population.**Subjects and methods:** A total of 292 children, 178 obese and
214 non-obese individuals, were included in this case-control study. Genotyping of FTO gene (rs17817449 and rs9939609) polymorphism for all subjects was performed by the PCR-RFLP method. We measured weight, height,

waist circumference, and triceps and subscapular skinfolds; body mass index (BMI [calculated as weight in kilograms divided by height in meters squared]). **Results:** The rs9939609 A allele was strongly associated with overweight and obesity in this population. The A allele of the *FTO* polymorphism was significantly associated with higher BMI and higher waist circumference.No significant association between FTO rs 17817449 genotype and adiposity was found.**Conclusions:** This study replicated the genetic association of SNP of FTO (rs9939609) with obesity in a romanian population and, to the authors' knowledge; this is the first such association study in a Romanian population. Acknowledgement This work was financially supported by Internal Research Grants of the University of Medicine and Pharmacy Tîrgu Mureș, România (grant number 1/30.01.2013).

J17.10

The effects of chemical mutagens on the chromosomes of the population living in the area of the oil and petroleum industries

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One of the major problems facing the world is the pollution of the environment and the study of its effect on the rate of mutation and inheritance rights. Petroleum products and petroleum industries affect pollution.
Aim: To evaluate the effects of prolonged exposure to chemical mutagens on chromosome population Zhylyoi district of Atyrau region, living in ecologically unfavorable regions.
Material for cytogenetic analysis: the culture of peripheral blood lymphocytes from 71 residents living in the area with the development of the oil industry. Culturing lymphocytes, cooking preparations, coloring G-method performed by standard methods. The control group consisted of 25 people in an ecologically clean region.
The results of the study. We studied the frequency and spectrum of chromosomal aberrations in the groups studied. Found a higher incidence of aberrant cells (1.75 per 100 cells) and the total frequency of chromosomal aberrations (1.9 per 100 cells) in affected individuals compared with controls (p <0,05).
Frequency paired fragments were 0,10±1,99 per 100 cells, dicentric 0,01,±0,23, ring chromosomes 0,14±0,38 frequency of stable chromosome aberrations 0,02±1,17 100 cells in people living in Zhylyoi area. The frequency of chromatid type aberrations was 0,87±0,01 100 metaphases and is represented mainly by single fragments. Findings suggest the influence of chemical factors on the chromosome of people living close to the oil companies and petroleum industries.
Conclusion. Cytogenetic studies of the population will be used to define a group of families of „high risk" for the prevention of genetic disorders in ecologically unfavorable regions.

J17.11

Association between C282Y and H36D mutations of the HFE gene with hepatic cirrhosis in Lithuanian population

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Introduction: Liver cirrhosis is one of the major gastrointestinal diseases. Cirrhosis is commonly caused by alcohol use, viral hepatitis B and C and many other possible causes. The search for epidemiological, biological or genetic factors that could help to select patients at higher risk of developing cirrhosis is necessary. Among these factors, the influence of excessive liver iron overload and HFE gene mutations have been debated over the past few years and up to now there is limited data on association between HFE gene mutations and cirrhosis.
The aim of this study: To determine the association between HFE gene C282Y and H63D mutations and liver cirrhosis.
Methods: A cohort of cirrhosis patients consisted of 209 participants. The diagnosis of cirrhosis was confirmed by clinical features, liver biopsy and radiological imaging tests. Control samples were randomly obtained from 1005 blood donors. HFE gene mutations were detected using PCR-RFLP method. Statistical analysis were performed using statistical software for genetic association studies PLINK v2.050.
Results: C282Y allele was associated with liver cirrhosis (5,02%, OR-2.074, p=0.00510) when compared with controls (2,49%). C282Y/wt genotype was linked with cirrhosis comparing with wt/wt genotype (OR-2.002, p=0.01239). A similar situation was observed in dominant model for C282Y mutation (wt/wt vs. C282Y/wt + C282Y/C282Y) which showed increased risk of liver cirrhosis (OR-2.065, p=0.00766). H63D mutation was not associated with cirrhosis in Lithuanian population.
Conclusion: HFE gene C282Y mutation is associated with liver cirrhosis and might cause a higher risk for progression of liver cirrhosis in Lithuanian population.

J17.12

Associations of genetic variants of CNR1 and CNR2 with hypertension or dislipidemia in the Chinese post-menopausal women

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Metabolic syndrome may occur in 40% of postmenopausal women and is largely determined by overweight and obesity. Obesity in menopause women occurs as a result of genetic background, hormonal changes and acquired changes in eating behavior and physical activity. Evidence showed that the endogenous cannabinoid system (ECS) plays a role in obesity and related metabolic disorders. To investigate the association between *CNR1* and *CNR2* gene polymorphisms and metabolic risk factors in the Chinese postmenopausal women, five hundred and fifteen post-menopausal women were recruited. Anthropometric measures (body mass index (BMI), waist circumference (WC)), blood pressures, and metabolic parameters were determined. Four SNPs from *CNR1* gene and 1 SNP from *CNR2* gene were selected for genotyping on these post-menopausal women. We found that post-menopausal women carrying TT+TC genotype of *CNR1* rs2023239 had increased risk of metabolic syndrome (p=0.047). We also found two SNPs were significantly associated with pre-hypertension or hypertension (OR= 1.72, 95% C.I.: 1.12-2.63 for rs806381 and OR= 1.77, 95% C.I.: 1.00-3.11 for rs1049353, respectively). We further observed that *CNR2* rs2229579 was significantly associated with hyper-triglycerides in our post-menopausal women (OR= 1.85, 95% C.I.: 1.06-3.23). Haplotype analysis also revealed that those women carry the CGTA haplotype of *CNR1* gene were less likely to develop pre-hypertension or hypertension (aOR= 0.67, 95% C.I.: 0.47-0.96). Our findings provide initial evidence that the *CNR1* gene variants may contribute to pre-hypertension or hypertension and *CNR2* gene variant may predict hyper-triglycerides in post-menopausal women in Taiwan.

J17.13

A study on the association of ERCC1 Asn118Asn polymorphism and colorectal cancer risk in West Algerian population

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Colorectal cancer (CRC) is one of the most common causes of death due to cancer in both men and women throughout the world. It has been suggested that sporadic CRC is most likely caused by lowpenetrance genes, including those involved in DNA repair mechanisms. Furthermore, the accumulation of DNA damage may contribute to colorectal carcinogenesis. Many epidemiological studies have explored the association between ERCC1 Asn118Asn (C/T, rs #3177700) polymorphism and colorectal cancer risk in various populations, its role in colorectal carcinogenesis in Algerian population has not been established. Therefore, we conducted this case-control study in a West Algerian population to assess the potential role of this genetic polymorphism on the risk of colorectal cancer. Peripheral blood samples of 90 sporadic CRC patients and 100 normal controls were collected, DNA extracted genotyped using pyrosequencing technique. The distribution of genotypes of ERCC1 Asn118Asn genotype and allele frequencies among CRC cases was not significantly different from those among controls (P>0,05). The variant genotype combinations did not show any significant association with CRC susceptibility risk suggesting that the ERCC1 codon 118 polymorphism does not convey moderate increase in susceptibility to CRC in West Algerian population. This is the first study on ERCC1 gene polymorphism in our population suggesting that the Asn118Asn polymorphism may not be associated with the colorectal cancer risk in West Algerian population. Further research with a larger sample size is needed to reveal more information about this polymorphism and the appearance of colorectal cancer in our population.

J17.14

Cystic Fibrosis in Arab populations: Identification of novel mutations and complex allele in Egyptian and Lebanese Patients

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Background: Cystic fibrosis occurrence in Arab populations is not common and still remains unidentified. Furthermore, the lack of disease awareness and diagnosis facilities mislead the identification of Cystic Fibrosis (CF) during many decades. The knowledge about cystic fibrosis (CF) in Egypt is very limited, and a few reports have draw attention to the existence of CF or CFTR-related disorders in Egyptian population as well as in Lebanese population. Therefore a comprehensive genetic analysis of the CFTR gene was realized in the Egyptian and Lebanese patients.
Methods: DNA samples of 56 Egyptian and 17 Lebanese patients were screened for the CFTR gene mutations. The 27 exons and their flanking regions of the CFTR gene were amplified by PCR using the published primer pairs and were studied by automated direct DNA sequencing to identify disease-causing mutations.
Results: CFTR screening revealed the identification of seven mutations: including two novel (c.3718-24G>A; c.2782G>C) and five previously reported mutations (c.1418delG, c.2997-3000delAATT, c.902 A>G, c.2620-15C>G, c.3877G>A). Furthermore, six polymorphisms were found: c.1408A>G, c.3870A>G, c.2562T>G, c.1584G>A, c.4389G>A, c.869+11C>T. These mutations and polymorphisms were not previously detected in the Egyptian population except for the c.1408A>G polymorphism. Whereas Lebanese patients have a complex allele c.[869+11C>T;3909C>G] not previously described, no other novel mutations were identified.
Conclusion: Identification of CFTR mutations will become increasingly important in undocumented populations. The current findings will help us to establish a panel of the CFTR gene mutations in the Egyptian and Lebanese populations for designing an appropriate strategy for future genetic diagnosis of CF.

J17.15

Is Elucigene CF30 Kit effective in detecting CFTR mutations on Algerian patients?

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Introduction: Cystic Fibrosis (CF) [MIM # 219700] is the most common autosomal recessive genetic disease, in Caucasian populations [1].
In Algeria, no information is available about the incidence of CF. In the Maghreb, there are few data about the molecular basis of CF, probably due to an under diagnosis.
This study evaluated the effectiveness of the ELUCIGENE CF30 Kit in a sample of Algerian CF patients.
Patients and methods: Subjects: Twenty four (24) unrelated CF patients were recruited from the Pneumology and Allergology Department of the specialized Hospital Center in Canastel Oran (Algeria).CF diagnosis was based on a clinical findings and repeated positive sweat chloride tests (>60 mmol/l).
DNA extraction and genotyping
- 10 ml of whole blood was collected from all participants in EDTA tubes.
- Genomic DNA was extracted using standard Salting Out procedure [2].
- Mutations were explored by the ELUCIGENE CF 30 Kit (Tepnel Diagnostics, Oxon, United Kingdom) which is base d on a PCR/ARMS technique.
In screening the twenty four CF patients (48 chromosomes) for 30 mutations available in ELUCIGENE CF30 kit, only five mutations were found:
- c.1521_1523delCTT (F508del).
- c.579+1G>T (711+1G>T).
- c.1624G>T (G542X).
- c.3909C>G (N1303K).
- c.1652G>A (G551D).
All the mutations found were validated.
„ The Elucigene CF30 detected 5 mutations in our sample, which is about 16.66%.
„ The detection rate seems low compared to European populations. **Conclusion:** The most interesting is to develop in collaboration with ELUCIGENE one specific Maghreb Kit and inclued mutations which frequency higher than 2%.

J17.16

Notification of death from cystic fibrosis in Brazil during 30 years from 1981 to 2010

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The aim of this work was to evaluate the notification of cystic fibrosis (CF) as primary cause of death in Brazil, from 1981 to 2010, and to compare with developed countries. The Brazilian data was obtained from SIM-Data/SUS and the American from the CDC WONDER. The Brazilian median age at death (MAD) was 3.9 years in 1981 and 12.4 years in 2010 for typical severe CF. In 1994, the Brazilian MAD was much lower (7.2y) than that (21y) of seven developed countries reported by Forgarty et al, 2000. We found no increase in MAD in Brazil from 1981 to 1985; from 1986 to 2010 it showed a three-fold increase. From 1981 to 1990, there were few deaths over 25 years of age in Brazil, mostly concentrated in younger age groups. During the sample period there was an increase in deaths in higher age groups in Brazil, which may reflect better patient survival rates due to increased knowledge of the disease, with repercussions for its diagnosis and treatment. We also observed an increase in the reported cases of CF deaths per 100.000 inhabitants in Brazil over the years, possibly due to the better knowledge of the disease and consequently more accurate death notification, since this increase is not typical of a genetic disease. For the USA we observed a decrease in notified CF deaths, despite the population increase which may be a reflection of a smaller number of people born with CF, due to appropriate genetic counseling and prenatal diagnosis.

J17.17

SERPINA1 gene polymorphism frequency in clinically confirmed cystic fibrosis patients

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Background - *SERPINA1* gene is known to be one of cystic fibrosis (CF) modifier gene. Patients who carry the Z allele are at greater risk of developing severe liver disease. The aim of this study - to assess whether PIZ (rs28929474), PIS (rs17580) M1 ala/val (rs6647) or M2 (rs709932) alleles are associated with liver disease in patients with CF. Materials and methods - 61 clinically confirmed cystic fibrosis patients (enrolled 1998- 2013) and 61 control patients (without confirmed cystic fibrosis) were analysed for M, S and Z alleles in *SERPINA1* gene. The methods used were multiplex PCR and RFLP. Results - M2 allele was more common in CF patients comparing to the control group, (MAF affected=0.2449, MAF controls = 0.123, p=0.018, OR=2,314, CI95%=1,138-4.705). Analysing haplotype rs28929474-rs17580-rs6647-rs709932 frequency in the *SERPINA1* gene in patients and controls significant association was found in CF patients with haplotypes N-N-val-M2 (MAF affected=0.244, MAF controls=0.123, p=0.0198) and N-N-val-wt (MAF affected=0.516, MAF controls=0.647, p=0.051). Comparing CF patients with confirmed liver damage (n=6) and CF patients without it statistically significant association was not found. Conclusions - Haplotypes in the *SERPINA1* gene do not predispose cystic fibrosis patients to liver disease/damage. M2 allele and its containing haplotypes are more common in cystic fibrosis patients than in control group.

J17.18

Population data of 11 DNA markers from a sample taken from South Romania

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In this work, were typed in 100 unrelated individuals (sex ratio 60:40) from South Romania eight DNA markers. Aim was to analyze the genetic variability and to establish the relation between this region and other European populations. We establish Hardy-Weinberg equilibrium, gene diversity, genetic distances and tree topology by PHYLIP 3.68 package. Polymorphism's frequencies were similar to the mean frequencies calculated for the whole set of populations included in the study. The mean value of Ht was 0,201, and for Hs was 0,18. The most affiliated population with our lot are Italy, Spain, Poland, Germany, Greece and Turkey the most distant population are United Kingdom, Sweden, Croatia and Slovacia.

J17.19

A new variant of Ehlers-Danlos syndrome with inborn errors of mucopolysaccharide metabolism in the mother and son

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Background: Connective tissue dysplasias are characterized by the clinical polymorphism and genetic heterogeneity. Each new patient with Ehlers - Danlos syndrome, according to our observations is potentially a new variant of the syndrome.
Care report: Family V. has been followed-up for 13 years. Proband V. Clinical manifestations - coarse facial features, a disproportionate body, keeled chest deformity, kiphoscoliotic spinal deformity, wing-like scapulas, an increased skin extensibility, its softness, velvet, hypotonia, hypermobility of joints, varicose veins, swelling of the lower extremities. Proband's mother M. - disproportionate body, coarse facial features, kiphoscoliotic spinal deformity, a soft, doughy, hyperelastic skin, hypermobility of joints, varicose veins, lymphatic edema of the lower extremities.
The examination echografically revealed hepatosplenomegaly, metabolic, dysplastic changes in the kidneys, mitral valve prolapse, an additional chord of the left ventricle. Biochemically - signs of an increased collagen degradation, daily urine oxyproline - 155.4 mg/day, increased urinary glycosaminoglycans up to 148 CPC U/g creat., hyperprolinemia, hyperglycinemia, hyperprolinuria, hyperhomocysteinemia - 26.6 µmol/l. Pedigree analysis showed that the pedigree is burdened by cardiovascular disease.
MTHFR G1793A/MTRR A66G polymorphism was revealed in the proband and his mother by the study of gene polymorphisms of folate cycle system.
Conclusion: A new variant of Ehlers-Danlos syndrome has been diagnosed in the mother and son with the phenotype associated with metabolism errors of mucopolysaccharides, hypermobility of joints, hepatosplenomegaly, an early common varicose disease, muscular hypotonia due to lower activity of MTHFR G1793A/MTRR A66G enzymes.

J17.20
Multidisciplinary study of Endometriosis as a common complex disorder

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Endometriosis (E) is a common multigenetic disorder affecting almost 10% of women of reproductive age. Comparative molecular, genetic, immunological analysis and endocrinology tests were applied in the studies of 257 women with E and in 117 women in the control. Participation of the genes responsible for steroid hormone activity, their receptors, inflammation, proliferation, cell migration, apoptosis, intercellular adhesion, angiogenesis as well as the genes regulating their activity have therefore been suggested as plausible candidates. A handful of very interesting new candidate genes involved in oncogenesis, metaplasia of endometrium cells and embryonic development of female reproductive system were identified by GWAS technology. In addition to alterations in the DNA sequence itself, differential expression levels of the candidate genes might be caused by different epigenetic modifications including methylation, heterochromatization, miRNA regulation etc. Complex genetic net of E, implies participation of epigenetic landscape of E. Origin of E could be provoked by any combination of both genetic and epigenetic risk factors with subsequent canalization of pathological processes (reverse epigenetic landscape), which become irreversible soon after it starts.

J17.21
Asthma related FCER2 variant in Roma and Hungarian populations

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Asthma is a complex respiratory disease, which can be caused by environmental factors and genetical predisposition. It is one of the most widespread diseases in the world; it has a high presence in most ethnic group. The low-affinity IgE receptor (FcεRII /CD23) encoded by the FCER2 gene (Fc fragment of IgE) plays important role in the regulation of IgE responses and inhaled corticosteroids (ICS) therapy in asthma. Corticosteroids influence FCER2 expression and FcεRII receptor function. The intronic rs28364072 polymorphism (T2206C) of FCER2 gene associated with elevated IgE levels, severe asthmatic exacerbations and decreased gene expression. The variation in the FCER2 gene contributes to variation in ICS treatment response in asthmatics. Our aim was to investigate the ethnic differences, allele and genotype frequencies of intronic variant of FCER2 in average Roma and Hungarian population. We examined 458 Roma (206 males, 252 females, mean age: 46.4±18.4) and 397 Hungarian subjects (222 males, 175 females; 37.8 mean age±12.6 years) with PCR-RFLP method. We found more than twofold increased homozygous CC genotype frequency in Hungarian group compared to Roma samples (5.8% vs. 2.8%, p<0,05). The C allele frequencies were similar in each group (24.8% in Romas and 24.6% in Hungarians). The current study demonstrated that unfavourable variants can diversely occur in Hungarian and Roma individuals. Genetic test of FCER2 variant are likely necessary to assess the outcome of asthma treatment. Homozygous 2206C allele carrier Hungarians have higher chance for insufficient response to corticosteroids compared with Roma subjects due to genotype higher risk frequency. This research was supported by TÁMOP-4.2.3-12/1/KONV-2012-0028.

J17.22
Common MEFV gene mutation profiles in Familial Mediterranean Fever patients in Canakkale Population

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Objective:In the current results it was aimed to find out of the current study is to determine the frequency of common MEFV gene point mutations in 741 patients who preliminary diagnosed as FMF Method:The genomic DNA was isolated by spin-colon method(Roche, Germany) from peripheral blood samples with EDTA and buccal smears. The MEFV gene profiles for the current FMF cohort were genotyped by Pyrosequencing and direct Sanger sequencing techniques for the target common point mutations. Results: Twenty-two different point mutations were identified in 363(49%) patients and no mutation was detected in 378(51%) current patients suspicious for FMF. The most frequent mutations were M694V(32.7%), E148Q(15.1%), R202Q(11.8%), M680I(9.9%), V726A(7.4%), P369S(6.3%) and K695R(4.4%) in the current FMF cohort. The M694V/E148Q mutation was the most frequent compound point mutation that detected in the current FMF cohort. The most common clinical finding was abdominal pain in the all MEFV mutation types that detected in the current mutated FMF patients. Median attack frequencies of untreated patients are ; 3.14 for M694V, 2.8 for E148Q, 2.75 for R202Q, 2.71 for P369S and 1.44 for K695R. Although attack frequencies were less than patients with M694V, all of the patients from Canakkale region with mutations E148Q, R202Q, P369S and K695R had FMF clinical diagnostic criterias. Conclussion:The current results showed that the R202Q point mutation frequency was higher than the other sub-populations that reported from different reigions of TURKEY. It was seen that the initiation of clinical symptoms were delayed in patients with R2020Q mutation when compared to the others.

J17.23
The association analysis of polymorphism the metabolism of lipids genes with bmi, waist circumference and blood lipidogramma's parameters at women before and after the Menopause

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Using the PCR-RFLP method we have studied polymorphism of 36 genes involved in lipid metabolism in 212 women residents of the North-West Region of Russia (St. Petersburg) at the age of 18 to 77. We found the association of polymorphisms in several candidate genes with body mass index, waist circumference, total cholesterol level, low density lipoprotein cholesterol level and very low density lipoprotein cholesterol level. We proposed a model for the prediction of examined parameters based on logistic regression method. Our findings confirm the possibility of primary assessment of body mass index, waist circumference, total cholesterol level, low density lipoprotein cholesterol level and very low density lipoprotein cholesterol level in women based on genetic markers. It is shown that women before and after a menopause have a contribution of genetics to determination of body mass index, waist circumference, total cholesterol level, low density lipoprotein cholesterol level and very low density lipoprotein cholesterol level is various.

J17.24
Genetic epidemiological study of hereditary disorders in Tatarstan Republic (Russia)

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Tatars - are the second sized ethnos of the Russia. The load and genetic diversity of monogenic hereditary disorders (HDs) in three major ethnographic groups of Tatars from Tatarstan Republic were analyzed (Kazan Tatars-3 Districts, Mishars-2 Districts and Teptyars-3 Districts. The size of the investigated populations was more than 270,000 inhabitants (213,000 Tatars). The total population was examined by standard protocol of medical

genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 3500 HDs of OMIM could be identified by this protocol. Clinical investigations were performed by neurologists, ophthalmologists, orthopedic, otolaryngology's, dermatologists, pediatricians and clinical geneticists, focused on diagnostic of HDs. Genetic diversity of HDs in the investigated population consisted of 256 disorders (1597 affected): 135 AD, 97 AR and 24 X-linked recessive. Genetic differentiation of load of Mendelian HDs between populations of different ethnographic groups was found. The average prevalence rates were 1:172 persons in Kazan Tatars; 1:120 persons in Mishars and 1:150 persons in Teptyars. Variation of prevalence of all HDs in districts was from 1:350 persons to 1:85 persons. Significant differences in the load and diversity of HDs was found between groups "Kazan Tatars- Mishars" and "Teptyares - Mishars".

J17.25

Medical and population genetic characteristics of the population genodermatosis Rostov region, Russia

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Genodermatosis is a genetic disorder of the skin usually generalized.
The medical and population genetic studies genodermatosis population 12 districts of the Rostov region were conducted. The total size of the investigated population was 497,460 persons, 20% of whom are children (101,845 children).
Data collection and processing were done according to the protocol of Genetic Epidemiology Laboratory of Research Centre for Medical Genetics in Moscow.
The total prevalence rate among the population genodermatosis Rostov region was 1:1709 and 1:1047 for the child population, respectively.
The incidence of autosomal dominant (AD) genodermatosis was high (5,15±0,32) and incidence of autosomal recessive (AR) and X-linked genodermatosis was low (0,28±0,08 and 0,84±0,18, respectively). The same tendency was among children (the incidence of AD, AR and X-linked genodermatosis, 7,81±0,90, 0,72±0,27, 2,05±0,65, respectively). Incidence was calculated per 10,000 populations.
Significant differences in the different districts of the incidence rates of AD and AR disorders were reviewed. The correlation with one of the main population genetics factor is genetic drift was found.
Spectrum genodermatosis represented 22 nosological forms, 15 of them with AD, 5 with AR and 2 with X-linked inheritance types. The «nucleus» of genodermatosis spectrum in Rostov region population are ichthyosis vulgaris (1:5025), palmoplantar keratoderma with autosomal dominant inheritance (1:13818), multiple lipomatosis (1:31091), neurofibromatosis type 1 (1:9212), tuberous sclerosis (1:29262), X-linked ichthyosis (1:15546) and anhidrotic ectodermal dysplasia (1:49746). The prevalence rate of these diseases has been frequently than 1:50000.
Foci accumulation such genodermatosis as neurofibromatosis type 1, vulgar and X-linked ichthyosis was determined.

J17.26

Allelic heterogeneity of GJB2 gene in Romanian population with congenital isolated hearing-loss

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Aimes: Different alleles within the same gene can cause a similar variant phenotype. Previously published studies showed the allelic heterogeneity of GJB2 gene as main genetic cause of isolated congenital hearing-loss phenotype. The proportional distribution of the different mutations within GJB2 gene varies in different ethnic groups. The aim of the present study was to provide a complete and updated spectrum of mutations in GJB2 and GJB6 gene and to identify the most prevalent mutations in Romanian population.
Material and Methods: To overcome our aims, we used clinical data from 80 unrelated persons with congenital hearing-loss and performed ARMS-PCR and DNA sequencing techniques for detection of known mutations and identification of mutations within GJB2 gene. Analysis of del(GJB6-D13S1830) and del(GJB6-D13S1854) was performed by multiplex PCR.
Results: Most prevalent mutation was c.35delG (40.0%) in both homozygotic and heterozygotic forms. The second mutant allele was W24X (8.75%) also founded in homo- or heterozygotic forms, followed by c.-23+1G>A originally named IVS1+1G>A and R127W mutations with lower frequencies.
Conclusions: The study reveals c.35delG mutation as most prevalent one, absence of GJB6 gene deletions, genetic background of congenital hearing-loss in local population and supports improvement of genetic counselling services.

J17.27

Alleles analysis of *GSTA1* and *GSTP1* genes in the Polish population

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Glutathione S-transferases A1 and P1 are the main enzymes involved in the biotransformation of drugs, carcinogens and toxins. Their activity may influence drug response as well as susceptibility to diseases. *GSTA1* and *GSTP1* genes coding for these enzymes, are important objects of many studies due to their genetic variability which may affect enzymes activity. The aim of our study was to determine the alleles *GSTA1*A/*B*, *GSTP1*A, *B, *C* distribution in the Polish population. We performed our analyses on the DNA of 160 subjects from the Polish population. In the *GSTP1* gene we have genotyped two polymorphisms (I105V and A114V) with pyrosequencing method and the *GSTA1* gene was screened for the changes in the promoter region using sequencing. The detected variants were subjected to the haplotype analysis. We found alleles *GSTP1*A* (c.313A, c.341C), **B* (c.313G, c.341C) and **C* (c.313G, c.341T) with 65.3%, 23.8% and 10.9% frequency, respectively. As a result of *GSTA1* gene promoter sequencing we detected 8 SNPs located in sites: G-52A, C-69T, C-115T, A-513G, T-567G, G-631T, A-1066T, G-1142C, G-1245A. The allele *GSTA1*B,* associated with the decreased enzyme activity was observed in our study with frequency of 43.1%. Finally, 4 haplotypes have been determined in 160 Polish individuals. Our study demonstrated that the distribution of the alleles *GSTA1*A/*B* and *GSTP1*A, *B, *C* in the Polish population corresponds to data for Caucasians. Furthermore, we found novel SNPs, excluding three well known changes (G-52A, C-69T, T-567G), which are linked to the alleles *GSTA1*A/*B* affecting enzymes activity.

J17.28

Genome-wide association analysis identifies a locus on DMD (dystrophin) gene for power athlete status in Russians

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Power athlete status is a heritable trait: around two-thirds of the variance in this phenotype is explained by genetic factors. Since power and endurance are located at the opposite extremes of a muscle performance continuum, a genome-wide association study (GWAS) of elite Russian power-oriented athletes (sprinters and strength athletes) and endurance-oriented athletes as controls was performed to identify common genetic variants associated with elite power athlete status. 102 sprinters, 86 strength athletes and 178 endurance-oriented athletes were genotyped using the Illumina® HumanOmni1-Quad BeadChips. When comparing sprinters and endurance-oriented athletes, the most significant association (P=6.2∗10-7) was shown for the rs939787 polymorphism. Interestingly, this association was replicated (P=2.9∗10-6) by comparing strength athletes and endurance-oriented athletes (P=3∗10-8 when sprinters and strength athletes were combined). The rs939787 is located in the DMD (dystrophin) gene which plays an important role in muscle contraction and strength, linking the intracellular cytoskeleton to the extracellular matrix. In conclusion, our data suggest that the DMD gene rs939787 polymorphism is associated with elite power athlete status in Russians.

J17.29

Human longevity and Alu-polymorphism

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It is assumed that genome instability can affect the lifespan. Alu-insertion is one of the causes of such instability.
The aim of study was to search association between age and polymorphic Alu-elements, localized within introns of PLAT, COL13A1, ACE, LAMA2 and TEAD1 genes.
DNA of 1611 unrelated individuals from 21 to 109 years, ethnic Tatars from Russia, was genotyped. Age dynamics of the genotype and allele frequency was estimated using logistic regression analysis (SPSS18.0).
Associations between age and polymorphic Alu-elements localized within introns of COL13A1 (Ya5ac1986), LAMA2 (Ya5-MLS19) and TEAD1 (Ya5ac2013) genes was found for females. Chances of achievement of longevity age above for females with COL13A1*I/*I (range 57 - 109 years, OR=1.046, P<0.001), LAMA2*I/*I (range of 56-109 years, OR=1.059, P<0.001), LAMA2*I/*D (range of 56-109 years, OR=1.016, P=0.002) and TEAD1*I/*I (range of 78-109

years, OR=1.055, P=0.012) genotypes. Chances of achievement of longevity age below for females with COL13A1*I/*D (range 57 - 109 years, OR=0.948, P<0.001) and LAMA2*D/*D (range of 56-109 years, OR=0.981, P<0.001) genotypes. Association between age and a polymorphic Alu-element within an intron 16 of ACE gene (Ya5ACE) was detected for males and females. Decrease of ACE*D/*D genotype frequency was observed among long-livers females (range of 77-109 years, OR=0.953, P=0.006) and long-livers males (range of 76-109 years, OR=0.935, P=0.040).
Thus, Alu-polymorphism of COL13A1, ACE, LAMA2 and TEAD1 genes, possibly, are associated with attainment of longevity age. And besides, positive association with age is traced for insertional allele of Alu-elements.
Supported by grants RFBR 13-04-01561a, 14-04-97094a, 14-04-01169a.

J17.30
Formation of the genetic structure of Uigurs in Kazakhstan
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The study of genetic structure of modern human population is one of key problems of human genetics. Genetic and demographic parameters of uighurs is presented. Marriage and migration structure of fourteen rural regions were studied. The mean value of ethnic assortative marriage was 1.68. Analysis of marriage structure suggests genetic differentiation of rural districts uighur region and is characterized by a high proportion of mononational marriages (86-97%), low intensity and radius of migrations, the share of external migration to an average of 0.99%, high level of inbreeding from 0.0005 in Chundzha rural district to 0.00946 in Kolzhat. Positive assortative mating on a national basis was 4.2. High index of endogamy found in Kolzhat districts (72%).
The mean number of children per woman constituted 3.98. Crow index of total selection (Itot) and its components (Im, If) were 0.25, 0.04 and 0.20 respectively. The size of the portion of the population before reproduction (40.4% of the total), the prevalence of reproductive parts of the population (43.8% of total), and family size is 4.0 allow us to classify the type of growing population of uighurs.
Recent social and economic changes have led to an increase in differentiation of rural districts uighurs of Kazakhstan. Thus, detected subdivision uighur rural population on number of interacting subpopulation units, the main insulating factor that is positive assortative mating on national basis, set growing type of reproduction, described nature of migration processes, analysis of components as possible of potential population screening for uighurs in Kazakhstan.

J17.31
Random Inbreeding in Karachay
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Karachays live compactly in 4 regions of Karachay-Cherkessia (Russia). Karachays are one of the Turkic-speaking North Caucasian peoples. Values of Wright random inbreeding (a quarter of the sum of squares of frequencies of surnames) are counted on the basis of distribution of adults surnames for rank of „district" population and „Village Council" population. Both of thees assessments are necessary for carrying out various types of analysis. District population rank F_{st} values are: 0.0035 (Malokarachaevsky district), 0.0014 (Prikubansky district), 0.0016 (Ust-Dzhegutinsky district), 0.0015 (Karachaevsky district). The average-weight values F_{st} for "Village Council" population rank are counted separately for rural and urban populations (where urban present) and make: 0.0056 (Malokarachaevsky, rural), 0.0033 (Prikubansky, rural), 0.0042 and 0.0010 (Ust-Dzhegutinsky, rural and urban), 0.0084 and 0.0016 (Karachaevsky, rural and urban), thus the general assessment of the average-weight F_{st} value for these areas without division into urban and rural people make: 0.0056, 0.0033, 0.0024, 0.0059, respectively.

J17.32
An early manifestation of LBSL syndrome, case description
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Background :Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation is an autosomal recessive disease. Mutation in gene DARS2 is associated with this syndrome. The gene is located on the long arm of chromosome 1 (1q 25.1), the disease is associated with deficiency of mitochondrial aspartyl - tRNA synthetase.
Care report: 1 year and 5 month old boy T., with static kinetic and psycho-speech development delay, excess body weight (16kg). The child is from I pregnancy against the background of threatened miscarriage. Delivery at 36-37 weeks by cesarean section. Birth weight - 3300g. At the time of examination we paid our attention at the decreased muscle tone, muscle strength tendon and periosteal reflexes are not observed, nystagmus, ataxia. ENMG - the neuropathic type of changes, myopathic syndrome. MRI of the brain - using T1, T2 and FLAIR- modes - white matter lesions of the brain and cerebellum. The karyotype - 46,XY. Amino acid levels, blood lactate are unchanged. Partial analysis of the DARS gene by sequencing: in 5 gene locus - mutation c492+2T-C in the heterozygous state.
Conclusion: The disease manifests in the age of 3-15 years. Cerebellar ataxia, spastic tetraparesis and cognitive impairment develop. Before the onset of the disease, psychomotor and speech development corresponds to age, movement disorders develop further, patients become disabled by the second to fourth decade of life. In our case, the disease manifested in the heterozygous carrier by one year of life and was accompanied by obesity.

J17.33
Association analysis of haplotypes of the leptin gene promotor region with BMI in Roma population
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Leptin, an adipocyte-derived protein, plays a key role in regulating energy intake and consumption. Increased circulating leptin levels, commonly found in obesity, indicate a failure to signal satiety and halt the progression of obesity. Aim of this study was to investigate the association of single nucleotide polymorphisms (SNP) and haplotypes of the leptin gene promotor region with body mass index (BMI) in Roma (Gypsy), a population known to be vulnerable to developing obesity presumably due to both their life-style and genetic background.
We sequenced 1500 bp region of leptin gene promotor in the sample of 377 Roma individuals from Croatia and identified 9 polymorphic sites. Haploview software was used to assess linkage disequilibrium (LD) among polymorphic loci as well as the Unphased version 3.0.13 software for haplotype association analysis.
All nine SNPs were grouped into single LD block due to their proximity. None of the single markers (rs1349419, rs146378188, rs13245201, rs185230264, rs791614, rs12535708, rs10487506, rs11770725 and rs12535747) showed individual statistically significant association with BMI. However, two haplotypes, A-A-G-G-A-C-G-T-C and G-A-A-G-G-A-A-T-A, showed significant association with BMI (p=0.005061 and p=0.03214). Both p-values remained significant after 1000 permutation tests.
Our data showed that haplotype association analysis provided advantage over individual SNPs analysis. Haplotypes spanning the leptin gene promotor region were found to be significant predictors of BMI in Croatian Roma.

J17.34
Germline variants of ARID5B as possible risk factors for childhood acute lymphoblastic leukemia in Latvia
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Background. Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy but etiology of pediatric ALL remains poorly understood. Data from GWAS provided convincing evidence that inherited genetic variation in ARID5B contributes to childhood ALL predisposition, the strongest association was found with SNP rs10821936, some studies show strong association with whole intron. **Aim.** To detect possible association of SNPs in intron 3 of gene ARID5B and ALL predisposition to childhood ALL in Latvia. Material and Methods. In study were enrolled 34 ALL patients and their biological parents. In study group were detected rs10821936 and 6 other SNPs as a closest three from both sides to SNP rs10821936, where MAF > 1%. Analyses were performed using PCR, RFLP and sequencing. **Results.** By TDT test strongest association were found with rs10821936 (OR=10, CI95% 1.28-78.12, p=0.0066), rs10821937 (OR=0.667, CI95% 1.172-8.735, p=0.0164) and almost significant with rs7908445 (OR3.333, CI95% 0.917-12.11, p=0.0522) Haplotype analysis TDT test - with sliding window 5 - rs7923074-rs77918077-rs10821936-rs12246030-rs10821937 (AGCCC) (transmitted frequency 5.959, untransmitted frequency 0.0284, p=0.0153); rs77918077-rs10821936-rs12246030-rs10821937-rs7896246 (GCCCA) (transmitted frequency 8.761, untransmitted frequency 1.042, p=0.0137). Haplotype analysis TDT test -with sliding window 4 - rs77918077-rs10821936-rs12246030-rs10821937 (GCCC) (transmitted frequency 5.929, untransmitted frequency 0.0279, p=0.0156); rs10821936-rs12246030-rs10821937-rs7896246 (CCCA) (transmitted frequency 6.917, untransmitted frequency 1.038, p=0.037). **Conclusions.** 1. We have found an association with SNP rs10821936 C allele and increased predisposition

to childhood leukemia. 2. We detected risk haplotypes of childhood ALL composed of 7 SNPs located in intron 3 of ARID5B gene.

J17.35
Genetic polymorphism and mRNA levels of TNFα and TGFβ genes in patients with chronic lower limbs infections

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Introduction: The wound healing process is important medical problem which still have gain no binding results. We choose two factors, strictly involved in inflammation, and connected in proper tissue healing.
Aim of study: The purpose of the study was to investigate SNP`s in TNFα (G-308A) and TGFβ (G-74C, T-29C) genes in patients and evaluate expression of mRNA levels in comparison with controls.
Material and methods: Group of 191 patients were divided into subgroups: A) chronic leg ulcers, B) chronic non-healing wounds, C) infected ischemic necrosis of foot, D) lymphatic ulcers symptoms of dermato-lymphangioadenitis (DLA). A control group comprised 129 blood donors. Detection of polymorphisms was performed using the PCR-RFLP method. The level of TNFα and TGFβ genes expression was performed by Real-Time PCR.
Results: Patients in subgroups showed higher frequency of genotypes TNFα-308GG and lower of GA than in controls. The homozygote TNFα-308AA was more frequent (subgroups B and C) than in controls (p<0,0001). TGFβ-74GG genotype was at highest values, in subgroup B. The GC genotype was at similar level in all subgroups being lower than in controls. TGFβ-29TT and TC genotypes was at similar level to controls. The presence of the polymorphic allele TNFα-308A correlated with an increased level of gene expression (subgroup A). In case of both TGFβ SNP`s the polymorphic allele C were correlated with increased gene expression.
Conclusions: The presence of polymorphic alleles could predispose to increased production of mentioned proteins. Protein overexpression may impair the proper conduct of wound healing contributing to formation of ulcers.

J17.36
The study of the populations of the Volga-Ural region of Russia in the context of Eastern Eurasian mtDNA haplogroups

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Volga-Ural region is a place of encounter of different peoples who vary in both linguistic and religious grounds. An emplacement in the crossroads of two continents, Europe and Asia, had a great influence on creation of this diversity.
The aim of our study was to analyze few lines that belong to the East Eurasian component of the genetic pool in the region. We focused on the lineages of haplogroup A namely A4b and A10.
Haplogroup A4b has previously been found in the study of Derenko et al., 2008 in the Evenk and Buryat populations from Siberia. In our research, we conducted a full genome sequencing of mtDNA samples with this haplogroup in Udmurt and Besermyan populations and found that mutations in the coding region do not correspond to those in the samples studied by Derenko et al., 2008. It is likely that these two lines are the two sub-branches of one subclade A4b. Although our populations showed similarity in difference with the Siberian A4b subclades on the other hand the presence of A4b in our populations itself shows some old connections with Siberian populations.
We also analyzed a full sequence of all known published examples of haplogroup A10 and compared with our results. We found that some new mutations that were not previously detected in Dolgan, Nganasan, Nogay and Tadjik populations. It is interesting that Tajik, Tatars from Kazan and Chuvash samples are likely belong to the one subgroup of this haplogroup, indicating the recent common ancestry of these populations.

J17.37
Polymorphisms in the RIG-I-like receptors (RLRs) genes and susceptibility to multiple sclerosis in the German population

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system potentially associated with viral infection. RIG-I-like receptors genes (*RIG-I*, *IFIH1* and *LGP2*) have been hypothesized as involved in the viral etiology of MS, since they are implicated in viral recognition. Previous studies have produced conflicting results regarding the association between *IFIH1* gene polymorphisms and MS. Here we examined the effects of 13 single nucleotide polymorphisms (SNPs) in the *RIG-I*, *IFIH1* and *LGP2* genes on MS in the German population. The study comprised 716 patients with MS and 706 healthy control individuals. Genotyping was performed using RFLP, TaqMan genotyping assays and high-resolution melting (HRM) analysis. There were no significant differences (p>0.05) between MS patients and healthy individuals for any of the investigated SNPs as well as haplotypes derived from these SNPs. Further, no differences were observed between healthy individuals and MS subgroups stratified according to disease characteristics. Our results suggest that variation in the *RIG-I*, *IFIH1* and *LGP2* genes does not exert a major influence on MS risk.

J17.38
Associations between MX2 polymorphism and the acquisition of HIV and co-infections in Caucasian intravenous drug users (IDUs)

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BACKGROUND: Until recently, myxovirus resistance protein 2 (MX2), a type I interferon-induced GTP-binding protein, was thought to be devoid of antiviral activity. Latest studies indicate MX2 to be involved in blocking of HIV-1 entry. We aimed to determine, whether C/T polymorphism of MX2 gene (rs45430) is associated with acquisition of HIV and co-infections (HCV, HBV, GBVC) in IDUs.
METHODS: Study included 345 IDUs and control group comprised of 301 blood donors (all HIV, HCV, and HBV negative). C/T polymorphism of MX2 gene was determined using TaqMan allelic discrimination assay.
RESULTS: Of IDUs, 50% were HIV+, 88% HCV+, 67% HBV+, and 33% GBVC+. In total 296 donors and 342 IDUs were successfully genotyped. C/T polymorphism was in Hardy-Weinberg equilibrium and allelic frequencies were similar in both groups (0.65 for T allele in IDUs, 0.6 for T allele in donors). No associations between C/T polymorphism and acquisition of HIV, HBV, and HCV were found. However, the prevalence of persons possessing at least one C allele was higher among GBVC- compared to GBVC+ (63% vs 51%, p=0.047). Persons with at least one C allele had decreased odds of being GBVC+ (OR=0.62, 95%CI=0.39-0.98), but after adjusting for co-variables associated with GBVC status (age, HIV positivity) it did not remain significant (OR=0.66, 95%CI=0.41-1.06), which might refer to underpowered groups.
CONCLUSIONS: This is the first study to investigate associations between MX2 (rs45430) polymorphism and acquisition of HIV and co-infections in high risk population. Whether this polymorphism plays a role in GBVC acquisition needs further evaluation.

J17.39
Do polymorphisms affecting the ornithine transcarbamylase (OTC) gene affect myocardial infarction risk and blood pressure in the West Algerian population?

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Purpose: Recently, it has been shown that the rs5963409 polymorphism of the gene encoding ornithine transcarbamylase (OTC) is associated with hypertension and coronary vasomotor. On the basis of these results, we chose to study the association of two polymorphisms rs5963409, located at the 5' region of the promoter and rs1800321, located in exon 2 of the OTC gene with the risk of the occurrence of myocardial infarction (MI) and the values of systolic and diastolic blood pressures. Methods: Concerning MI, the polymorphisms were characterized in a case-control study (69 cases vs 67 age-matched controls) based on the male population originating from Oran, Algeria. The associations with blood pressure were assessed in an enlarged control group including 115 male subjects. Genetic characterization of two polymorphisms was performed by PCR (Polymerase Chain Reaction) followed by enzymatic digestion. Results: We neither showed associations of the rs5963409 and rs1800321 polymorphisms with variations in blood pressure values. However, we observed a significant interaction between the rs5963409 polymorphism and Body Mass Index (BMI) on the risk of the occurrence of MI. Conclusion: Our results indicate the two polymorphisms of the OTC are not associated with the MI in the West Algerian population.

J17.40

NLRP3 genotype CG increases the risk of HIV-1 in Caucasian intravenous drug users (IDUs)

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BACKGROUND: The increased stability of NLRP3 (Nod-like receptor family, pyrin domain containing 3) by rs10754558 G could lead to earlier immune response against viruses and it has been associated with decreased susceptibility to HIV in mother-to-child transmission. Whether and how the NLRP3 polymorphism is associated with susceptibility to HIV in IDUs is unknown.
METHODS: We studied 172 HIV+ IDUs and 20 highly exposed HIV- IDUs (HESNs). 301 blood donors negative for HIV, HCV and HBV were used as controls. The NLRP3 polymorphism rs10754558 (C/G) was determined using TaqMan allelic discrimination assay.
RESULTS: Altogether 191 IDUs were genotyped successfully. Of HIV+ 98.3% were HCV+ and 82.0% HBV+. Of HESNs 80.0% were HCV+ and 45.0% HBV+. NLRP3 C was the major allele in all groups and did not differ between groups (allelic frequencies 0.68 in HESNs, 0.63 in HIV+ and 0.60 in donors). The polymorphism was in Hardy-Weinberg equilibrium in all groups. The most common NLRP3 genotype was CG (52.9% in HESNs, 55% in HIV+ and 44.2% in donors). The distribution of NLRP3 polymorphisms did not differ between HESNs and HIV+ IDUs. However, HESNs possessed more NLRP3 CG genotype than donors (70.0% vs 44.2%; p=0.0373). No differences were found in CG heterozygotes in HIV+ (50.3%) compared to other groups.
CONCLUSIONS: Similar to mother-to-child transmission NLRP3 CG genotype might be associated with increased risk of HIV infection in Caucasian IDUs.

J17.41

Molecular screening of PTPN11 hotspot exons in moroccan children with Noonan syndrome

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Noonan syndrome is an autosomal dominant disorder with an estimated incidence of 1 in 1 000–2 500 live births. It is characterized by short stature, typical facial dysmorphia and congenital heart defects. This disorder results in almost 50% of patients from protein-tyrosine phosphatase, non-receptor type 11 (*PTPN11*) mutations, that lead to gain of function in the non-receptor protein tyrosine phosphatase (SHP-2) into the signaling RAS- mitogen activated protein kinase (RAS–MAPK) pathway.
Till now, mutational screening of *PTPN11* has been carried out in different populations. Thus, the aim of this study was to screen the *PTPN11* mutations in series of Moroccan Noonan Syndrome patients.
Twenty-three patients were recruited in HASSAN II university hospital of Fez within the last five years. We have extracted genomic DNA from Blood samples. Then, we used PCR and bidirectional direct sequencing of the PTPN11 hotspot exons reached by mutations for 23 Moroccan children.
We detected 5 heterozygous missense mutations in 23 sporadic patients (21.7%). Those patients share the characteristic facial traits of Noonan syndrome. Most of them suffer from pulmonic stenosis.
The present study allowed identification of mutations clustered in hotspot exons of *PTPN11* gene in Moroccan Noonan syndrome cohort, and enabled us to give an appropriate genetic counseling to the mutation-positive patients.

J17.42

Role of the Neuregulin 1 gene(NRG1) in multiple sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system, characterized by inflammation, demyelination and axonal injury, probably caused by environmental factors in genetically susceptible individuals.
Neuregulin 1 (NRG1) is involved in neuronal specification and migration, gliogenesis, neuron-glial signalling. Viehover et al. (2001) found that the expression of Neuregulin is dramatically reduced in MS lesions and hypothesized that the absence of this protein contribute to the paucity of remyelination in the disease. For these reasons, the NRG1 gene could represent a good candidate for a research on patients with immuno-related and demyelinating disordes. We focused our attention on it, aiming at evaluating its role in Southern Italy by means of a case control study.
We examined 226 patients with MS and 200 healthy controls. Genotyping was performed by amplification and subsequent digestion with the restriction enzyme CviQI.
We found significant differences of allele and genotype distributions (p=0.011 and p=0.05, respectively) between cases and controls. To our knowledge, this is the first study performed on NRG1 in patients with MS. On the basis of the preliminary data obtained in our population, we could hypothesize an involvement of the gene in the susceptibility to the disease. In particular, the presence of the T allele, under-represented in the patients, seems to be a protective factor against MS. However, due to the small number of participants, the confirmation of the role of NRG1 requires further studies extended to a larger sample of subjects and also to other populations.

J17.43

Variability of Genes Associated with Obesity in Populations of Russia and Neighboring Countries

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The prevalence of obesity and related diseases reached a high level throughout the world. The role of genetic factors in the disease development has been proved by numerous studies on twins and adopted children by testing candidate genes predisposing to obesity. Genome-wide association studies (GWAS) is one of the advanced methods used to detect mutations associated with overweight. We selected 20 SNPs associated with obesity with high level of significance from GWAS-catalog. Allele frequencies for these SNPs, were analyzed in 14 populations of Russia (Yakuts, Buryats, Tuvans, Komi, Mordovians, Russians, Khanty, Kets, Chuvashes, Kabardinians, Karachai, Ossetians, Tatars, Udmurts and Karelians) and neighboring countries (Kazakhs, Uzbeks, Kyrgyz, Megrelians and Moldavans). Data on 7 native non-admixed populations from HapMap and "1000 Genomes" project were also included into analysis. Correlation analysis of allele frequencies and genetic diversity with geographic (latitude, longitude, elevation) and climatic (mean annual temperature, average temperature in January and in July, temperature range, maximum and minimum temperature and precipitations) parameters was performed. Allele frequency of 16 from 20 studied SNP correlated with climate and geography. 5 SNP showed correlation with latitude only (rs5762430, rs1805081, rs7138803, rs2531995 and rs11042023), 6 - with latitude and climate (rs1704198, rs9299, rs10182181, rs6110577, rs999943 and rs7603514) and 5 – with climate only (rs3101336, rs7784447, rs259067, rs2275848 and rs7474896). The revealed relationships between genetic and geo-climatic parameters may indicate the presence of positive selection on genes associated with obesity and adaptive changes in the gene structure in human populations during human dispersal out of Africa.

J17.44

The common polymorphism Val109Asp in the omentin gene is associated with daily energy intake in the Central-European population

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Omentin was originally first described in 2003 and was reported to be expressed specifically in human omental adipose tissue. So far, very little is known about the relationship between the genetic variability of the omentin gene and pathophysiology of obesity. The aim of the study was to investigace two common polymorphisms in the omentin gene (rs2274908 and rs2274907) and dietary composition and anthropometric parameters of obesity in the Central European population.
Material and methods.The total of 495 subjects were included into the study that were further dividend into the non-obese, obese and morbidly obese cohorts. Dietary habits were established using the 7-d food records and selected anthropometric parameters were measured.
Results: There were significant differences in genotype distributions of rs2274907 between the obese and morbidly obese cohorts (Pg = 0.01). In the multivariate modelling, the rs2274907 polymorphism expressed independent prediction role for the daily energy intake, independently on the age and gender distribution (p = 0.03); the TT genotype being associated with the lowest average energy intake (7877± 2780 J/day) and the AA genotype with the highest daily intake of energy (8764 ± 2467 J/day). Significant association of the rs2274907 were also observed for the daily consumption of fat and proteins.
Conclusion: This is so far the first study to investigate the polymorphisms in the omentin gene in a large population cohort of obese and non-obese individuals. Based on our results, the rs2274907 polymorphism is associated

with the daily energy intake as well as daily intake of fat and protein.

J17.45
Associations between polymorphisms in PAX9 gene and congenital missing teeth

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The congenital missing of teeth is one of the most common developmental disorders in dental pathology with values between 4.4% and 8%. Autosomal dominant hypodontia is caused by abnormalities in genes MSX1 and PAX9. PAX9 gene plays a key role in ondontogenesis and gene polymorphisms were found in both familial forms but also in sporadic cases with dental agenesis. Recent studies show model PAX9 gene polymorphisms in patients with dental agenesis.
Our study group consisted of 180 adolescents aged 18-22 years and one hundred controls. In this group were found a total of 10 cases (5.5%) with specific agenesis, without signs of other disorder, and no other dental anomaly, of which 7 cases were sporadic and 3 cases with familial aggregation. In these families, besides the index, agenesis was also found in five individuals. In two families agenesis was present in grandmother, mother and daughter.
All patients were investigated clinically oral and extraoral and X-ray was performed. Selection of patients for the genetic analysis of gene PAX9 was based on the type of hypodontia clinically found, because families bearing PAX9 mutation had tooth agenesis as the only clinical sign. Heterozygous mutation C503G was detected in the female proband, in her mother and grandmother. One polymorphic A240P mutation site was found, in PAX9 gene, in two sporadic cases. This variant was found in subpopulations of African American and Europe, but had no relevance to the ethnic Chinese population.

J17.46
Association of the MMPs genes single nucleotide polymorphisms with gastric and duodenal ulcer in Volga-Ural region of Russia

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Peptic ulcer disease (PUD) is a chronic disease, which is based on recurrent gastric (GU) or duodenal ulcer (DU). Recent studies have indicated that gastric ulceration is associated with cleaving and remodeling of the extracellular matrix (ECM) by matrix metalloproteinases (MMP). DU and GU has a genetic background and the aim of this study was to investigate the allele and genotype distribution of SNPs in the MMP1 (rs49437), MMP2 (rs2285053), MMP3 (rs3025058), MMP9 (rs3918242, rs17576) and MMP12 (rs2276109) genes in patients with PUD and healthy donors from Volga-Ural region of Russia. The patient group consisted of 260 individuals with PUD, the control group included 272 unrelated non-ulcer individuals with different ethnic origins (Russians, Tatars, Bashkirs). Genomic DNA was extracted from peripheral blood leucocytes by standard phenol/chloroform method. Genotyping was performed by polymerase chain reaction - restriction fragment length polymorphism analysis. The analysis has revealed that Russians have significant higher frequency of rs494379*A/G and rs3025058*5A/5A genotypes in patients than in control group (P=0.02;OR=1.86 and P=0.0007;OR=5.31, respectively). We have also found the association of rs494379*A/G genotype with PUD in Tatars (P=0,001;OR=2,58). Genotype rs17576*A/G is a marker of the increased risk of PUD development in males (P=0,005;OR=1,79), whereas rs3918242*T/T and rs17576*A/A were protective (P=0.037;OR=0.10; P=0.01;OR=0.61, respectively). The association analysis of the rs228505 and rs2276109 with PUD has not revealed significant differences between patients and healthy donors (p>0,05). Thus, we have determined statistically significant association between MMPs genes polymorphisms and peptic ulcer in Volga-Ural region of Russia.

J17.47
Sequence analysis of *PMS2* exon 11 and homologous region in pseudogene *PMS2CL*

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PMS2 gene product is involved in DNA mismatch repair process. Besides the gene, there are several pseudogenes in the human genome, showing high sequence homology. To describe and distinguish sequence polymorphism in highly homologous loci which could be co-amplified in PCR assays, we have sequenced "short" 243 bp fragment containing co-amplifying regions chr7:6026862-6027104 (part of *PMS2* exon 11) and chr7:6776854+6777096 (part of *PMS2CL* pseudogene) in 50 DNA samples. In 14 DNA samples, additional "long" fragment encompassing region chr7:6026862-6027488 (part of intron 10 and exon 11 *PMS2*) has been sequenced. The same heterozygous positions in both fragments, together with peak height differences in heterozygous positions within the short fragment allowed us to determine their location (gene/pseudogene) and to calculate MAF estimates. Altogether 6 variable positions were identified and most of them had been registered as SNPs in *PMS2* in public databases (NCBI). Among them were rs111255573 (C/T) in intron 10 (MAF=0,036) and rs15020462 (A/G, Gly460Asp) in exon 11 (MAF=0,080). High frequency was determined for rs1805321(C/T, Pro470Gly, allele T frequency 0.525) in additional 134 samples by restriction analysis. We have found that rs63750685 polymorphism (C/G, MAF=0.120) is actually located in the pseudogene (rs199736163), as well as novel SNP A/G in position chr7:6777067 (MAF=0.090). The previously known SNP rs1805320 (C/T) turned out to be a difference between the gene (C) and the pseudogene (T) sequences. Our results refine SNP list in the regions and emphasize careful probe design for SNP studies in the *PMS2* gene.

J17.48
The association of REN gene polymorphism with athlete status and muscle mass

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The renin-angiotensin system (RAS) is supposed to be one of the regulators of skeletal muscle growth and differentiation (Zhang et al. 2003; Johnston et al. 2011). Renin (encoded by *REN* gene), as a component of the RAS, activates the renin-angiotensin cascade by catalyzing the conversion of angiotensinogen to angiotensin I (Rupert, 2006). The aim of present study was to investigate the association between the intron 8 83A/G (rs2368564) polymorphism of the *REN* gene, athlete status and muscle mass in Russians. Two hundred and sixty eight Russian athletes (90 females and 178 males) from different sporting disciplines were involved in the study. *REN* genotype and allele frequencies were compared to 151 controls (74 females and 77 males). Genotyping for the *REN* polymorphism was performed by RT-PCR. Muscle mass parameters were assessed by bioelectrical impedance analyzer Tanita MC 980 (Japan) in 125 athletes (44 females and 81 males). We found that the frequency of the *REN* G allele was significantly higher in power-oriented athletes (78 vs 68%; P=0.021) compared to controls and this difference was even more pronounced in elite power-oriented athletes (89%; P=0.018). Furthermore, the *REN* G allele was positively correlated with fat-free mass, absolute muscle mass, muscle mass of trunk and left/right legs in elite athletes. In conclusion, we have shown that the 83A/G polymorphism of the *REN* gene is associated with power athlete status and skeletal muscle parameters in Russians.

J17.49
The association of *PPARD* and *PPARA* gene variants with physical performance in Lithuanian elite athletes

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Peroxisome proliferator-activated receptor (PPAR) delta and PPAR alpha (encoded by the *PPARD* and *PPARA* genes) play a role in energy homeostasis, vascular biology, mitochondrial function. Functional SNPs in the *PPARA (*rs4253778, intron7 G/C) *and PPARD* (rs2016520, 5'-UTR region of the exon4, +294T/C) have been associated with mRNA and/or protein activity. The aim of this study was to determine the allele/genotype frequency distributions among 130 Lithuanian elite athletes (endurance-oriented (n=40), power-oriented (n=52), mixed endurance/power athletes (n=38)) and 175 healthy non-athletes controls. Genotyping was performed by PCR-RFLP. Results showed the genotype distribution was in Hardy-Weinberg equilibrium within all groups (P>0.05). Genotype frequency differed significantly between the male athletes and the male controls (*PPARA* GG/GC/CC: 58.4/35.7/5.9% vs 76.2/22.9/0.9%; P=0.012; *PPARD* TT/TC/CC: 88.1/11.9/0% vs 75.2/21.9/2.9%; P=0.031). The *PPARA* GG genotype was more frequent among the athletes in endurance(67.5%) and mixed sports(71.1%) than the power (50%). In power-oriented events group significantly elevated frequencies of *PPARA* GC and CC genotypes were determined, compared to the endurance-orientated (P=0.039), mixed athletes (P=0.0005) and controls (P=0.008). These results support the positive as-

sociation of the *PPARA* C allele with power performance. *PPARD* genotype distribution in athletes with mixed endurance/power activity showed significant difference compared to controls (TT/TC/CC: 92.1/7.9/0% vs 53.1/40/6.9%; P=0.023). In conclusion, the *PPARA* C allele may help athletes to attain elite status in power-oriented sports, and the *PPARD* C allele is a factor unfavourable for athletics. This finding is relevant of physical performance; it may also be informative for the targeted prevention of diseases associated with low fitness.

J17.50
A polymorphism in a possible regulatory region of PGR associated with risk of spontaneous preterm labor with intact membranes (PTB-I)

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Background. Progesterone has been used to prevent recurrences of preterm labor suggesting that the progesterone receptor gene (*PGR*) may play a critical role in the maintenance of pregnancy. The aim of this work was to investigate the association between a single nucleotide polymorphism (SNP) located in a possible regulatory region of *PGR* (rs1942836) and two different clinical subtypes of preterm birth: spontaneous (PTB-I) and premature rupture of membrane (PTB-PROM). This SNP has been previously studied in a heterogeneous group of PTBs.
Methods. The sample included 412 triads (proband, mother, father) recruited at the Nuestra Señora de la Merced Maternity Hospital in Tucumán, Argentina. Of these triads, 200 had probands from PTB-I and 212 had probands from PTB-PROM. Genotyping was performed using Applied Biosystems Taqman probes and the Fluidigm genotyping platform. Both maternal and fetal genetic effects were analyzed using a log-linear method for analysis of case-parent–triad data (Weinberg et al. 1998).
Results. We found a significant association between rs1942836 (maternal effect) and PTB-I (OR: 2.01; IC 95%: 1.12-3.61; p= 0.01), but not between this SNP and PTB-PROM (OR: 1.05; IC 95%: 0.57-1.93; p= 0.85).
Conclusions. These results would suggest a specific association between the *PGR* gene (maternal effect) and PTB-I, but not with PTB-PROM. These findings may have implications in understanding the pathophysiology of clinical subtypes of preterm birth and the potential therapeutic role of progesterone in prevention of PTB-I.

J17.51
The diversity and prevalence rate of monogenic hereditary pathology among the children of Tatarstan Republic (Russia)

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Results of prevalence rate and genetic diversity of monogenic hereditary disorders (MHDs) among the children's of eight investigated Districts of Tatarstan Republic (Russia) was submitted. The total size of investigated populations was made 268894 people, including 57648 (21.44%) children. The ethnic structure of considered sample on more that 80 % is presented by Tatar population. The study was conducted on the original protocol examined by standard protocol of medical genetic research elaborated in laboratory of genetic epidemiology, Research Centre for Medical Genetics. About 3500 MHD and syndromes of OMIM could be identified by this protocol. Clinical investigations were performed by pediatricians, clinical geneticists, neurologists, ophthalmologists, orthopedists, audiologist, and dermatologists, focused on diagnostic of MHD. The spectrum of MHP detected in the eight Districts comprises 158 nosologycal forms - 84- autosomal dominant (AD), 54- autosomal recessive (AR) and 20- X-linked. The total prevalence rate of children's population by all types MHD - AD, AR, and X-linked, separately for urban (1:187 children) and rural (1:89 children) populations was calculated. In rural populations, the prevalence is higher in 2 times higher than in urban. The total prevalence of MHD was 1:103 children. Given that this study can detect only half of hereditary diseases, the total prevalence of hereditary disorders in children more than 1,2%. In this study, we studied the prevalence of hereditary diseases among children Republics Bashkortotan, Udmurtia, Rostov Region and Chuvashia, in which the burden of hereditary diseases was 1.4%, 1.2%, 1.3% and 1.1%, respectively.

J17.52
Birth defects in Chile: Implementing a National Registry and Surveillance System

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Chile is an upper middle income country, has an infant mortality rate of 7.7 per 1000 births in 2011. Birth defects (BD) are an important public health problem, account for 35% of the infant mortality, being the second leading cause after prematurity. Aware of that Chilean Ministry of Health has designed a registry and surveillance system for BD (REVINACH) in stillbirths, newborns and infants less than a year in all public hospital in Chile. We report herein the characteristics of this national registry system.
The Department of Statistics at Ministry of Health will be in charge of clearing, encoding, statistical analyses and dissemination of data. Data collection will be in charge of any professional that detects a BD. This registry has 3 instruments for data collection (1) Online Birth Certificate with an open field named: „Congenital Malformations Anomaly" (2) Online database ex-post to collect information after birth named "REVINACH" (3) Online database to collect complex information named "Genetics Module" as a second step. All three systems are linked to a three partite system involving the Civilian Registry and the National Statistic Institute.
This system will be a source of information for surveillance of BD and will be essential to the planning, implementation, evaluation of public health practice and policies as well as prevention.

J17.53
Genetic predisposition to the development of restenosis in Kazakh population

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Restenosis is a renarrowing of the stented vessel that occurs after stent placement. At the moment, genetic factors of restenosis were studied mostly in Caucasian population. However, ethnical variability of genetic markers is well known. The purpose was to study association of genetic variation in candidate genes of patients diagnosed with restenosis in the Kazakh population.
This case-control study included patients with a diagnosis of coronary heart disease (CHD) who developed restenosis. The control group consisted of patients with CHD who have not developed restenosis within 6 months after stenting. Genomic DNA was extracted from peripheral whole blood samples. SNP genotyping was performed on QuantStudio 12K Flex. Allele frequencies in both case and control groups were in compliance with Hardy-Weinberg equilibrium. Odds ratios (OR) and p-values have been calculated for all studied SNPs. Confidence interval (CI) was set at 95% significance level.
Sampling data shows that men undergo stenting at an earlier age than women. In addition, an increased BMI was found in all patients. The results showed that known associations of polymorphisms with the risk of restenosis, shown by J.W. Verschuren in the European population, were not observed in Kazakh population. However, a statistically significant association with the risk of restenosis was found with fibrinogen beta-chain gene polymorphism rs1800790 (OR - 2.1, P-value - 0.009) and thrombomodulin (THBD) gene polymorphism rs1042579 (OR - 1.7, P-value - 0.01).
It can be inferred that both polymorphisms are likely to be predictors of restenosis, while THBD polymorphism is specific for Asian population.

J17.54
Dimorphism -23HphI of the INS gene (rs689): association with type 1 diabetes in several populations of the Russian Federation

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After HLA, the next-strongest genetic association with Type 1Diabetes (T1D) is seen for the *INS* gene. The aim of this study is cross-ethnic group comparisons of frequencies and analysis of associations with T1D -23*HphI INS* dimorphism (in promoter region of the gene, rs689) in Bashkir, Buryat, Udmurt, Yakut and Russian ethnic group of the Russian Federation. Case-control design was applied for assessment of 528 patients with T1D and 374 healthy sex and ethnos matched individuals. Allele identification was performed with RFLP or Real-Time PCR technique. Association of genetic markers with pathology was evaluated according to odds ratio index (*OR*), association was considered statistically significant when p-value<0,05). Allel A and genotype AA of rs689 are associated with T1D in Russian (*OR*=2,5 and 2,7 respectively), Bashkir (*OR*=5 and 6 respectively), Udmurt (*OR*=2,4

and 2,6 respectively) and Yakut (*OR*=3 and 4 respectively) populations. Allel T and genotype T+ of rs689 are protective markers in these populations (0,16<*OR* <0,4). Association of rs689 with T1D has not been identified in Buryat population (with low incidence of T1D). Cross-ethnic comparison of frequencies of alleles and genotypes showed statistically significant differences. Buryat population differs from all other examined populations by much higher frequency of allele A (87% vs. 69-75%, p<0,006) and of genotype AA (77% vs. 45–60%, p<0,01). It is concluded that in the Buryat ethnic group rs689 is not diabetogenic marker unlike all other examined groups.

J17.55

Spinocerebellar ataxias (SCAs) in Europe: updating current situation

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Spinocerebellar ataxias (SCAs) represent a clinically, genetically and pathologically heterogeneous group of rare hereditary untreatable neurodegenerative disorders, which affect the cerebellum and its connections. As far as we known, there are more than 30 subtypes reported in literature. In Europe, it's estimated that one to three per 100 000 individuals suffer from a SCA subtype, but this number could vary among different ethnic and geographic groups. Epidemiological data on SCAs is considered scarce and fragmentised, with only their relative frequency within a population usually being better known. The goal of this study was to systematically compile and update the available information regarding prevalence of SCAs, as well as several indicators, namely number of published reports, expert centres, diagnostic tests, patient's organizations, available biobanks, as well as performed and ongoing clinical trials. Data were collected considering literature reports from 2000 until 2013, and using several databases, such as Orphanet and EuroGentest. Updating SCAs current data could be an important tool for needs assessment in specialized molecular diagnosis resources, planning future health and community services and better resources allocation, being helpful to evaluate if the available resources are in accordance with the real requirements in different European countries.

J17.56

The SHBG gene polymorphism (rs12150660) is associated with elite power athlete status and muscle mass

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Testosterone regulates muscle mass and strength, bone mass, fat distribution and the production of red blood cells. Sex hormone-binding globulin (SHBG) is the key protein responsible for binding and transporting of testosterone. SHBG regulates its bioavailability and therefore its effects in the body. Polymorphism at the SHBG gene locus (rs12150660 G/T) has been associated with testosterone concentrations. Since individuals with the TT genotype have higher serum testosterone concentrations in comparison with carriers of the G allele (data from GWAS), we hypothesized that the carriage of the T allele may give some advantage for strength and power performance. The aim of the study was to investigate the association between the SHBG G/T polymorphism, athlete status and muscle mass. A total of 363 Russian athletes and 130 controls were genotyped using RT-PCR. Muscle mass was measured by body composition analyzer Tanita MC-980. The frequencies of the T allele in power-oriented athletes (n=143, 20.3%; P=0.7462), endurance-oriented athletes (n=220, 15.0%; P=0.2054) and a whole cohort of athletes (17.1%; P=0.5078) were not significantly different from controls (18.8%). However, the frequency of the T allele in elite power-oriented athletes (n=65, 26.2 vs. 12%, P=0.0061) was significantly higher as compared with elite endurance-oriented athletes (n=58). Furthermore, correlation analysis showed positive association between the T allele and muscle mass among non-elite female athletes (n=8, P=0.0072, r= 0.8729). Although more evidence is needed, one might suggest that the SHBG gene G/T polymorphism is associated with power athlete status.

J17.57

Extreme Carrier Frequency Of The Splice Site Ivs1+1g>A Mutation In Gjb2 Gene In The Eastern Siberia Is Comparable To Carrier Frequency Of The Sickle Cell Anemia In Africa

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This study presents data on the carrier frequencies of the splice site mutation IVS1+1G>A in GJB2 gene, causing an autosomal recessive form of deafness among various ethno-geographical groups of Yakut population and in a random sample of Yakut individuals. 350 DNA samples of hearing individuals from various ethno-geographical groups of Yakut population: Central group (n=60), Vilyui group (n=60), Northern group (n=60), and random samples of Yakut individuals (n=170) were obtained from the DNA Bank of the Department of Molecular Genetics of Yakut Research Center of Complex Medical Problems of RAMS (Yakutsk, Russian Federation). The detected average carrier frequency of IVS1+1G>A mutation in Yakut population (n=350) was - 10.3%. Extremely high carrier frequency of the splice site mutation IVS1+1G>A in GJB2 gene in Yakut population is comparable to carrier frequency of the sickle-cell anemia in Africa, that may indicate a possible selective advantage of carriers of this IVS1+1G>A mutation in a subarctic climate.

Study was supported by RFBR (#12-04-00342_a, #12-04-98520_r_vostok_a, #12-04-97004_r_povolzhye_a, #14-04-01741_A), SB RAS Integration project #92 «Ethnogeny of indigenous peoples in Siberia and North Asia: comparative, historical, ethno-social and genomic analysis», the Sakha Republic President grant for Young Researchers for 2014 (RP#80), RAS Program «Fundamental Sciences for Medicine» (#30 for 2013-2015), and «Scientific and Educational Foundation for Young Scientists of Republic of Sakha».

J17.58

Variations in nuclear genes are associated with elite sport performance in the Polish population

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Objectives: Single nucleotide polymorphisms are the most common type of human genetic variation. It is widely recognized that genetic factors located in mitochondrial and nuclear genomes influence sport performance. The aim of our study was to assess whether selected nuclear DNA variants are associated with athlete performance in the Polish population.
Methods: The study group comprised 413 unrelated elite athletes and the control group consisted of 451unrelated sedentary individuals. The athletes were stratified into two subgroups: the power athletes (n=188) and the endurance ones (n=225). The study group included 284 participants of Olympic and International Games and the remaining 129 athletes were national–level athletes. The DNA was isolated from peripheral blood lymphocytes using standard procedures. Genotyping of 10 nuclear DNA variants (*ACE*, rs4341; *ACTN3*, rs1815739; *GABPB1*, rs12594956; *CHRNB3*, rs4950; *AGT*, rs699; *FAAH*, rs324420; *PPARG*, rs1801282; *TFAM*, rs1937; *TFAM*, rs 2306604; *PGC1α*, rs 8192678) was conducted using TaqMan method. All statistical analyses were performed using Statistica ver. 10.
Results: We showed that six polymorphisms were associated with outstanding results in power (*TFAM*,rs 2306604, *FAAH*, *ACE*, *ACTN3*) or endurance sports (*CHRNB3*, *GABP1*). Gender and sport level of athletes were also significant
Conclusion: Our study indicates that in the Polish population genetic background could influence sport performance.

J17.59

Gene pool of the Kazakhs according MTDNA and of ten X-chromosomal STR markers

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The aim of the present research was: to study the polymorphism of mitochondrial DNA and 10 X-linked microsatellites (DXS8378, GATA172D05, DXS7132, DXS9898, DXS7423, DXS8377, DXS101, DXS6809, DXS6789, HPRTB) in population of Kazakh.
Genetic and geographic analysis of the genetic structure of Kazakh populati-

on using data of the frequencies of mtDNA haplogroups was conducted. The high degree of intensity of gene exchange between the Kazakh population and frontier populations of Russia was identified on the north-west, north, northeast and east of Kazakhstan. The phylogenetic tree was constructed for Kazakhs on female lineage and was detected position of the studied population between ethnic groups in Europe and Asia.
Ten STR loci demonstrate the high level of genetic diversity in Kazakhs. The average genetic diversity in a combined sample is quite high (mean diversity for 10 STRs, H=0.768). Rare alleles were detected for DXS8378 (7 repeats), DXS8377 (57, 59, 60), DXS101 (31), DXS6789 (12) loci. All rare alleles were confirmed by direct sequencing of PCR product. DXS8377 proved to be the most polymorphic locus (H=0.905). Three another STRs (DXS101, DXS6809 and DXS6789) also have genetic diversity index above 0.8 in a combined sample. The least polymorphic locus in Kazakhs is DXS7423 (H=0.662).
The area of Kazakhstan has been a place of interaction of many ethnic layers during a historically long period. Molecular-genetics methods give an objective criteria to study the origin, migration and ethno genesis of human populations.

J17.60
Polymorphism of the lipoprotein lipase gene as genetic markers for stroke in Colombian population

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Recently, scientists are redirecting their researchers to find the genetic origin of diseases; stroke is part of them. Polymorphisms of the lipoprotein lipase gene have been extensively studied in different populations finding variations in results among societies. Showing that the HindIII and PvuII polymorphisms can act as risk markers for the development of cerebrovascular disease by increasing levels of tiacilgliceroles and decreasing HDL. However, Ser447X can be a protective marker causing an increase of HDL levels and reducing triglycerides levels. The aim of the study was to analyze if there is an association between the presence of polymorphisms in the LPL gene (HindIII , PvuII and Ser447X) with development of ischemic stroke in Colombian population. Sample size was 347 stroke patients (clinical diagnosis and x-ray CT) and 347 healthy subjects. PCR -RFLP technique was used to detect Ser447X , HindIII and PvuII polymorphisms in the LPL gene. Results were analyzed by Stata12 and Arlequin software. As results, allele and genotypic frequencies of the studied polymorphisms did not show a statistically significant difference between the cases and controls. In the present research was not found any association between any of the LPL gene polymorphism and stroke in the population sample used. These results suggest that in the Colombian sample used, LPL gene polymorphisms are not genetic markers for the development of stroke

J17.61
Recurrent spontaneous abortion - importance of testing the thrombophilic mutations. The experience of Genetic Center, Romania on 627 consecutive cases

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Introduction: Recurrent spontaneous abortion represents a frequent and important medical problem. There are a lot of possible causes, but during last years a great proportion of the women with recurrent spontaneous abortion have been shown to harbor thrombophilic mutations.
Material and methods: Five thrombophilic mutations, namely Factor V Leiden, Prothrombin G20210A, PAI-1 4G/5G, MTHFR C677T and A1298C have been tested in 627 consecutive women with recurrent spontaneous abortion of unknown etiology (other causes, such as anatomical or endocrine factors, antiphospholipid syndrome, or chromosomial anomalies were excluded by specific investigations).The methods used were - PCR-based assays (PCR-RFLP, ARMS-PCR, tetra-primer PCR).
Results: Fifty-eight patients (9.2%) harboured the Factor V Leiden mutation, while the Prothrombin G20210A was seen in 37 patients (5.9%). Four patients (0.6%) harboured both mutations. At least one 4G allele of the PAI-1 mutation was seen in 482 patients (77%), of which 184 (29.3%) were 4G/4G homozygotes. At least a variant allele of the MTHFR mutations was seen in 546 patients (87%), of which 291 patients (46.4%) were either homozygotes for C677T or A1298C mutations, or compound heterozygotes. There were only 13 patients (2.1%) homozygous for the wild-type allele in case of all 5 mutations analyzed.
Conclusions: A great proportion of women with recurrent spontaneous abortion harbour thrombophilic mutations. It is important to test these mutations in these cases, since the results can guide towards appropriate therapeutic measures during next pregnancies - low-molecular-weight heparines, acetylsalicylic acid or folic acid, with favorable outcomes in most of the cases.

J17.62
Association between TLR8 and TLR9 gene polymorphisms and Pulmonary Tuberculosis

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Background: Tuberculosis is steel a health problem through the world. Both genetic and environmental factors may contribute the susceptibility to tuberculosis. Toll-like receptors play a critical role in the recognition of Mycobacterium Tuberculosis (TB). The aim of this study was to evaluate the possible association between TLR8 rs3764880 and TLR9 rs148805533 polymorphisms and PTB in a sample of Iranian population.
Materials and methods: In this study, blood samples of 320 subjects including 160 PTB patients and 160 healthy subjects were collected. DNA was extracted and TLR8 rs3764880 polymorphism was analysed by Tetra Amplification Refractory Mutation System-Polymerase Chain Reaction (TARMS-PCR) and TLR9 rs148805533 polymorphisms was analysed by Allele specific PCR.
Results: The allelic and genotypic frequencies of the TLR8 rs3764880 not differ significantly between PTB and the controls. No significant difference was found between the groups regarding TLR9 rs148805533 polymorphism.
Conclusion: Our finding suggests that TLR8 rs3764880 and TLR9 rs148805533 polymorphism may not be a risk factor for susceptibility to tuberculosis in a sample of Iranian population.Larger studies are required to validate our findings.

J17.63
Admixture of Turks, Hungarians and Romas in the Carpathian Basin

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Hungary was a part of the Ottoman Empire between the 16th and 17th centuries for about 150 years. It is also known that Roma, most significant ethnic group of Hungary, migrated through Turkey in the 12th century on their way to Europe. Genetic relationship of Turks with Hungarians and Roma living in the Carpathian Basin was investigated based on genome-wide autosomal single nucleotide polymorphism (SNP) data. Computational methods were carried out with pruned datasets containing 111,928 SNPs. In order to understand Turk, Hungarian and Roma relationship in context to other European and Middle East populations, principal component analysis was applied. Our collection of 27 Roma samples, 20 Turkish and 20 Hungarian samples were merged with 20 Stanford-HGDP populations and 13 populations from the Caucasus obtained from public domain. In order to infer population relationships in a model-based manner, clustering algorithm based on maximum likelihood estimation method was applied, also with 11 HapMap3 populations. The admixture events detected, based on these methods, were investigated with formal tests of admixture. The date of admixture event was inferred based on the exponential decay of admixture linkage disequilibrium. Our results show that extents of Hungarian genetic elements in Turks are significant, and admixture between Turks and Hungarians occurred during the 17th century. The proportion of Hungarian ancestry in Turks was 2 times higher than the share of ancestry from several groups of the Caucasus region, Balkars, Druze and Kurds. Interestingly, contribution of Roma genetic elements in Turks is difficult to detect.

J17.64
Association of Polymorphism Gene for TGF-B1 with the Forming of Kidney Scars among the patients with vesicoureteral reflux (VUR) in children

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Introduction: Transforming growth factor beta (TGF-B) family members are multi-functional cytokines that play a key role in differentiation, cellular growth, and proliferation. TGF- B1 stimulates the production of extracellular matrix protein and induces fibrosis in different tissue. Over expression of TGF-B1 is characteristic of all human and experimental models of kidney fibrosis.

Aim: To prove the role of polymorphism in the promoter region of TGF-B1 C-509-T gene as a possible risk factor in forming kidney scars within the patients with VUR.
Material and method: Samples of DNA were extracted by phenol-chloroform method from the peripheral blood of 50 children with VUR and 70 controls. The gene typing was performed by the PCR /RLFP method and detected after restriction enzyme digestion.
Results: Proportions of C homozygote, heterozygote, and T homozygote for TGF-B1 gene polymorphisms were 21.4/77.1/1.5% in the control and 14/64/22% in the patient group. Statistical analysis indicates that homozygote gene type T/T is obviously more frequent in the patients group ($\chi 2$ = 13,92, p = 0,0009), and that there is corelation of the genotype T/T with the kidney scars forming risk (TT vs.CT + CC: OR = 19.4615; CI 2.420-156.477; p = 0.0003).There wasn't statistical significance between genotypes with T alleles and the genotype C/C (T- vs. CC: OR = 1.675; CI 0.63-4.47; p>0,20).
Conclusion: The presence of the genotype T/T on the position -509 in the promoter region TGF-B1 gene is risk factor for the kidney scars development in patients with VUR in children.

J17.65
Characteristics of Y-chromosome Polymorphisms in Kazakh Populations from the Perspective of Tribal-clan structure

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The characteristic feature of the Kazakh nomadic society was the presence of a hierarchically organized and widely branched tribal-clan structure called "Shezhire", which reflected complex system of ethno-social organization. In the context of the Shezhire, Kazakh populations are divided into three ethno-territorial association of tribes called „Zhuz" (Great, Middle, and Small Zhuzes) and a group of aristocratic tribes (Tore, Kozha, Sunak).
This study aims to compare Y-chromosomal polymorphism of three Kazakh Zhuzs and group of aristocratic tribes (total sample size N= 1407). We analyzed 40 SNP and 17 STR Y-chromosomal markers. Summary statistics were calculated using Arlequin 3.5. Neighbor-joining tree was constructed by the program MEGA 5.0. Multidimensional scaling plot was drawn by the software package Statistica v.7.1.
Population pairwise FST values were calculated from the Y-chromosomal haplogroup frequencies to assess the genetic similarity among studied groups of Kazakh tribes. The most distant ones were the tribe of Sunak and the Small Zhuz (0.393), whereas the shortest distance was found between the tribe of Tore and the Great Zhuz (0,021). These genetic distances are associated with the geographic distances between studied populations. The distribution of Y-chromosomal haplogroups is strongly correlated with the tribal-clan structure of Kazakhs. Presence of certain haplogroups at high frequency at particular tribes is in favor to the hypothesis that many tribes go back to one biological founder, confirming the link between Kazakh family tree Shezhire with the genetic composition.

J17.66
A mitogenomic phylogeny of haplogroups U2e and U3: revealing the phylogenetic signals for population expansions in the Slavs prehistory

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To resolve the phylogeny of some uncommon and poorly studied West Eurasian mitochondrial DNA (mtDNA) haplogroups, we sequenced 32 U2e and 19 U3 complete mitogenomes of Central and Eastern Europeans (Czechs, Slovaks, Poles, Russians, Ukrainians and Belarusians) and re-analysed the available at the present time data on 74 U2e and 80 U3 complete mtDNAs. Molecular dating suggests that the coalescence time estimates are ~21 and ~35 thousand years (ky) for haplogroups U2e and U3, respectively. Detailed analysis of about 500 Slavic complete mitogenomes belonging to different haplogroups allowed us to identify a number of lineages that seem specific for Central and Eastern Europe (U3b1b, U4a2a1, U5a2a1c, U2e1b1a, U2e1b1, U3a1a, H5a1f, U5a1a1a1, U5a1c1, U2e2a1a, U4a2a, H5a2, U2e2a1d and U5a1b1b). These subhaplogroups consist of similar haplotypes revealed in different ethnic groups of modern Slavs, thereby proving the existence of ethnolinguistic community of Slavs through DNA testing. Evolutionary age of Slavic-specific subhaplogroups is calculated to approximately 3.9 ky (from 2.3 to 5.9 ky, according to the mutation rate proposed by Soares et al. (2009) for the entire mtDNA molecule). This indicates that the ancestors of modern Slavs inhabited areas of Central and Eastern Europe from the times of Bronze and Iron Ages, *i.e.* earlier than it was estimated on the basis of archaeological, historical and linguistic data. This study was supported by Russian Foundation for Basic Research (grant 14-04-00131) and the Program of Presidium of Russian Academy of Sciences (grant 12-I-P30-12).

J17.67
Incidence of alpha globin gene defect in the Lebanese population: a pilot study

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Background*:* Inherited hemoglobin disorders are the most common monogenic defects described worldwide. It is well established that Mediterranean and Arab populations are at high risk for thalassemias in general, and for α-thalassemia in particular. Prenatal as well as premarital screening programs in countries with high prevalence have already been founded. In this study, we aim at assessing the incidence of alpha thalassemia deleterious alleles in the Lebanese population. **Methods:** DNA was extracted from 200 newborns dried blood cards remaining from routine neonatal screening at the American University of Beirut Medical Center. DNA samples were screened for the 21 most common α-globin deletions and point mutations reported worldwide, through multiplex Polymerase Chain Reaction (PCR) and Reverse-Hybridization technique. **Results:** The carrier rate of -thalassemia in our sample population was 8% which is higher than that reported from Jordan (2-4%). This finding is comparable to Mediterranean countries (Israel: 5-9%, Greece: 7%, Adana-Turkey: 7.5%) but lower than that reported in other Arab countries (UAE: 16.5%; Oman: 38.6%; Saudi Arabia: 50%). Two mutations were detected: the -3,7del single gene deletion (75%) and the non-gene deletion 2 IVS1 [-5nt] (25%). These mutations are common worldwide. Interestingly, the -α 4.2 and MED mutations, particularly common in Arab and Middle Eastern populations, were absent in our survey **Conclusion:** This study is the first dedicated to investigate α-thalassemia genotype incidence in Lebanon. Data obtained demonstrates a high carrier rate in a relatively, highly consanguineous population. These results may impact premarital and newborn screening policies in our country.

J17.68
5-HTT genotype and sexual behavior traits in healthy female subjects

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Serotonergic neurotransmission affects a large range of behaviors, from food intake to sensory processing and motor activity, cognition, emotion regulation, social behavior and most important to our proposal, reproductive activity. A key regulator for serotonergic neurotransmission is the serotonin transporter (5-HTT), which removes serotonin released into the synaptic cleft. The 5-HTT protein is encoded by a single gene, SLC6A4. Transcriptional activity of this gene is modulated by several variations, including a repetitive sequence, the SLC6A4 linked polymorphic region (5-HTTLPR). Specifically, the 5-HTTLPR short allele (s) has reduced transcriptional efficiency compared with the long allele (l), and individuals carrying the s allele tend to have, among other, an exaggerated amygdala response to threatening visual stimuli, biased processing of emotional information, likely resulting from altered functional connectivity within a corticolimbic circuit, impulsivity and increased anxiety related temperamental traits.
We used a new instrument for the measure of sexual behavior: "Sex-Promiscuity-Infidelity-Questionnaire" on a cohort of 154 female students recruited at the University of Chieti, in order to test the possible interactions between s carriers individuals and sexual behavior. Through an Explorative Factor Analysis (EFA) we explored the factor structure of the questionnaire, composed by three factors: "Demographic Informations", "Sexual Promiscuity" and "Sexual Instability". Furthermore the EFA showed significant s carriers' factor loadings on sexually instability behavior factor (-.35).
Our findings support the possible association between serotonergic neurotransmission, mediated by 5-HTTLPR affect, and the sexual behavior in female healthy subjects.

J17.69
Screening for mutations in genes underlying familial atypical mycobacteriosis

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Several mutations in the IL12B, IL12RB1, IFNGR1, IFNGR2, STAT1, and IKBKG genes lead to the development of rare syndrome of atypical familial mycobacteriosis (OMIM # 209950). We set out to test whether rare variants and polymorphisms in these genes can predispose to tuberculosis. These genes were studied in two Siberian populations of Russians (304 patients, 265 controls) and Tuvinians (238 patients, 263 controls). First, we carried out the screening for most common mutation variants of the genes (IL12RB1 Gln32Ter, Gln376Ter, Arg213Trp; IFNGR1 Ile87Thr, 4-bp Del NT818, 1-bp Del NT818; IFNGR2 2-bp Del 278AG, Thr168Asn, 663Del27; STAT1 Leu706-Ser, Gln463His, Glu320Gln). All these variants appeared as "wild-type". Then we performed the search for rare variants of the studied genes by Sanger's sequencing in 10 individuals suffered from aggressive forms of TB. Direct sequencing did not reveal any mutations causing atypical familial mycobacteriosis; however, 15 previously established single nucleotide polymorphisms were identified (IL12RB1 rs11086087, rs11575934, rs17852635, rs401502, rs12461312, rs17882555, and rs3746190; IL12B rs919766; IFNGR1 rs2234711, rs17181457, rs7749390, rs11754268, and rs11914; IFNGR2 rs17883129; and STAT1 rs2066797). Using "case-control" design, we identified an association between the rs2066797 variant of the STAT1 gene and TB in Russians (p=0.02). Association of this polymorphism with TB was detected for the first time.
Our results suggest that rare mutations responsible for atypical familial mycobacteriosis are unlikely associated with TB.

J17.70
Pathway-VEGAS: A post-GWAS method in genetic epidemiology

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Genome wide association studies (GWAS) have revolutionised the field of gene mapping. As the GWAS field matures it is becoming clear that for many complex traits, a proportion of the missing heritability is attributable to common variants of individually small effect. Detecting these small effects individually can be difficult and statistical power would be increased if relevant variants could be grouped together for testing. We propose grouping markers together based on pre-specified biological pathways. We have implemented this pathway-based test in the program Pathway-VEGAS. The method is based on prior calculation of gene-based p-values using the existing Versatile Gene-based Association Study (VEGAS) software. Pathway-VEGAS uses the gene-based P-values to construct a pathway test based on a set of pre-specified pathways. The method appropriately takes into account situations where neighbouring genes are present in the same pathway - results for relevant regions are calculated by accounting for linkage disequilibrium between markers using simulations from the multivariate normal distribution. Pathway size is taken into account via a re-sampling approach. Importantly, since the approach only requires summary data, the method can be easily applied in all GWASs, including meta-analysis, singleton based, family based and DNA-pooling based designs. We found statistically significant findings using pathway-VEGAS on a number of traits including endometriosis, height and educational attainment. The approach identifies biologically relevant pathways, offering insights not possible with single marker approaches.

J17.71
Epidemiology of consanguineous families in Autism

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Analyses of large autism datasets have provided statistical and functional evidence for the role of rare point mutations (O'Roak, 2012, Sanders, 2012; Yu, 2013) and transmitted and de novo copy number variants (CNVs) (Morrow, 2008; Pinto, 2010; Levy, 2011; Sanders, 2011), and offer crucial insights into the diverse genetic mechanisms that can lead to Autism Spectrum Disorders (ASDs). Here we present CNV analysis for a cohort of 183 consanguineous families with one or more children affected with ASD. We provide new insights into the genetic architecture of ASDs as our cohort is uniquely enriched for recessive loss of function variants. We follow up findings and draw comparisons with additional large ASD and control datasets: the Simons Simplex and the Autism Genetic Resource Exchange (AGRE) collections (2,670 affected individuals; 9681 total individuals). Comparing across these cohorts, we demonstrate that ascertainment can lead to selection of different underlying genetic mechanisms causing ASDs. These differences are reflected in metrics such as the affected male:female ratio and the relative contribution of de novo CNVs versus inherited homozygous deletions. Specifically, we find that de novo CNVs play a significant role in non-consanguineous families with a single affected child (p=0.04), but a lesser role in multiplex families, and they are no more common in ASD cases than controls in multiplex consanguineous families. In contrast, we present the strongest statistical evidence (p=0.013) to date that homozygous deletions, are a major contributor to ASD disease burden in consanguineous families, contributing to as much as 5-10% of cases.

J17.72
Cost-effective designs for genotype imputation in sequencing based genome-wide association studies

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Genotype imputation is now an essential tool in genetic studies to systematically infer missing and untyped genotypes. Accordingly, progressively larger reference panels are being constructed based on whole-genome sequencing in various populations. Developing general guidelines for optimally cost-effective imputation designs, however, requires evaluation of performance issues that include the relative utility of population-specific compared to general/multi-population reference panels; genotyping with various array scaffolds; and effects of different ethnic backgrounds. Here we compared the effectiveness of a Sardinian specific (SardSeq) panel, derived from whole-genome sequencing of 2,120 Sardinians, to the 1000 Genomes Project (1000G) reference panels, using combinations of two genome-wide and three custom arrays as baseline genotype, in both Sardinians and other Europeans. In Sardinians, the SardSeq panel provided better coverage and genotype imputation accuracy than the 1000G panels, particularly for low frequency and rare variants (mean squared correlation with true genotypes 0.95 vs 0.68, respectively, at variants with MAF >1% and <5%). In particular, when imputing with the SardSeq panel, even gene-centered custom arrays interrogating a smaller number of variants (on the order of 200,000, as the Illumina CardioMetaboChip) provided highly informative content across the entire genome. Notably, in Sardinians a combined panel including both the SardSeq and the 1000G sequencing data did not provide substantial improvement. By contrast, we showed that a combined panel could be advantageous in other Europeans. We expect our results to be useful in planning future studies and in current sequencing efforts that are not part of the 1000 Genomes Project.

J17.73
Association of single nucleotide polymorphism in SLC30A8 gene with aging in Tehran lipid and glucose study

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Background: Genome-wide association studies have identified thousands of variants that are associated with numerous phenotypes. One such variant, rs13266634, in Solute Carrier family 30 (zinc transporter), member 8, has been reported in several studies that has association with type-2 diabetes as such in European and Japanese population. Here we confirm association of rs13266634 with aging in Tehran lipid and glucose study (TLGS).
Method: 600 individuals in range of 20-35 years old as young group and 2347 individuals in range of 45-85 as old group were selected from participant of TLGS cohort and enrolled in the study and rs13266634 has been genotyped using the Centaurus (Nanogen) platform in DeCODE genetics. Association of G-allele with aging was tested using plink software.
Results: Apart from replicating previous findings, TLGS participants showed that T-allele of rs13266634 showed association with aging after comparison young and old groups ($p < 0.0025$).
Conclusion: Our findings showed the association between the presence of T allele in rs780094 and aging and related traits among Iranian population. However to address this issue, length of telomere as one of the aging factor in this cohort will of interest to investigate more in detail.

J17.74
Lack of association between the rs2304391 GABRR2 polymorphism and Temporal Lobe Epilepsy

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Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain, acting primarily via the GABA receptors. Multiple lines of evidence indicate that dysfunction of GABA B receptors may be involved in the pathogenesis of temporal lobe epilepsy (TLE). The aim of this study was to determine whether a single nucleotide polymorphisms (SNPs), +8A>G (rs2304391) at 3'UTR of subunit 2 of the GABA B receptor gene (GABBR2), contributes to TLE. We used a case-control approach comparing the frequency of the above-mentioned GABBR2 polymorphism between patients with TLE and controls. We enrolled 303 consecutive patients (167 women; mean SD age: 47.08 18.10), and 271 healthy controls (137 women; mean SD age: 64.20 ± 17.06), matched for age, sex and ethnicity. All patients had a diagnosis of non-lesional TLE, based on comprehensive clinical, neuropsychological, electroencephalographic, and brain MR evaluations. All patients and controls were Caucasian and were born in Italy. Patients and controls gave written informed consent prior to participation in the genetic studies. Patients and controls were genotyped for detection of the rs2304391 polymorphism using TaqMan Allelic Discrimination assays, on an Applied Biosystems PCR platform. The genotype distributions for both healthy subjects and epileptic patients were consistent with the Hardy-Weinberg equilibrium. Analysis of genotype or allelic frequencies between patients and controls showed no statistically significant difference, moreover the polymorphism did not influence severity and age at onset of epilepsy. Our data suggest that GABBR2 gene polymorphism does not act as a susceptibility factor for sporadic non-lesional TLE.

J17.75
Gene-gene interaction analyses of variants in the GABA system and alcohol use

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Epistatic or gene-gene interaction refers to the phenomenon that the joint effect of two genes is different from the sum of the individual effects of each gene. Recently, gene-gene interactions have been implied as a potential solution to the problem of missing heritability in the detection of genes for complex disorders. Furthermore, combinatorial approaches based on principal components analysis with the generalized multifactor dimensionality reduction method to study G x G and G x E interactions uniting nuclear families and unrelated individuals have been proposed. We apply methods of G x G interaction to a sample of 7224 individuals who have been phenotyped for their drinking and alcohol abuse and genotyped on 970 SNPs in 34 GABA system genes including 20 GABA receptor subunit genes. Results from applying both combinatorial approaches, PGMDR and Random Forest approaches based on classification trees, to the alcohol use data will be presented. We further discuss and compare the power to detect these interactions using these two methods and the resulting outcome. This should shed light into the functionality of these variants implied in the GABA receptor signaling pathway.

J17.76
Unsupervised Clustering Analysis of Gene Expression in an extended pedigree from Norfolk Island Population

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Norfolk Island belongs to the Commonwealth of Australia and it comes from a settlement of 194 inhabitants resettled from the Pitcairn Islands in 1856. All of them were descendants of nine "Bounty" mutineers' men and twelve Tahitian women. The Island has been isolated and strict immigration and quarantine legislation restrict new founder from migration. In an attempt to understand and identify gene interactions in this unique population we performed a microarray analysis using an Illumina HumanHT-12 v4 array in venous blood samples from 335 related individuals. In order to compare the transcriptome structure from Norfolk Island population, we have taken previously published microarray data from the Atlanta CHDWB study. An unsupervised clustering analysis using k-means and principal component analysis (PCA) were performed using R. PCA showed that, in the Norfolk Island population, approximately 22% of the total variation is explained by its first principal component, against 14% of variation in the CHDWB population. On the other hand, the k-means analysis identified 24 clusters in the Norfolk Island population and 18 in the CHDWB population where approximately 31% of the transcripts could not be assigned to any of the clusters due to the lack of correlation. Enrichment analysis and Network visualization was performed. Our study supports a homogeneous structure of the Norfolk Island population and suggests a specific set of genes to explain different phenotypic traits. It also contributes to the identification of possible biomarkers in common diseases such as migraine and cardiovascular disease, common pathologies in the Norfolk Island population.

J18.01
Chromosomal microarray analysis in paediatric patients with cognitive impairment/behavioural abnormalities/epilepsy and congenital anomalies: well-known syndromes, novel syndromes, parental contribution. A clinical perspective

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Chromosomal microarray analysis (CMA) has emerged as a powerful new tool to identify genomic abnormalities associated with a wide range of developmental disabilities and congenital anomalies.
The detection of submicroscopic copy number variants (CNVs), not visible by conventional karyotyping, helped the genetic diagnosis in our paediatric patients who had already received, with normal results, standard karyotype analysis, metabolic screening, single gene sequencing. Since 2008, 212 patients, aged 2 months-16 years, were analyzed using SNP-array technology and clinically significant submicroscopic imbalances were detected in 70 cases (33%). De novo imbalances were identified in 26 children (12 %), while inherited imbalances were recognized in 34 children (16 %). In 10 patients it was not possible to study both parents. In 59 patients (27,8%) a CNV was causative for the phenotype: in these cases, known microdeletion/microduplications syndromes and chromosomal region with a likely pathogenic correlation were identified. In 11 patients (5%) two or more CNVs were presents (2 CNV= 7 patients; 3 CNV= 4 patients): parents' analysys revealed that the rearrangements were inherited and contributed to child's clinical phenotype. Clinicians who evaluate children with developmental disabilities and congenital anomalies have the role to attempt to establish a genetic etiology. Furthermore, an analysis of parents or family members becomes a routine recommendation, given that incidental CNVs findings have a significant impact on risk counseling for future pregnancies and other family members at risk.

J18.02
Bioethical, Humanistic And Technocratic Resources Of Enhancing Of Civilian Control Of Human Genetics

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Background: Civilian control on human genetics represents one of main practical leverages to increase benefits and reduce risks for society as well as for healthcare system at global, local and community levels, in context of scientific and technological progress in genetic research, for melioration of general-social acceptance rate of the most ambiguous biotechnological innovations. The approach of civilian control in decision-making for better exploitation of human genetics potential for sustainable and long-term improving of healthcare, individual and global security and innovation climate in society, represents a new mainstream in development of medicine, public healthcare, genetic research and human neuro- and bio- enchantment.
Materials and methods: The study used statistical data analysis and synthesis, observation, social survey, individual questioning.
Results: There are several categories of resources to increase importance of civilian control on human genetics: 1) stimulation of professionalism, deontological ethics and humanistic capacity of geneticists; 2) supremacy of bioethical committee expertise and normativity; 3) rising biotechnocratic social leadership class, involved into genetic decision-making.
Conclusion: A coordinated application of the full spectrum of available resources is required to streamline public monitoring on human genetics as a lever to ensure a high level of protection of society against abuses and distortions, based on a new concept of civilian control in decision-making in human genetics as a combination of bioethical and humanistic expertise and auditing, in the context of modernizing of society, where the protection of individuals and promotion of progressive innovations in human genetics are prerequisites for a global welfare.

J18.03
Clinical outcome and genetic counselling in a rare complex small supernumerary marker chromosome with dup(9p21->pter) and

21(pter->21q21): case report

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Abnormal unique complex small supernumerary marker chromosomes (sSMC) have been found in 0.9% of patients, making the correlation between the patient's phenotype and genotype particularly difficult. Some characteristic syndromes have been described; i.e., Pallister Killian [i(12p)], Cat-eye [i(22p~q)] and Emanuel [der(22)t(11;22)]. Genetic counselling for these new marker chromosomes may be challenging, both for the patients and the parents, particularly when the sSMC is a derivative of a maternally inherited translocation, resulting in partial trisomy of two chromosomes. The authors present the case of a 9 month female, referred for karyotyping with high resolution banding presenting with microcephaly, slow physical development and facial dysmorphisms. Classical cytogenetics revealed an abnormal sSMC. The father's karyotype was normal but the mother had a t(9;21) balanced translocation. Analysis of Fluorescence in situ hybridization (FISH - whole chromosome painting and centromeric probes, Cytocell) and Multiplex Ligation-dependent Probe Amplification (MLPA, MRC Holland) results in the proband allowed us to conclude that she has partial trisomy for chromosomes 9 (9p21->pter) and 21(pter->21q21). Final Karyotype: 47,XX,+mar.ish der(21)t(9;21)(p21;q21)(D13Z1/D21Z1+,wcp21+)mat. mlpa 9p,21psubtel(P036E1, P070E2)x3
As in the present case some publications have noted that sSMC resulting from apparently balanced translocations present in one of the progenitors will influence the phenotype by the presence of specific partial trisomies. As far as we know this is the first case found with an sSMC that originates in chromosomes 9 and 21. The authors emphasise the need for good characterization of these markers in order to define a reasonable/probable clinical outcome and allow appropriate counselling for the family.

J18.04
Inconsistency between molecular and clinical data in subjects with a D4Z4 reduced allele should prompt to further investigations

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Out of 171 consecutive myopathic index cases recruited from the Italian National Registry for FSHD and carrying of a D4Z4 reduced allele (DRA) with 4-8 repeats, we identified 148 subjects completely fulfilled the clinical diagnostic criteria for FSHD and 23 subjects that presented myopathic features suggestive of an alternative diagnosis. In this group we observed a wide clinical heterogeneity, including 1) involvement of muscles that are not usually affected 2) sparing of muscles that are typically affected in FSHD, 3) non autosomal dominant inheritance, 4) additional clinical features. Molecular analysis of the 4q35 subtelomeric haplotype did not reveal any molecular element enabling us to differentiate these patients from classical FSHD cases. Further investigations in two patients with overlapping syndromes allowed us to identify additional mutations, respectively a heterozygous CAV-3 gene mutation and a heteroplasmic transition of mitochondrial tRNALeu. In an elderly woman the muscle biopsy resulted suggestive for the diagnosis of inflammatory myopathy with inclusion bodies. Three cases presented a bent spine syndrome: histological and immunohistochemical analysis of muscle biopsies failed to detect other pathological conditions and additional genetic testing are ongoing. Collectively, our analysis highlight the necessity to re-evaluate the significance and the predictive value of DRA, not only for research but also in clinical practice. Further clinical and genetic analysis of FSHD families will be extremely important for studies aiming at dissecting the complexity of FSHD. This approach will favor correct diagnosis and genetic counseling.

J18.05
Constructing a family tree. Frequent mistakes and new needs

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Analyzing more than 1500 pedigrees some frequent mistakes were shown: a) the absence of a horizontal line for an offspring, b) symbol location between generation lines, c) displacement of children birth order, d) wrong numbering of generations, e) incorrect numbering of symbols in each generation. Human infertility is an important problem. 10 to 15 percent of couples all over the world are infertile. Assisted reproductive technology (ART) is modern effective therapy for overcoming infertility. To draw a pedigree with ART it is necessary to use adequate standard genetic symbols that are absent so far. The author offers to discuss the following symbols to construct a family tree with ART: 1) bold symbols of biological parents, 2) a broken line to image germinative cell donation, 3) a symbol of substitutive, ersatz-mother can be denoted by a circle that contains a pregnancy symbol inside. All particular facts of medical history and different kinds of medical treatment ought to be in legend. Some family trees that are drawn with different symbols are compared. Symbols suggested are founded by the author.

J18.06
Legislative and ethical peculiarities of human genetic data protection

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Genetic science related to the individual as the main subject of the research is exposed to a wide range of ethical and legal issues. From the developments in genetic science other science have evolved, thanks to which the modern world is able to protect the genetic information of data, and to receive the sanction while ignoring the laws of such data. However, there are still many problems related to the protection of personal genetic information, such Regulatory standards, the inviolability of an individual, the assurance of freedom and privacy of information.
Genetic discrimination is strictly forbidden by international conventions and declarations (Convention on Human Rights and Biomedicine, Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research, Directive 95/46/EC of the European Parliament), which means that discrimination on grounds of personal genetic information (diseases, abnormalities) is not available in all areas, including employment and insurance. However, individuals face some problems unable to get a job due to the publicity and information disclosure to their employers, or increasing the amount of insurance premiums. It is essential to protect genetic rights, because of that any form of discrimination against a person on grounds of his genetic heritage is prohibited and intervetion in the health field or disclosure of such information may only be carried out after the person concerned has given free and informed consent to it.

J18.07
The impact of genetic counselling on prevention of mental retardation in south of Iran

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Introduction: Mental retardation has high prevalence in south of Iran because of consanguineous marriages. As uncurable nature,prevention is the best way.This study was designed to evaluate the impact of genetic counseling on prevention of mental retardation. Methods: This case control study was done between 2010-2013 and 120 women with mental retarded children were participated.All of them had another pregnancy after their mental retarded child.60 of them had pregnancy after genetic counseling (case group) and 60 women had pregnancy without doing genetic counseling (control group).The study data was analyzed using SPSS19. RESULTS: In case group mean maternal age was 33±4.9 and mean age of mental retarded children was 7.8±4.5 years.In control group it was 34±5.2 for mothers and 10±2.9 for mental retarded children.Genetic counseling before pregnancy was protective factor for having mental retarded child.(Odds Ratio 4.261, 95% confidence interval 1.312-13.834). 71.7% of parents in case group and 55% in control group had consanguineous marriages.Screening tests in pregnancy were done in 78.3% of mothers in case group and 21.7% in control group. Down syndrome was the most common cause of mental retardation in both groups. 3.3% of mothers in case group were referred for abortion because of abnormal amniocentesis. Conclusion: Genetic counseling is effective in prevention of mental retardation.

J18.08
Hereditary Hyperferritinemia Cataract Syndrome in two Italian patients.

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We describe two unrelated Italian patients with a combination of congeni-

tal nuclear cataract and hyperferritinemia without iron overload. Affected patients had normal serum iron, transferrin saturation, and transaminases, but high serum ferritin levels, 1819 and 823 ng/ml, respectively. The first patient was referred to us with the suspect of thalassemia trait. Only asking a detailed clinical story, a previous surgical intervention (at the age of 15) for cataract was referred. Pedigree analysis showed a brother with cataract. After this, in the brother hyperferritinemia levels were discovered too. The second one was referred, during pregnancy, because of the presence of cataract and hyperferritinemia. Pedigree analysis showed bilateral cataract and hyperferritinemia in several relatives. The association of hyperferritinemia in patients with congenital cataract and positive family history led us to consider a diagnosis of Hyperferritinaemia-Cataract Syndrome (HHCS). HHCS is an autosomal dominant syndrome characterized by cataract and high ferritin serum levels, not related to iron overload. The syndrome is caused by heterozygous mutations in the iron-responsive element (IRE) of the ferritin light chain gene (FTL). Direct sequencing of 5'UTR of FTL showed, in the first patient, an heterozygous deletion of 16 nucleotides (-178_-162 del 16 nt, NM_000146.3) and, in the second one, an heterozygous missense mutation (-163C>G), both causative of HHCS (Luscieti et al, 2013) We conclude that in patients with recurrent congenital cataract and hyperferritinemia without iron overload, HHCS should be explored in order to provide genetic counseling and targeted follow up.

J18.09
Assessment of diagnostic criteria for HNPCC by comparative study of overall survival of CRC patients

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HNPCC is a clinical diagnosis, based on the Amsterdam criteria I (ACI) or revised Amsterdam criteria II (ACII). Due to cultural, socio-economical reasons and small size of families, in clinical practice often is hard to fulfil ACII and obtain an appropriate anamnesis for patients diagnosed with CRC. Therefore, based on different publications several modified criteria are in use. Studies also showed overall survival of CRC patients with HNPCC is better than sporadic CRC patients.
The aim of this study is to evaluate diagnostic criteria for HNPCC used in clinical practice by comparative study of overall survival of CRC patients.
The retrospective case-record data of 1423 patients were analysed. Patients were classified in eight groups by clinical diagnosis, family anamnesis, age of diagnosis, stages by TNM, criteria matching. HNPCC group was selected based on ACII. The mortality was analyzed using the Cox proportional hazards models. The diagnosed group, age, and stratified tumour stages were tested with multivariable linear regression models adjusted for covariates.
Patients group matching to ACII criteria (HNPCC) did show significantly better survival prognosis vs. any other patient group with cancer anamnesis in family and CRC patients without reported cancer anamnesis in family (sporadic CRC). All groups, except HNPCC, did not reach statistical significance for better overall survival compared to sporadic CRC group.
Our data suggest that despite small families and lack of appropriate family anamnesis, ACII criteria is still the most sensitive clinical diagnostic guideline for HNPCC if microsatellite instability testing is not available.

J18.10
Patient's use of the Internet for Medical Genetics Informations

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Clinical experience suggests that the Internet is increasingly becoming an important resource for patients seen in medical genetics. Patient-provider relationships will probably change and medical providers will face new challenges as patients obtain health informations from the Internet. Objectives To determine thene percentage of patients enrolled in a medical genetics practice who use the internet health information, to describe the types of information and to evaluate patient's perception of the quality of this information. Material and methods A prospective study was performed about patient use of the Internet before attending a medical genetics appointment . We analysed 61 questionnaires assessing : frequency of patient use of the internet for medical genetics informations, perceptions of the quality of this information, if patients discuss this with their doctors. Results Results show that 72,13 % (44/61) of patients have acces to the Internet , of which 63,6 % (28/44) report searching the Internet for genetic informations. 85,71% (24/28) of the patients found the information useful. Of those using the Internet for medical genetics informations 46,4% (13/28) did not discuss this informations with their doctor. Conclusions Medical genetics care providers should recognize that patients are using the Internet as a source of medical information and should be prepared to offer suggestions for Internet resources and to assist patients in evaluating the quality of medical information available on the web sites. The role of medical genetics professionals is changing as a result of the development of the Internet.

J18.11
Genetic counseling for a pregnant woman with Marfan syndrome

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Marfan syndrome is well known autosomal dominant disorder of connective tissues and is caused by mutation of *FBN1*. Most cases are inherited from parents but 25% cases are caused by de novo mutation.
A pregnant lady aged 27 with Marfan syndrome was referred to genetic outpatient at 10 gestational weeks. She visited to us with her younger brother. Her main concern was the risk of Marfan syndrome for her baby, however she did not realized that neonatal presentation of severe and rapidly progressive disease in multiple organ systems. She knew she could take genetic test, but she was not into it and prenatal test, either.
She admitted maternal ward from 25 weeks because of her heart condition. She needed keeping rest and monitoring with cardiovascular team. At 27 weeks, Valsalva sinus expanded to 43mm and complained slight dyspnea gradually. At 31 weeks, we decided to perform Caesarean section because the circulating plasma volume usually increases at 30-32 weeks in pregnancy. She delivered a girl weighed 1532g with Apgar score
5/5. The girl is now 2 years old and does not show any symptoms.
Generally, pregnancy should only be considered after appropriate counseling from a medical geneticist or cardiologist familiar with this condition. However this lady did not have any counseling before pregnancy because the cardiologist asked her not to be pregnant. It is important to consider how we could share the genetic information with female patients who is not allowed to conceive, but might happen to be pregnant.

J18.12
The application of the Illumina NextSeq500 sequencing platform to whole human, exome and RNA sequencing

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Here we will present data and analyses obtained on the Illumina NextSeq500 sequencing platform with whole human genome resequencing of clinically relevant samples, together with data from indexed exome and RNA samples.
Further developments by Illumina of their sequencing by synthesis chemistry together with advances in instrumentation design have enabled the production of the first desktop high throughput sequencing system.
The NextSeq500 is capable of producing up to 120G of high quality data with 400 million tags in <29 hours from paired 150 cycle runs. Shorter paired and single 75 cycle runs are also possible together with the availability of mid throughput configuration delivering 40G from 130 million tags with paired 150 cycle reads.
The system has a number of innovative features including the use of:
- Miniaturized optics
- Novel 2 channel chemistry
- Dry flowcells
- Simplified workflow with reagent cartridges

Connectivity to BaseSpace for cloud analysis or an onsite version enables simple secure storage and manipulation of data. Data can be analyzed using a variety of tools relevant to the sample such as BWA/GATK or the Illumina iSAAC pipeline for DNA samples. RNA data can be analyzed by the Tophat and Cufflinks applications.

J18.13
Silver-Russell syndrome - novel (epi)genotype-phenotype correlations

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Silver-Russell syndrome (SRS) is a congenital imprinting disorder with hypomethylation of ICR1 on chromosome 11p15.5 or maternal uniparental disomy of chromosome 7 (mUPD7), characterized mainly by severe pre- and postnatal growth retardation, relative macrocephaly and a small triangular face.
Retrospective and prospective assessment included 77 SRS patients (36 fema-

les and 41 males), at the mean age of 4.75 years (range 0.1-18.5 years), with the molecular confirmation of ICR1 hypomethylation in 63 (82%) and mUPD7 in 14 (18%) subjects.
Anthropometric measurements were performed using standard techniques and variables were expressed by number of standard deviations (SDS) in reference to the calendar age. Mean number of sessions per patient was 8 (range 1-35). Growth parameters, BMI, head characteristics and developmental anomalies were analyzed and scored.
Statistically significant differences were shown with regard to following parameters: mean SDS for weight, height, BMI and lower extremities length were lower in the mUPD7 group of patients but head circumference, length and breadth of head, forehead and bizygomatic breadth were larger compared to ICR1 hypomethylation group. Asymmetry (face/body/limbs) and major congenital anomalies were more frequent in patients with ICR1 hypomethylation. Interestingly, genital anomalies (underdevelopment of the uterus, gonadal dysgenesia/ambiguous genitalia, cryptorchidism, hypospadias) were scored only in patients with the loss of ICR1 methylation.
Systematic and long-term follow-up of both SRS groups enabled assessment novel correlations of the phenotype depending on epigenetic modulations.
The study was supported by National Science Centre, Poland (grant NN 407 285339).

J18.14
Floating Harbor syndrome - two additional cases, phenotype / genotype correlation

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Floating-Harbor syndrome (FHS; OMIM #136140) is a rare genetic condition characterized by short stature, delayed bone age and speech development, and typical facial dysmorphism. The face of FHS is the most distinctive aspect for making the right diagnosis; otherwise it can be difficult. The typical FHS phenotype consists of triangular facial shape, deep set eyes with long eyelashes and low set, large, often posteriorly rotated ears, nose narrow at the root broadening to the tip, low hanging columella, large nares, short philtrum and wide mouth with thin upper and everted lower lip.
Whole-exome sequencing revealed heterozygous truncating mutations (nonsense or frameshift) in the *SRCAP* gene in several affected persons (Hood at al., 2012). The mutations are *de novo*, some of them are recurrent and all are tightly clustered within the final exon, with the exception of a recently identified stop mutation in the penultimate exon 33 (Kehrer et al., 2013).
Here we present two patients with typical FHS facial features, although they slightly differ one from the other. In the first patient a nonsense mutation c.7330C>T (p.Arg2444*) was detected. This is a recurrent mutation described in several other patients. The parents of the patient did not harbour this mutation, thus supporting its *de novo* origin. A frame shift mutation c.7257_7258delAA (p.Gln2419*fs**23) was detected in the second case. To our knowledge this mutation has not been described yet. Phenotype / genotype correlation is further discussed.
Supported by 00064203 and CZ.2.16/3.1.00/24022.

J18.15
Two supernumerary marker-chromosomes in a healthy woman: a case report

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Presence of supernumerary marker chromosome (SMC) is a rare chromosomal abnormality with broad-spectrum of possible clinical consequences. Most carriers are healthy people, but some SMCs could be associated with infertility, especially in males. They are also more frequent in intellectual disability and cases with various congenital defects. Genetic counselling in cases of prenatal detection of SMCs is rather challenging since prognosis differs widely depending on the SMC chromosomal origin and presence of specific segments. Thus, every new published case is valuable for decision-making.
Here, we report a case of the 33-year-old healthy woman referred to clinical geneticist as parent of the deaf child. She was cytogenetically examined due to chromosomal aberration in the pregnancy of her mother. In her peripheral blood lymphocytes two SMCs in a mosaic form were detected by various FISH methods. Our analysis revealed that the first SMC is derivative chromosome 7 containing Williams-Beuren region (7q11.23) which belongs to considered dosage-sensitive area of this chromosome but was clinically silent. Besides the larger SMC we also recognized a smaller SMC, where due to its low frequency and size it was impossible to determine its origin (likely from constitutive heterochromatin). Our observations are compared with similar case reports.
Supported by the GAUK-264811 and TACR-TA01010931.

J18.16
Mind as epigenetic modifier in mood disorders

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Epigenetic modifications of DNA might be crucial for understanding the molecular basis of mood disorders. One reason for this is that epigenetic factors are sometimes plastic enough to react the external and internal environments. New scientific studies suggest, that these environmental factors can be not only food or chemicals, but also spiritual: positive emotional state, optimism, reaction to stress. The aim of this manuscript is to provide a conceptual background for studies by reviewing key findings from different forms of investigation. In order to provide an understanding the role of genetic and environmental (spiritual) factors in the causation of mental disorders here is a simplified account of some of the key features of how genes 'work'. Results of that review indicate that of particular interest, traumatic events or negative mind content may potentially alter our DNA methylation pattern and induce abnormal brain gene expression and ultimately depression, and other mood disorders. In summary, this review demonstrates that an epigenetic state of a genes responsible for mental health can be established through life experience and thinking manner and is potentially reversible.

J18.18
The analysis of reasons and expectations of women attending genetic counselling

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Backround: Pregnant women are referred to genetic counselling when there are increased risk factors to a fetus. Often this is associated with increased stress or lack of information. The aim of the study was to identify the reasons why women attend genetic counselling, and their actions if a genetic disease would be found in their fetus.
Methods: A prospective analysis was based on original genetic questionnaire given to 277 pregnant women who were attending genetic counselling in LSMU central policlinic. The gathered data was analyzed using statistic program SPSS 17. The results' significance were assessed by Chi square test, with the level of confidence at 95%
Results and conclusions:
1. Most women attend genetic counselling due the older age, family history with hereditary disease.
2. Half of the patients would choose amniocentesis if there is found an increased risk for genetic disease. One third would choose amniocentesis because of the genetic decision.
3. Women, who do not agree with abortion, would like to get more information about keeping pregnancy before conception.
4. Most women (52%) would consult firstly with their husbands about the keeping the pregnancy. 16% would choose pregnancy termination, 10% would keep pregnancy.
5. More than half of women would choose termination of pregnancy when fetus is diagnosed with disease incompatible with life. Other would choose to give birth and leave everything to natural course.

J18.19
The meaning of informed consent in paternity testing

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The following objectives have been set: to frame the concept and the requirements for informed consent, to analyse the legal acts of the Republic of Lithuania covering the issues regarding informed consent, to analyse types of and indications for paternity testing and to name legal and ethical problems arising from application of the informed consent.
The concept of informed consent is based on the principle of autonomy. The obligation of doctors to inform patients is inseparable from the requirement to receive informed consent. The two parts are mandatory for any medical procedures and interventions. The main requirements for the informed consent include rationality, sufficient and clear information, free will and the form of consent conforming to the legal acts. However, the informed consent is not an absolute requirement as the patient has a right to remain uninformed. Additionally, under certain circumstances it might be impossible to

inform patients or to receive consent from patients or their duly authorised representatives. The main problems of the informed consent in paternity testing, by outlining two stages of the process: conveyance before testing and interpretation of the results with it's effectivenes.

J18.20
Ethical aspects of medical genetic consulting and conducting of genetic research and testing

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Introduction: An important point of the genetic consulting is fixing a correct diagnosis, which most often requires the appointment and conduct of genetic research. Because of the hereditary nature of diseases and predispositions in our clinical practice when working with patients may result moral and ethical, psychological, social and legal cases and problems. **Goals:** The aim of our study is to investigate and analyze peoples personal position on the moral and ethical aspects of medical genetic consulting and conducting of genetic research. Materials and methods: In the study were included in general 140 people, aged from19 to 61 years, dominated by people aged between 20 and 22 years. The study was conducted on complete and voluntary and anonymous principles. **Discussion:** Significant contingent of respondents are positive about the conducting of genetic testing and genetic consultation. Most of them think that good, empathic attitude of the physician to the patient and the strict observance of medical confidentiality is very important. For 57% of respondents application of genetic test is a serious stressful situation, associated with a number of psycho-emotional and moral ethical factors. Therefore, consistent with the characteristics of the patient's presentation and interpretation of the results of the conducted genetic tests is crucial for the beneficial effect from the counseling itself. **Conclusion:** Genetic counseling requires abilities and approach to communicate, establishment of confidence and partnership between doctor and patient, correct presentation and interpretation of genetic information with respect for autonomy and the right of personal choice of the patient.

J18.20
Teaching workshops of Medical Genetics to students

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Today the major guide for educators in establishing their teaching goals and objectives is Bloom's Taxonomy of the Cognitive Domain revised by Krathwohl. From only knowing the specific concepts, students should then develop more and new skills, so that they could analyze, synthesize and even critically evaluate their own work or their colleagues.
During each workshop that I taught from October 2013 to January 2014 students had to apply their knowledge to either create an original presentation (short story) or solve different problems. 100 first year students from the Medical and Pharmacy University 'Carol Davila' answered 20 questions at the beginning of their training and corrected these during the last workshop, to prove that they have learned fundamental genetics. The answers were then corrected by a peer and in the end by the teacher. Workshops were intended to strengthen students' knowledge by means of creative thinking and also a lot of comparative approaches. Because I replaced the frontal teaching with group or individual activity, during workshops students had to apply the concepts of the lectures to explain a certain practical situation, showing they were able to use under new circumstances what they had previously learned.
In the paper I discuss the comparative results and the degree to which students discovered and corrected their own mistakes, being thus aware of their personal improvement in knowledge besides connecting with peers and learning to work in groups.
The study demonstrates the achieved ability in understanding and interpreting genetic information of the medical students.

J19.1
Predictive genetic testing in families with growth hormone deficiency: an Indian experience

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Growth is a multifactorial and complex process, yet its pattern is predictable in children. Deviations from normal pattern results in short stature which has serious psychological and social repercussions. Growth hormone (GH)/insulin like growth factor-I (IGF-1) axis has an established role in somatic growth regulation. Alterations impairing synthesis, secretion or biological action of GH results in a pathological phenotype called Growth Hormone Deficiency (GHD). GHD is eminently treatable, if supported by an early diagnosis and timely commencement of recombinant GH therapy which is highly expensive and prescribed over several years. In addition to the stigma of short stature, this exorbitant remedy is considered a burden by the patients in developing countries. High risk of inheritance in the next generation is also a matter of great concern. The present report on 100 families describes the impact of the disorder and socio-economic concerns which are a decisive factor in opting for treatment. Families with one child affected with GHD were ardent to discern the recurrence in subsequent pregnancies based on which were willing for termination. The solution to this problem in developing countries is predictive genetic testing enabling early detection for treatment and prenatal diagnosis for prevention. Timely genotyping can be provided if the genetic spectrum is known in the population as variations in mutation type and prevalence are documented worldwide. Equipped with this knowledge identification of common mutations will be easier and faster reducing anguish, improving risk perception, providing quality life and thereby eliminating the burden of morbidity.

J19.2
Population genetic carrier screening for cystic fibrosis, fragile X syndrome and spinal muscular atrophy: Exploring experiences of carriers identified through the VCGS Reproductive Genetic Carrier Screening program

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With advancing genetic technologies, carrier screening for multiple inherited conditions can now be offered within the population. This research aimed to explore how women experience undergoing carrier screening for three common inherited conditions; cystic fibrosis (CF), spinal muscular atrophy (SMA) and fragile X syndrome (FXS), through the Victorian Clinical Genetics Services Reproductive Genetic Carrier Screening program. Adopting a qualitative approach using phenomenology as the theoretical framework, the study utilised in-depth semi-structured interviews, which were transcribed verbatim. The transcripts were coded using thematic analysis to identify emerging themes. Eight female participants took part: five received a carrier result for SMA and three for CF. The majority of participants were pregnant during screening and described the decision to have the test as straight forward. Participants experienced emotional responses such as anxiety and stress whilst waiting for their partner's test result and also completed online research to find out more about the relevant condition during this time. Participants were in favour of population carrier screening, preferably offered prior to conception. The findings of this study elucidated that genetic counsellors (GCs) play an essential role within this program to adequately support couples after they receive a carrier result given the varying consent processes undertaken prior to screening. The implementation of Internet resources and GC facilitated guidance to access reliable online information is crucial to help empower couples and assist the coping process. Improving awareness of the availability of population carrier screening within the community will also help improve knowledge levels and facilitate preconception screening.

J19.3
The "utility" of incidental findings seen through the eyes of parents with children in genetic research

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The use of new generation sequencing technologies has added complexity to the issues surrounding the return of research results to participants. Guidelines are in flux in both the research and clinical settings. In ongoing scientific and bioethical discussions about a possible "duty" to return incidental findings (or not), the criteria of clinical utility plays a central role. Some authors have been arguing for a multi-dimensional concept of utility, including utility as perceived by patients as well (Foster et al., 2009, Grosse et al., 2010, Bollinger et al., 2012). But what does the concept of "utility of an incidental finding" actually entail from a research participant's standpoint?
Our presentation aims to shed light on this issue, drawing on semi-structured in-depth interviews conducted in Québec (Canada) with parents of children undergoing oncology treatment and participating in research. Following an overview of the concept of clinical utility under professional guidelines, we will analyse the multifold reasons as revealed behind the willingness of parents to know their child's incidental findings in four case-scenarios. Parents expressed very practical reasons, even though they do not necessarily rely on risk factors or on the "clinical" actionability of results. Finally, we propose some limits to the adoption of this broader concept of utility by parents in the hopes of informing bioethics guidelines on return of research incidental findings.

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