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Abstracts

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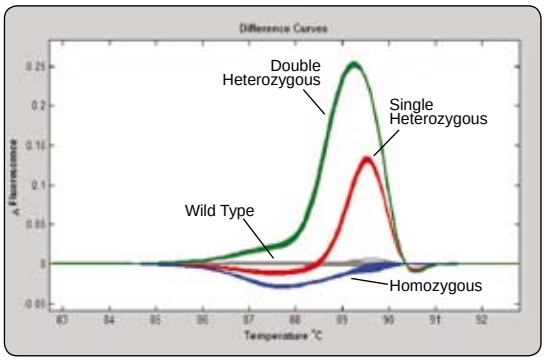

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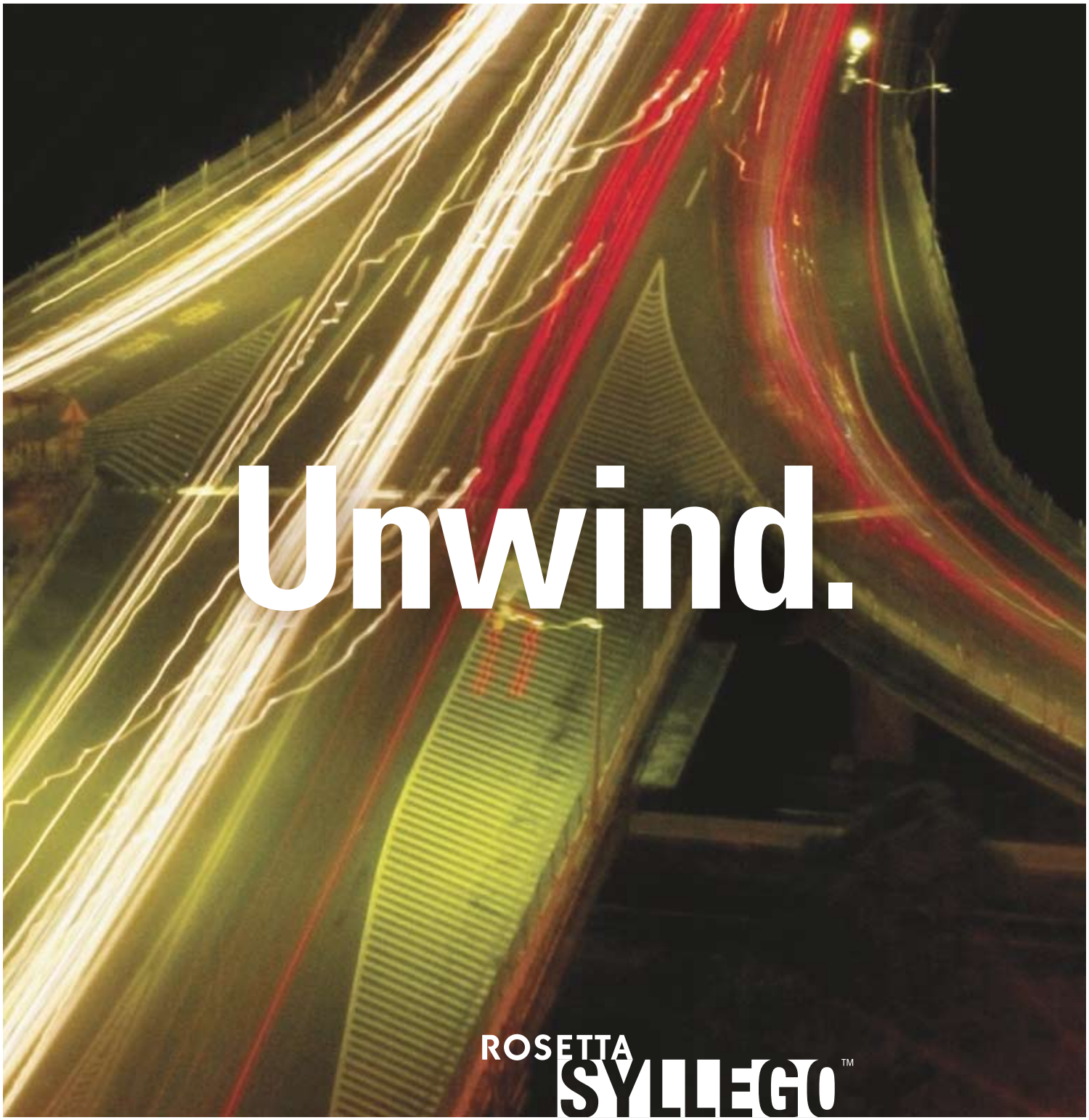
Table of Contents



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Plenary Lectures

PL01. Establishment and hereditary transmission of epigenetic modifications and pathological developments: the mouse model

F. Cuzin;
Unité 636 de l'Institut de la Santé et, de la Recherche Médicale, Nice, France.

No abstract available as per date of printing. Please check www.eshg.org for updates in the online database.

PL02. DNA Repair, genome maintenance and human disease

M. Radman;
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No abstract available as per date of printing. Please check www.eshg.org for updates in the online database.

PL03. Human neuroimaging - contributions to neurogenetics

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Voxel based morphometry (VBM) is a new way of analysing structural MR images. VBM can characterise differences in structural MRI scans of diseases influenced by genetic variation. In X-linked Kallmann's syndrome there is selective hypertrophy of the pyramidal tract in patients with mirror movements compared to those without. In a dominantly inherited, dyspraxic, language-impaired family, gene penetrance is full and associated with abnormal structure and function of the caudate nucleus and other areas. Atrophy of the caudate in affected family members is associated with task-related hyperactivity, suggesting functional compensation. Presently unaffected individuals from families of Huntington's patients show caudate atrophy that correlates with genetic status. Caudate atrophy correlates with clinical score and CAG codon repeats on chromosome 4. Studies with Turner's and partial Turner's patients have identified focal structural brain abnormalities. Candidate regions on the X-chromosome have been found that influence amygdala and orbital frontal cortex development. A structural amygdala abnormality in patients predicts failure to recognise fear in photographs of faces; a prediction that is now confirmed. These studies suggest that imaging is an efficient way of associating candidate genes with quantitative measures of brain structure and function and that informative intermediate phenotypes can be described that predict future disease in asymptomatic at-risk individuals.

PL04. Neurodegeneration in lysosomal storage diseases is associated with impairment of autophagy

A. Fraldi[1], *C. Settembre*[1], *C. Spampanato*[1], *R. de Pablo*[1], *D. L. Medina*[1], *L. Jahreiss*[2], *A. Lombardi*[1], *C. Venturi*[3], *C. Tacchetti*[3], *D. C. Rubinsztein*[2], *A. Ballabio*[1,4];
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Autophagy is the mechanism responsible for the turn-over of intracellular organelles and digestion of protein aggregates which are sequestered by autophagosomes and degraded upon the fusion of the autophagosome with the lysosome. Several neurodegenerative disorders, such as Alzheimer, Parkinson and Huntington diseases are associated with an impairment of autophagy. We have analyzed the autophagic pathway in two different murine models of lysosomal storage disorders (LSDs), Multiple Sulfatase Deficiency (MSD) and Mucopolysaccharidosis type IIIA (MPS-IIIA). Western blotting, immunofluorescence and immunohistochemical analyses using anti-LC3 antibodies demonstrated a significant intracellular accumulation of autophagic (LC3-positive) vacuoles in MEFs as well as in several brain regions of both MSD and MPS-IIIA mice. Accumulation of autophagosomes was also confirmed by ultrastructural analysis. Co-staining of MEFs using both anti-LC3 and anti-LAMP2 antibodies demonstrated that autophagosomes do not co-localize with lysosomes, suggesting the presence of a fusion defect. As a consequence of an impairment of autophagy, a massive intracellular accumulation of ubiquitin-positive aggregates and an increased number of mitochondria with altered membrane potential were detected in the brain of both MSD and MPS-IIIA mice. Interestingly, the build-up of polyubiquitinated proteins and dysfunctional mitochondria has been associated with neuronal cell death in neurodegenerative diseases. Taken together our data indicate that accumulation of storage material, due to the lysosomal enzyme deficiency, causes a lysosomal dysfunction which affects the autophagic pathway, and more specifically the formation of autophagolysosomes. We postulate that neurodegeneration in LSDs is caused by secondary storage of toxic protein aggregates due to an impairment of autophagy.

PL05. Identification of 3 novel genes that cause X-linked mental retardation; AP1S2, CUL4B and ZDHHC9.

F. Raymond[1], *P. Tarpey*[2], *G. Turner*[3], *R. Stevenson*[4], *C. Schwartz*[4], *J. Gecz*[5], *P. Futreal*[2], *M. Stratton*[2], *on behalf of the GOLD (Genetics of Learning Disability) study;*
[1]*Cambridge Institute for Medical Research, Cambridge, United Kingdom,* [2]*Sanger Institute, Cambridge, United Kingdom,* [3]*University of Newcastle, Newcastle, Australia,* [4]*JC Self Research Institute of Human Genetics, Greenwood, SC, United States,* [5]*University of Adelaide, Adelaide, Australia.*

Phenotypic variability, genetic heterogeneity and the high number of disease genes estimated to be involved has made the identification of causative gene abnormalities in X-linked mental retardation complex. To identify further novel disease genes we have used a systematic mutational screen of the X chromosome in 250 families with multiple affected members with mental retardation.

We report the identification of 3 novel genes that cause X-linked mental retardation.

AP1S2 encodes the sigma 2 subunit of the adaptor protein 1 complex. We have identified 3 truncating mutations associated with mental retardation, hypotonia, delayed walking, absent speech and aggressive behaviour. The AP1 complex is present in endosomes and the trans-Golgi network and binds vesicle cargo proteins destined for transport into different cellular compartments.

CUL4B is an ubiquitin E3 ligase subunit involved in targeting of proteins for intracellular degradation. We have identified 8 mutations, 5 truncating and 3 conserved missense mutations. Additional clinical features include macrocephaly, central obesity, hypogonadism, pes cavus and tremor were observed in some of the affected individuals but not all.

ZDHHC9 is a palmitoyltransferase that catalyses the post-translational modification of NRAS and HRAS. The phenotype associated with the 2 truncating and 2 conserved missense mutations found were mental retardation and a Marfanoid habitus.

This systematic strategy has identified 3 entirely novel disease causing mechanisms which would not have been predicted by a candidate gene approach and illustrates that defects in many different cellular processes may be sufficient to cause a mental retardation phenotype.

PL06. Mutations in *LRP2*, coding for the multi-ligand receptor megalin, cause Donnai-Barrow and Facio-oculo-acoustico-renal (FOAR) syndromes

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Donnai-Barrow syndrome (DBS) is an autosomal recessive disorder characterized by hypertelorism, myopia, corpus callosum agenesis, deafness, omphalocele, and congenital diaphragmatic hernia. There is phenotypic overlap with Facio-oculo-acoustico-renal syndrome (FOAR). Genetic mapping using Affymetrix 10K SNP arrays on four DBS children from one large consanguineous kindred identified a 17 cM region of homozygosity-by-descent on chromosome 2q23.3-q31.1. This was refined and confirmed by microsatellite marker analysis in three additional multiplex DBS families.

Sequencing of candidate genes in six DBS and one FOAR kindreds revealed homozygous or compound heterozygous mutations in the 79 exon *LRP2* gene in all cases. *LRP2* encodes megalin, a ~600 kDa

transmembrane protein that mediates receptor-mediated endocytosis for re-uptake of numerous ligands including lipoproteins, sterols, vitamin-binding proteins, and hormones in the renal proximal tubules and other sites, and may also play a role in Sonic hedgehog signaling.
The DBS/FOAR phenotypes mirror those of megalin knock out mice which have perinatal lethality due to respiratory insufficiency, and malformations of the forebrain and eye (agenesis of the corpus callosum, holoprosencephaly, microphthalmia). Among surviving mice, low-molecular weight proteinuria with spillage of retinol binding and vitamin D-binding proteins has been demonstrated. We confirmed comparable urinary abnormalities in our patients, which may serve as a valuable diagnostic marker.
We suggest that malformations of DBS/FOAR result from mutations in *LRP2* and absence of functional megalin leading to embryonic failure to uptake and deliver key lipophilic compounds required for normal development. This identifies pathways important in neural and diaphragmatic development and suggests potential targets for therapy.

PL07. RAB23 mutations in Carpenter syndrome imply an unexpected role for Hedgehog signaling in cranial suture development and obesity

D. Jenkins[1], *D. Seelow*[2], *F. S. Jeehee*[3], *C. A. Perlyn*[4], *L. G. Alonso*[5], *D. F. Bueno*[6], *D. Donnai*[7], *D. Josifiova*[8], *I. M. J. Mathijssen*[9], *J. E. V. Morton*[10], *K. Orstavik*[11], *E. Sweeney*[12], *S. A. Wall*[13], *J. L. Marsh*[14], *P. Nurnberg*[2], *M. Passos-Bueno*[15], *A. O. M. Wilkie*[1];
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Carpenter syndrome is a pleiotropic disorder with autosomal recessive inheritance, the cardinal features of which include craniosynostosis, polysyndactyly, obesity and cardiac defects. Using homozygosity mapping, we found linkage to chromosome 6p12.1-q12 and, in 15 independent families, identified five different mutations (4 truncating, 1 missense) in RAB23, which encodes a member of the RAB GTPase family of vesicle transport proteins and acts as a negative regulator of Hedgehog (HH) signaling. In 10 patients the disease was caused by homozygosity for the same nonsense mutation, L145X, which resides on a common haplotype, indicative of a founder effect in patients of northern European descent. Surprisingly, nonsense mutations of Rab23 in open brain mice cause recessive embryonic lethality with neural tube defects, suggesting a species difference in the requirement for RAB23 during early development. The discovery of RAB23 mutations in Carpenter syndrome implicates HH signaling in cranial suture biogenesis, an unexpected finding given that craniosynostosis is not usually associated with mutation of other HH pathway components, and provides a new molecular target for studies of obesity.

PL08. Y chromosome detection by Real Time PCR and pyrophosphorolysis-activated polymerization using free fetal DNA isolated from maternal plasma.

H. B. Schlecht[1], *E. M. J. Boon*[2], *P. Martin*[3], *G. Daniels*[3], *R. H. A. M. Vossen*[4], *J. T. den Dunnen*[4], *B. Bakker*[2], *R. Elles*[1];
[1]*National Genetics Reference Laboratory, Manchester, United Kingdom,* [2]*Leiden University Medical Centre, Leiden, The Netherlands,* [3]*International Blood Group Reference Centre, Bristol, United Kingdom,* [4]*Leiden Genome Technology Centre, Leiden, The Netherlands.*
The discovery of cell-free fetal DNA in the maternal blood plasma has potential for the development of non-invasive prenatal diagnosis of fetal sex and genetic traits. Current fetal genetic testing relies mainly on invasive testing by chorionic villus sampling or amniocentesis, which carry a significant risk of fetal loss (1-2%). Detection of Y chromosomal sequence of fetal origin is highly advantageous for early fetal sex determination where there is a risk of X-linked genetic disorders or conditions such as congenital adrenal hyperplasia. Highly sensitive and specific techniques are required to accurately detect the very low levels of cell free fetal DNA in the maternal plasma. This study is to validate the use of Real Time PCR, a widely used technique that can detect very low levels of Y chromosomal sequence, and to assess the use of a highly sensitive PCR technique, pyrophosphorolysis-activated polymerisation (PAP), for fetal sex determination. Both techniques detected Y chromosome sequence at very low levels with high specificity and sensitivity. Furthermore, the PAP technique was shown to be more robust than the Real Time PCR as none of the samples tested failed to meet the acceptance criteria. Combining the two techniques for male fetal sex detection from maternal blood plasma increases the sensitivity and specificity in this series. This study shows that the PAP assay can be used for Y chromosome detection using ffDNA. Furthermore, by using PAP in combination with Real Time PCR more reliable early non invasive prenatal sexing can be performed.

PL09. Neurodegenerative dementias

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Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB; Laboratory of Neurogenetics, Institute Born-Bunge; and University of Antwerp, Antwerpen, Antwerpen, Belgium.
The neurodegenerative dementias are characterized by different clinical and pathological phenotypes and are diagnosed according to specified criteria. In brain the pathology shows different neurodegenerative profiles due to the deposition of defined proteins in defined areas that are characteristic for the disease e.g. tau within neurons in tauopathies. Neurodegenerative dementias are known to have a genetic aetiology though multifactorial in nature meaning that both genetic and environmental factors contribute to the expression of the disease. Since almost 20 years molecular and epidemiological geneticists are aiming at identifying the genetic variations that underlie the disease process. Initial results were obtained in families in which the disease was apparently only genetically since the disease was transmitted as an autosomal dominant trait. As in most multifactorial diseases, also in all dementia subtypes rare families with a monogenic trait have been identified. In these families causal genes were identified that when mutated produced a 100% risk for the carrier of the mutated gene. The proteins involved are being studied in detail and have resulted in important biological hypothesis that are currently being pursued for the development of suitable and more effective treatments e.g. the amyloid cascade in Alzheimer's dementia. For the majority of patients where the disease is multifactorial, not many susceptibility genes have been identified i.e. genes that carry a genetic variation that predisposes to disease in conjunction with environmental factors. One reason is that each one of these genetic variants contributes a small fraction of the risk and that not yet the right instruments are available for finding these small effects contributed by several distinct genes. Nevertheless, the current knowledge of genetics and biology of neurodegeneration in dementias indicates that there is substantial crosstalk between the different pathways that lead to disease substantiating the complexity of brain and disease.

PL10. Selective reduction of cerebellar iron accumulation in Friedreich's Ataxia obtained by a moderate defriprone chelation treatment

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Background. Friedreich's ataxia (FA) is caused by a deficiency in frataxin needed for mitochondrial Fe-S cluster (ISC) formation and heme protein synthesis. The resulting dysfunctional respiratory chain activity and ensuing oxidative damage are accompanied and possibly associated with mitochondrial iron accumulation. Previous attempts to overcome the above deficiencies have led to the successful application of the CoQ_{10} analog Idebenone for reducing cardiac complications. Failure of Idebenone to slow-down neurodegeneration has led to us to consider the removal of regionally accumulated iron as a possible adjunct therapy in FA. The rationale used in this work rested on the concept that oxidative damage is generally caused by iron accumulated in chemically labile (redox-active and chelatable) forms, but also that

labile iron can be safely relocated from areas of accumulation to areas of deprivation. We therefore considered the possibility of: a. selectively dissipating clusters of labile-iron with chelators that demonstrably reach the CNS and that can remove labile iron from cell organelles such as mitochondria and relocate it to cellular and extracellular targets b. applying chelation regimens that minimally affect the iron status of individuals known to have no systemic iron overload by using chelators that can safely transfer labile tissue iron to plasma apotransferrin.
Methods. An efficacy-toxicity phase I-II open trial was conducted for 6 months on 11 FA adolescents (13-23 years) with deferiprone (20-30 mg/kg/day) administered together with antioxidants (Idebenone). The patients were monitored: monthly or every other month by MRI, so as to gain information about regional iron levels from R2*=1/T2* values; bimonthly by biochemical and haematological tests and at entrance and at exit for ataxia by ICARS and Purdue pegboard test. Nine of the eleven FA patients completed the study.
Results. Brain iron content monitored by MRI, indicated that at entrance FA patients had smaller and irregularly shaped dentate nuclei and proton relaxation rates R2* significantly higher ($p<0.027$) than age-matched controls. Deferiprone (DFP) intake led to a progressive and significant decrease in R2* ($p<0.002$) in the dentate nuclei of 8/9 DFP-treated patients and none in untreated patients. After 6 months treatment the relative decrease in R2* was proportional to the value at the onset of treatment, indicating the labile nature of iron in FA dentate nuclei but not in other brain areas and the possible attainment of an objective clinical endpoint. Clinical benefits were manifested especially in the youngest patients as: disappearance of constipation and incontinence, improvement of manipulative dexterity and of ataxic gait, fluency of speech, subjective signs of neuropathy but no changes in hematological indices.
Conclusion. Deferiprone evoked removal of labile iron accumulating in spinocerebellar tracts of FA patients. The ensuing clinical improvements indicate for the first time that local iron chelation might reduce and possibly reverse oxidation-induced brain injury, particularly at early stages of the disease. The results of the clinical pilot study warrant controlled prospective studies aimed at assessing iron chelation alone and in combination with antioxidants for treating FA and other diseases of regional ion accumulation, NBIA (neurodegeneration with brain iron accumulation).

Acknowledgments: The clinical aspects of the study were supported by APHP, GE (France) and Association Francaise contre les Myopathies (AFM) and the basic studies by the Israel Science Foundation ISF (Israel) and AFIRN. Deferiprone was a kind gift from Apopharma (Canada).

PL11. Huntington's disease : Intracellular dynamics and neuronal apoptosis

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Huntington's disease (HD) is a fatal neurodegenerative disorder that affects 1 in 10000 individuals of European origin. The neuropathology of HD involves neuronal dysfunction and the selective death of striatal neurons in the brain. The mutation that causes disease is an abnormal expansion of a polyglutamine (polyQ) stretch in the N-terminus of the 350 kD protein huntingtin. The mechanisms by which huntingtin induces dysfunction and death of neurons in the brain are not clearly understood. These could involve the gain of a new toxic function as well as the loss of the beneficial activities intrinsic to wild type huntingtin. Indeed, huntingtin possesses anti-apoptotic properties as observed in cell culture and animal models.
We have used 3D fast video microscopy techniques to study the intracellular dynamics in normal and pathological situations. Using this approach we have unravelled a function of huntingtin in the microtubule-based transport of neurotrophic factors such as BDNF. In the pathological situation, huntingtin-stimulated BDNF transport is altered. Reduced BDNF transport leads to a decrease in neurotrophic support and to neurotoxicity that are both rescued by wild-type huntingtin. Our results demonstrate that the anti-apoptotic properties of huntingtin are linked to the ability of huntingtin to promote transport of BDNF in the brain.
The development and the use of relevant neuronal models of HD allowed us to identify molecular mechanisms and signal transduction pathways that control neuronal death in HD. We will report recent findings that explain how these signaling pathways inhibit the toxicity of mutant polyQ-huntingtin in disease.

Concurrent Symposia

S01. The increasing impact of internet on genetic services

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With the widespread dissemination of the internet and the multiplication of web-based information services, the question of to what extent this affects the behaviour of the stakeholders needs to be addressed. For the patients, potential benefits are a better understanding of their disease and its mode of inheritance, improved dialogue with health care professionals, the ability to more easily locate the expert service that they can be referred to, and occasionally to be able to participate in clinical research. For the patients support groups, the benefits are improved visibility, which leads to an expansion of their membership and allows them to help more people. For the non-specialised healthcare professional, the benefits are similar to those for the patients with the ultimate benefit being to deliver better care. At the beginning of the internet era, experts feared they would no longer be consulted, due to the wide availability of what they considered as their specific added-value. Experience shows that this fear was unfounded. Taking Orphanet (www.orpha.net) as an example of a website, and looking at the behaviour of the site visitors during the past ten years and at the impact in terms of referral to listed services, it seems like the positive effects outweigh the negative ones. Additional services are expected from the community. The physicians and biologists would like to access mutation databases by population, the physicians are expecting more practical information and the patients would like to be partners in the development of an encyclopaedia concerning their own experience.

S02. Is there a role for the Human Geneticist in the genomic revolution

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With the completion of the human genome project, the belief that virtually all human diseases and responses to therapy are the products of both genetic and environmental factors has firmly taken hold, and an extensive search for mutations/variants that influence susceptibility to disease and the efficacy and toxicity of drugs is well underway. The anticipated outcomes of this research are a better understanding of disease pathogenesis and the development of genetic tests to predict who will be at risk for what. It is believed by many that genetic risk assessment or profiling will lead to a genomic or "personalized" medicine in which individualized strategies will be used to prevent the onset of disease and to improve the efficacy of therapeutic agents. Although human geneticists will certainly be involved in the research, the role of the medical (clinical) geneticist in the delivery of this genomic medicine, if it actually comes to pass, is less certain. Given their small numbers, medical geneticists would certainly not be in a position to become the principal providers of genetic testing and risk assessment for the greater population, and this responsibility would fall to others. Nevertheless, geneticists do have a special knowledge of genetics and human disease that should be brought to bear on the provision of these services. This can be accomplished by playing a role in the education in genetics of other physicians and health providers and by serving as designated referral sources for problems that may be too complex for those without substantial knowledge of genetics to handle alone. At the same time, medical geneticists need to continue to provide and, indeed, to expand the services that are uniquely theirs to give: the diagnosis, management (including treatment), and counseling of patients and families with Mendelian, chromosomal, and mitochondrial disorders, malformations, and syndromes.

S03. What use the 1000 euro genome?

***M. E. Pembrey**;*
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Whatever this use of the word 'genome' includes in terms of coverage and DNA sequence information, the 1000 euro genome will detect a vast amount of personal genetic variation including rare mutations. It will become commercially available in less than 20 years was the conclusion of a 2005 UK report on genetic profiling by a group chaired by John Sulston (www.hgc.gov.uk/Client/news_item.asp?NewsId=38) and probably used by individuals, although not as state-funded screening. However being used is not the same as being useful! Software claiming to interpret your genome will become the new palmistry. There is a pressing need to develop research programmes within existing and in new cohort studies that include intermediate as well as disease phenotypes and environmental exposures to discover what it means prospectively to carry a particular genotype. The cheap genotyping techniques behind the drive for the 1000 euro genome will help in this research although more useful in epidemiology may be selected gene by gene sequencing of many thousands of samples in parallel. The first fruits of such cohort research could be the introduction of clinically useful newborn genetic screens sequencing 100 genes or so. Detection of filaggrin null mutations might be an example where simple strategies might prevent eczema related allergies. One of the problems with the idea of genetic profiling at birth, or at any other time for that matter, is that most genetic effects are likely to be *conditional* on the person's developmental experience and/or the prevailing environment. Thus the value of knowing just one's genotype is also conditional. By the time the 1000 euro genome arrives we may have more useful tests based on epigenetic/methylation profiling to detect prenatal developmental programming or based on gene expression patterns in peripheral blood cells under challenge from specific stressors.

S04. Better education for medical geneticists and non-geneticists in the new genomics era

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No abstract available as per date of printing. Please check www.eshg.org for updates in the online database.

S05. MicroRNAs as oncogenes and tumor suppressors

***S. M. Hammond**;*
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MicroRNAs (miRNAs) are short, non-coding RNAs that post-transcriptionally regulate gene expression. Over 450 miRNA genes have been identified in the human genome. We have undertaken the study of miRNA function in mammals. Using a custom microarray platform, we investigated miRNA expression patterns in mammalian development and in cancer. We found that many miRNAs are down-regulated in tumor cell lines. This downregulation is not due to decreased transcription, but is due to reduced maturation during miRNA biogenesis. On the other hand, several miRNA genes are over-expressed in tumor cell lines and primary tumors. Seven of these cancer-associated miRNAs are clustered in a single primary transcript termed chr13orf 25 or OncomiR-1. This cluster is located in a region amplified in lymphoma and several solid malignancies. Ectopic expression of these miRNAs in a mouse model of lymphoma accelerated disease progression. In addition, the lymphomas had reduced apoptosis and were more disseminated into secondary regions. This work establishes non-coding RNAs, and specifically miRNAs, as oncogenes in human cancers.

S06. Small RNAs in gametogenesis

***A. Girard**, A. Aravin, R. Sachidanandam, M. Carmell, G. Hannon;*
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Argonaute proteins and associated small RNAs have critical role in development by regulating messenger RNA stability, protein synthesis, chromatin organization and genome structure. In animals, Argonaute proteins segregate into two subfamilies. Ubiquitously expressed members of Argonaute subfamily bind 21-23 nt RNA and act in RNA interference and in microRNA-mediated gene regulation. The Piwi subfamily is involved in germline-specific events such as germline stem cell maintenance and meiosis. Particularly, three members of the Piwi subfamily in mouse are critical for successful spermatogenesis. Recently we identified a new class of 25-30 nt RNAs (piRNAs) as a binding partner of Piwi proteins in the mammalian male germ cells. piRNAs are highly abundant in germ cells and accumulate at the onset of meiosis. Thousands of identified piRNAs show distinctive localization patterns in the genome, being predominantly grouped into 20-90-kilobase clusters scattered throughout genome. We explore biogenesis of piRNAs and function of Piwi-piRNA complexes in the mammalian germline.

S07. Vertebrate miRNA diversity

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MicroRNAs are 20- to 23-nucleotide RNA molecules that can regulate gene expression. Currently over 400 microRNAs have been experimentally identified in mammalian genomes, whereas estimates go up to 1000 and beyond. We have used bioinformatic and experimental (microarray-based detection and massively parallel sequencing) approaches to get insight in the vertebrate microRNA repertoire and evolutionary dynamics.

Analysis of the microRNA content of human and chimpanzee brain regions resulted in the identification of hundreds of novel microRNAs genes, many of which are not conserved beyond primates, indicating their recent origin. Others are expanded in one species through duplication events, suggesting that evolution of microRNAs is an ongoing process and that along with ancient, highly conserved microRNAs, there are a number of emerging microRNAs that could contribute to evolutionary processes and differences in human and chimpanzee brain function.

S08. SLE susceptibility genes

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SLE is a generalised autoimmune disease affecting a wide range of tissues including skin, joints, bone marrow and kidneys, which afflicts ~10,000 individuals in the UK. The disease is characterised by the production of autoantibodies to nuclear and cell-surface antigens. The cause of the disease is poorly understood although genetic factors contribute. The strategy adopted is to use both family-based and case-control methods to ascertain genetic association with SLE. To achieve this we have established large collections of SLE cases (n=500) and families (n=850). We have analysed approximately 100 different genes (based on functional and positional candidacy) and found convincing evidence to support the role of genetic variation in five of these in SLE susceptibility.

How nuclear autoantigens are targeted in SLE has been a subject of controversy. Nucleic acids can bind Toll-like receptors on antigen presenting dendritic cells, which stimulates an interferon response and thereby augments dendritic cell function. Interferon regulatory genes (IRFs) are key molecules within this amplification loop and we have shown that patients with lupus are almost twice as likely to carry genetic variants in IRF5 that generate more transcripts, in part due to differential polyadenylation. Autoantibodies in SLE are of the IgG isotype and bind with high affinity to their targets, implicating the adaptive immune system in pathogenesis. We examined a number of candidate genes acting at the T-B lymphocyte interface and identified associations with SLE at the CTLA4 locus and a stronger signal arising from the tumour necrosis factor family member, OX40L. Finally, the IgG Fc receptor locus on human chromosome 1q23 has been extensively studied in autoimmunity genetics. We have provided strong evidence that the locus does contribute to the risk of SLE as a result of copy number variation. Reduction in neutrophil FCGR3B expression predisposes to end organ damage in the kidney, probably through defective removal of immune complexes.

In conclusion, we have identified lupus susceptibility genes operating in both the adaptive and innate immune systems. Gene effects promote initial autoantigen targeting, aberrant regulation of the immune response and finally accentuate end organ damage.

S09. New pathways in pathogenesis of allergic asthma: *ADAM33* and end organ susceptibility genes

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While asthma is an inflammatory disorder of the airways usually associated with atopy, an important additional component is involvement of the epithelium and underlying mesenchyme acting as a trophic unit (EMTU). In addition to allergens, a wide range of environmental factors interact with the EMTU, such as virus infections, environmental tobacco smoke and pollutants, to initiate tissue damage and aberrant repair responses that are translated into remodelling of the airways. While candidate gene association studies have revealed polymorphic variants that influence asthmatic inflammation, positional cloning of previously unknown genes is identifying a high proportion of novel genes in the EMTU and revealing mechanistic pathways behind the remodelling response. A disintegrin and metalloproteinase (ADAM)33 is one such susceptibility gene strongly associated with asthma that is preferentially expressed in the airway mesenchyme. Furthermore, recent results suggest that variation in the gene may play a significant role in other diseases that involve tissue remodelling in response to chronic inflammation, and that this variation may act early in life, altering developmental processes, rendering tissue innately susceptible to remodelling.

S10. Genetic and environmental factors in celiac disease

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Coeliac disease (CD) is an intestinal disorder which develops as a result of interplay between genetic and enviromental factors. Particular HLA genes together with non-HLA genes predispose to the disease. The concordance rate among monozygotic twins is about 75% whereas the concordance rate among HLA identical siblings is about 30%. This suggests the involvement of non-HLA genes, but the many attempts to identify non-HLA genes has been met with limited success. There is evidence for susceptibility genes located on chromosomes 5q32, 2q33 and 19p13. By contrast, the HLA susceptibility genes in CD are well characterised. The primary HLA association in the majority of CD patients is with DQ2 and in the minority of patients with DQ8. DQ2 (DQA1*05/DQB1*02 can be encoded in *cis* in DR3DQ2 individuals and in *trans* in individual being DR5DQ7/DR7DQ2 heterozygous). Among the multifactorial disorders with involvement of HLA genes CD is unique, as a critical environmental factor has been identified, namely dietary gluten. Evidence suggests that CD4+ T cells are central in controlling a multifaceted immune response to gluten that causes the characteristic disease pathology. Gluten reactive T cells can be isolated from small intestinal biopsies of coeliac patients but not from non-coeliac controls. DQ2 or DQ8, but not other HLA molecules carried by patients, are the predominant restriction elements for these T cells. Lesion derived T cells mainly recognise deamidated gluten peptides, and this the deamidation is mediated *in vivo* by transglutaminase 2 (TG2). A number of distinct T cell epitopes exist within gluten. DQ2 and DQ8 bind the epitopes so that the glutamate residues introduced by TG2 are accommodated in pockets of the binding site which have preference for negatively charged side chains. Notably, TG2 can also generate complexes between gluten and TG2. These complexes may permit gluten reactive T cells to provide help to TG2 specific B cells thereby explaining the occurrence of gluten TG2 auto-antibodies which is a characteristic feature of CD patients exposed to gluten.

S11. The genetic architecture of Skin pigmentation

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No abstract available as per date of printing. Please check www.eshg.org for updates in the online database.

S12. Novel Insights into melanoma genetics

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Melanocytic neoplasms display significant phenotypic variation.We used a genetic approach to compare primary melanomas from mucosal membranes, the non-hair bearing skin of the palms and soles (acral melanomas), and sun-exposed skin with and without signs of chronic sun-induced damage (CSD and non-CSD). We found differences in the degrees of genomic instability, the specific genomic regions that are gained and lost, frequencies of mutations in specific genes, and effects of genetic variants in the germline on melanoma risks. Melanomas on acral skin and mucosa had distinct patterns of frequent amplifications and deletions involving very small segments of the genome, indicating a unique type of genomic instability. By contrast amplifications were rare in melanomas arising on some-exposed skin. Among these, BRAF mutations occurred in~ 60% of non-CSD melanomas but

were significantly less frequent in CSD melanomas. We showed that germline variants of MC1R are a major susceptibility factor for BRAF mutations among non-CSD melanomas. Further analysis our array CGH data suggested a genomic region harboring KIT to be important in melanomas on mucosa, acral skin and skin with CSD. Oncogenic mutations or focused copy number increases in the gene were found in 40% of acral and mucosal melanomas, and 30% of CSD melanomas. These results suggest that the existing drugs such as imatanib and new are inhibitors against KIT could be useful for certain melanoma types. More recent studies correlating phenotypic alterations with the underlying genotype have shown that mutations in BRAF are associated with distinct histopathological features of the primary tumor that can be combined to simple algorithms that can predict the mutation status of BRAF with high accuracy. Our data show that melanoma is comprised of genetically and phenotypically distinct subtypes with different requirements for treatment stratification.

S13. Comprehensive analysis of the SRY-box 10 locus (SOX10): Implications for diseases of neural crest derivatives

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The transcription factor SOX10 is a key regulator of genes involved in neural-crest derived melanocytes, enteric neurons, and myelinating Schwann cells. Mutations in the SOX10 gene are associated with human diseases that affect these cell populations, including impaired pigmentation, enteric aganglionosis (e.g., Waardenburg-Shah syndrome), and demyelinating peripheral neuropathy. While SOX10 targets have been identified, the mechanisms involved in the transcriptional regulation of SOX10 remain elusive. Recently, we used comparative sequence analysis to identify highly-conserved, non-coding segments upstream of SOX10. We demonstrated that three of these segments are encompassed by a 15-kb deletion residing ~ 50 kb upstream of Sox10 in a mouse model of Waardenburg-Shah syndrome. Importantly, this deletion does not completely compromise Sox10 expression in all tissues. Thus, other sequences at the Sox10 locus are likely involved in transcriptional regulation, and mutations in these sequences may contribute to neural crest disorders in humans.

One goal of our lab is to fully characterize SOX10 regulatory sequences and to establish if mutations in these sequences contribute to human disease. Our studies involve four distinct research areas: (1) Testing conserved segments for enhancer activity in cell lines; (2) Determining if these conserved segments drive reporter gene expression in a tissue-specific manner in zebrafish and mouse; (3) Deleting these conserved segments in BACs and testing for altered reporter gene expression in transgenic mice; and (4) Screening these conserved segments for sequence variations in a panel of >600 DNA samples isolated from patients with enteric aganglionosis (Hirschsprung disease). These studies will provide important clues about the transcriptional regulation of SOX10 and the full role of the transcription factor in human development and disease.

S14. Cilia in Skeletal Development: identification of Evc as a mediator of Ihh signalling

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Cilia are projections from the cell made of a core of microtubules and associated proteins. Cilia are classified according to their microtubule components: 9+2 motile cilia and 9+0 primary cilia, which are usually immotile. The importance of primary cilia in development, e.g. left-right development, and postnatally, e.g. in the kidney, is increasingly recognised. Intraflagellar transport (IFT) is required to generate cilia. IFT mutants are embryonic lethal but generation of conditional alleles enables studies regarding requirement for cilia in later processes and have shown that they play a critical role in endochondral bone formation. A limitation of this approach is that removal of cilia disrupts all of their many functions. We identified two causative genes for the human chondrodysplasia Ellis-van Creveld syndrome (EvC, OMIM:225500) by positional cloning. We have inactivated *Evc* in the mouse and show that *Evc*$^{-/-}$ mice develop an EvC-like syndrome, including short ribs, short limbs, and dental abnormalities. *LacZ* driven by the *Evc* promoter revealed that *Evc* is expressed in the developing bones and the orofacial region. We developed antibodies that localized the Evc protein to the base of the primary cilium. Recent studies of IFT mutants have revealed that primary cilia are integral to Hedgehog signalling. Analysis of the growth plate of *Evc*$^{-/-}$ mice shows delayed bone collar formation and advanced maturation of chondrocytes. By in situ hybridization we detected that *Indian hedgehog (Ihh)* is expressed normally in the growth plates of *Evc*$^{-/-}$ mutants but expression of the Ihh downstream genes *Ptch1* and *Gli1* were markedly decreased. Cilia formation is not affected in *Evc* mutants and western blot analysis indicates that Gli3 processing is normal. We conclude that Evc is required for Ihh signalling in endochondral bone formation.

S15. Molecular basis of primary ciliary dyskinesia

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Defects in structure and function of cilia have recently been associated with a growing number of rare diseases. The phenotypic features of these so-called ciliopathies reflect the diverse biological properties of these evolutionarily conserved structures that protrude from the apical surface of most eukaryotic cells. Cilia, which contain a microtubule cytoskeleton (axoneme) structurally related to the flagella of spermatozoa, can be classified according to their axonemal components: primary cilia, with sensory function, are involved in a wide range of disorders, whereas motile cilia are involved in the most prominent ciliopathy called primary ciliary dyskinesia (PCD). PCD is a rare respiratory disease, affecting approximately 1/16,000 individuals, due to impaired mucociliary clearance. This disorder, usually transmitted as an autosomal recessive trait, is characterized by chronic airway inflammation, male infertility, and, in approximately half of the patients, abnormal left-right asymmetry, defining the Kartagener syndrome. The disease phenotype results from various axonemal defects involving the motile respiratory and embryonic nodal cilia, as well as the flagella of spermatozoa. Its molecular basis is just beginning to be elucidated, with, to date, 5 genes known to be involved in that condition (i.e. *DNAI1*, *DNAH5*, *DNAH11*, *RPGR*, and *TXNDC3*). These results were obtained by various means, including original candidate-gene approaches based on data from a one-billion-year-old unicellular flagellar algae (*Chalmydomonas reinhardtii*) and from see urchin. The current challenges are to identify new PCD genes and to characterize the molecular and cellular mechanisms involved in ciliary dyskinesia. Flagellated protists like trypanosomes represents promising models to achieve these goals. These lower eukaryotes possess genes for flagellar motility whose inactivation reproduces the motility and ultra-structural defects seen in patients with PCD. In addition, in spite of the evolutionary distance between these unicellular organisms and humans, the complementation of genetically modified strains of trypanosomes with human orthologous sequences seems conceivable.

S16. Making sense of cilia: Bardet Biedl syndrome and other ciliopathies

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Despite knowledge of primary (non-motile) cilia for over a century, recently ascribed functions have rekindled interest in these sessile cellular appendages. Less than a decade ago, cilia were associated with renal cyst formation (through observations in mouse models of polycystic kidney disease) although the mechanism is still hotly debated. More recently, developmental biologists have discovered the origins of left-right asymmetry common to vertebrates and demonstrated the involvement of specialised “spinning” primary cilia. Our own area of study, the Bardet-Biedl syndrome along with nephronophthisis, both causes of renal cyst formation have revealed the importance of primary cilia in human disease.

In this lecture, I will describe the evidence for ciliary dysfunction in Bardet-Biedl syndrome and how this is leading us to define extended roles for cilia in developmental pathways. I will also describe an approach we have taken to discover the causes of other diseases of cilia function thus widening the scope of this emerging class of ciliopathies.

S17. Towards a gene expression map of human brain development

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Discoveries of human-specific gene expression or gene regulation or gene content are being made at an accelerating pace and have sparked interest in molecular answers to the question of "what makes us human". Studies of the human brain and its development are expected to provide at least some of these answers.

Characterising gene expression patterns is a crucial step towards understanding the molecular determinants of development and the roles of genes in disease. However, human brain development involves transformations from a simple tube to a highly organised and complex 3-dimensional (3-D) structure. We have used a recently developed method, optical projection tomography (OPT), to generate digital 3-D models of early human brain development. These models can be used both as frameworks, on to which normal or experimental gene expression data can be mapped, and as objects, which provide a valuable means for visual interpretation and overview of complex morphological data and within which morphological relationships can be investigated in silico. Together these models, mapped gene expression patterns and the sophisticated software to manipulate and analyse them are being expanded towards the generation of an electronic atlas of human brain development (www.ncl.ac.uk/EADHB). This should be a mechanism for systematically correlating human gene expression results with corresponding data in mouse and provide a scientific basis for extrapolation from mouse model to human disease which is crucial if we are to safely use the mouse (or other animal models) to investigate the mechanisms underlying human disorders and for testing therapeutic agents or interventions.

More generally, human developmental studies bring with them ethical and technical challenges and will need a large-scale, trans-national effort in order to make the maximum effective use of the limited human tissue (suitable for gene expression studies) that is being collected. DGEMap (Developmental Gene Expression Map) is an EU-funded Design Study with a multidisciplinary team which aims to define the molecular genetic & informatics technologies and the organisational & collaborative structures necessary for a new research infrastructure to meet these challenges within an appropriate ethical framework (www.dgemap.org)

S18. Genetic and genomic studies of patterning in the human cerebral cortex

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The human cerebral cortex is distinguished from that of other species by its remarkable size, its subdivision into lobes and regions with distinct functions, and the relative specialization of the left and right hemispheres for complementary functions, with the left usually dominant for language and mathematical ability. Remarkably, genetic malformations of human cerebral cortex can affect different cortical regions preferentially, potentially identifying genes involved in generating area-specific patterns in human cortex. Mutations in *GPR56,* which encodes an unusual G-protein coupled receptor, cause a recessively inherited disorder that preferentially affects the frontal lobe (bilateral frontoparietal polymicrogyria), and specific *GPR56* alleles cause remarkably localized frontal defects. Understanding the regulation of GPR56 expression may reveal additional factors that pattern the cortex. In order to identify genes with potential differences in expression between left and right hemisphere, we used SAGE to compare right and left perisylvian cortex at 12-14 weeks gestation, and found substantial numbers of genes with differential levels of expression, notably *LMO4*. We are presently extending these studies to earlier developmental ages. Finally, a recently described human brain malformation preferentially disrupts the right perisylvian region but not the left, suggesting a gene that may be involved in hemisphere-specific development. Supported by the NINDS, HHMI, the NLM Family Foundation and the Simons Foundation. C.A.W. is an Investigator of the Howard Hughes Medical Institute.

S19. Signalling Pathways Deduced from a Global Analysis of Spatial Gene Expression Patterns

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Automated *in situ* hybridization (ISH) permits construction of comprehensive atlases of gene expression patterns in mammals. When web-accessible, such atlases become searchable digital expression maps of individual genes and offer an entryway to elucidate genetic interactions and signaling pathways. An atlas housing ~1,000 spatial gene expression patterns of the mid-gestation mouse embryo was generated. Patterns were textually annotated using a controlled vocabulary comprising 90 anatomical features. Hierarchical clustering of annotations was carried out using distance scores calculated from the similarity between pairs of patterns across all anatomical structures. This ordered hundreds of complex expression patterns into a matrix that reflected the embryonic architecture and the relatedness of patterns of expression. Clustering yielded twelve distinct groups of expression pattern. Because of similarity of expression patterns within a group, members of this group may be components of regulatory cascades. We focused on group 7 which is composed of 80 genes, many of which encoded regulatory proteins such as Pax6, an evolutionary conserved transcriptional master mediator of the development. By combining ISH on *Pax6*-deficient embryos, bioinformatics-driven Pax6 binding site selection, and Pax6 binding site validation by means of electromobility shift assays, we identify numerous new genes that are transcriptionally regulated by Pax6 in the developing neocortex. Hence cluster analysis of annotated gene expression patterns derived from ISH is a novel approach to unravel components of signaling cascades regulating critical aspects of mammalian development, physiology and pathophysiology.

S20. New mechanisms of human genetic disease

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We describe a new mechanism underlying human genetic disease by identifying a gain of function regulatory SNP (rSNP) that causes a form of alpha thalassaemia which occurs at polymorphic frequencies in Melanesia. Association studies localised the mutation to a 168kb segment of the genome including the alpha globin locus but conventional analyses failed to detect any molecular defect. After re-sequencing this region and using a combination of chromatin immunoprecipitation and expression analysis on a tiled oligonucleotide array, a regulatory SNP (rSNP) was identified in a nondescript region of the genome lying between the alpha globin genes and their highly conserved, remote, upstream regulatory elements. The rSNP creates a new promoter-like element which interferes with normal activation of all downstream alpha-like genes. This not only demonstrates a new mechanism of human genetic disease but also illustrates an important general strategy for distinguishing between neutral and functionally important rSNPs.

S21. Chromosome dynamics in cytokine gene expression

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In the last few years, we have found that regulatory elements on one chromosome associate with genes on another chromosome. The system that we study is the differentiation of naïve, precursor CD4 T cells into different kinds of effector helper T cells. We first observed inter-

chromosomal association of the TH1 *ifnγ* locus and TH2 loci, which are mutually exclusively expressed by two subtypes of differentiated T helper cells, precedes differentiation and may act as a checkpoint for T cell fate determination. Since this primary observation, we have found several other cases of interchromosomal associations between different cytokine loci in the naïve T cell precursors. Interestingly, upon TH cell differentiation, these associations are terminated and replaced by interactions between expressed loci. Regulatory elements in one locus appear to play an active role in orchestrating processes across these chromosomes. The interchromosomal interactions we describe appear to direct either activatory or silencing roles in gene transcription.

S22. Molecular mechanisms of congenital hyperinsulinism

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Persistent hyperinsulinemic hypoglycemia of infancy (HI) is the most important cause of hypoglycemia. HI is a heterogeneous disorder which may be divided into two forms on the basis of the histopathological lesion, but which are clinically indistinguishable: diffuse (DiHI) and focal (FoHI).

FoHI is characterized by somatic islet-cell hyperplasia, which is associated with hemi- or homozygosity of a paternally inherited mutation of the sulfonylurea-receptor (*ABCC8* or *SUR1*) or the inward rectifying potassium channel genes (*KCNJ11* or *Kir6.2*) on chromosome 11p15, and loss of the maternal allele in the hyperplastic islets. The focal lesion is a sporadic event, as indicated by the somatic molecular abnormality in the pancreas, the observation of discordant identical twins and our experience. However, FoHI occurring in consanguineous family can repeat with a diffuse form.

DiHI is a heterogeneous disorder which can be caused by various defects in the regulation of insulin secretion by the pancreatic beta-cell. The same genes can be implicated in neonatal diabetes or MODY. These include:

i) channelopathies affecting either the *SUR1* or the *KIR6.2* channel; Recessive *ABCC8* mutations and, more rarely, recessive *KCNJ11* mutations, are responsible for the majority of diffuse and severe neonatal HI resistant to medical treatment. Dominant *ABCC8* mutations are responsible for less severe HI sensitive to diazoxide;

ii) metabolic HI since anaplerosis appears to play an important role in the secretion of insulin, including *GCK*, *GDH* and probably *SCHAD* deficiencies; the two first causes are dominantly inherited or caused by a *de novo* mutation;

iii) defects of insulin transcription factors (*HNF4A* mutations described in MODY and HI) or insulin receptor, with de novo or dominantly-inherited mutations, or defect in proinsulin processing.

Syndromic HI (CDG, HI/HA by overactivity of GDH with hyperammonemia, Sotos, Beckwith-Wiedemann and Kabuki syndromes) is rare in our recruitment. Symptoms associated to diazoxide-sensitive hypoglycaemia are mental retardation, diarrhea, liver abnormalities, gigantism, or malformations. The differential diagnosis is facticious hypoglycemia secondary to Munchausen by proxy syndrome.

S23. Title not available as per date of printing

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No abstract available as per date of printing. Please check www.eshg.org for updates in the online database.

S24. Stem cell renewal and the Nanog gene

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No abstract available as per date of printing. Please check www.eshg.org for updates in the online database.

S25. The role of Pax5 in B-cell commitment and lymphomagenesis

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Lineage commitment and differentiation to a mature cell type are considered to be unidirectional and irreversible processes under physiological conditions. Mature B lymphocytes critically depend on the transcription factor Pax5 for their differentiation and function. Here we show that conditional *Pax5* deletion allowed mature B cells from peripheral lymphoid organs to dedifferentiate *in vivo* back to early uncommitted progenitors in the bone marrow, which rescued T-lymphopoiesis in the thymus of T cell-deficient mice. These B cell-derived T-lymphocytes carried not only immunoglobulin heavy- and light-chain gene rearrangements, but also participated as functional T cells in immune reactions. Notably, mice lacking *Pax5* in mature B cells also developed aggressive lymphomas, which were identified by their gene expression profile as progenitor cell tumours. Hence, the complete loss of Pax5 in late B cells could initiate lymphoma development and uncovered an extraordinary plasticity of mature peripheral B cells despite their advanced differentiation stage.

S26. Copy number variation in the human genome

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The advent of genome-scanning technology and comparative DNA analysis has uncovered a significant extent of structural variation in the human genome. Structural variants can include microscopic and more commonly submicroscopic deletions, duplications, insertions, and large-scale copy number variants - collectively termed copy number variants (CNVs) or polymorphisms - as well as, inversions and translocations. CNVs are the most prevalent form of structural variation and they can comprise millions of nucleotides of heterogeneity within every genome, having an important contribution to human diversity and disease susceptibility. We have been applying numerous experimental and data mining approaches to catalogue the complete complement of CNVs in worldwide populations, as well as to characterize the genomic features and affects of CNVs. Our collective data, integrated with all other available information, is released in the 'Database of Genomic Variants' (http://projects.tcag.ca/variation/), which we are continually curating and upgrading. The database serves as a resource to assist numerous clinical research studies. Our latest data assessing CNV content for involvement in disease will also be presented.

S27. Destabilization of the NFAT Signaling Pathway in Down Syndrome

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Trisomy 21 results in Down Syndrome (DS), but little is known about how a 1.5-fold increase in gene dosage produces the pleiotropic phenotypes of DS. Studies of patients with partial trisomy 21 have defined a DS critical region (DSCR) on chromosome 21q. We have recently shown that increased dosage of two genes, found within the DSCR, cooperatively reduces the activity of the calcineurin/NFAT signaling pathway, which is a critical regulator of vertebrate development (1,2). At the centromeric border of the DSCR is *DSCR1*, a member of a family of closely related inhibitors of the protein phosphatase calcineurin. DSCR1 is blocks the calcineurin dependent nuclear localization of the NFATc proteins in response to Ca^{2+} influx. *DYRK1a* is telomeric to *DSCR1* and encodes a nuclear kinase that blocks NFAT signaling by rapid nuclear export of NFATc proteins. We found that mice harbouring mutations of the four genes encoding NFATc transcription factors, individually and in combinations, exhibit many of the characteristics of DS. Transgenic expression of DYRK1a and DSCR1 produces features

at E13.5 similar to those seen in DS and *NFATc* mutant mice. Mathematical modelling of the NFAT pathway, which includes positive and negative feedback loops, predicts that a 1.5-fold increase in DSCR1 and DYRK1a levels will reduce NFAT activity and alter the expression of target genes. These studies raise the question that perturbation of the NFAT genetic circuit by increased dosage of these genes may explain many of the developmental phenotypes in DS. More generally this suggests that developmental defects may arise from the specific susceptibilities of genetic regulatory circuits.

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S28. The Tc1 mouse, an aneuploid mouse with a human chromosome that models aspects of Down syndrome

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Down syndrome (DS) arises from trisomy human chromosome 21 (Hsa21) and is the most common known genetic cause of mental retardation, and also results in increased susceptibility for other disorders, such as heart defects. DS is a complex genetic disorder likely involving several 'major effect' dosage sensitive genes on Hsa21 and their interaction with the rest of genome/environment. To help towards our understanding of DS we generated a mouse model in which an almost complete Hsa21 segregates through the germline. This trans-species aneuploid mouse strain, 'Tc1', has widespread novel phenotypes including in behaviour, synaptic plasticity, cerebellar neuronal number, heart development and mandible size, that relate to human DS. Transchromosomic mouse lines such as Tc1 could be useful genetic tools for dissecting other human aneuploidies and syndromes arising from dosage sensitivity of multiple genes.

S29. Polymorphic miRNA-mediated gene regulation: contribution to phenotypic variation and disease

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The expression level of at least one third of mammalian genes is post-transcriptionally fine-tuned by ~ 1,000 microRNAs assisted by the RNA silencing machinery comprising tens of components. Polymorphisms and mutations in the corresponding sequence space (machinery, miRNA precursors and target sites) are likely to make a significant contribution to phenotypic variation including disease susceptibility. We herein review basic miRNA biology in animals, survey the available evidence for DNA sequence polymorphisms affecting miRNA-mediated gene regulation and hence phenotype, and discuss their possible importance in the determinism of complex traits.

S30. Epigenetics and X-inactivation

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Some 150 years after the emergence of genetics, epigenetic mechanisms are increasingly understood to be fundamental players in phenotype transmission and development. In addition, epigenetic alterations are now linked to several human diseases, including cancers. A common feature of many epigenetic phenomena, for which X-chromosome inactivation (XCI) is the paradigm, is the implication of non-coding RNAs. The X-inactivation centre, which controls the initiation of X-inactivation, hosts several such non-coding RNAs, of which at least two play essential roles in the process in the mouse. The *Xist* gene produces a nuclear RNA that, when expressed in sufficient amount, coats the chromosome in *cis* and induce its silencing. *Tsix*, a transcript antisense to *Xist*, is a negative regulator of its sense counterpart, whose chromatin-remodelling activities have been shown by us and others to be important for the epigenetic programming of *Xist* expression.

Although X-chromosome inactivation has been adopted as a dosage compensation mechanism in all therian mammals, phenotypic divergences are known to exist between species and to correlate with genotypic differences, in which non-coding RNAs are particularly concerned. As essential as it is in placental mammals, *Xist* was recently found to have no homolog in marsupials and to be derived from a protein-coding gene with ancestral functions unrelated to X-inactivation. Likewise *Tsix*, which is clearly involved in some aspect of X-inactivation in the mouse, has seen its existence in human actively debated. The observation that X chromosome inactivation can be achieved in different species through distinct pathways, most of which remaining to be deciphered, underlies the mechanistic plasticity of epigenetic processes during evolution.

S31. DNA methylation signatures in colorectal cancer

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Cancer cells are characterized by a generalized disruption of the DNA methylation pattern involving an overall decrease in the level of 5-methylcytosine together with regional hypermethylation of particular CpG islands. The extent of both DNA hypomethylation and hypermethylation in the tumor cell is likely to reflect distinctive biological and clinical features. We have analyzed DNA methylation profiles in sporadic colorectal carcinomas, synchronous adenoma-carcinoma pairs and their matching normal mucosa using different techniques. All tumors displayed altered patterns of DNA methylation in reference to normal tissue. Genome-wide hypomethylation and hypermethylation associate with different features in colorectal tumorigenesis suggesting that DNA hypermethylation and hypomethylation are independent processes and play different roles in colorectal tumor progression. While hypermethylation is associated with patient's sex, tumor staging, and specific gene hypermethylation, hypomethylation is an early event, associated with chromosomal instability and poor prognosis.

S32. Testing and estimation of genotype and haplotype effects in case/control and family-based association studies

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A variety of methods are used for the analysis of data generated in genetic association studies. Most methods focus on the detection of genetic effects using case/control or family (pedigree) data, although arguably a more interesting question, once a region of disease association has been identified, is to estimate the relevant genotypic or haplotypic effects and to perform tests of complex null hypotheses such as the hypothesis that some loci, but not others, are associated with disease. We previously developed a regression-based approach (Cordell and Clayton 2002; Cordell et al. 2004) that provides a unified framework for detection or estimation of effects using case/control or family data. This approach allows genotype and haplotype analysis at an arbitrary number of linked and unlinked multiallelic loci, as well as modelling of more complex effects such as gene-gene interactions (epistasis), gene-environment interactions, parent-of-origin and maternal genotype effects. In practice, many genetic studies contain moderate to large amounts of missing genotype data, either arising from individuals who have not been fully genotyped, or from the inability to infer phase (alleles received in coupling from a single parent), given unphased genotype data. We have recently been exploring different approaches to deal with this missing data problem in the context of case/control (Cordell 2006) or family (Croiseau et al. 2007) data. In particular, multiple imputation approaches, in which the missing data is repeatedly filled in using the correct posterior probability distribution (given the observed data), appear to represent a promising approach that has some advantages over missing data likelihood methods with regards to model flexibility and ease of use.

S33. Genomics of bipolar affective: from multiply affected families to the general population

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Bipolar affective disorder (BPAD) is characterized by severe episodes of mania and depression and represents a common disorder affecting approximately 1% of the world's population. Therefore, BPAD is considered to be one of the top public health problems associated with a significant morbidity. Although formal genetic studies consistently provide strong evidence for a major genetic contribution to BPAD, the underlying genetic architecture is poorly understood.

Using family samples from different European populations we implicated several chromosomal regions in the development of BPAD, including regions on chromosomes 1, 4, 6, 8, and 10 (e.g. Cichon et al. Hum Mol Genet 2001, Schumacher et al. Am J Hum Genet 2005). A genome-wide interaction linkage scan provided strong interaction evidence between BPAD genes on chromosomes 2q22-q24 and 6q23-q24, and 2q22-q24 and 15q26. Focusing on specific candidate genes, the best evidence was obtained for G72/G30 and tryptophan hydoxylase 2 (e.g. Schulze et al Am J Psychiatry 2006). The recent first genome-wide association study was a further progress in the genetic study of BPAD showing that several genes, each of modest effect, reproducibly influence disease risk (Baumer et al. Mol Psychiatry 2007). Specifically, the gene DGKH showed significant association even after conservative experiment-wise correction.

The causative mutations in the implicated genes for BPAD, however, remain to be identified. As soon as these will be known, samples of patients collected from the general population will allow estimation of parameters such as relative risks and etiological fraction.

S34. Large population studies

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Approaches in Genetic Epidemiology have now advanced technically to such as extent that it is increasingly possible to capture longitudinal real-time clinical phenotype and exposure data relating to massive population samples. When this is combined with relentless advances in high-throughput and low-cost technologies to define either laboratory-based phenotypes/biomarkers or genetic/transcriptomic profiles, vast meta-databases are being generated. Analysis and mining of such multi-level datasets will reveal important insights into the health and disease at both a population and individual level. This will particularly relate to disease progression, and gene-environment interactions relating to drugs (pharmacogenetics) and nutrients (nutrigenomics). Such longitudinal population-based cohort studies are now being developed in a range of countries, taking advantage of advances in health service infrastructure for electronic clinical record capture. UK Biobank (www.ukbiobank.ac.uk) is such a study, designed to investigate both health and disease in the UK.

At the same time, large national and international collections of samples and data are being assembled for case-control studies of specific diseases (www.dna-network.ac.uk), www.genomeutwin.org). These are being used in whole genome association studies using high density genotyping (>500,000 SNP loci). Examples of such projects include the WTCCC (www.wtccc.org.uk) and GAIN (www.fnih.org/GAIN/GAIN_home.shtml).

Such massive initiatives are required to generate the levels of power and replication studies to detect small genotype relative risks (<1.2). However, initiatives of this complexity and size are only possible through increasing international collaboration and data/sample sharing.

New International and European initiatives are directed at such needs and these will specifically address what framework has to be put in place for greater standardisation and harmonisation of data/sample collection, management and exchange, and what measures of quality assurance/control are required. Further important considerations are the ethical, legal and sociological frameworks that these studies need. The context and advances being made in the field of large population studies will be discussed.

S35. Cell division and modelling cancer in Drosophila

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Asymmetric stem cell division is crucial to ensure cellular diversity, tissue repair and organ homeostasis during embryo development and adult life. The key issue during stem cell asymmetric division is the unequal segregation of cell fate determinants into each of the two resulting cells, thus priming one of the daughter cells to differentiate and contribute tissue mass, while the other retains stem-cell identity, and can go into another round of asymmetric mitosis. Loss-of-function of any of several genes that control the fate of the daughters of Drosophila larval neuroblasts -the stem cells that originate the fly´s CNS- results in the growth of malignant neuroblastomas. One of the most conspicuous asymmetric clues displayed by larval neuroblasts is the mechanism of spindle assembly and spindle dynamics, which indeed governs the marked size asymmetry of the neuroblast's daughters. I will present and discuss recent results obtained in our laboratory regarding the connexion between spindle dynamics, segregation of fate determinants and tumor suppression in these model stem cells.

S36. How is time controlled during embryonic development? Lessons from the chick embryo.

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Throughout the Animal Kingdom, the time of embryonic development is maintained and strictly controlled. Each step of the process is successful only when it occurs at the right time and place. The importance of time control during embryo development is particularly evident during somitogenesis, the process by which the vertebrate presomitic mesoderm is segmented along its anterior-posterior axis to form somites. These are formed in a highly controlled way, both temporally and spatially, and later give rise to definitive segmented structures, including vertebrae and ribs. In 1997, chick presomitic cells were shown to undergo several cycles of *hairy1* gene expression, providing the first molecular evidence for the existence of a molecular clock underlying the rhythm of somitogenesis (Palmeirim *et al*., Cell 1997). We currently know that this molecular clock operates in all vertebrate groups studied, in more than one embryonic tissue, with different time-periods (Pascoal et al., 2007; Pascoal and Palmeirim, 2007) and an increasing number of genes belonging to the Notch, FgF and Wnt signalling pathways are being implicated in this clocked mechanism (reviewed by Andrade et al., 2007, *in press*).

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S37. Conditional gene targeting to model human diseases in mice

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Conditional gene targeting using the Cre/loxP recombination system allows one to introduce mutations into the mouse genome in a cell type-specific or inducible manner, and thus to model acquired human diseases in mice. In addition to the generation of loss-of-function mutations, Cre-mediated recombination can also be used to conditionally turn on gene expression. This latter approach is particularly useful for the analysis of the role of oncogenes in the pathogenesis of cancer. I will exemplify this approach by recent experiments addressing the role of the NFκB signaling pathway and of Epstein-Barr-Virus in lym-

phomagenesis. I will also draw attention to pitfalls of the conditional gene targeting approach, which can lead to serious misinterpretations of experiments.

S38. On principal & modifying genes in Hirschsprung disease

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Hirschsprung disease (HSCR), or congenital aganglionosis, is a classical multifactorial disorder that has continued to teach us important lessons in non-Mendelian inheritance and the genomics of complex disorders. To date, we know of 9 genes that harbor rare mutations, all incompletely penetrant but with greater effects in males than females. One of these genes, the RET tyrosine kinase, also harbors a polymorphism in an enhancer leading to reduced RET transcription and high association with short segment HSCR (S-HSCR). Interestingly, the enhancer polymorphism modifies the genetic effects of rare mutations not only for RET in HSCR but many other HSCR-related traits such as Down syndrome, Congenital Central Hypoventilation syndrome and Bardet-Biedel syndrome. Thus, a single gene can have a diversity of mutations with differential effects but the same mutation can also have differential effects depending on its interactions with other (mutant) genes. In HSCR, the genetic modifiers can be both allelic and non-allelic. I will describe the genetic tests needed to distinguish between these scenarios.

S39. Why use the candidate gene approach to find CF modifiers?

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Genes that do not cause, but modify, a clinical phenotype are of medical interest as these genes provide information about the biology causing the phenotype and will potentially suggest therapeutic strategies to treat the disease phenotype. There are various strategies to identify these modifying genes and one of the key design features is the choice of variants selected. Technologic advances are making genome scans more feasible and affordable, and these approaches allow one to test hypotheses about candidate genes, as well as identify genes not previously considered to contribute to the phenotype. However, these scans are still costly and therefore candidate gene approaches are still useful. The candidate gene approach relies on knowledge of the pathophysiology of the disease to be effective, as genes whose products lie in relevant pathways will be assessed. Consequently, when a candidate gene is found to associate with the phenotype, some information is already known about the gene's role in the phenotype. Therefore, this approach is most likely to verify a pathway's involvement in the disease phenotype, rather than to identify new pathways. We have taken the candidate gene approach to identifying modifiers of cystic fibrosis. In doing so, we have incorporated several strategies to identify candidates. One approach has been to examine genes in pathways thought to contribute to the pathophysiology of the disease, such as epithelial ion transport mediators, inflammatory cascade components, endocrine pathways and innate defense, to name a few. A second approach has been to examine genes that have been reported to modify or cause related disorders, such as asthma and chronic obstructive pulmonary disease (COPD), as CF, asthma and COPD are likely to have overlapping biology. Using this candidate approach, we have tested apparent associating genes in at least two populations of CF patients to verify associations. These approaches have identified transforming growth factor beta1, and genes in inflammatory pathways and airway function as genes contributing to disease severity.

S40. Genes that modify iron loading in mice

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Adult onset hemochromatosis, an iron overload disorder affecting the liver, heart and pancreas, is usually caused by mutations in HFE. However, only a fraction of patients homozygous for disease-associated mutations develop clinical hemochromatosis. A wide range in the severity of iron loading and its complications can be explained by both genetic factors (modifier genes) and environmental factors (e.g., alcohol intake, dietary iron consumption, and menstruation). Iron physiology in mice closely resembles that in humans, making the mouse a valuable genetic model. We undertook a quantitative trait locus (QTL) analysis in mice to identify modifier genes that might influence the severity of hemochromatosis. We identified a strong QTL on mouse chromosome 9 that differentially affected macrophage iron burden in C57BL/10J and SWR/J mice. A C57BL/10J missense allele of an evolutionarily conserved gene, Mon1a, co-segregated with the QTL in congenic mouse lines. We present evidence that Mon1a is a cytoplasmic protein involved in trafficking of ferroportin, the major mammalian iron exporter, to the surface of iron-recycling macrophages. Differences in amounts of surface ferroportin correlate with differences in cellular iron content. Mon1a is also important for trafficking of cell surface and secreted molecules unrelated to iron metabolism, suggesting that it plays a fundamental role in the mammalian secretory apparatus.

S41. Genetic Modulation of Sickle Cell Anemia

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Sickle cell anemia, a Mendelian disease caused by homozygosity for a beta-globin gene mutation (*HBB,* glu6val)), has notorious phenotypic variability. We are conducting candidate gene and genome-wide association studies (GWA) to understand the relationships between genetic heterogeneity and the phenotype of disease.

Fetal hemoglobin (HbF) is the most powerful modulator of sickle cell anemia. HbF levels are regulated by at least three quantitative trait loci (QTL) on 8q, Xp and 6q and by elements linked to *HBB*. When panels of single nucleotide polymorphisms (SNPs) were used to study the association of variability in these QTLs in two independent sickle cell anemia patient groups, SNPs in *TOX* (8q12.1) were associated with HbF. *TOX* belongs to a high mobility group box protein family that binds DNA with high sequence specificity. Many potential *TOX* binding sites, including one in the *HBG2* promoter are found near the *HBB* gene cluster. GWA using pooled DNA confirmed the association on SNPs in 6q and 8q associated with HbF and identified promising new areas for further study.

Stroke is a common complication of childhood sickle cell anemia. Using Bayesian network modeling to evaluate the interactions between many candidate gene SNPs and the risk of a stroke, we developed a prognostic model for stroke. SNPs in 11 genes and four clinical variables, interacted in a complex network of dependency to modulate the risk of stroke. Case-control association studies examining candidate genes in other subphenotypes of sickle cell anemia showed associations with several genes of the TGF-beta/BMP pathway.

To study the genetic association with a global estimate of disease severity, we developed a model predicting which patients with sickle cell disease are at risk for near-term death and validated this model in two independent patient groups. Using this severity score as a phenotype of disease, SNPs in *EDN1, ECE1, KDR, EGF* and *NOX3* were associated with overall disease severity.

Understanding the genetic modulation of the hemolytic, vascular and inflammatory components of this disease could provide important prognostic information and suggest novel approaches to treatment.

S42. Inborn errors of mitochondrial fatty acid beta-oxidation: From newborn screening to diagnosis and treatment

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The mitochondrial fatty acid (FA) beta-oxidation deficiencies constitute an expanding group of clinically and genetically heterogeneous disorders. Originally, diagnosis of patients suffering from a fatty acid oxidation (FAO) disorder was difficult, but the introduction of (tandem) mass-spectrometry and in particular its use for the analysis of acylcarnitines in plasma from patients, has revolutionized the diagnosis of FAO deficiencies. In fact, since tandem mass spectrometry has turned out to be so robust and reliable, existing neonatal screening programs have been extended in many countries around the world and now include different FAO disorders, using tandem mass spectrometric analysis of acylcarnitines in blood spots. The best known FAO disorder is medium-chain acyl-CoA dehydrogenase (MCAD) deficiency with an

incidence of 1:5-10.000, at least in most Western countries. Inclusion of MCADD in new neonatal screening programs is obvious since it is known that 25% of MCADD patients die upon the first hypoglycemic attack. Follow-up diagnostic studies in patients with a positive acylcarnitine screening result are usually based on the analysis of metabolites, including acylcarnitines in plasma. Since metabolites may be erroneously normal in affected patients, we have investigated whether it would be possible to measure the candidate enzymes directly, i.e. in blood cells. Our results show that all enzymes of mitochondrial fatty acid oxidation are expressed in lymphocytes and that lymphocyte analysis is a reliable and quick method to discriminate between false and true positives.
The second part of our research program is focused on the generation of new therapeutic options, especially for defects in the oxidation of long-chain fatty acids, using selective knockout mice.

S43. Glycosylation defects

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Congenital Disorders of Glycosylation (CDG) are an expanding group of inherited metabolic diseases caused by defects in the synthesis and processing of glycoproteins. The genetic defect in CDG-Ia, the most common type of the disease, was reported 10 years ago. Since then, more than 20 novel defects have been described, mainly in the endoplasmic reticulum (type I) but also in the Golgi (type II) (Freeze, Nat Rev Genet.7:537-51, 2006).
The systematic search towards other defects in the endoplasmic reticulum is based on the analysis of intermediate structures of the dolichol-linked oligosaccharides and takes advantage of the strong homology with yeast. The corresponding human genes have now been identified, and for nearly also steps in the synthesis of dol-PP-GlcNAc-2Man9Glc3, at least one or a few patients with a defect have been identified. On the contrary, the number of type II cases with a known defect in the Golgi compartment, is limited. Up till now, only a few defects in the processing of the glycans were identified.
Whereas these CDG cases have defects in genes that encode enzymes or transporters that are directly involved in the glycosylation process, recent work has shown that the abnormalities may also be due to abnormal intracellular trafficking of resident Golgi enzymes or transporters involved in glycosylation. Even though the mechanisms which allow the selective retention of glycosyltransferases in certain cisternae are still poorly understood, it is clear that multiprotein complexes, like the Conserved Oligomeric Golgi (COG) complex, are key determinants in their localization. Wu et al. (Nat Med.10:518-23, 2004) described a form of CDG caused by a defect in COG7. We have now also identified mutations in 2 different COG subunits (Foulquier et al. PNAS 103:3764-9, 2006; Hum Mol Genet. 16, 717-730, 2007). These observations open a new era of research into CDG.

S44. Pyridoxine metabolism

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Some infants have epilepsy that is poorly controlled by antiepileptic drugs (AED's) but responds dramatically to treatment with pyridoxine or pyridoxal phosphate (pal-P). Mutations in the gene encoding pyridox(am)ine phosphate oxidase (PNPO) lead to defective conversion of pyridoxine to pyridoxal phosphate. These infants do not respond to treatment with pyridoxine but their fits cease when they are given pal-P. The first patients identified as having PNPO deficiency had biochemical changes in their cerebrospinal fluid that suggested reduced amounts of pal-P in the brain. These included raised levels of 3-methoxytyrosine, threonine and glycine. However, more recently mutations have been found in the *PNPO* gene in patients ascertained on the basis of their response to pal-P treatment and in some of these, no abnormality of CSF biochemistry was found.
Seizures responsive to pyridoxine treatment can occur when an inborn error of metabolism leads to accumulation of an intermediate that complexes with pyridoxal phosphate, thus increasing the body's requirement for vitamin B6. The first described example of this was hyperprolinaemia type 2 which leads to accumulation of Δ^1-pyrroline-5-carboxylate. The latter forms a condensation product with pal-P. We were able to show that the majority of patients with pyridoxine-dependent epilepsy (PDE) have mutations in the *ALDH7A1* gene encoding antiquitin. Antiquitin catalyses the conversion of an equilibrium mixture of α-aminoadipic semialdehyde / Δ^1-piperideine-6-carboxylate (P6C) to α-aminoadipic acid. In the absence of this enzyme, P6C accumulates and forms a condensation product with pal-P, thus inactivating it. We can now diagnose PDE by measuring α-aminoadipic semialdehyde in the urine and confirm the diagnosis by sequencing the *ALDH7A1* gene; a few mutations are found quite frequently. It is becoming clear that the phenotypic spectrum of PDE due to antiquitin deficiency includes patients with late onset seizures, cortical malformations, endocrine dysfunction and a partial response to AED's.

S45. The adenomatous polyposis coli protein as a regulator of the cytoskeleton

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Mutations in the adenomatous polyposis coli protein (APC) are common to most colorectal cancers and occur extremely early during tumourigenesis. The most prominent function of APC is its ability to support the assembly of a protein complex that regulates the degradation of beta-catenin in a Wnt-regulated manner. Accumulating beta-catenin causes changes in the activity of TCF/Lef transcription factors. This has been implicated in the transformation produced by truncations mutations in APC that disrupt the ability of APC to support a functional beta-catenin degradation complex. However, APC is also an important regulator of the cytoskeleton. Inactivating APC in cells causes a decrease in cell migration in cultured cells and enterocytes in the intestine. In cells, this change is accompanied by changes in post-translationally modified microtubules and cell shape. Loss of APC also disrupts mitotic spindles and compromises the spindle checkpoint. This leads to defects in cell division and produces tetra- and polyloidy in cells and tissues lacking APC. Another immediate consequence of APC loss is a reduction in apoptosis. Importantly, the mitotic and apoptotic defects induced by loss of APC do not require transcriptional changes induced by the accumulation of beta-catenin.
In summary, loss of APC leads to the inappropriate accumulation of cells with altered differentiation and increased genetic instability to provide an efficient means of increasing the number and survival of potential tumour cells.
I will describe our work in a number of experimental systems to illustrate the effect of APC on the cytoskeleton and discuss possible implication of these findings for tumour initiation and progression.

S46. Wt1 acts at multiple stages during kidney development

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Wilms' tumour or nephroblastoma is a paediatric kidney cancer that is believed to arise from nephrogenic rests, embryonic cells of the kidney that have failed to differentiate into nephrons. *WT1* encodes a zinc-finger transcription factor that has been shown to be mutated in up to 15% of Wilms' tumours. However, mutations in *WT1* are also found in other congenital diseases affecting urogenital development, most notably Denys-Drash (DDS) and Frasier Syndrome. Using *in vitro* and *in vivo* models we are studying the cellular and developmental function of Wt1. Here we will discuss the role Wt1 plays at various stages of kidney development, its direct target genes and the mode of action of its various spliced isoforms. We have shown earlier that nephrin represents one of the direct downstream targets of Wt1. Analyzing nephrin knockout mice we now demonstrate a novel function of this gene in heart development. Finally, we will report on an unusual mutation in *WT1* that is associated with a rare form of congenital nephrotic syndrome with an almost complete absence of glomeruli and severe hypertension.

S47. HSP90 modulates developmental and cancer phenotypes

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HSP90 is a highly-expressed molecular chaperone with roles in maintaining normal developmental regulators, as well as in the folding of mutant proteins. Partial inhibition of HSP90, previously reported to un-

cover cryptic mutations in Drosophila and Arabidopsis, has now also been shown to modulate phenotype in zebrafish. Using carefully titrated inhibition of HSP90, we identified novel structural eye anomalies reminiscent of human microphthalmia and anophthalmia in one strain, showing frequent unilaterality, and illustrating the variable penetrance that is so often observed with some developmental malformations. Some phenotypes with Mendelian inheritance patterns were also modulated in response to inhibitors. Interestingly, responsive phenotypes were associated with missense mutations. Some showed increased severity, others were ameliorated. The proposed molecular mechanism for this HSP90 function also provides insight into the promising cancer therapeutic role of HSP90 inhibitors.

Concurrent Sessions

C01. A novel ciliary gene is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome

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Cerebello-oculo-renal syndrome (CORS), also called Joubert syndrome (JS) type B, and Meckel (MKS) syndrome belong to the ciliopathy group of developmental autosomal recessive disorders associated with primary cilium dysfunction. Nephronophthisis (NPHP), the most frequent genetic cause of renal failure in children and young adults, is associated with retinal degeneration and cerebellar vermis aplasia in CORS. MKS is characterized by renal cystic dysplasia, central nervous system malformations and hepatic developmental defects. Using SNP mapping, we identified missense and truncating mutations in a novel gene, NPHP8, in both CORS and MKS, and we show that inactivation of its mouse orthologue recapitulates the cerebral, renal, craniofacial and limb defects of MKS. We further demonstrate that NPHP8 protein co-localizes at the basal body/centrosomes with nephrocystin-6 and nephrocystin-4, the protein products of both NPHP6 and NPHP4, known disease genes for NPHP. In addition, missense mutations of NPHP8 protein found in CORS patients diminishes its interaction with nephrocystin-4. Mutations of this novel ciliary in both CORS or MKS syndromes identify novel JS (JBTS7) and MKS loci (MKS5) and further demonstrate that these phenotypes are a continuum of the same underlying disorder.

C02. Pleiotropic effects of *CEP290 (NPHP6)* mutations extends to Meckel-Gruber syndrome

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Meckel syndrome is a rare autosomal recessive lethal condition characterized by the association of central nervous system malformations, postaxial polydactyly, multicystic kidney dysplasia and ductal proliferation in the portal area of the liver. MKS is genetically heterogeneous and 2 genes have been identified: *MKS1/FLJ20345* in Finnish kindreds and *MKS3/TMEM67* in families from Pakistan and Oman. The gene at the *MKS2* locus remains unknown.

A genome wide linkage scan was performed by Affymetrix 10K SNP chips or 10cM-resolution microsatellite markers in consanguineous MKS families. Homozygozity on chromosome 12q was observed in four families, in an interval containing *CEP290*, a gene recently identified as causative for Joubert syndrome and isolated Leber congenital amaurosis. In view of the clinical overlap between MKS and JS, and our recent findings of allelism at the *MKS3* locus between these two disorders, *CEP290* was considered as a good candidate. Homozygous truncating mutations were identified in 3/4 families, confirming that *CEP290* is the gene for MKS on chromosome 12. Sequencing of additional cases identified compound heterozygote *CEP290* mutations in 2 additional MKS cases and in 3 families presenting a "cerebro-reno-digital" syndrome, with a phenotype in between MKS and JS, further demonstrating that Meckel and Joubert are the variable expression of the same disorder. These data identify a fourth locus for MKS (*MKS4*) and *CEP290* as a gene responsible for MKS, and extend the phenotypic spectrum of *CEP290* mutations to severe and lethal cystic kidney dysplasia with bile duct proliferation of liver.

C03. A novel vertebrate specific chaperonine related protein (BBS12) is involved in Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is primarily an autosomal recessive ciliopathy defined by progressive retinal degeneration, obesity, cognitive impairment, polydactyly and kidney anomalies. BBS is genetically heterogeneous with 11 BBS genes identified to date, which account for about 70% of affected families. We have combined SNP array homozygosity mapping with *in silico* analysis to identify a new BBS gene, *BBS12*. Patients from two gypsy families were homozygous and haploidentical in a 6 Mb region of chromosome 4q27. *FLJ35630* was selected as a candidate as it was predicted to encode a protein sharing similarity to members of the type II chaperonin superfamily, which includes BBS6 and the recently identified BBS10. Pathogenic mutations in both gypsy families, as well as in 14 other families from various ethnic backgrounds were found. *BBS12* accounts for about 5% of all BBS. *BBS12* is vertebrate specific and together with *BBS6* and *BBS10* define a novel branch of the type II chaperonin superfamily. These three genes are characterized by unusually rapid evolution and are likely to perform ciliary functions specific to vertebrates that are important in the pathophysiology of the syndrome, and together they account for about one third of the total BBS mutational load.

C04. Mutations in *SCN9A* cause a congenital inability to experience pain

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The complete inability to sense pain in an otherwise healthy individual is a very rare phenotype. In three consanguineous families from northern Pakistan, we mapped the condition as an autosomal-recessive trait to chromosome 2q24.3. This region contains the gene SCN9A, encoding the alpha-subunit of the voltage-gated sodium channel, Na(v)1.7, which is strongly expressed in nociceptive neurons. Sequence analysis of SCN9A in affected individuals revealed three distinct homozygous nonsense mutations (S459X, I767X and W897X). We show that these mutations cause loss of function of Na(v)1.7 by co-expression of wild-type or mutant human Na(v)1.7 with sodium channel beta(1) and beta(2) subunits in HEK293 cells. Our data suggest that SCN9A is an essential and non-redundant requirement for nociception in humans.

C05. Identification of mutations in Cytokine Receptor-Like Factor 1 (CRLF1) in Crisponi syndrome.

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Crisponi syndrome is an autosomal recessive disorder characterized by congenital contractions of facial muscles with trismus, dysmorphic features, camptodactyly, feeding and respiratory difficulties with access of hyperthermia leading to death in the first months of life. Up till now, the disease has been reported in a total of 19 newborns from sardinian, italian and portuguese families. The overlap of Crisponi syndrome with Stüve-Wiedemann syndrome (SWS) is striking. However, congenital bowing of the lower limbs, which is a cardinal feature of SWS, has never been reported in Crisponi syndrome. Studying four children (from three unrelated families) with Crisponi syndrome, we

first excluded the Leukemia Inhibitory Factor Receptor gene (responsible for SWS) in Crisponi syndrome. We then considered the Cytokine Receptor-Like Factor 1 (CRLF1) gene as a candidate gene based on the identification of CRLF1 mutations in cold-induced sweating syndrome. By direct sequencing, we identified CRLF1 mutations in the four children. The mutations were located in the Ig-like and type III fibronectin domains and three of them predicted premature termination of translation. Using real time quantitative PCR, we found a decrease of CRLF1 mRNA expression in patient fibroblasts, suggestive of a mutation-mediated decay of the abnormal transcript. CRLF1 forms a heterodimer complex with Cardiotrophin Like Cytokine Factor 1 and this heterodimer competes with Ciliary Neurotrophic Factor for binding to the ciliary neurotrophic factor receptor complex (CNFTR). The identification of CRLF1 mutations in Crisponi syndrome supports the key role of the CNTFR pathway in the function of the autonomic nervous system.

C06. Crisponi Syndrome is Caused by Mutations in the *CRLF1* Gene and Shows Allelism to Cold-Induced Sweating Syndrome Type 1

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Crisponi syndrome (CS) is a severe autosomal recessive condition, characterized by abnormal, paroxysmal muscular contractions resembling neonatal tetanus, large face, broad nose, anteverted nares, camptodactyly, hyperthermia and sudden death in most cases. We performed homozygosity mapping in five Sardinian and three Turkish families with CS using high-density SNP arrays and identified a critical region on chromosome 19p12-13.1. The most prominent candidate gene was *CRLF1*, recently found to be involved in the pathogenesis of cold induced sweating syndrome type 1 (CISS1). CISS1 belongs to a group of conditions with overlapping phenotypes, also including cold induced sweating syndrome type 2 (CISS2) and Stüve-Wiedemann syndrome (SWS). All these syndromes are caused by mutations of genes of the ciliary neurotrophic factor receptor (CNTFR) pathway, which is known to be involved in motor neuron survival. Here we describe the identification of four different *CRLF1* mutations in eight different Crisponi families, including a missense mutation, a single nucleotide insertion, a nonsense and an insertion/deletion (indel) mutation, all segregating with the disease trait in the families. Comparison of the mutation spectra of CS and CISS1 suggests that neither the type nor the location of the *CRLF1* mutations point to a phenotype/genotype correlation that would account for the most severe phenotype in CS. Our findings provide further evidence for the importance of the CNTFR pathway in the development of autonomic and motorial functions of the nervous system.

C07. The novel neuronal ceroid lipofuscinosis gene *CLN7* encodes a putative lysosomal transporter protein

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Neuronal ceroid lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative disorders with a variable age of onset. Within the NCLs, the late-infantile onset group (LINCLs) is genetically the most heterogeneous with mutations identified in *CLN1, CLN2, CLN5, CLN6,* and *CLN8*. A variant form of LINCL (vLINCL) present in Turkish patients is clinically characterized by onset at 2-7 years of age, epileptic seizures, myoclonus, psychomotor deterioration, loss of vision, and premature death. Initially, Turkish vLINCL was considered a distinct genetic entity (CLN7). However, we recently reported mutations in the *CLN6* and *CLN8* genes accounting for the disease in a subset of Turkish vLINCL patients, whereas in the majority of families, Turkish vLINCL is not linked to any of the known NCL loci. By performing a genome-wide single nucleotide polymorphism scan and homozygosity mapping in nine Turkish and one Indian family, we mapped a novel vLINCL locus, *CLN7*, in five of these families. Subsequently, by positional candidate gene sequencing, we identified the causative gene with six different homozygous mutations found in patients in six families. The gene encodes a novel polytopic membrane protein that belongs to the major facilitator superfamily, members of which have various transporter activities. The *CLN7* transcript is expressed ubiquitously with several alternatively spliced variants. Like most of the known NCL proteins, CLN7 localizes mainly to the lysosomal compartment. The cellular function of the protein encoded by this novel NCL gene remains to be elucidated.

C08. Identification of a new gene mutated in autosomal recessive centronuclear myopathies, and functional links with the dominant form

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Centronuclear (myotubular) myopathies (CNM) are characterized by muscle weakness and abnormal centralisation of nuclei in muscle fibres which is not secondary to regeneration. The severe neonatal X-linked form (myotubular myopathy, XLMTM) is due to mutations in the phosphoinositide phosphatase myotubularin (MTM1), while mutations in dynamin 2 (DNM2) have been found in some autosomal dominant cases. By direct sequencing of functional candidate genes, we identified homozygous mutations of the amphiphysin 2 gene (also called BIN1) in three families with autosomal recessive inheritance. Two different missense mutations in the BAR (Bin1/Amphiphysin/RVS167) domain disrupt its membrane tubulation properties in transfected cells, while a partial truncation of the C-terminal SH3 domain abrogates the interaction with dynamin 2 and its recruitment to the membrane tubules. Our results suggest that mutations in amphiphysin 2 cause centronuclear myopathy by interfering with membrane remodeling at T-tubules, and that the functional interaction between amphiphysin 2 and dynamin 2 is necessary for normal muscle function and positioning of nuclei.

C09. A protein sharing similarity with an ancestral prokaryotic kinase is mutant in a new form of recessive ataxia

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A SNP-based genome-wide scan in a large consanguineous family allowed us to identify a new locus for autosomal recessive ataxia at 1q41 (LOD score : 3.9). We searched for nucleotide change in genes of this region coding for mitochondrial proteins and found deleterious mutations in CABC1 in 6 patients from 3 families. All patients have child-

hood-onset cerebellar ataxia with slow progression and little additional signs. The CABC1 yeast homologue is mutated in the ubiquinone (or coenzyme Q) deficient S. cerevisiae strain COQ8. Likewise, we found that ubiquinone synthesis was partially impaired in muscle biopsy and fibroblasts of one of the patients (additional patients are under study). Albeit COQ8 seems to be involved in one of the monooxygenation steps of the hydroxy benzoic ring of the coenzyme Q precursor, its role is likely indirect, since COQ8/CABC1 belongs to a small family of ancestral prokaryotic kinases. Coenzyme Q10 deficiency was previously identified in severe encephalopathy-nephrotic syndromes with direct blocks in the biosynthetic pathway and, surprisingly, in ataxia-oculomotor apraxia 1 (AOA1) which is caused by a defective nuclear DNA repair protein. The identification of CABC1 mutations emphasizes the role of coenzyme Q10 in the physiopathology of degenerative ataxias and leads to direct supplementation therapeutic possibilities.

C10. Prenyldiphosphate synthase (*PDSS1*) and OH-benzoate prenyltransferase (*COQ2*) mutations in ubiquinone deficiency and oxidative phosphorylation disorders

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Coenzyme Q_{10} (CoQ_{10}) plays a pivotal role in oxidative phosphorylation (OXPHOS), as it distributes electrons between the various dehydrogenases and the cytochrome segments of the respiratory chain. We have identified two novel inborn errors of CoQ_{10} biosynthesis in two distinct families. In both cases, enzymologic studies showed that quinone-dependent OXPHOS activities were in the range of lowest control values, while OXPHOS enzyme activities were normal. CoQ_{10} deficiency was confirmed by restoration of normal OXPHOS activities after addition of quinone. A genome-wide search for homozygosity in family 1 identified a region of chromosome 10 encompassing the prenyldiphosphate synthase gene (*PDSS1*) which encodes the human ortholog of the yeast *COQ1* gene, a key enzyme of CoQ_{10} synthesis. Sequencing *PDSS1* identified a homozygous nucleotide substitution modifying a conserved amino acid of the protein (D308E). In the second family, direct sequencing of the OH-benzoate prenyltransferase gene, the human ortholog of the yeast *COQ2* gene, identified a single base pair frameshift deletion resulting in a premature stop codon (c.1198delT, N401fsX415). Transformation of yeast Δ*coq1* and Δ*coq2* strains by mutant yeast *COQ1* and mutant human *COQ2* genes, respectively, resulted in defective growth on respiratory medium showing that these mutations are indeed the cause of OXPHOS deficiency

C11. Mitochondrial DNA depletion is a major cause of multiple respiratory chain defects

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Mitochondrial DNA depletion syndrome (MDS) is a clinically and genetically heterogeneous condition characterized by reduction in mtDNA copy number responsible for multiple oxidative phosphorylation (OXPHOS) enzyme deficiency. In order to determine the actual incidence of mtDNA depletion in multiple respiratory chain deficiency, we have carried out the real-time PCR quantification of mtDNA in liver or muscle tissue of 100 of 270 children with unexplained multiple OXPHOS deficiency. Half of the patients presented a reduction in mtDNA copy number below 50% of control values in liver and/or muscle (50/100). Most patients (16%) presented a neonatal form with severe liver involvement, a second group was represented by Alpers syndrome (4%) and 20% of the patients presented encephalomyopathy. DGUOK or POLG mutations could be identified in 22% of patients with liver disease, POLG mutations were consistently found in all but one patient with Alpers syndrome. Two patients carried a homozygous TK2 or MPV17 mutation respectively. Our findings show that mtDNA depletion is a very frequent cause of multiple respiratory chain deficiency with a incidence of at least 18% of children with unexplained OXPHOS deficiency

C12. Identification of a new gene of mitochondrial DNA depletion

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The mitochondrial DNA (mtDNA) depletion syndrome (MDS) is characterized by a severe and tissue-specific reduction of mtDNA copy number. Until now, mutations of five nuclear genes are known to result in MDS (TK2, POLG, DGUOK, SUCLA2 and MPV17). They are either involved in mitochondrial dNTP salvage pathway (TK2, DGUOK, SUCLA2) or in mtDNA replication (POLG) whereas MPV17 has a yet unknown function. However, only part of patients (36%) presents mutations in these genes suggesting the occurrence of mutations in other yet unknown genes. A genome-wide linkage analysis in a large inbred Moroccan family with a severe MDS in muscle revealed a region of autozygosity on chromosome 8q21.3-q22.3 with a maximal lod score at 3,331. We identified a homozygous non-sense mutation in a gene mapping in this region and involved in nucleotide metabolism as well as in three additional families of French origin.

C13. Gender-specific association of a novel polymorphism in the *TNFSF4* gene with allele-specific promoter activity and risk of myocardial infarction.

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The OX40L/OX40 system, along with other receptor-ligand pairs, has been shown to be involved in T-cell activation and might be therefore proatherogenic. We previously showed that genetic variants in *TNFSF4*, the gene encoding for OX40 ligand, are associated with the risk of developing precocious coronary artery disease (CAD) and myocardial infarction (MI) in women from two independent cohorts. Our approach was to use data from a mouse atherosclerosis model to positionally identify candidate genes in a human context.
Based on a combined strategy including a systematic screening by sequencing of the *TNFSF4* genomic region and a bionformatic evaluation of genetic variants affecting potential regulatory regions, we here report a novel promoter polymorphism (-921C>T) and a new haplotype, conceivably involved in gene regulation. The -921T-allele was shown to be associated with increased risk of MI in women. Haplotype-specific chromatin immunoprecipitation (haploChIP) of activated polymerase II, as a measure of transcriptional activity *in vivo*, suggested that the -921C>T polymorphism is functionally important, the -921T-allele being associated with lower transcriptional activity. Electromobility shift assay (EMSA) showed that the -921C>T polymorphism affects the binding of nuclear factors in allele-specific manner, thus suggesting that the lower transcriptional activity associated with the -921T-allele is due to binding of one or more transcriptional repressor(s) to the T-allele.
In conclusion, our results strongly reinforce the contention that the haplotype carrying the -921T-allele may be causally related to MI and/or CAD.

C14. Genome-wide copy number variation in schizophrenia

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Background: Recently, considerable attention has focused on the surprisingly common degree of structural variation in the human genome. While excess rates of psychiatric illness have been reported in cytogenetically-defined chromosomal alterations, whole-genome microarray technology has not yet been utilized to identify sub-microscopic structural variation underlying psychiatric phenotypes. We report results of a novel approach to examining genome-wide structural variation in a case-control schizophrenia (SZ) cohort.
Methods: SZ cases (65F/113M) and controls (63F/81M) were examined. All subjects self-identified as Caucasian non-Hispanic; testing

of 210 ancestry informative markers revealed no population stratification. Single nucleotide polymorphisms (SNPs) were assayed using the Affymetrix 500K array. Quality control procedures yielded mean call rates of 97%, reliability (concordance across repeated samples) > 99%, and 439,511 high-quality SNPs available for analysis. A novel algorithm was implemented to identify and quantify extended runs of homozygosity (ROHs), which can signify chromosomal microdeletions and/or ancestral autozygosity.
Results: A total of 339 "common" ROHs (observed in >3% of subjects) were identified across the 22 autosomes. In genome-wide analysis, SZ patients had significantly more ROHs than controls (32±12 vs.28±13, p=0.009). Frequency of nine specific ROHs significantly differed between patients and controls at a nominal p<.01; all demonstrate greater frequency in cases. Two prominent ROHs overlapped genes for *DTNBP1* and *DISC1* binding partners.
Conclusions: To our knowledge, this is the first study of structural variation in schizophrenia using genome-wide SNP data. Results suggest that extended homozygosity, and possibly microdeletions are both more frequent in schizophrenia and specific to critical risk loci.

C15. A genome-wide assessment of the genetic basis of type 1 diabetes

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The strong familial clustering of autoimmune type 1 diabetes (T1D) in families remains only partially explained. The six confirmed genes or chromosome regions with convincing statistical support in large, and multiple, populations, namely the major histocompatibility complex (MHC), the insulin gene (INS), CTLA4, PTPN22, IL2RA/CD25, and IFIH1/MDA5 can explain only about 50% of familial aggregation. Nonetheless, their identification has provided many insights into the biology and pathways of T1D. Recent expansions in sample collections, including the Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory (JDRF/WT DIL) British T1D case sample (n = 8,000), and advances in affordable, genome-wide, high throughput single nucleotide polymorphism (SNP) genotyping technologies (Affymetrix), has allowed an association analysis of 500,000 SNPs across the genome in 2,000 T1D cases and 3,000 controls as part of the Wellcome Trust Case-Control Consortium (WTCCC). Attesting to the quality of the SNP map, positive results were obtained for all six susceptibility loci reported previously. However, follow-up genotyping studies of 14 other chromosome regions showing P < 10-5 in the WTCCC scan in over 6,000 cases and 6,000 controls, and in approximately 2,000 families, supported in a convincing way (P < 10-15 and odds ratios approximately 1.2) four regions associated with T1D in both the family and case-control samples, on chromosomes 16p13, 18p11, 12q13 and 12q24. These results illustrate the power of genome-wide association approach to characterise the genetic basis of T1D.

C16. Identification of *TGFB1* as the first gene associated with otosclerosis

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Otosclerosis is a progressive hearing loss characterized by an abnormal bone homeostasis leading to stapes fixation. Although its etiology remains unknown, otosclerosis can be considered a complex disease. *TGFB1* was chosen for a case-control association study, because of several non-genetic indications of involvement in otosclerosis. SNP analysis in a large Belgian-Dutch case-control group gave significant results (p = 0,0044) for an amino acid changing SNP in *TGFB1*, T263I, remaining significant after multiple testing correction. Analysis of an independent large French case-control group replicated this significant association for the same SNP T263I (p = 0,00019), again surviving multiple testing correction. Haplotype analysis and the independent effect test using WHAP in both populations were both compatible with SNP T263I being the only causal variant. The variant I263 is underrepresented in otosclerosis patients and hence protective against the disease. Combining the data of both case-control groups for SNP T263I with a Mantel-Haenszel estimate of common odds ratios gave a very significant result ($p = 9.2 \times 10^{-6}$). Functional analysis of this SNP T263I with a luciferase reporter assay showed that the protective variant I263 of *TGFB1* is more active than the WT variant T263 ($p = 1,6 \times 10^{-6}$). On the basis of our results, including very low p-values for association, replication in two independent populations and a demonstrated functional effect of the causative variant, we conclude that *TGFB1* is the first gene that influences the susceptibility for otosclerosis, and that the I263 variant is protective against the disease.

C17. Origin of the Etruscans: novel clues from the Y chromosome lineages

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Three hypotheses have been proposed on the origin of the distinctive Etruscan civilization and language that flourished ca. 3,000 years before present (BP) in Central Italy: 1) an external Anatolian source (Lydia and Lemnos) as claimed by Herodotus, 2) an autochthonous process of formation from the preceding Villanovan society as firstly proposed by Dionysius of Halicarnassus and 3) an influence from Northern Europe. A synthetic geographical map summarizing 34 classical genetic markers in Italy differentiates a genetically homogeneous Central Italian region between the Arno and Tiber rivers (ancient Etruria) from the rest of Italy. While this fact was tentatively interpreted as a genetic footprint of the Etruscans, its verification remained a challenge due to lack of data on differentiation of such markers and its calibration with time.
Here we show the genetic relationships of modern Etrurians, who mostly settled in Tuscany, with other Italian, Near Eastern and Aegean peoples by comparing the Y-chromosome DNA variation in 1,264 unrelated healthy males from: Tuscany-Italy (n=263), North Italy (n=306), South Balkans (n=359), Lemnos island (n=60), Sicily and Sardinia (n=276). The Tuscany samples were collected in Volterra (n=116), Murlo (n=86) and Casentino Valley (n=61).
We found traces of recent Near Eastern gene flow still present in Tuscany, especially in the archaeologically important village of Murlo. The samples from Tuscany show eastern haplogroups E3b1-M78, G2*-P15, J2a1b*-M67 and K2-M70 with frequencies very similar to those observed in Turkey and surrounding areas, but significantly different from those of neighbouring Italian regions. The microsatellite haplotypes associated to these haplogroups allow inference of ancestor lineages for Etruria and Near East whose time to the most recent common ancestors is relatively recent (about 3,500 years BP) and supports a possible non autochthonous post-Neolithic signal associated with the Etruscans.

C18. Epidemics of viral haemorrhagic fever in Medieval times as a possible selection pressure for CCR5del32 in Europe: new insights from Croatian island isolates

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Aim. The CCR5del32 mutation is protective against HIV infection. The possible historic selection pressure that gave rise to its increased frequency in Europe remains a matter of conjecture. The hypotheses in-

clude plague epidemics, childhood infections or random genetic drift.
Materials and methods. We sampled 1001 examinees from 9 isolated Dalmatian villages which had differing history of epidemics during the period between 11th-20th centuries. The demographic history of these isolates during the past 1,000 years was reconstructed in great detail from historic records. The frequency of CCR5del32 mutation was determined in each population.
Results: CCR5del32 mutation frequency in the Croatian general population is 7.1%. The villages with no history of epidemics had a CCR5del32 mutation frequency between 1.5% and 3.9% ($p<0.01$ in all cases in comparison to general population). The villages with a history of a major 15th-century epidemic (with lethality rates of about 70%) had CCR5del32 frequencies between 6.5% and 11.0% ($p<0.01$ in all cases in comparison to epidemic-free villages). Further research on the historic records suggested that epidemics of viral haemorrhagic fever (possibly originating in Russia) and with an exceptionally long incubation period may have spread to Baltic states and then through the 'route of Amber' to southern Europe and the Republic of Venice, from where it affected some village populations on the Croatian islands.
Conclusion. The extensive available historical and genetic data from these Croatian island isolates supports the hypothesis that an unknown viral haemorrhagic fever was reponsible for the selection pressure on the CCR5del32 mutation in Europe.

C19. Reconstructing functional gene loci using PCR

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A very long PCR method has been established for 30-65 kb sized human genomic products from PAC/BAC clones for human artificial chromosome development. Within PAC libraries, gene loci rarely are represented in an applicable form. The available segments often contain more than one gene, or parts of adjacent genes, or are incomplete. Long PCR can introduce adequate restriction sites via primers, however, up to now it was unknown if long PCR within this size range can produce functional genomic molecules.
Using a novel DNA quality termed grade 1, quantitative amplification is robustly achieved within 7-9 PCR cycles, resulting in a very low error rate. For locus reconstruction via subcloning of PCR products, a large fraction of resulting molecules needs to be free of errors.
Here we have subcloned single 32 kb molecules of the human genomic HPRT gene locus in a telomerized PAC vector. The constructs were transfected into HT1080 (HPRT-) cells and checked in a HAT assay. Out of five bacterial clones obtained, 2 showed large deletions. Out of the 3 showing the correct PFGE size, 2 turned out to be functional. FISH analysis of HAT resistant cell lines from each construct showed presence of the transgene locus randomly integrated in a host chromosome. Cotransfection of a centromere resulted in the incorporation of the synthetic locus in a human artificial chromosome (HAC). This technology represents a breakthrough for the assembly of HAC constructs based on conventional cloning and for the development of therapeutic HACs containing entire gene loci.

C20. Single cell microRNA and mRNA profiling reveals unique gene expression signatures and heterogeneities in mouse ES cells

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We describe a new method for simultaneously quantifying 237 mouse microRNAs (miRNAs) and 21 messenger RNAs (mRNAs) from single cells including embryonic stem (ES) cells, embryoid bodies (EBs), 3T3 cells, or splenocytes. The method is based on multiplex RT, multiplex preamplification, and singleplex real-time TaqMan® PCR assays. Assays are quantitative for a dynamic range of at least three logs. Single cell expression signatures could classify individual ES, EBs, or somatic cells. Significant inter-cell variations of both miRNA and mRNA expression within ES cell lines indicated the heterogeneity of ES cells. The highest variability was observed among EB cells (CV = 139%), demonstrating that EBs undergo differentiation at different stages. Interestingly, ES marker gene Oct4 and signaling gene Tdgf1 were highly co-expressed. Both were absent in 3T3 and splenocyte cells, highly expressed in ES cells, and significantly reduced in EB cells. Results indicated that Oct4 and Tdgf1 might be co-regulated during ES differentiation. The total number of expressed miRNA genes in ES, EB, and somatic cells remained constant. However, their expression levels were significantly elevated during differentiation, further suggesting the involvement of miRNAs in cellular development and specification. Furthermore, there was no correlation in the expression levels between miRNAs and their predicted target mRNAs, thereby supporting a translational repression model. Our results provide new insight into both miRNA and mRNA expression patterns at the single cell level.

C21. A High Resolution Oligonucleotide CpG Island Microarray

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CpG islands are areas of DNA containing multiple CpG dinucleotides. When CpG dinucleotides within these islands are methylated, especially in promoter regions, expression of the corresponding downstream genes is often repressed. Aberrant CpG island methylation is implicated in cancer.
We developed an oligonucleotide microarray that represents the CpG islands in the human genome. This microarray contains ~230,000 oligo probes tiling the 21 megabases of 27,800 CpG islands, with an average spacing between probes of 95 base pairs. The microarray is compatible with several published methods for the genome-wide detection of methylated CpG islands. To demonstrate the ability of this microarray to detect methylated DNA, we performed analysis of human genomic DNA samples after methylated DNA immunoprecipitation (mDIP). We also developed "spike-in" control DNA that was *in vitro* methylated to varying degrees.
The mDIP method combined with CpG island microarray analysis accurately differentiated between partially and fully methylated spike-in DNAs. We then applied the whole-genome assay to the prostate cancer cell line PC3 and detected methylated CpG islands upstream of cancer genes including CDKN2A/p16. We studied other cancer cell lines and determined relative methylation at multiple cancer-related genes. Finally, using male and female embryonic fibroblast cells, we demonstrate that many CpG islands on the female X chromosome are more methylated than the corresponding islands on the male X chromosome. Comparitively, methylation of CpG islands on autosomes is essentially the same for the male and female samples. This supports a role for CpG methylation in silencing the inactive X chromosome in females.

C22. Cost-effective screening of deletions and duplications of up to 1500 loci in one assay: a bead-based approach

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Copy Number Variation (CNV), deletions and duplications, is frequently involved in genetic disease. Historically, methodologies detecting quantitative changes were laborious, costly and consequently neglected. Now genome-wide analyses can be used to study CNV, revealing extensive variation in the human genome, both pathogenic and non-pathogenic. However, in diagnostic setting cost prohibits application of these methods to screen all incoming patients. Focused on clinical application, we developed a flexible targeted method using Illumina's GoldenGate® assay, facilitating cost-effective screening of 1420 loci in parallel in hundreds of samples. The assay should detect all trisomies, telomere-end rearrangements and known micro-deletion syndromes and perform a rough whole genome scan. Analysis of 48 control cases succefully identified all known rearrangements, from trisomy-21 to a single DMD-exon deletion. Analysis of 320 patient samples (mental retardation of unknown etiology) revealed many new rearrangements, including non-pathogenic CNV (present in >20% of cases). MLPA, FISH or whole genome SNP-array analysis thus far confirmed other changes as pathogenic in 29 cases. The assay designed is reliable, cost-effective, can be easily modified to include new regions of interest and used to screen up to 1500 loci in parallel.

C23. Literature-aided interpretation of microarray data: a compendium study on muscle development and disease

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Lists of differentially expressed genes from microarray studies are difficult to interpret. Comparative analysis of related microarray studies may help to improve the understanding of the molecular and cellular processes involved. We analyzed 102 microarray studies published in the field of muscle development and disease. The overlap in the lists of differentially expressed genes was generally small. Analysis at the level of biological processes should be more rewarding, since different genes may hint at the same process. Currently available methods rarely performed well due to low sensitivity and their dependence on the limited annotation of gene function and pathways in curated databases. As an alternative, we developed a literature-based annotation system. For all genes, species-independent concept profiles were derived, linking all biological concepts mentioned together with the gene in Medline abstracts in a weighted fashion. The concept profiles for all possible gene pairs from two microarray studies were matched and evaluated for significance of overlap. Our algorithm discovered biologically meaningful associations between studies that had no genes in common. The biological processes shared between studies were easily recognized. For example, mouse and human studies on limb-girdle muscular dystrophy 2B were brought together by non-overlapping macrophage-expressed genes. In vitro and in vivo studies into muscular atrophy were connected by decreased mitochondrial activity, and found closely related to mitochondrion-related myopathies. In conclusion, our approach facilitates finding common biological denominators in microarray studies in an unsupervised way, and without the need for raw data analysis or annotations of biological functions in curated databases.

C24. Producing a reference human gene set

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The Havana group (www.sanger.ac.uk/HGP/havana/) produces manual annotation of finished sequence from vertebrate genomes, concentrating on finished human, mouse and zebrafish genomes. Manual annotation is needed for the accurate determination of duplicated gene clusters, pseudogenes and non-coding genes, polyA features, splice variation and accurate nomenclature. Currently, annotation of genes on the human genome is provided by multiple public resources. To provide a standard uniform human gene set Havana is collaborating with NCBI, UCSC and Ensembl to produce a consensus CDS (CCDS) database containing a core set of mouse and human protein-coding regions that are consistently annotated. The next release of the human CCDS set will contain around 19000 coding transcripts compared to 13372 currently in mouse.

Havana also collaborates with Ensembl to improve the genebuild on the finished genome and produce a merged gene set that is visible in Ensembl. This merged set contains only full-length coding transcripts predicted identically and independently by both groups and is coloured gold in Ensembl. This enables Ensembl users to highlight errors within the automated genebuild allowing Havana to correct the genes manually. The resulting corrections get re-incorporated into the new genebuild. The merged set consists of approximately 12,000 Ensembl and Havana transcripts with identical exon coordinates and splice sites. It differs to the CCDS approach in that UTR, as well as protein-coding regions, must be the same. A new graphical interface called Zmap has recently been implemented which enables multiple genomes to be annotated simultaneously and thus should improve the consistency of annotation.

C25. How healthy are children born after preimplantation genetic diagnosis?

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Preimplantation genetic diagnosis (PGD) was introduced in the clinic in 1990 as a very early form of prenatal diagnosis (PND) for couples at high risk to transmit a genetic disease. Preimplantation genetic screening (PGS), a variant of PGD was introduced later in order to improve the selection of embryos for transfer in in vitro fertilisation (IVF).

The requests for PGD or PGS of infertile as well as of fertile couples were evaluated and accepted for treatment whenever possible. As soon as the PCR-based or FISH-based single cell assays were ready, the IVF treatment was planned .A prospective data collection on pregnancies and children was organised by giving questionnaires to the couples on the day of transfer. Additional questionnaires were send to the patients and their physicians (gyneco- logists, pediatricians) during pregnancy, at delivery and later on. Children were examined at 2 months and 2 years of age whenever possible. Between 1993 and 2005, 2756 PGD and PGS cycles were performed for many different indications including HLA-typing. After transfer of 'fresh' embryos, 567 children were born .Of these, 548 were liveborn, 19 were stillborn. And 9 children died neonatally. The perinatal death rate (5 from singleton pregnancies and 23 from multiple pregnancies) of 4.9 %.should be further investigated.. The mean term and birth weight in live born singletons is respectively 38.8 weeks and 3268 grams. The major malformation rate is 3.6%. Embryo biopsy does not increase the major malformation rate when compared to children born after IVF/ICSI without PGD.

C26. The use of proteomic methods to search for biomarkers in maternal plasma from trisomy 21 pregnancies.

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Objective: To use proteomic methods to search for biomarkers for trisomy 21 (T21) in maternal plasma.

Methods: Surface Enhanced Laser Desorption Ionisation Mass Spectrometry (SELDI) detects small mass proteins (<30kDa) that are differentially expressed. Using protein array chips specific to proteins with different properties maternal plasma samples matched for gestational age were analysed. Plasma from pregnancies with normal male and female fetuses, and 8 T21 fetuses were analysed. Higher molecular weight analyses on albumin and IgG depleted plasma samples were performed using 2D Differential Gel Electrophoresis (2D-DiGE) in the pH 4-7 range. Further analysis of interesting areas was performed in a narrower pH range.

Results: Initial SELDI analysis showed differences between normal and T21 pregnancies and subsequent studies were controlled for gestational age. IMAC30 arrays in T21 samples showed potential biomarkers with masses of ~24kDa (p=0.0087) and 24.3kDa (p=0.046). A Q10 Chip array showed a potential biomarker of higher mass, 123kDa (p=0.027). These proteins were expressed in samples analysed at 11-13 weeks' and 18-19 weeks' gestation. 2D DiGE results showed three possible biomarker proteins in T21 pregnancies, one of 54kDa (p=0.021) with a 2.3 fold increase in spot intensity and two smaller proteins with 1.8 (p= 0.01) and 2.1 (p= 0.024) fold increases. Initial work in a narrower pH range shows better resolution of proteins of interest. Studies are underway to confirm and identify these proteins.

Conclusions: Proteomic methods may be useful to identify biomarkers of T21 that could be used clinically for non-invasive diagnosis of Down's syndrome

C27. Investigation of New Markers for Improved Isolation of Fetal Trophoblast Cells in Maternal Peripheral Circulation

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Trophoblasts present an attractive target cell type for non-invasive prenatal diagnosis in that they can be isolated from maternal blood early in the first trimester.

The aim of this study is to identify specific Trophoblast cells markers for application in retrieval of rare fetal cells from maternal peripheral blood.

Trophoblast and peripheral blood cell samples were obtained from women, undergoing pregnancy termination in the first trimester. Trophoblast isolation was efficient and the cell populations showed high purity for trophoblasts (85-94% positive cells), with low fibroblast contamination (0-12%- positive cells).
Isolation and identification of proteins was performed by proteomics technology. The results of 2-D gel analysis revealed many differences in protein expression between the maternal blood cells and placenta-derived trophoblasts. The most obvious protein spots that appeared in the placenta samples but were absent in the blood samples were isolated, purified and identified.
The proteins identified were: cytokeratin 7, cytokeratin 8, keratin 19, Zeta globin, A-gamma globin, G-gamma globin, and annexin IV.
Antibodies against cytokeratin 7 (an intracellular antigen) and annexin (a cell-surface antigen) were tested in trophoblast cell lines (JAR and BeWo) and Chorionic Villus (CVS) cultures. Annexin staining was positive in the JAR cell line only whereas both lines as well as CVS cultures were positive for cytokeratin 7. The remainder of the proteins detected by proteomics are currently being screened and the most promising markers will be applied in trophoblast isolation from maternal blood samples, the ultimate goal being culture expansion and karyotype analysis of the isolated cells.

C28. APEX microarrays for the non-invasive prenatal diagnosis of beta-thalassaemia

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The recent discovery of relatively abundant quantities of cell free fetal DNA in maternal plasma and serum has opened up new possibilities for the non-invasive prenatal diagnosis. In Cyprus, seven different beta-thalassaemia mutations account for over 98% of all cases with IVSI-110 representing the 81% of the total. Therefore, the development of a non-invasive method for the Cyprus population is based on the detection of paternally inherited Single Nucleotide Polymorphisms (SNPs) as well as the direct detection of paternal beta-thal mutations.
One of the approaches that are being developed is the Arrayed Primer Extension (APEX) method on the Genorama® QuattroImager™ . We developed a DNA chip called “thalassochip” that contains 29 beta-thal mutations and 10 SNPs linked to the beta-globin locus. More informative SNPs are being added constantly.
The specificity and the sensitivity of the approach were tested using genomic DNA. We were able to detect as low as 30pg/µl of genomic DNA. Fetal DNA in maternal plasma is about 90pg/µl. Moreover, the specificity of the approach was determined using genomic DNA. Following that, the procedure was applied on maternal plasma DNA for 2 SNPs and we were able to detect the paternal allele of the fetus for those SNPs.
Although the results are preliminary, the approach is very promising. All the SNPs and mutations that are currently on the thalassochip need to be standardized. Moreover, more informative SNPs need to be added validated and standardized.

C29. Prenatal diagbosis in Denmark after the introduction of nuchal translucency screening

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In 2004, the National board of Health in Denmark recommended new guidelines for prenatal diagnosis. Instead of restricting the offer of prenatal diagnosis mainly to pregnant women over the age of 35 years, all pregnant women shall have the possibility to opt for 1. trimester screening including nuchal translucency measurement in the fetus and a maternal blood sample (doubletest), allowing a combined risk calculation that the fetus has Down syndrome (or other trisomies). If the risk is over 1:300, an invasive procedure is offered with chromosome analysis of fetal cells from chorionic villus sample or amniocentesis.
We have surveyed the outcome of prenatal diagnosis in 3 counties in Denmark after gradual implementation of the new guidelines during 2004, 2005 and 2006. The area covers approx 1.1 mill inhabitants, 1/5 of the total population. A comparison was also made with national figures obtained from the Central Cytogenetic Registry. Main findings were: An overall reduction in invasive procedures (from approx. 11% of all pregnancies to approx. 5%), a doubled number of trisomy 21 fetuses diagnosed prenatally, and a reduction in the number of children born with Down syndrome by approx 50%. The maternal history of the children born with Down syndrome revealed: decline of offer of screening, risk calculation above cut-off limit, too late appearance in pregnancy. Sex chromosome abnormalities were apparently not detected more frequently prenatally, but triplodi and possibly trisomy 18 were diagnosed more often than previously.
Selected cases with mosaicim and pseudomosaicim will be presented.

C30. Prenatal detection of partial chromosome imbalance by Quantitative Fluorescent PCR (QF-PCR)

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The QF-PCR assay allows prenatal diagnoses of chromosome aneuploidies in a few hours after sampling.
Several markers are needed on chromosomes X, Y, 21, 18 and 13 to minimize homozygous or uninformative samples. However, if microsatellites are selected along the examined chromosomes, QF-PCR may also allow detecting partial trisomies.
We developed a QF-PCR assay including up to 10 markers on the sex chromosomes, 8 on chromosomes 21 and 18 and 6 markers on chromosome 13.
In the course of its application on over 30.000 clinical cases the test allowed detecting 100% aneuploidies without false positive results. On the other end, it was also possible to detect 11 different partial aneuploidies resulted from unbalanced translocations, duplications, insertions and other structural rearrangements. Chromosome 18 was involved in 4 cases, 6 fetuses had either X or Y derived extra sequences and in two more samples partial trisomies 21 or 13 were also readily detected as trisomic patterns for two or more STRs. In 6 cases QF-PCR result was crucial to achieve the correct diagnosis.
Rapid prenatal diagnosis by QF-PCR has proven to be efficient and reliable in detecting major numerical abnormalities. In this study we demonstrate that an appropriate marker selection can also allow identification of partial chromosome imbalance.
The main advantages of the assay are its low cost, speed and automation allowing high throughput of samples. QF-PCR reaches the purposes of relieving anxiety of most parents within 24 hours from sampling or to accelerate therapeutical interventions in case of abnormal result.

C31. Screening and maternal transmission instability of intermediate-size and premutation FMR1 alleles in 24,446 mother-newborn pairs from the general population

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Background: To study the instability of FMR1 gene triplet repeats in the general population, we screened a large prospective sample of 24,446 anonymized mother-offspring pairs to determine transmission instability of intermediate-size (40-58 triplets) and premutation-size FMR1 alleles (59-230 triplets) in the general population.
Methods: We used a mini-Southern blotting method to screen all mothers for FMR1 alleles of 40 to 58 triplets (n=676) or premutation-size (59+ triplets, n=22). We studied transmission of these alleles to their offspring using triplet repeat PCR with validation of the mother-child link using six highly polymorphic microsatellite markers.
Results: Out of a subsample of 343 transmissions of normal-size alleles (<40 triplets) we observed three(1%) unstable transmissions. There were 11 unstable alleles out of 191 transmission (6%) of intermediate-size alleles (40 to 58 repeats) and 6 unstable premutation-size alleles out of 11 transmissions (55%). One mother with a 100 triplet allele had

a boy with a typical full mutation. The incidence of fragile-X syndrome in this population was thus 1:24446 (upper limit of 95% C.I. 1/7065). Intermediate-size alleles were significantly more unstable than normal-size alleles (Fisher exact p : p=0.0012) but much more stable (about ten-fold) than premutation-size alleles (Fisher exact p = <0.0001). The major fragile-X haplotype (T50-T42-T62) was present in all unstable premutation-size alleles.
Conslusion: Empirical measures of incidence and instability are of interest to determine the feasibility and cost-effectiveness of putative FMR1 screening programs and to counsel carriers of large FMR1 alleles with no family history of the disease.

C32. Familial inherited microtia caused by a benign CNV amplification at chromosome 4pter

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Recently, large scale benign copy number variations (CNVs) were uncovered, encompassing over 12% of our genome. Deletions and duplications of these regions present in normal individuals contain genes considered to be dosage tolerant for human development.
Here we present a 4 generation family with autosomal dominant inheritance of microtia, eye coloboma and lacrimal duct obstruction, a novel syndrome. This phenotype is linked (lod >3) with a cytogenetically visible alteration at 4pter but was never described in patients with 4pter deletions nor duplications. We demonstrate by array CGH, qPCR, FISH and Southern blot that the disorder is caused by the amplification of a 780kb region on 4pter, encompassing the olfactory receptor gene cluster. This is the first example in which the amplification of a well-known copy number variable region causes a phenotype and hence is a novel genetic disease mechanism causing a dominant disorder.
In addition, we investigated the organization of the amplified fragment in order to understand the mechanism by which this amplification occurred. We show that perfect copies of the 780kb fragment are amplified in tandem.

C33. Gene dosage, craniofacial and neurological development and Williams-Beuren syndrome.

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Segmental duplications play an important role in disease by creating instability that leads to genomic rearrangements in important regions. The consequences can be dosage imbalance of gene(s) critical for normal human development. Abnormal gene dosage is involved in the aetiology of the microdeletion disorder Williams-Beuren Syndrome (WBS) which displays characteristic cardiovascular, craniofacial and neurological phenotypes. 28 or more genes are heterozygously deleted and upsetting the balance of specific gene(s) affects human speech and language as well as cognitive capabilities. Genotype-phenotype correlations have been difficult to assign and position effects involving genes outside the critical region cannot be excluded. We have investigated rare individuals with atypical deletions in the WBS region, displaying partial or more severe features of the classic cases, supplemented by the study of mouse models. The combination of clinical, psychological and 3D-face morphometric analyses alongside detailed molecular profiling on a genome-wide scale has narrowed down the critical region harbouring genes important for the WBS phenotypes as well as highlighting new regions associated with craniofacial and neurological development. Reciprocal WBS duplications also exist and the clinical and molecular profile is still being defined. Using aCGH analyses on the Sanger Institute 30K WGTP arrays we have generated a genomic footprint for patients with different sized deletions and duplications and compared their genome architecture on a global scale. Our patients with atypical deletions and duplications involving the WBS locus present with a spectrum of phenotypes for which we will attempt to link genotype to pathological phenotype.

C34. Long-range conserved non-coding SHOX sequences regulate expression in developing chicken limb and are associated with short stature phenotypes in human patients.

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Defects in long-range regulatory elements have recently emerged as previously underestimated factors in the genesis of human congenital disorders. Léri-Weill dyschondrosteosis is a dominant skeletal malformation syndrome caused by mutations in the short stature homeobox gene *SHOX.* We have analyzed four families with Léri-Weill dyschondrosteosis with deletions in the pseudoautosomal region but still with an intact *SHOX* coding region. Using FISH and SNP studies, we identified an interval of ~200 kb that was deleted in all tested affected family members but retained in the unaffected members and in 100 control individuals. Comparative genomic analysis of this interval revealed eight highly conserved non-genic elements between 48 kb and 215 kb downstream of the *SHOX* gene. As mice do not have a *Shox* gene, we analyzed their enhancer potential in chicken embryos using a GFP reporter construct driven by the ß-globin promoter, by *in ovo* electroporation of the limb bud. We observed cis-regulatory activity in 3 of the 8 non-genic elements in the developing limbs argueing for an extensive control region of this gene. These findings are consistent with the idea that the deleted region in the affected families contains several distinct elements that regulate *Shox* expression in the developing limb. Furthermore, the deletion of these elements in humans generates a phenotype apparently undistinguishable to those patients identified with mutations in the *SHOX* coding region and, for the first time, demonstrates the potential of an *in vivo* assay in chicken to monitor putative enhancer activity in relation to human disease.

C35. Chromosomal imbalances detected in patients with cerebral cortex malformations and epilepsy through array−CGH technique

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A cohort of 72 patients (37 males and 35 females) with cerebral cortex malformations and/or epilepsy, and with a normal karyotype was analysed trough array-CGH at a resolution of about 75Kb (Agilent platform 44B). Most of the patients had also mental retardation and a few presented congenital anomalies. All have been analyzed for brain MRI, behavioural tests and types of epilepsy. Array-CGH resulted diagnostic in 10 out of 72 patients analysed (14%). The size of the imbalance ranged from 350Kb to 10Mb. The following chromosomal anomalies were found: duplication of regions 7q11.23, 22q12.3-13.2 and Xp22.2; deletion of regions 2q22.1-q23.3, 5q14-21, 7q11.23, 15q13.2-q13.3 and 20q13.33. All chromosomal aberrations have been confirmed by microsatellite analysis and the parental origin determined. Three cases with 7q11.23 duplication, reciprocal to the Williams-Beuren deletion, have been detected in a girl, a male child and his mother. All suffered from severe speech impairment; the girl and the boy had cerebral cortex malformations of different type whereas the mother had not been tested for brain MRI. The 15q13.2-q13.3 deletion was mediated by the same type of segmental duplications (BP1, BP2 and BP3) responsible for instability of the PWS/AS region but located at BP4 and BP5. These

preliminary results represent the first step to identify novel genes involved in biological mechanisms important for human cerebral cortex development and for processes causative of epilepsy.

C36. Activating SOS1 mutations cause Noonan syndrome

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Noonan syndrome (NS) is a relatively common, genetically heterogeneous Mendelian trait characterized by short stature, facial dysmorphisms, congenital heart defects and skeletal anomalies. Accumulating evidence supports the idea that NS is caused by enhanced RAS-MAPK signaling. Indeed, gain-of-function mutations in PTPN11, the gene encoding SHP-2, a protein tyrosine phosphatase that positively controls RAS signaling, and KRAS have been documented in 50% of NS cases. Here, we demonstrate that approximately 20% of NS patients without PTPN11 or KRAS mutation have missense mutations in SOS1, which encodes a RAS-specific guanine nucleotide exchange factor (GEF). SOS1 mutations cluster at residues implicated in the maintenance of SOS1 in its autoinhibited form and expression of two NS-associated mutants induced enhanced RAS and ERK activation. The phenotype associated with SOS1 defects is distinctive, although within NS spectrum, with a high prevalence of ectodermal abnormalities but generally normal development and linear growth. Our findings implicate for the first time gain-of-function mutations in a RAS GEF in inherited disease and define a new mechanism by which upregulation of the RAS pathway can profoundly change human development.

C37. Mutations in the SPG11 gene, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum

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Autosomal recessive hereditary spastic paraplegia (ARHSP) with thin corpus callosum (TCC) is a common and clinically distinct form of familial spastic paraplegia, linked to the SPG11 locus on chromosome 15 in most families. It clinically manifests as spastic paraplegia usually beginning during infancy or puberty, preceded by learning difficulties. Some patients develop a pseudobulbar involvement, with dysarthria, dysphagia and upper limbs spasticity, associated with bladder dysfunction and signs of peripheral neuropathy. MRI shows thin corpus callosum (TCC), with hyperintensities in periventricular white matter, and cerebral cortical atrophy predominating in the frontal region.

We collected 12 ARHSP-TCC families. All available members were genotyped using 34 microsatellite markers covering the candidate interval for SPG11. Linkage analysis and haplotype reconstruction in 10 informative families restricted the SPG11 region to a 3.2-cM homozygous region. Eighteen genes were analyzed in SPG11 index patients by direct sequencing, but no disease-causing mutations were found in 17 of them. Ten mutations in 11 of the 12 families were found, however, in a novel gene (KIAA1840/FLJ21439) expressed ubiquitously in the nervous system but most prominently in the cerebellum, cerebral cortex, hippocampus and pineal gland. The mutations were either nonsense or insertions and deletions leading to frameshifts, suggesting a loss-of-function mechanism.We have identified the SPG11 gene that accounts for the majority of the ARHSP-TCC families studied here (11/12). The identification of the function of the gene will provide insight into the mechanisms leading to the degeneration of the corticospinal tract and other brain structures in this frequent form of ARHSP.

C38. OST3 mutation in non-syndromic mental retardation: expanding the spectrum of congenital disorders of glycosylation?

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Congenital disorders of glycosylation (CDG) is a group of inherited disorders that affect glycoprotein biosynthesis. The Eighteen different CDG types are characterized by a central nervous system dysfunction and multi-organ involvement. Group I CDG results in the failure of assembly or transfer of the N-glycan chain while group II is defined as defects in the processing of the protein-bound glycan.

Here, we report on a non-syndromic mental retardation (MR) in two sibs born to first cousin French family. Homozygosity mapping in the two affected and two healthy children led to the identification of a unique homozygous region of 8 Mb on 8p23.1-p22. This interval encompassed the gene TUSC3/OST3 (Ost3 S.cerevisae homologue) encoding a protein involved in the oligosaccharyltransferase complex which catalyses the transfer of an oligosaccharide chain on nascent proteins, the key step of the N-Glycosylation process. Sequencing the OST3 gene identified a one base-pair insertion in exon 6, 787_788insC. The mutation co-segregated with the disease and resulted in a premature stop codon 37 codons downstream of the coding sequence, N263fsX300. Extensive work-up (Caryotype analysis, liver function, clotting factors, brain MRI, EEG) was unremarkable with normal isoelectric focusing and Western blotting assay of serum N-glycoproteins.

Recent studies of glycoprotein fucosylation and polysialic acid modification of neuronal cell adhesion molecules have shown the critical role of glycoproteins in synaptic plasticity. However, our results provide the first demonstration that a defect in N-Glycosylation can result in non-syndromic MR providing therefore new insights in the understanding of the pathophysiological bases of MR.

C39. A defect in the ionotropic glutamate receptor 6 gene (GLUR6) causes autosomal recessive mental retardation

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Mental retardation (MR) is among the most common forms of genetic handicaps. So far, however very little is known about the gene defects underlying this disorder. Especially the contribution of autosomal recessive hereditary defects is largely unresolved and to date, only three genes have been found to be directly associated with non-syndromic autosomal recessive MR (NS-ARMR).

We have previously identified 8 new genomic loci for NS-ARMR (MRT6-11) and report here a complex deletion-inversion mutation in the glutamate receptor 6 gene (GLUR6, GRIK2), a member of the kainate receptor family. GLUR6 maps to the MRT6 locus, a 10Mb linkage interval on Chr6q16.3, which we have recently identified in a large consanguineous Iranian family with moderate to severe non-syndromic ARMR. As shown by Southern blotting, inverse PCR, array-CGH and cloning experiments, the sequence changes include a deletion of approximately 120Kb including exons 7 and 8, as well as an inversion of about 80Kb that encompasses exons 9 to 11. This mutation was not found in 172 controls, and the integrity of the coding sequence of 7 other potential candidate genes in the interval was verified by direct sequencing.

Glutamate receptor signalling has previously been implicated in the pathogenesis of the fragile-X-syndrome and may also play a role in autism. However, our studies provide the first direct evidence for the importance of glutamate receptors in human cognition. Moreover, they have implications for the diagnosis and prevention of NS-ARMR and may shed new light on the role of ion channels in the human brain.

C40. Mutation of a potassium channel-related gene in progressive myoclonic epilepsy

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Objective: We investigated a large consanguineous Moroccan family with progressive myoclonic epilepsy (PME) consistent with autosomal recessive inheritance, in order to describe the phenotype and identify the causal gene.
Methods: We recorded the clinical course of the disease and the response to drug therapy, while carefully excluding known causes of PME. We then linked the disease by homozygosity mapping using microsatellite markers and SNP microarrays (11K GeneChip®) and studied candidate genes in the critical linkage region.
Results: Epilepsy started between 16 and 24 months of age after normal initial development. Seizures were multifocal myoclonus aggravated by movements, and generalized tonic-clonic seizures in two patients. EEG showed slow dysrythmia, multifocal and occasionally generalized epileptiform discharges, and photosensitivity. Brain MRIs were normal. All patients were demented. Two had refractory epilepsy and a severe course. Seizures were controlled in the third patient, whose disease course was less severe. Linkage analyses identified a new locus on 7q11.2, with a maximum multipoint LOD of 4.0 at D7S663. In the critical linkage region, we found a C to T mutation in exon 2 of the Potassium Channel Tetramerization Domain-containing 7 gene (KCTD7). The mutation affected a highly conserved segment of the predicted protein, changing an arginine codon into a stop codon (R99X).
Interpretation: Neurodegeneration in PME presented by our patients paralleled therefractoriness of epilepsy. The disease was transmitted as an autosomal recessive trait linked to a novel locus at 7q11.2, where we identified a mutation in KCTD7.

C41. Mutations in *TCF-4*, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction.

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Pitt-Hopkins syndrome (PHS) is a rare syndromic encephalopathy characterised by severe psychomotor delay, epilepsy and daily bouts of diurnal hyperventilation starting in infancy, mild postnatal growth retardation, postnatal microcephaly and distinctive facial features. We have ascertained 4 cases of PHS and carried out a systematic 1Mb resolution genome wide BAC array screening for microdeletions or duplications in all cases. Using this approach, we have identified a 1.8 Mb *de novo* microdeletion on chromosome 18q21.1 in 1/4 case. We subsequently identified two *de novo* heterozygous missense mutations of a conserved amino acid in the basic region of the *TCF4* gene in three additional PHS cases. These findings demonstrate that TCF4 anomalies are responsible for PHS and provide the first evidence of a human disorder related to class I basic helix-loop-helix transcription factor defects (also known as E-proteins).
Differential diagnoses include Rett, Angelman, Mowat-Wilson and Goldberg-Shprintzen syndromes. The facial gestalt of patients with PHS is extremely valuable for clinicians to consider the diagnosis before the onset of distinctive features such as bouts of hyperventilation and epilepsy. EEG and brain MRI may also give valuable clues that will be discussed. Patients diagnosed with PHS dysplay a broad spectrum of dysautonomic features (hyperpneic episodes, dilated pupils with sluggish responses to light, short-segment Hirschsprung disease or severe gastrointestinal dysfunction). These data may shed new light on the normal processes underlying autonomic nervous system development and maintenance of an appropriate ventilatory neuronal circuitry.

C42. Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome)

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Pitt-Hopkins syndrome is a rarely reported syndrome of so far unknown etiology characterized by mental retardation, wide mouth and intermittent hyperventilation. By molecular karyotyping using GeneChip Human Mapping 100K SNP arrays we detected a 1.2 Mb deletion in 18q21.2 in one patient. Sequencing of the TCF4 transcription factor gene, which is contained in the deletion region, in 30 patients with significant phenotypic overlap revealed heterozygous stop, splice and missense mutations in 5 further patients with severe mental retardation and remarkable facial resemblance. Thus we establish the Pitt-Hopkins syndrome as a distinct, but probably heterogeneous entity caused by autosomal dominant de novo mutations in TCF4. Due to its phenotypical overlap Pitt-Hopkins syndrome evolves as an important differential diagnosis to Angelman and Rett syndromes. As both, null and missense mutations impaired the interaction of TCF4 with ASCL1 from the PHOX-RET-pathway in transactivating an E-box containing reporter construct, hyperventilation and Hirschsprung disease in patients with Pitt-Hopkins syndrome might be explained by altered development of noradrenergic derivatives.
Our study shows, that molecular karyotyping is not only able to disclose novel microdeletion syndromes and the underlying gene defect in well known disorders like CHARGE and Peters-Plus syndromes, but also to resolve the etiology in very rarely reported phenotypes. However, Pitt-Hopkins syndrome is probably widely under-diagnosed like the now clinically recognizable Mowat-Wilson syndrome was until the identification of the underlying gene defect. To our knowledge, Pitt-Hopkins syndrome is the first constitutive phenotype in which the etiology was identified by molecular karyotyping using genome wide SNP arrays.

C43. Powerful methods for whole genome association analysis of quantitative traits in samples of related individuals

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Genome-wide association (GWA) analysis is a tool of choice for identification of genes responsible for complex human disorders. GWA analysis using closely or remotely related individuals from genetically isolated populations (e.g. EUROSPAN consortium) or families from general population (e.g. Framingham study) may have certain advantages over designs using apparently unrelated individuals from the general population.
Most of the methods suitable for GWA analysis of pedigree data are based on variants of the Transmission-Disequilibrium test (TDT). These methods utilise within-family variation and are therefore robust to false positives potentially induced by population stratification. However, these methods lose power because they ignore between-family variation and also require precise knowledge of pedigree and sampling of closely related individuals, which may be not always possible and cost-effective.
Here, we suggest a set of powerful methods suited for analysis of samples of (potentially) related individuals. We show that when pedigree relationships between the study subjects are not known or are only partly known, genomic control (GC) may be used to correct for relatedness and stratification. As expected, for a wide range of scenarios the

power of this method is much higher compared to TDT-based methods. When pedigree structure is at least partly known, utilisation of this information allows even more powerful analysis. The baseline method is extended to include covariates, interactions and more complex genetic effects, such as parent-of-origin and epistasis. We demonstrate the utility of the newly developed methods using real data from a young genetically isolated population.

C44. Optimizing information for linkage genomescreen in a large and inbred pedigree with a high density SNP map

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Large genealogies available in isolated populations are potentially very informative for linkage analyses, in particular when considering high density SNP maps.

Here, we investigate different methodological aspects of linkage analysis in a large and inbred 1840-member Hutterite pedigree, phenotyped for asthma and asthma-related traits. Starting with a 5cM microsatellite map, we first identify genome-wide significant regions (1p13, 1p31, 5q33, 6q21-23, 12q14, 13q13 and 14p11-q11), after a carefully optimized breaking of the pedigree. Our approach is interesting since these regions had not been significantly detected in previous analyses of the same dataset but have been described by others.

We further analyse these linked regions using a 500K SNP map. Because SNPs are much closer than microsatellite markers, they present important linkage disequilibrium (LD), which bias classical nonparametric multipoint analyses. This problem is even stronger in population isolates where LD extends over larger regions with a more stochastic pattern. The only method that models LD in the NPL analysis is limited in both the pedigree size and the number of markers (Abecasis and Wigginton, 2005) and therefore could not be used. Instead, we propose methods that identify sets of SNPs with maximum linkage information content in our pedigree and no LD-driven bias. Both algorithms that directly remove pairs of SNPs in high LD and clustering methods are evaluated. Preliminary results suggest that in such population isolates, a careful marker selection is necessary to obtain information content for linkage higher with SNPs than that already available with microsatellites.

C45. Epistasis for physiological variables in admixed populations

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The metabolic syndrome and diabetes mellitus are highly influenced by genetic factors. These conditions are heterogenous and polygenic in nature for the vast majority of cases, each gene supposed to have only a minor impact of the phenotypic variance in the general population.

Therefor large populations are required to identify and elucidate the genetic structure of these conditions. A major obstacle is the heterogeneity of most study populations, i.e. the populations consist of a mixture of yet unidentified physiological and genetic homogenous subpopulations. To define these subpopulations a latent class analysis is performed. This was done in a structural equation modelling framework. Basic physiological variables previously shown to be related to the metabolic syndrome were included in analysis as indicators and covariates. No genetic model is assumed. This model defined 19 subpopulations with an entropy measure of approximately 0.9. Less than 0.1%. of all possible single-gene heritabilities were present. In contrast, including two-gene interactions revealed that 28 of the 30 genetic markers are involved in one and usually several epistatic interactions. On average approximately 1/3 of all possible two-gene interactions were significant for all the traits included in the model. Most notably, stratifying the basic study population revealed approximately 30% more interactions masked in the physiological mixed study population. These results supports the notion that genome wide single-gene association studies generally will be futile. For a genome wide screening to be successful the populations should be physiologically stratified and at least two-gene interactions should be included.

C46. A powerful approach to detect parent-of-origin effects in whole-genome association scans of quantitative traits

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Parent-of-origin effect (POE) is a widespread genetic phenomenon extensively studied in model animals. For the genes exhibiting POE, the trait value in a heterozygous offspring depends on the parental origin of the alleles. There exist evidences that POE is important for such human traits as weight, type 2 diabetes, and others. For these traits the power of genome-wide association (GWA) analysis may be increased by incorporating POE into the analysis model.

To study POE in GWA scans of human quantitative traits, different variants of TDT-based methods may be applied. These, however, exploit only within-family variation in order to avoid population stratification bias. In homogeneous populations methods utilizing both within- and between-family variation, such as the measured genotype (MG) approach, are shown to have greater power. In MG analysis, genetic polymorphism is included as a fixed effect or covariate in a mixed linear model along with a polygenic component. To utilize POE information, we suggest using probabilities indicating parental origin as a covariate in the MG analysis. We suggest a fast approximation to the full MG analysis, which is suitable for the analysis of GWA scans and a method to estimate probabilities of parental origin in arbitrary complex pedigrees. Using simulated data, we compare our approach to the TDT-based methods. We show that our approach is more powerful. For many scenarios the sample size can be reduced two times when using our approach and the same significance level as by using the TDT-based methods is still achieved.

C47. Quantifying the increase in human individual genome-wide heterozygosity through isolate break-up and admixture

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Aim. The human population is undergoing a major transition from a metapopulation structure (subdivision in many relatively isolated communities) to a more admixed structure such as that which occurs in large cities. We attempted to quantify the magnitude of increase in average individual genome-wide heterozygosity (IGWH) that has occurred through isolate break-up and admixture.

Materials and methods. We sampled 100 examinees from 9 isolated villages on 5 Croatian islands, and an additional 101 immigrants into those villages. Out of the larger sample of 1001 examinees, we carefully selected 6 samples each of 23 individuals with a predicted increasing level of genome-wide heterozygosity, which was then measured studied using 1,200 STR markers. The first sample was from the most isolated island, the second from less isolated island, the third from the least isolated island, the fourth included individuals admixed between villages, the fifth immigrants from mainland cities and the sixth extremely outbred individuals.

Results: Relative to the mean IGWH estimate in the first sample, the increase in the percentage of the genome that is heterozygous in the remaining 5 samples was found to be: 2.3%, 3.8%, 5.8%, 6.7% and 7.9%. These differences are statistically highly significant ($p<0.0001$), and their gradual increase follows the predictions based on a priori hypothesis.

Conclusion. The human population is undergoing substantial outbreeding through breakup of isolate communities and rural-urban migration, with the potential to increase average genome-wide heterozygosity by up to 10%. The health consequences of this major change in genetic structure merit further study.

C48. CHROMSCAN: Genome-wide association mapping

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Many genome-wide association mapping studies using high density arrays of single nucleotide polymorphisms (SNPs) are underway. Typically these involve 100s to 1000s of DNA samples collected as case-control studies screened for several hundred thousand SNPs across the genome. Efficient analysis and interpretation of these vast data sets raises considerable difficulties. If significance is tested at individual SNPs, large numbers of false positives make interpretation difficult. Models which utilise information from multiple SNPs simultaneously offer advantages by reducing the number of tests and providing increased power. However, the reliable computation of significance levels remains an issue, given the high density of non-independent SNPs and consequent auto-correlation. Other concerns include the difficulty of incorporating information on the underlying linkage disequilibrium (LD) structure when testing association with disease. Including such information has been shown to substantially increase power and precision of mapping.

CHROMSCAN models association with disease in non-overlapping sliding windows and rapidly and robustly determines candidate regions and maximum likelihood locations and standard errors for putatively causal polymorphisms. The program employs a composite-likelihood based model, estimating a small number of parameters in each region, thereby reducing the number of tests made. A permutation test ensures the P-value distribution is not distorted due to autocorrelation caused by extensive LD. Association with disease is modelled on an underlying map in linkage disequilibrium units (LDUs). *CHROMSCAN*, which runs on UNIX and Linux platforms, is also implemented for parallel computing on either a Linux-based cluster.

C49. Screening for splice defects: application to *BRCA*1 and *BRCA*2 unknown variants (UV)

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Nearly half of *BRCA1* and *BRCA2* sequence variations remain of uncertain clinical significance (UV) and are candidates for splice alterations e.g. by creating cryptic splice sites. Since an out-of-frame splicing defect leads to severe reduction in the level of the mutant mRNA, we developped a cDNA-based test to evidence such allelic imbalance.

Seventy patients without *BRCA1/2* mutation and bearing consecutively identified UVs were included in the study. Following RNA extraction, DNAse treatment and RT-PCR, 3 exonic SNPs on both *BRCA*1 and *BRCA*2 were asked using a semiquantitative single-nucleotide primer extension approach.

Data were collected with an ABI3130XL then analysed using GeneMapper (Applied Biosystems). cDNA allelic ratios were corrected using genomic DNA ratios from the same sample. This approach was combined with *in silico* predictions using Splice Site Finder, Splice Site Prediction, MaxEntScan, ESE Finder and Rescue ESE.

Allelic imbalances were found in 4/21 and 11/49 cases for *BRCA*1 and *BRCA*2, respectively. However, the corresponding UVs did not show *in silico* pathogenic effects.

In order to determine UVs' impact on mRNA instability, sequencing of their cDNA flanking regions has started, using cDNA from cells treated with puromycin.

Analysis of the first 5 patients excluded UVs' involvement therefore complete cDNA sequencing was performed, evidencing in one case an out-of-frame deletion of *BRCA*2 exons 12 and 13. These preliminary results showed that allelic imbalance screening is a simple way to detect splicing defects but they also demonstrated that changes in allelic expression may be due to other cis- or trans-acting factors.

C50. HNPCC-associated missense mutations in MSH2 may lead to failure of the protein to bind mismatched DNA

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Inherited mutations in mismatch repair (MMR) genes, MLH1 MSH2, MSH6 and PMS2 predispose to hereditary non-polyposis colorectal cancer (HNPCC). A significant proportion of all identified MMR gene mutations are non-truncating, which complicates the interpretation of their clinical relevance.

We have previously conducted functional studies on non-truncating MSH2 mutations to clarify their role in the pathogenesis of HNPCC. In this study we characterized the biochemical defects of mutated MSH2 proteins in more detail, by assessing the ability of the mutated proteins to bind base-base mismatches, and to dissociate from mismatched DNA in vitro.

Wild-type and fifteen mutated MSH2 proteins, twelve of which had showed functional defects in our previous studies, were expressed in the baculovirus system together with his-tagged wild-type MSH6. The recombinant heterodimeric MSH2/MSH6 complexes were purified by fast protein liquid chromatography or partially purified on Ni-NTA columns. The heterodimers were then used in bandshift assays to examine their affinity for homo- and heteroduplex DNA. In addition, their ATP-mediated dissociation from the mismatches was assessed.

None of the proteins showed detectable binding to homoduplex DNA. Preliminary data showed that some of the mutants, also those defective in other functional assays, displayed mismatch binding comparable to the wild-type protein complex. Some failed to bind mismatches, and some weak binders could also be identified. The ATP-mediated dissociation from DNA was not impaired in any of the proteins analysed thus far. This study will provide additional information for interpreting the pathogenicity of HNPCC-associated missense mutations.

C51. MUTYH-associated polyposis (MAP): spectrum and frequency of extracolonic lesions

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MUTYH-associated polyposis (MAP) is a recently discovered autosomal-recessive precancerous condition of the colorectum which is caused by germline mutations in the base excision repair (BER) gene MUTYH. MAP is associated with a colorectal cancer lifetime risk of up to 100%, comparable to familial adenomatous polyposis. However, there are only sporadic descriptions of extraintestinal manifestations. Here we report on a systematic evaluation of the tumour spectrum in German MAP patients, based on medical records and anamnestic information. The study is part of a collaborative European trial performed together with two centres in the Netherlands and UK. To date, 83 biallelic German MUTYH mutation carriers were included. The median age at evaluation was 54 years (range 28-85). In 17% of the patients duodenal polyps are reported; 49% had extraintestinal lesions (10 patients had two, 6 had three different tumours), 23% had at least one extracolonic malignancy. The following tumours were observed more than once:

Tumour	Frequency	Age of diagnosis
Breast cancer	10% (4/42 females)	49-60
Endometrial cancer	5% (2/42 females)	32-54
Ovarian cancer	5% (2/42 females)	45-56
Skin cancer	5% (4/83)	32-68
Bladder carcinoma	2,4% (2/83)	45-62
Teratoma	2,4% (2/83)	28-33
Lipomas	8% (7/83)	30-50
Benign skin tumours	15% (12/83)	15-62

Compared to the age-related population-based tumour risk these preliminary data indicate an increased incidence of gynaecological cancer (endometrium, ovary) and cutaneous tumours in MAP patients. The risk of breast cancer is close to the age-related female population risk. The results may influence future surveillance recommendations and contribute to the understanding of the underlying pathophysiological mechanisms in MAP.

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C52. A genome-wide SNP screen for bowel cancer susceptibility alleles

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We present the results of a genome-wide screen using the Illumina Hap550 SNP arrays to search for susceptibility alleles for colorectal carcinoma. 1,000 familial cases and 1,000 controls from the UK Caucasian population were screened in stage 1 of this survey and we re-

port the findings of this screen, including SNPs that are significantly associated with increased risk of bowel cancer. These SNPs are now being tested in a phase 2 study involving 3,000 unselected cases and 3,000 controls. Further collaborative studies involving large sample sets will be needed to confirm that SNPs from our phase 1 and 2 data are truly associated with disease.

C53. Hereditary Diffuse Gastric Cancer (HDGC) patients and CDH1 mutations: a systematic review of the literature

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Worldwide, gastric cancer is the second cause of cancer-related death. Despite an overall trend for decrease, incidence of gastric cancer in young patients and cases with familial clustering remains stable. Despite being an uncommon disease, HDGC is a major health problem and extremely difficult to address in clinical and therapeutic grounds, due to its severity and unavailability of early diagnosis. A single gene was identified with a causative role in HDGC, E-cadherin. We performed a systematic review of *CDH1* mutation carriers clinical presentation and its association with frequency, localization and type of *CDH1* germline mutations. We collected information on 99 *CDH1* mutation carriers, described to date in the literature. The age of onset of *CDH1* mutation carriers varied between 16 and 73 years old (mean=41.8±14.4), and 75% of cases were diagnosed before 50. Male:female ratio was near 1.0. Although *CDH1* mutations were distributed along all gene sequence, exons 2,3,7 and 11 were preferentially affected. Most mutation carriers (87.9%-87/99) harboured *CDH1* truncating mutations. The type of mutations (truncating and missense) was significantly different between families with complete and incomplete criteria for HDGC, previously established by the IGCLC (p=0.0001). Carriers from families with complete criteria harboured preferentially truncating mutations (92%), while carriers from families with incomplete criteria harboured truncating and missense mutations in similar frequencies (50%). Moreover, truncating mutations carriers were significantly younger (40.5±14.3) than missense mutation carriers (50.7±12.4) (p=0.02). This systematic analysis is of crucial importance to help genetic counselling and to direct molecular analysis in suspected HDGC mutation carriers.

C54. The identification of (ETV6/)RUNX1-regulated genes in lymphopoiesis using histone deacetylase inhibitors in ETV6/RUNX1-positive lymphoid leukaemic cells

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Chimeric transcription factor ETV6/RUNX1 (TEL/AML1) is believed to cause pathological block in lymphoid cells development via interaction with corepressor complex and histone deacetylase. We wanted to demonstrate regulatory effect of ETV6/RUNX1 and its reversibility by histone deacetylase inhibitors (HDACi) and to identify potential ETV6/RUNX1-regulated genes. We used luciferase assay to demonstrate the interaction of ETV6/RUNX1protein, ETV6/RUNX1-regulated gene and HDACi. In order to identify ETV6/RUNX1-regulated genes we employed expression profiling and HDACi in the lymphoid cells. Next using the flow cytometry and qRT-PCR we measured changes in gene and proteins expression after HDACi treatment. Luciferase assay showed repression of granzyme B expression by ETV6/RUNX1 protein and reversibility of this effect by HDACi. Proving this regulatory role of ETV6/RUNX1, we used complex statistical analysis to identify 25 genes that are potentially regulated by ETV6/RUNX1 protein. In 4 selected genes with known role in the cell cycle regulation (JunD, ACK1, PDGFRB and TCF4) we confirmed expression changes after HDACi by quantitative analysis. After HDACi treatment, ETV6/RUNX1-positive cells showed immunophenotype changes resembling differentiation process compared to other leukaemic cells (BCR/ABL, ETV6/PDGFRB-positive). Moreover, ETV6/RUNX1-positive leukaemic cells accumulated in G1/G0 phase after HDACi while other B-lineage leukaemic cell lines showed rather unspecific changes including induction of apoptosis and decreased proliferation. Presented data support the hypothesis that HDACi affect ETV6/RUNX1-positive cells via direct interaction with ETV6/RUNX1 protein, and that treatment with HDACi may release aberrant transcription activity caused by ETV6/RUNX1 chimeric transcription factor.

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C55. Analysis of the genomic insertion sites of viral gene therapy vectors using next generation sequencing technologies.

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Viral vectors commonly used for somatic gene therapy can cause insertional mutagenesis, activating oncogenes in the patient, potentially leading to oncogenic transformation. After transduction, the viruses integrate into the genome, preferentially upstream of actively expressed genes. The potential therefore exists to alter the expression of oncogenic or tumor suppressor genes which occasionally leads to changes in stem cell self-renewal and oncogenic transformation. Clonal expansion of a cell with an integration event that promotes cell division, and possibly oncogenic transformation, leads to the dominance of a few (or single) integration events in hematopoietic progenitor cells.

By using LAM-PCR, DNA-sequencing and bioinformatic analysis, we can identify the viral integration sites in the genome. Because the integration site can differ in each cell, it can be difficult to identify all genes potentially influenced by the integration events. Whereas cloning and Sanger sequencing only allow identification of several hundred integration events, next generation sequencing methods allow the identification of ten thousands of individual integration events in parallel. The ability to identify more integration events early in the gene therapy treatment procedure, and follow changes in the distribution of cells having different integration sites, will allow to better assess the safety of somatic gene therapy, and develop new and safer viral vectors and transduction methods.

We present the results of a systematic insertion site analysis for a retroviral vector and show that the broad range of insertion sites found using next generation sequencing technology is a vast improvement over the traditional Sanger method.

C56. Correction of VLCAD deficiency and prediction of mutation severity with bezafibrate: how to kill two birds with one stone.

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We recently showed that fibrates could restore FAO in patient cells harboring inborn defects in Very-Long-Chain-AcylCoA-Dehydrogenase (VLCAD; mitochondrial β-oxidation), by stimulating residual enzyme activity. Given the variety of reported VLCAD gene point mutations, we investigated the response to drug as a function of genotype. 34 VLCAD-deficient fibroblast with distinct genotypes representing 50 different mutations were treated with 400µM bezafibrate for 72h and FAO was measured using tritiated palmitate. Untreated cells exhibited FAO rates much lower (-30 to -90%) than control. Bezafibrate induced a marked increase in FAO in 60% of the genotypes tested, and a complete correction in 15 cell lines. These data allowed to identify three groups: - severely deficient cells with nonsense mutations, or missense mutations affecting residues essential for catalysis (G222, G441, R469), that were drug-resistant -a 2nd group with missense mutations compatible with a moderate response to bezafibrate - a 3rd group which harbored genotypes compatible with a full restoration of FAO by bezafibrate, pointing to mild mutations (V283A, G441D, R615Q). We also characterized changes in VLCAD mRNA and residual enzyme activity levels induced by bezafibrate, as a function of genotype. The mutations were reported in a predictive VLCAD 3-D model allowing to confirm the mild or severe mutations that were characterized in the "bezafibrate test". The response to bezafibrate can therefore predict the severity of VLCAD point mutations that was not documented yet and might help to identify patients for a future clinical trial.

C57. RNAi-based allele specific silencing of the ataxin-7 gene in South African patients with SCA7

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RNA interference (RNAi) is a mechanism that occurs naturally in eukaryotes and mediates post-transcriptional gene silencing and has been shown to have application to treatment of many debilitating genetic disorders. One such disease is the polyglutamine disorder, spinocerebellar ataxia 7 (SCA7). SCA7 has a higher frequency in South Africa and has only been diagnosed here in black African patients to date. A recent study demonstrated that an exonic single nucleotide polymorphism (SNP) is tightly linked to the disease-causing $(CAG)_n$ expansion within *ataxin 7*. The aim of this project is to exploit the RNAi pathway to achieve specific knockdown of the mutant mRNA transcript of *ataxin 7*. A panel of 14 different Pol III U6 promoter-encoded short hairpin RNAs (shRNAs) that target the *ataxin 7* SNP were designed. Knockdown effects against mutant and wild-type targets linked to a luciferase reporter gene were measured in triplicate. Knockdown of the targets was measured in triplicate. Luciferase emissions were reduced in cell cultures containing the mutant target compared with those of the wild-type, although the differences were for the most part, small. However, one shRNA showed nearly 30% discrimination between the wild-type and the mutant target cell cultures. Unexpectedly, shRNAs with secondary mismatches abrogated discrimination and knockdown with one exception. These preliminary results indicate that discriminatory knockdown of *ataxin 7* can be achieved using a weak mismatch. Further shRNAs have been designed to maximise this initial discrimination. This investigation shows promise for development of a gene therapy-based approach to treating a disease of South African importance as well as other similar conditions.

C58. Nonsense-mediated mRNA decay regulates response of cystic fibrosis patients to gentamicin

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Aminoglycosides can readthrough premature termination codons (PTCs), permitting translation of full-length proteins. Previously we have found variable efficiency of readthrough in response to the aminoglycoside gentamicin among cystic fibrosis (CF) patients all carrying the W1282X nonsense mutation. Here we demonstrate that there are patients in whom the level of CFTR nonsense transcripts is markedly reduced while in others it is significantly higher. Response to gentamicin was found only in patients with the higher level. We further investigated the possibility that the nonsense-mediated mRNA decay (NMD) might vary among cells and hence governs the level of nonsense transcripts available for readthrough. Our results demonstrate differences in NMD efficiency of CFTR transcripts carrying the W1282X mutation among different epithelial cell lines, even derived from the same tissue. Variability was also found for β-globin transcripts carrying a disease-causing PTC as well as for five physiologic NMD substrates, RPL3, SC35 1.6 kb, SC35 1.7 kb, ASNS and CARS. Importantly, our results demonstrate existence of cells in which NMD of all transcripts was efficient, while others in which the NMD was less efficient. Downregulation of NMD in cells carrying the W1282X mutation increased the level of CFTR nonsense transcripts and enhanced the CFTR chloride-channel activity in response to gentamicin. Together our results show that the efficiency of NMD might vary and hence regulate the response to treatments aiming to promote readthrough of PTCs in many human genetic diseases.

C59. Harmless selection of genetically manipulated human stem keratinocytes

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Ex-vivo gene therapy of monogenic and recessively inherited genodermatoses prone to cancer require the selection of transduced epidermal keratinocytes in a manner compatible with skin graft perspectives. We have set up a selection system which aims at : i-preserving growth and differentiation potentials of transduced keratinocytes, ii-reduce the risk of immune response in grafted patients, iii-maintain sustained expression of the corrective gene. In this system, selection is based on ectopic expression of the small cell surface marker CD24 in proliferative keratinocytes. In human epidermis, CD24 is normally expressed in post mitotic, differentiated keratinocytes. Several primary strains of normal keratinocytes could be successfully transduced using a CD24-IRES-GFP MoMLV retroviral viral vector. CD24-selected cells could be passaged serially over more than one year, attesting the conservation of stem cell growth potential. Reconstruction of organotypic skin cultures using transduced cells, indicated normal differentiation and proliferation capacity. Transduced cells were grafted onto the nu/nu athymic mouse and regenerated a full thickness, normally differentiated epidermis, over a period of 20 weeks. Expression of the GFP reporter gene was maintained without attenuation. The encouraging results strongly stimulate our prospects of genetic correction of epidermal keratinocytes from patients suffering from the DNA repair deficient / cancer prone disease, xeroderma pigmentosum or for any other genodermatose candidate for ex vivo cutaneous gene therapy. In addition, our system now allows any application of long term and harmless gene transfer such as gene extinction or mutation expression in human primary cells.

C60. Development of liver disease despite mannose treatment in two patients with CDG-Ib

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Congenital disorders of glycosylation (CDG) result from a defect in N-glycosylation. Phosphomannose isomerase (PMI) deficiency is the only treatable CDG (CDG-Ib). The first clinical description of CDG-Ib was made by Pelletier et al. in 1986, as a lethal disease in 4 patients from the “Saguenay-Lac St-Jean” area in Quebec. Since this description, mannose therapy improved the general condition and the digestive symptoms in all reported patients as well as in ours.
We report here the 6 year follow-up of two patients with CDG-Ib treated with oral mannose, who were diagnosed at 2 months of age with digestive symptoms, liver involvement and hyperinsulinemic hypoglycemia. Both developed portal hypertension while general condition improved and other symptoms disappeared. #The development of the characteristic histological lesions of congenital hepatic fibrosis was observed despite an early onset of treatment and several years on mannose. In both patients, improvement of the transferrin profile was noted, although complete normalization was never observed. We can speculate that persistently abnormally glycosylated proteins interfere with the normal development of the liver, either by accumulation of an abnormal product, or more probably by a loss of function.
In conclusion, the efficacy of mannose might transform this lethal disease into a treatable one. However, in some patients this treatment does not seem to protect against the liver disease.

C61. Acetylcholinesterase deficiency with neuromuscular junction remodeling in a mouse model of Schwartz-Jampel syndrome

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Schwartz-Jampel syndrome (SJS) is a rare autosomal recessive disorder characterized by permanent and generalized muscle stiffness (myotonia), and chondrodystrophy. First symptoms appear during early childhood, and the disease is slowly progressive until adulthood. SJS is due to hypomorphic mutations in the gene encoding perlecan, a ubiquitous heparan sulfate proteoglycan secreted within basement membranes. We have developed a mouse model of SJS by a knock-in approach, introducing one missense mutation into the perlecan mouse gene by homologous recombination, to explore the pathophysiological mechanism of this human disorder.
Homozygous mutant mice were viable with unaffected life span, but showed a reduced growth and a distinct neuromuscular phenotype with delayed opening of the eyelids, and flexion of the hind paw when suspended by the tail. EMG recordings revealed a sustained bursting activity at rest. Histological analyses of skeletal muscles were suggestive of denervation-reinnervation events. Major modifications of NMJs with lack of pretzel-like shape and acetylcholinesterase deficiency were observed. However, *ex-vivo* electrophysiological analyses did not reveal abnormal synaptic transmission. Our results argue for the accuracy of our mutant mouse line as a model of the disease, demonstrate that the incomplete perlecan deficiency which characterized SJS is responsible for major modifications of NMJs, and suggest that acetylcholinesterase deficiency alone is not responsible for the muscle hyperexcitability observed in SJS.

C62. Trinucleotide repeat "big jumps" in DM1 transgenic mice: large CTG expansions, splicing abnormalities and growth retardation

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Trinucleotide repeat expansions are the genetic cause of numerous human diseases, including fragile X mental retardation, Huntington disease and myotonic dystrophy type 1. Disease severity and age-of-onset are critically linked to expansion size. Previous mouse models of repeat instability have not recreated large intergenerational expansions ("big jumps"), observed when the repeat is transmitted from one generation to the next, and have never attained the very large tract lengths possible in humans/patients. We now describe dramatic intergenerational CTG•CAG repeat expansions of several hundred repeats in a transgenic mouse model of myotonic dystrophy type 1, resulting in increasingly severe phenotypic and molecular abnormalities. Homozygous mice carrying over 700 trinucleotide repeats on both alleles display severely reduced body size and splicing abnormalities, notably in the central nervous system. Our findings demonstrate that large intergenerational trinucleotide repeat expansions can be recreated in mice, and endorse the use of transgenic mouse models to refine our understanding of triplet repeat expansion and the resulting pathogenesis.

C63. Recessive severe/lethal osteogenesis imperfecta is caused by deficiency of proteins comprising the 3-hydroxylation complex

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Osteogenesis imperfecta (OI) is well-known to be caused by dominant mutations in the genes that code for type I collagen, *COL1A1* and *COL1A2*. Collagen defects cause about 85% of OI cases. Mutations that alter the type I collagen primary structure delay helix folding and allow overmodification of the collagen helix by prolyl 4-hydroxylase, lysyl hydroxylase and glycosylating enzymes. We have discovered that essentially all cases of lethal/severe OI without a primary collagen defect, but with overmodification of the collagen helix, are caused by null mutations in *LEPRE1*, encoding prolyl 3-hydroxylase 1 (P3H1), or *CRTAP* (cartilage-associated protein), two members of a complex in the endoplasmic reticulum that 3-hydroxylates only α1(I)Pro986 in type I collagen. We identified 3 OI probands with null *CRTAP* mutations and 7 with null *LEPRE1* mutations. Five patients with P3H1 defects have a common *LEPRE1* mutant allele, which apparently originated in West Africa and is also present in African-Americans. All probands have defects in both alleles and heterozygous carrier parents. Proband mRNA and protein from the mutant gene is absent or severely reduced. Mass spectrometry demonstrated absent or residual hydroxylation of α1(I)Pro986. Interestingly, excess lysyl hydroxylation of proband collagen helices is comparable to that caused by defects in the primary structure of the collagen helix, suggesting that helix folding is delayed. Proband collagen secretion is moderately delayed but total collagen secretion is increased. These recessive null mutations of *CRTAP* and *LEPRE1* delineate a novel skeletal paradigm and demonstrate that the 3-hydroxylation complex is crucial for normal bone development.

C64. Left-sided embryonic expression of the BCL-6 corepressor, BCOR, is required for vertebrate laterality determination

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Oculofaciocardiodental (OFCD) syndrome is an X-linked male lethal condition encompassing cardiac septal defects, as well as ocular and dental anomalies. The gene mutated in OFCD syndrome, the *BCL-6 corepressor* (*BCOR*), is part of a transcriptional repression complex whose transcriptional targets remain largely unknown. We reviewed cases of OFCD syndrome and identified patients exhibiting defective lateralization including dextrocardia, asplenia and intestinal malrotation, suggesting that BCOR is required in normal lateral determination and that the frequent heart problems occurring OFCD syndrome may be due to defects in this process. To study the function of BCOR we used morpholino oligonucleotides (MOs) to knockdown expression of *xtBcor* in *Xenopus tropicalis*, thus creating an animal model for OFCD syndrome. The resulting tadpoles had cardiac and ocular features characteristic of OFCD syndrome. Reversed cardiac orientation and disorganized gut patterning was seen when MOs were injected into the left side of embryos, demonstrating a left-sided requirement for xtBcor in lateral determination in the frog. Ocular defects displayed no left-right bias and included anterior and posterior segment disorders such as microphthalmia and coloboma. Expression of *xtPitx2c* was shown to be down regulated when xtBcor was depleted, providing a mechanism by which xtBcor is required for lateral specification and an explanation of how BCOR mutation may disrupt cardiac septal development via the left-right laterality pathway.

C65. USF1 and Dyslipidemias; Insulin dependent expression of a transcription factor in muscle and fat results in adverse regulation of target genes

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We recently reported association in Finnish families of allelic variants of USF1 with FCHL, a common dyslipidemia predisposing to cardiovascular disease (CVD). This association with dyslipidemia has since

been replicated in numerous studies in different populations. However, evidence for the underlying mechanism how USF1 variants relate to dyslipidemia remains limited.
We here show for the first time, allele-specific differences in USF1 transcript levels in two relevant tissues. In muscle biopsies from 142 twins, following insulin challenge only subjects homozygous for the non-risk allele responded with significantly increased USF1 transcript levels, as measured by RT-PCR. Essentially the same phenomenon was observed in fat-biopsies of FCHL family members as insulin levels correlated with allelic imbalance. Quantitative sequencing of genomic- and transcribed RNA from subjects heterozygous for the best associating SNP, located in the 3'-UTR of USF1 revealed an average ~20% lower expression of the risk-allele in fat.
In a set of 47 expression arrays of fat-biopsies, differential expression of numerous USF1 target genes was evident -among them many genes of lipid metabolism and inflammatory-response. Interestingly, the neighboring F11R gene recently implicated in development of atherosclerotic plaques also showed allele-dependent expression, suggesting the presence of multiple, potentially co-regulated CVD associated genes in this chromosomal region.
In summary, common allelic variants, defined by non-coding SNPs of the USF1 transcription factor gene and associated with dyslipidemia risk, seemingly eradicate the insulin response of transcript levels. Subsequently, they result in differential expression of numerous relevant target genes predisposing carriers of risk alleles to dyslipidemia and CVD.

C66. The cohesion protein NIPBL recruits histone deacetylases to mediate chromatin remodeling

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Cornelia de Lange Syndrome (CdLS) is a rare malformation disorder with multiple congenital anomalies, a characteristic face, growth and mental retardation as well as gastrointestinal and
limb abnormalities. About 50 % of the patients with CdLS carry mutations in the *NIPBL* gene.
NIPBL encodes a homologue of the fungal Scc2-type and Drosophila Nipped-B protein and is part of the chromatid cohesion complex. Recent studies show an association of chromatid cohesion with chromatin-remodeling complexes, either in the recruitment of cohesion to particular sequences along chromosome arms or in the establishment of cohesion.
In yeast-two hybrid assays we could identify the chromatin remodeling factors histone deacetylases 1 and 3 (HDAC1 and 3) as potential NIPBL-interacting proteins. Using different fragments of NIPBL in liquid β-galactosidase assays, we could narrow down the interacting region for HDAC1 and 3 to a stretch of 162 amino acids (aa) within a highly conserved region of NIPBL which was predicted to be a HDAC-interacting domain by *in silico* studies. In luciferase reporter gene assays we could show that this HDAC-interacting domain of NIPBL fused to the GAL4-DNA-binding domain (GAL4-DBD) is able to repress reporter gene transcription. Moreover, cotransfections of HDAC1 and 3 enhance this effect significantly. To confirm whether this effect is based on histone deacetylation we used an antibody recognizing the acetylated histone 3 in chromatin immunoprecipitation assays and could identify specific deacetylation of histone 3 adjacent to the NIPBL-GAL4-DBD binding sites. Our data show that NIPBL is able to recruit chromatin remodeling enzymes mediating histone deacetylation.

C67. The Human Epigenome Project (HEP)

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Epigenetic processes play an essential role in biology with wide-ranging implications for human health and disease. To understand and harness these processes we need to read and interpret the epigenetic code with the same rigour and vigour that made reading the genetic code one of the greatest scientific achievements. To this end, a number of efforts have already been initiated of which the EU-funded HEP was among the first to be set up in 2000 with the aim to map one of the epigenetic marks, DNA methylation. On behalf of the HEP Consortium, I will present our findings to date, discuss some of the lessons learnt and give an outlook on how the data and technology may be used in an integrated (epi)genetic approach to common disease.
www.epigenome.org

C68. Mechanism of Alu integration into the human genome

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LINE-1 or L1 has driven the generation of at least 10% of the human genome by mobilising Alu sequences. Although there is no doubt that Alu insertion is initiated by L1-dependent target site-primed reverse transcription, the mechanism by which the newly synthesised 3' end of a given Alu cDNA attaches to the target genomic DNA is less well understood. Intrigued by observations made on 28 pathological simple Alu insertions, we have sought to ascertain whether microhomologies could have played a role in the integration of shorter Alu sequences into the human genome. A meta-analysis of the 1624 Alu insertion polymorphisms deposited in the Database of Retrotransposon Insertion Polymorphisms in Humans (dbRIP), when considered together with a re-evaluation of the mechanism underlying how the three previously annotated large deletion-associated short pathological Alu inserts were generated, enabled us to present a unifying model for Alu insertion into the human genome. Since Alu elements are comparatively short, L1 RT is usually able to complete nascent Alu cDNA strand synthesis leading to the generation of full-length Alu inserts. However, the synthesis of the nascent Alu cDNA strand may be terminated prematurely if its 3' end anneals to the 3' terminal of the top strand's 5' overhang by means of microhomology-mediated mispairing, an event which would often lead to the formation of significantly truncated Alu inserts. Furthermore, the nascent Alu cDNA strand may be 'hijacked' to patch existing double strand breaks located in the top-strand's upstream regions, leading to the generation of large genomic deletions.

C69. Evolution of mammalian gene expression promoted by retroposons

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Expression of eukaryotic protein-coding genes is strongly affected by specific sequence motifs which concentrate near transcription start sites (TSS) and bind transcription factors. These transcription factor binding motifs (TFBM) may arise in promoters by slow accumulation of base changes in a random sequence but recent data indicate that promoter TFBMs can also arise rapidly through insertion of mobile elements already having functional motifs or having sequences which may be converted to TFBMs by a small number of mutations. For example, single C to T transition at specific position can create functional estrogene-response element in human Alu retroposons. We studied abundance of retroposon-derived sequences in human and mouse promoters (-1000 to +200 bp segments relative to TSS) and found that >15000 of human promoters and >11000 of mouse promoters have Alu-derived or B1/B2-derived elements, resp. Global distribution of these retroposons in human and mouse chromosomes strongly correlates with clusters of CpG islands present in promoters of ~75% genes. In active genes CpG islands are not methylated but major fraction of retroposons, especially LINEs and LTRs, are heavily methylated. It is unknown how promoter CpG islands are protected from spread of methylation initiated at adjacent LINEs and LTRs. Human Alu and mouse B1 elements can be transcribed by RNA polymerase III because their internal promoters bind transcription complex TFIIIC. This complex can limit spread of repressive histone methylation in *S.pombe* and we suggest that Alu- and B1-associated TFIIIC sites have similar function in mammals.

C70. Prominent use of distal 5' transcription start sites and discovery of a large number of additional exons in ENCODE regions

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The aim of the ENCODE project (http://genome.gov/10005107) is to identify all the functional elements of the human genome. The pilot phase focuses on a specified 1% of its sequence. As part of this project, the GENCODE consortium by combining manual annotation with experimental validation, produced a high quality annotation to be used as the "reference set" by the ENCODE consortium.

To uncover further the complex architecture of the human transcriptome and potentially identify new gene elements (exons), we combined 5'Rapid Amplification of cDNA Ends in twelve adult human tissues and three cell lines with high-density 22 nucleotides-resolution tiling arrays. We identified previously unannotated and often tissue/cell line specific transcribed fragments (RACEfrags), both 5' distal to the annotated 5' terminus and internal to the annotated gene bounds for the vast majority (81.5%) of the tested genes. Half of the distal RACEfrags span large segments of genomic sequences away from the main portion of the coding transcript and often overlap with the upstream-annotated gene(s). Several lines of evidence suggest that these sequences correspond to bona fide exons. First, the 5' distal RACEfrags exhibit a statistically-significant trend to map in the vicinity of Transcription Start Sites identified using independent methods such as CAGE tags, 5'PETs, promoter mapping and/or hypersensitivity to DNAse. Second, the splice site strength of the novel exons appears as high as that of GENCODE UTRs and CDSs. Third, the transcripts that contain novel exons could be independently isolated. Fourth, these novel exons show some conservation in the mammalian lineage.

C71. Mapping of small RNAS (smRfrags) in the human encode regions

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The elucidation of the largely unknown transcriptome of small RNAs is of considerable interest. We report here the results of an analysis of the small RNAs (<50nt) in the ENCODE regions of the human genome.

Size fractionated RNAs (between 19 and 50 nts) from 4 different cell lines (HepG2, HelaS3, GM06990, SK-N-SH) were mapped using the forward and reverse ENCODE tiling arrays of 22 bp resolution. The small RNA probe was prepared by labelling a newly synthesized poly(A)tail.

Our smRfrags (small RNA fragments; ~7500 positive signals; top 1% signal) overlap only 8% of the exons of known genes (Gencode annotation), whereas the majority map to intergenic (34%) and intronic (53%) regions. As much as 18% of the 3' or 5' UTRs contain smRfrags. In addition, 9.6% and 16.8% of smRfrags in the 5'UTR regions overlap significantly with His.PolII.TAF250 and DNase I Hypersensitive sites respectively (compared to 5.3% and 9% expected). Interestingly, 19-26% of smRfrags show evidence of overlapping transcription on both strands. Approximately 4.8% and 1.7% of smRfrags in non-annotated regions overlap transfrags in HeLa and GM06990 cell lines respectively. We hypothesized that a fraction of the identified smRfrags corresponded to microRNAs. We tested by northern-blot a set of 30 high-likelihood predictions of potential microRNAs candidates that overlap with smRfrags. We validated 3 novel microRNAs (~20nt length); however most of the remaining candidates showed a larger band (~100nt) likely to be a microRNA precursor.

C72. Human miR-155 on chromosome 21 differentially interacts with its polymorphic target in the *AGTR1* 3'UTR - a mechanism for functional SNPs related to phenotypes

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Animal microRNAs (miRNAs) regulate gene expression through base pairing to their targets within the 3' UTR of protein coding genes. Single Nucleotide Polymorphisms (SNPs) located within such target sites can affect miRNA regulation.

We mapped the human and mouse SNPs from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) onto the 138 annotated entries for the human and/or mouse experimentally supported miRNA target sites from TarBase database (http://www.diana.pcbi.upenn.edu/tarbase.html). This mapping revealed only five human miR-target sites that harbor SNPs.

We further experimentally investigated one of these target sites, an hsa-miR-155 target site within the 3' UTR of the human *AGTR1* gene that contains SNP rs5186. Using reporter silencing assays, we show that hsa-miR-155 downregulates the expression of only the 1166A, and not the 1166C allele, of rs5186. Remarkably, the 1166C allele has been associated with hypertension in many studies. Thus the 1166C allele may be functionally associated with hypertension by abrogating regulation by hsa-miR-155, thereby elevating *AGTR1* levels.

We have also shown that miR-155that maps to chromosome 21. This could explain the observation that individuals with Down syndrome have reduced blood pressure compared to controls.

C73. Unravelling the diploid genome - The synaptonemal complex as a molecular machine.

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Generating haploid gametes from diploid germ cells is central to both genetics and reproduction. Around 10-15% of couples are classed as infertile at some point in time and the genetic contribution to this is thought to be about half. To expand our understanding of the basic biology and to provide new candidate fertility/infertility genes we have used transcriptional profiling to identify new genes involved in meiosis.

Two of these genes encode proteins of the Synaptonemal Complex (SC). In mammals including humans this structure, which holds the aligned sister chromatids together prior to recombination, is essential for the completion of repair of meiotic double strand breaks and for meiosis. Failure of this process in some individuals results in infertility.

We have modelled the effects of mutation of both of these genes (SYCE1 and SYCE2) by targeted mutagenesis in mouse. Heterozygous animals are fertile but homozygous null mutants are sterile with females producing occasional abnormal follicles and males showing a Stage IV arrest of spermatogenesis. At the chromosomal level homologues align but complete SC's are not formed, double strand break repair is incomplete and crossover is does not occur. Based on a detailed examination of the biochemical interactions between these proteins and other SC proteins coupled with immunocytochemistry in the mutant mice we propose a molecular basis for the zipping up of the Synaptonemal Complex.

C74. Low copy repeats: few make the most noise

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We demonstrated that some recurrent rearrangements of chromosome 8p such as the inv dup del(8p), the supernumerary analphoid der(8p) and the interstitial deletion of 8p23.1 are mediated by the two homologous segmental duplications (proximal and distal repeats: 8p-REPP and 8p-REPD) constituted by olfactory receptor gene clusters (ORGC). We also demonstrated that the t(4;8)(p16;p23) usually detected in Wolf-Hirschhorn individuals carrying the der(4) is a recurrent translocation mediated by two pairs of REPs, highly homologous to those at 8p23, that are located at 4p16. We demonstrate by *in silico* analysis that a 200 Kb portion of the 8p-REPs share over 95% identity with several other chromosomal locations, including 4p16, 11p15, 11q13, 3p12, 3q21, 16p13. We report a new series of constitutional rearrangements mediated by some of these segmental duplications and we confirm the mechanism leading to the t(4;8)(p16;p23) translocation in two new cases. We also demonstrate that the same mechanism is

involved in an apparently non-recurrent t(8;11)(p23.2;p15.5) translocation and we describe a cryptic 11q13 deletion mediated the same class of repeats. We also analyze the evolutionary origin of these segmental duplications and demonstrate that they originate from a sequence related to human chromosome 16p13. The genome of *M. mulatta* has only one copy of the region, but its sequence contains a 2.3 Kb inverted repeat localized to one end of the region generating the different duplications. This repeat is not found in non-primate species and may be the first step in the genesis of ORGC segmental duplications.

C75. Diagnostic DNA copy number screening in patients with unexplained mental retardation using whole genome tiling-resolution array CGH

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Genome-wide array based Comparative Genomic Hybridization (array CGH) using tiling path DNA microarrays was diagnostically implemented at our department in 2005. Since then we have tested 350 individuals with unexplained mental retardation. This is the largest clinically well-defined cohort analyzed by array CGH so far. We detected 51 DNA copy number alterations in genomic regions that are not known to harbor copy number variation and were able to proof *de novo* occurrence in the majority of these. In total 26 copy number losses and 25 copy number gains, varying in size from 0.1 to 12.4 Mb, were identified. Interestingly, the average size of the losses in our series was larger compared to the gains (2.5 Mb versus 1.7 Mb) and 20 cases showed alterations smaller than 1 Mb. Although the vast majority of all aberrations is non-recurrent, several recurrent aberrations have now been identified. The first and most frequent novel microdeletion identified by array CGH is characterized by a 600 kb deletion at 17q21.31 and is associated with a clearly recognizable phenotype[1]. The 17q21.31 region contains several low copy repeats that might act as recombination substrates for nonallelic homologous recombination and a common inversion polymorphism. Other loci at which recurrent genomic aberrations and a recognizable clinical phenotype were identified are 2q23.1 and 15q24.

In conclusion, array CGH technology considerably increases the diagnostic yield in patients with mental retardation and reveals novel recurrent microdeletion syndromes for which the frequency remains to be established.

1) Koolen, et al. Nat Genet 2006;38:999-1001.

C76. The chromosomal constitution of an embryo changes during preimplantation development

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Introduction: Preimplantation genetic screening (PGS) using single-cell FISH analysis is proposed for selecting euploid embryos in recurrent IVF failures. An additive value for this procedure is the ability to analyze early embryonic development at different stages, allowing to characterize post zygotic chromosomal changes.

Aim: To track post zygotic chromosomal changes during preimplantation development.

Materials & Methods: FISH analysis was performed on day 3 of development. For each embryo, 1-2 blastomeres were analyzed, using probes for 3 to 9 chromosomes (13, 15, 16, 17, 18, 21, 22, X, Y). Chromosomally normal embryos were transferred to the uterus on day 4 or 5. Aneuploid embryos were re-analyzed on day 5, using the same probe panels.

Results: A total of 52 aneuploid embryos (as determined on day 3), were reanalyzed on day 5. In 25 embryos (48%), the day 5 reanalysis confirmed the results observed on day 3. In 14 embryos (27%), „self correction“ of the chromosomal aberration was observed, in some or in all of the blastomeres. In 13 embryos (25%) different chromosomal aberrations were noted than those observed on day 3.

Discussion: PGS provides the opportunity to study the chromosomal constitution of preimplantation embryos. Our results demonstrate that embryos, designated as “aneuploid” on day 3, may demonstrate a high rate of mosaicism for chromosomal aberration. Some of these mosaic embryos are capable of developing into normal embryos by „self correction“. However, some embryos accumulate further chromosomal anomalies. These findings suggest that PGS results must be interpreted with caution.

C77. Multiple cryptic deletions are a common finding in “balanced” complex chromosome rearrangements: a study of seventeen cases.

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We report array-CGH findings in seventeen carriers of *de novo* complex chromosome rearrangements, involving from 3 to 8 chromosomes, all but one interpreted as balanced through conventional cytogenetic examinations. Thirteen of them were detected in individuals with abnormal phenotypes and mental retardation, two in normal females with repeated abortions, the remaining two in fetuses with normal ultrasonographic findings investigated for advanced maternal age.Two rearrangements, one present in one of the females with repeated abortions and the other in a male with mental retardation, were balanced. The remaining 15 cases had up to four deletions both at the breakpoints and elsewhere. All the deletions had occurred *de novo* as confirmed in all cases by the array-CGH performed in the parents and by the paternity tests performed in eight of the cases. Ten of these deletions could be tested for the parental origin and all turned out to be paternal. Using a customized array for seven patients we have narrowed the deletion breakpoints at few hundreds of bp; no peculiar motif of DNA sequences associated to the imbalance was detected. Our findings demonstrate that 1) phenotypic abnormalities, reported in half of the cases of apparently balanced de novo complex rearrangements are mainly due to cryptic deletions mostly but not exclusively at the breakpoints, 2) in order to exclude cryptic imbalance with impact on the phenotype, detection of a CCR in prenatal diagnosis requires array-CGH analysis and 3) male gametogenesis is more prone to create multiple chromosomal imbalances than female one.

C78. External Quality Assessment (EQA) in Europe

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The Forum of Cytogenetic EQA providers, assembled under the umbrella of the EuroGentest Network two years ago, agreed it would be desirable to establish a European EQA scheme independent of National schemes. It is not intended that this European scheme would replace existing schemes but rather provide opportunities for laboratories that have no access to a National scheme. Currently there are about 700 cytogenetic laboratories in Europe of which less than 50% participate in or have access to EQA.

CEQA (Cytogenetics European Quality Assessment) consists of a web-based EQA providing images for analysis which can be evaluated on line or downloaded to an image analysis system. This EQA has been developed to closely mimic the diagnostic situation and overcomes some of the limitations of other forms of EQA. It allows the participant to choose appropriate follow-up tests (for example, FISH)

to elucidate the chromosome abnormality. This is a major improvement on conventional distribution of slides or images where the inclusion of additional test material inevitably reveals the answer. Participants can submit reports in Czech, English, French, Finnish, German, Italian or Spanish. A prenatal and postnatal pilot EQA was completed by 19 laboratories from 18 countries. Seven laboratories had never participated in EQA before. The reports reflected the variation in interpreting and reporting cytogenetic results within Europe. Some poor performance was identified.
The pilot will be expanded to allow more laboratories to participate next year. Participation in EQA will improve the provision of genetic services for the benefit of the patient.

C79. Regulations and practices of genetic counselling in 38 European countries

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Background: As genetic tests are increasingly offered across the borders of the European countries, EuroGentest, a NoE aiming at improving the quality of testing, also aims at harmonizing the quality of genetic counselling.
Objective: To review what kinds of regulations and practices related to genetic counselling there are in different European countries.
Methods: An electronic survey was performed among the National Societies of Human Genetics in 29 countries and contact persons in the 9 countries where a Society could not be traced. The president or the board of the society, or a selected expert provided the answers to the questions about existing legislation, guidelines and generally applied practices of genetic counselling. The respondents also estimated how well genetic counselling is organized in their country and what future changes can be predicted.
Results: There is legislation related to counselling in 13 and guidelines in 21 countries. It was hoped that the amount of regulations would increase. The topics that were most often covered in legislation and guidelines, and also considered as generally applied practices, were counselling in the context of prenatal testing, informed consent, confidentiality, training of the person who performs counselling, and non-directiveness. The seldom-covered topics were counselling in the context of predisposition testing for multifactorial diseases, duty to re-contact the patient afterwards, and counselling persons from ethnic minorities. Of the respondents, 70 % considered that national regulations are necessary, and 90 % thought that some improvements are needed in the organization of genetic counselling in their country.

C80. Attitudes regarding genetic testing in minors. A survey of European clinical geneticists

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Background Various professional guidelines have discussed genetic testing in minors and provided recommendations on the topic. As we have described earlier (Borry et al. 2005, 2006) professional guidelines have shown to disagree on a various issues. Regarding carrier testing guidelines varied regarding (a) the role of genetic services in ensuring that children are informed about their carrier status and associated risks when they are older; (b) exceptions to the general rule of withholding or deferring carrier testing; (c) the communication of incidentally discovered carrier status. In the case of predictive and presymptomatic genetic testing ambiguity exists for childhood-onset disorders for which preventive or therapeutic measures are not available and for the timing of testing for childhood-onset disorders.
Objective The aim of our study was to gather information from clinical geneticists about their practices and attitudes with regard to carrier testing in minors.
Method 316 medically qualified specialists in genetics who have offered genetic counseling to patients in the last year were asked to complete a survey of items assessing their attitudes and practices regarding genetic testing in minors. The data collection took place between October 2006 and March 2007. All respondents completed a 28-item questionnaire. The measures were based on ethical issues related to genetic testing in minors identified in the literature and from previous research.
Results At this moment we have a response rate of 60%. We just started a preliminary statistical analysis of the data using non-parametric statistics. (22 February 2007) Results are planned in March and April.

C81. An operational checklist for evaluation of molecular genetic tests destined to clinical use: development and description.

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Background: The completion of the Human Genome Project has increased the pace of genetic markers of disease. Despite tremendous fundamental research efforts, clinical applications are still lagging behind expectations, partly due to the lack of effective tools to summarize published data relative to the clinical assessment of new diagnostic molecular tests.
Methods: We used a collaborative process using published tools and an expert panel to develop a detailed checklist of the evidence that needs be collected or produced to evaluate the potential usefulness of a new molecular diagnostic test in medicine.
Results: We present a checklist that allows 1)stakeholders to collect data related to a given molecular test and improve their decision making process and 2)researchers to summarize known evidence and direct research efforts towards studies to fill knowledge gaps. This checklist comprises 29 clearly defined items, grouped into 10 categories, including an overview of disease epidemiology and genetics, available diagnostic tools and their analytical and clinical performances, availability of quality control programs, methodological and clinical best practice guidelines, clinical utility, impacts on health care, psycho-social, ethical and legal aspects of the analysis. It also comprises a summary of the evidence available and the identification of research priorities to fill the knowledge gaps relative to the test.
Interpretation: This systematic checklist is intended to streamline collection of the available evidence to appraise the potential for clinical application of new molecualr diagnostic tests and help prioritize research to complete the evidence base relative to the clinical implementation of molecular tests.

C82. Public Perception of Genetic Medicine - A survey of attitudes in the United Kingdom

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The UK's North West Genetics Knowledge Park (Nowgen) sets out as one of its aims *to act as a forum for education, engagement and dialogue around human genetics that will empower people to make decisions about health management*. In August 2006, Nowgen commissioned a telephone interview based survey amongst a sample of 1006 subjects from around the United Kingdom. The survey questions were designed to establish public perception of the role genetics plays in health, how genetic medicine is seen in relation to genetic modification of food, the level of acceptability amongst the population to undergo a genetic test for health reasons and to gauge public expectations in relation to future genetics and healthcare.
The research carried out by polling organisation ICM is broken down into standard demographic categories of age, gender, social class and region, with the intention of providing an evidence base for Nowgen's continuing public engagement work.
The survey results indicated that just over 70 per cent of the popula-

tion considers that their genes significantly influence their health status whereas over 40 per cent feel that an individual's genetic makeup guides their behaviour. Just under half of the sample felt that heart disease and cancer are significantly caused by genes, with a third considering mental illness to have a high heritable dimension. The figure of 40 per cent who said that they would not consider having a pharmacogenetic test to see whether a medicine would work for them, poses some interesting questions for future public engagement work.

C83. Teaching genetics to medical trainees: grounding genetics in clinical practice at each level of training

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To highlight the relevance of genetics to the clinical practice of medical practitioners outside specialist genetics services, it is important to work with them to define where and how genetics impacts on their practice. This knowledge can then be used to define educational outcomes for trainees.

Genetics knowledge, skills and attitudes needed by medical students, general practice trainees and specialty trainees for their clinical roles have been defined by the UK NHS National Genetics Education and Development Centre in partnership with a number of groups. These have been used to develop genetics learning outcomes.

Learning outcomes for medical students were developed in consultation with UK medical school genetics leads based on topics previously agreed as important for medical students. These lay the foundation of core genetic concepts which can be built upon in later training. Learning outcomes for general practice trainees are outlined in 'Genetics in Primary Care', curriculum statement 6 of the Royal College of General Practitioner's new Training Curriculum. These learning outcomes are based on genetics items identified by GP trainees and trainers, programme directors and geneticists as important for general practice (N=60). Learning outcomes for specialty trainees are based on the findings of a similar needs assessment involving geneticists and specialty consultants (N=66).

The Centre has defined genetics learning outcomes relevant to clinical practice for different stages of medical training in the UK. These outcomes, available from www.geneticseducation.nhs.uk, may be a useful tool for those involved in genetics education across Europe.

C84. Implementation of a medical genetics distance education programme for registered nurses

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The South African National Department of Health Policy Guidelines for the Management and Prevention of Genetic Disorders, Birth Defects and Disability, recommended the development and implementation of a postgraduate distance-learning programme for primary health care nursing staff in South Africa. With reference to this, the aim of this study was to produce a Medical Genetics Education Programme (MGEP) incorporating a birth defects manual (BDM) to assess the knowledge and skills of Primary Health Care Registered Nurses, and to evaluate the results after offering a distance education course. The sample for the study was selected from registered nurses working in rural and semi-rural areas in South Africa. The instrument used was a pre-test/post-test questionnaire. Combined with distance learning, four contact days with lectures and "hands-on" workshops with "Recognising Birth Defects" as a consistent theme were held. *Pre-course* knowledge of the six groups was poor with an average of 48%. *Pre-course* skills, including the drawing and interpretation of a three-generation family tree scored an average of 4,5%. *Post-course knowledge* increased to an average of 75%, and *post-course* skills improved to an average of 86%. The long-term goal of this study is to produce at least one certificated "genetic specialist" nurse at each primary care facility, with the aim to improve management and care of children and families with genetic conditions. It is planned that after successful completion of the BDM, a two-week "hands-on" course in basic genetic counselling be offered and implemented.

Posters

Po01. Clinical genetics

P0001. A 17q21.31 microduplication (including *MAPT*) and a 2q22.3q23.1 microdeletion (including *ACVR2A*) detected by routine array-CGH analysis using 244k Agilent oligoarrays in two patients with mental retardation and dysmorphic features

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Two cases with *de novo* chromosomal imbalances detected by routine screening using 244k Agilent oligoarrays are presented. In case 1, a microduplication of 17q21.31 was found (approximately 0.5 Mb). The duplication is reciprocal to the recently described 17q21.31 microdeletion and includes the *MAPT* gene. The patient is a nine-year-old girl with severe psychomotor developmental delay. Her facial dysmorphism includes a low anterior and posterior hairline, a small nose with a flat root, a long philtrum, and small widely spaced teeth. In addition, she has microcephaly, abnormal fingers and toes, and hirsutism with very long hairs on her back. In case 2, a microdeletion of 2q22.3q23.1 was found (approximately 0.4 Mb). The deletion includes the *ACVR2A* gene, which has been associated with cranio-facial development in mice. The patient is a three-year-old girl. Her psychomotor development is approximately 12 months delayed. Her facial dysmorphism includes lateral extension of eyebrows, a low anterior hairline, long palpebral fissures, a high nasal bridge giving the impression of deep-set eyes, a bulbous nasal tip, a thin upper lip, and micrognathia/retrognathia. In addition, she is microcephalic, hypermobile and hypotonic. Beside the two patients an outline will be presented from seven months of routine CGH-analysis using 244k Agilent oligoarrays of patients with mental retardation and dysmorphic features.

P0002. Clinical and cytogenetic aspects in two children with trisomy 18

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Objective: The authors present two cases of trisomy 18 with different clinical and cytogenetic characteristics.

Material and methods: The children were admitted to the Department of Pediatric Neurology of the Clinical Hospital Al. Obregia, Bucharest for evaluation of a delayed psychomotor development. They were included in a large study, part of a national research program, which investigate the cytogenetic causes of MR in children. First case, an eight years old girl, showed: dysmorphic features, severe MR, hyperkinesia with self-injurious behavior. Second case, a four months old girl, displayed the clinical phenotype characteristic of Edwards syndrome (including dysmorphic features, failure to thrive, arthrogryposis, epileptic seizures, brain malformation). The children were investigated cytogenetically by karyotype with GTG-banding.

Results: In the first case, the cytogenetic investigation revealed a partial trisomy 18pter-18q21. In the second case, the diploid karyotype showed a duplication of the long arm of chromosome 18.

Conclusions: The phenotype of the patients with trisomy 18 can be different depending on the specific triplicated chromosomal region. For a better description of the sequences involved in the breakpoints in this two cases, we intend to extend the study by applying molecular genetic methods.

P0003. CYP21A2 genotyping in an Italian population with 21-hydroxylase deficiency: molecular characterization of 1050 alleles

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Steroid 21-hydroxylase deficiency (21OHD), an autosomal recessive disorder caused by mutations in the CYP21A2 gene and responsible for >90% of cases of congenital adrenal hyperplasia, presents a wide phenotypic spectrum ranging from a severe classical form (CL, 1/15000) with or without salt wasting, through to a mild nonclassical form (NC, 1/100), to a hyperandrogenic adult form. The active gene and the inactive pseudogene CYP21P are organized in a complex locus on chromosome 6p21.3. Most of the genetic lesions that cause 21OHD are deletions/conversions (often giving rise to chimeric CYP21P/CYP21 genes) or various gene conversion-type mutations resulting in CYP21 genes carrying one/multiple pseudogenic mutations. Mutations arising independently of the pseudogene are rare.

A molecular strategy analysis that combines specific PCR (designated to avoid allele dropout phenomena caused by pseudogene sequences and to discover a chimeric gene), sequencing of the entire CYP21A2 gene region (from nt-420 to nt+2907), as well as intragenic polymorphism segregation analysis, enabled us to characterize a total of 1050 alleles of: 383 21OHD patients (162 CL, 231 NC) and 136 hyperandrogenic subjects with 98.8% of mutation detection. Mutations were confirmed in the parents in 85% of the cases. Single/multiple pseudogene-derived mutations account for 93.2% of the alleles, whereas 5.6% carry a rare/new mutation, mostly missense mutations affecting a conserved residue. Functional studies of 3 new mutations confirmed their implication in the phenotype observed. The genotype/phenotype relationship was generally good. Further studies will focus on rare variants in the non coding regions associated with mild forms.

P0004. A boy with 3q29 microdeletion and congenital mitral valve stenosis

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We present an 8 year old boy with congenital mitral valve stenosis and mental retardation. Clinical findings: progressive microcephaly, epicanthus, antimongoloid eye shape, oedematous eyelids, prominent nasal tip, irregularly and widely spaced teeth. He has hypotonic musculature with pronounced joint laxity both elbows. He has overriding 2nd toes, fetal pads toes and fingers. He is developmentally retarded. He started to walk at 23 months of age. His language is severely delayed, and he has autistic-like features.

Array CGH using CytoChip™ (BlueGnome Ltd.) revealed an interstitial microdeletion of chromosome 3q29. This microdeletion is reported in only 7 patients worldwide. The clinical phenotype in the reported cases is variable despite an almost identical deletion size. It includes mild/moderate mental retardation and progressive microcephaly with mildly dysmorphic facial features (long and narrow face, short philtrum and high nasal bridge).

The phenotype in our patient is very similar to the other cases, but mitral valve stenosis is not reported in the 7 other patients. However, early death (at 3mo-5y) caused by cardiac events has been reported in some of the other patients with 3q29 microdeletion, and terminal deletions of chromosome 3q are associated with congenital heart defect. The microdeletion encompasses several genes including PAK2. This gene is the autosomal homologue of a known X-linked mental retardation gene (PAK3), and might be the cause of mental retardation in the patients. The specific gene causing the heart defect reported in our patient is unknown. Our patient's phenotype broadens the clinical spectrum of the 3q29 microdeletion syndrome.

P0005. Subtelomeric 6p deletion: clinical and array-CGH findings in two patients

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Approximately 30 patients with subtelomeric 6p deletion have been reported. A recognizable phenotype, including hypertelorism, downslanting palpebral fissures, flat nasal bridge and dysplastic ears, has been delineated. In addition to variable mental retardation and potential hearing deficit, there is a distinctive malformation pattern, which comprises anterior eye chamber malformations, posterior fossa/cerebellar anomalies, and heart defects.

Patient 1 is an 8-month-old female born with normal growth parameters, typical facial features of 6pter deletion, bilateral corectopia, and protruding tongue. She has severe developmental delay, profound bi-

lateral neurosensory deafness, poor visual contact and hypsarrhythmia from 6 months. Patient 2 is a 5-year-old moderately mentally-retarded boy born with normal growth parameters and unilateral hip dysplasia; he has a characteristic facial phenotype and bilateral embryotoxon.
Routine karyotypes were reported as normal. Subtelomeric MLPA or multi-FISH and parental testing revealed de novo 6pter deletions. Microarray testing (Agilent Human Genome kit 244K) showed that patient 1 has a 7.8 Mb 6pter-6p24.3 deletion and a contiguous 5.5 Mb 6p24.3-6p24.1 duplication. Investigations to explain the origin of the duplicated segment are ongoing. Patient 2 has a 5.7 Mb 6pter-6p25.1 deletion, partially overlapping that of patient 1.
Few 6p subtelomeric deletions have been characterized by microarray analysis and at present it is not possible to make accurate conclusions concerning genotype-phenotype correlations. The identification of the contiguous duplication in patient 1 may provide a clue to the mechanism of the deletion.

P0006. Molecular analysis of ABCA4 and CRB1 genes in one mixed Spanish family segregating Stargardt disease (STGD) and Leber Congenital Amaurosis (LCA)

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Stargardt disease (STGD) is the most common juvenile macular dystrophy, characterised by central visual impairment. All recessively inherited cases are thought to be due to mutations in the ABCA4 gene. Leber Congenital Amaurosis (LCA) is the most severe inherited retinopathy with the earliest age of onset. LCA has been associated with mutations in ten genes, being CRB1 the most mutated gene in Spanish population. The aim of this study is to describe one mixed pedigree segregating two different retinal dystrophies, STGD and LCA.
This family was identified through the course of a conventional mutational screening performed on 223 Spanish families. Mutational analyses were performed by screening on genotyping microarray, dHPLC and direct sequencing.
The patient with STGD was homozygous for the p.Asn1805Asp mutation in the ABCA4 gene. Therefore, the involvement of ABCA4 in the etiopathogenesis of the STGD patient was clear but not for the case of LCA. Further analyses showed that the LCA patient was double heterozygous for the p.Cys948Tyr and p.Trp822ter mutations in the CRB1 gene, the second mutation was found in our laboratory. These mutations are consistent with her retinal phenotype.
A correct ophthalmologic examination is necessary for providing accurate clinical diagnoses to the patient and therefore an appropriate molecular analysis can be concluded. Therefore, identifying the mutation responsible of the disease is helpful for confirming diagnosis and counselling. Note that in one family different phenotypes and genetic causes can coexist, so it is very important to study all affected members.

P0007. A case of achondrogenesis in Kaunas Medical University Hospital

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Achondrogenesis is a rare disorder subtyped according to radiologic and histopathologic characteristics. The are three subtypes of achondrogenesis: achondrogenesis type 1A , achondrogenesis type 1B and achondrogenesis type 2 .Within the achondrogenesis group, clinical and radiologic distinction between subtypes of achondrogenesis is not always possible.
We are presenting a case of achondrogenesis type 2/hypochondrogenesis in Clinics of Kaunas University of Medicine. The diagnosis was confirmed by clinical, radiological and histological data. The patient was born from the first unaffected pregnancy, 36 weeks gestation age. The prenatally preformed ultrasound detected polyhydramnion, regression of normal genesis of sacrococcygea. Newborn was born hypotrophic, with dysplastic signs: large head, flattened face, small narrow eyes openings, short neck and displastic ears. Some skin areas had blisters. After a few day patient dead of pulmonary insufficientia. The radiological data was a marked delay in bone maturation with severe vertebral dysplasia, some metaphyseal involvement, and severe epiphyseal delay. There was no ossification of the pubis or ischiadic bones. The histology shows a conspicuous lack of cartilage matrix with ballooning and vacuolization of chondrocytes.
All data indicate that the fetus had achondrogenesis type 2/hypochondrogenesis. The monitoring with ultrasound was decided to perform in case of another pregnancy in this family.

P0008. Acro-oto-ocular syndrome: a malformation disorder with psychiatric features

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Acro-oto-ocular syndrome (OMIM #264475) is an autosomal recessive disorder characterized by mixed hearing loss, pseudopapilledema, malformation of the face, ears, hands, and feet. We extend the phenotype to include psychiatric symptoms. The propositus was born to consanguineous parents and had minor facial and limb anomalies. His facial features were remarkable for a high prominent forehead with bitemporal narrowing, broad nasal base, malar hypoplasia, and blepharophimosis. His ears, which had thick over-folded helices, were small, posteriorly rotated, and low set. His mouth had a high arched palate with crowded dentition, shallow eruption of the teeth from the gingiva, and abnormally shaped molars. His hands and feet were distinctive. He had unusual palmar creases, marked fifth finger clinodactyly, broad fingertips, mild soft tissue syndactyly of fingers 3 and 4, and mild hypoplasia of the thenar, hypothenar, and inter-digital areas. He had brachydactyly of his toes, increased space between the first and second toes, mild soft tissue syndactyly and camptodactyly of toes 2-5, valgus deviation of the halluces, and bilateral pes planus. Additionally he displayed bilateral gynecomastia, diffuse café-au-lait spots, and ataxia as well as moderate global developmental delay. However, in contrast to previously described individuals, he presented with auditory hallucinations and behavioral problems. Our observations confirm the previous hypothesis that psychiatric features are a component of Acro-oto-ocular syndrome. To better understand the underlying pathophysiology and phenotypic spectrum, we seek other patients with this disorder.

P0009. Wide clinical spectrum of Adams Oliver syndrome in a new family

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Introduction: Adams-Oliver syndrome (AOS) is a rare congenital disorder, characterized by scalp defects and distal transverse limb reduction anomalies.
Case presentation: a 14 month-old boy, the 2nd child of phenotypically normal parents, who was born at 36 weeks' gestation, SGA. Current clinical examination shows growth retardation and central hypotonia. The patient presents an area 2x4cm of hairless atrophic skin, large anterior fontanel, nail dystrophy of hands and feet, camptodactyly of the right thumb and 3rd finger, and left inguinal hernia. X-rays show a variety of hypoplasia or aplasia in different phalanges in thumbs and in fingers of the hands and feet. Cardiac sonogram reveals a wide atrial septal defect. Brain MRI shows bilateral asymmetrical dilatation of lateral ventricles, periventricular leukomalacia, and hypoplasia of corpus callosum. The karyotype is normal. The father has a hairless scar over his posterior scalp. Father's father presented cutis aplasia and nail dystrophy.
Discussion: Variable clinical expression of the AOS syndrome has been reported in the literature. In our patient the combination of aplasia cutis congenita, distal limb anomalies and transverse dystrophy of the nails indicated the diagnosis of AOS. A vertical transmission is recognized in this new AOS family, which is consistent with autosomal dominant inheritance. Our patient presents cardiac defects, which are present in approximately 20% of cases. Some affected children have intracranial anomalies including hypoplasia of corpus callosum, or periventicullar leucomalacia, such as in our patient. This may be the result of abnormal small vessel structures manifesting in embryogenesis.

P0010. Adrenal hypolasia congenita (AHC) in monozygotic twins-a contiguous gene syndrome

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Mutations in the DAX1 (NR0B1), which is encoded in the Xp21 chromosomal region, can lead to adrenal insufficiency with glucocorticoid and mineralocorticoid deficiency. These mutations also cause hypogonadotropic hypogonadism, which is consistent with the expression of DAX1 in the hypothalamus and pituitary.

DAX1 belongs to the nuclear receptor family and acts with a number of transcription factors in a transcription regulatory network under its control.

In some patients the deficiency is part of an Xp21 contiguous gene deletion The deletion extends proximal and distal to the DAX1 gene. (Gene order Xper...IL1RAPL1- DAX1-GK_DMD... Xcen.)

Usually there is considerable phenotypic variability associated with DAX1 mutations even within the same family. This is due to genetic and environmental influences.

We report on monozygotic twins with a contiguous gene syndrome, who presented with an identical clinical course. The male twins were born to a 23 years old mother with low normal IQ. Caesarean section was performed at 36/6 weeks (BW 1st twin 2580g, BW 2nd. twin 2090g)

Both twins developed a moderate respiratory distress syndrome and were on CPAP for 4 days. Within the second week of life they presented with feeding problems, lethargy and weakness - adrenal insufficiency was diagnosed. The adrenals were severeley hypoplastic on ultrasound.

Genetic investigation revealed a large deletion of at least 3Mb comprising the IL1RAPL1, DAX1, GK (Glycerol kinase), and the distal part of the DMD gene. The deletion is one of the largest ever reported.

P0011. Molecular analysis in two siblings African patients with severe form of Hunter syndrome: Identification of a novel (p.Y54X) nonsense mutation

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Hunter syndrome (or Mucopolysaccharidosis type II, MPS II) is an X-linked recessive disorder due to the deficiency of the iduronate-2-sulfatase (IDS) enzyme, resulting in the accumulation of heparan and dermatan sulfates in the lysosomes. The heterogeneity of clinical phenotypes, ranging from mild to severe forms, is a result of different mutations in the IDS gene. We report here a novel nonsense mutation (p.Y54X) in two siblings MPS II African patients affected with a severe form of the disease. We postulated that the p.Y54X mutation which causes a loss of the IDS region highly conserved among sulfatase enzymes, could be predicted as a severe disease-causing mutation for Hunter syndrome.

P0012. Alpha-Thalassemia prevalence and hematological findings in Iranian population

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Thalassemia is the most prevalent monogenic disorder in the world. This paper reports our experience of molecular screening of α-thalassemia as a part of thalassemia prevention program in Iran. To determine statues type (α or β thalassemia) we performed a total of 750 couples at risk for thalassemia. Those couple with low MCV (<80), low MCH (<27) and normal A_2 are most probably α-thal carriers. To investigate α-globin genes mutations we can either test for deletions or point mutations. Deletions can be detected by Multiplex gap PCR and point mutation by ARMS PCR or DNA sequencing.

DNA samples are usually tested for deletions like 3.7, 4.2, 20.5kb and Med deletions by multiplex PCR. The samples subjected to point mutation tests such as point mutation in polyA (2 types), Codon19, 5Nt Del, Constant Spring, if no deletion detected in previous tests.

In 20% of the cases tested the CBC values were normal after iron treatment .Deletions and point mutations were found in reaming 70% and in 10% of the cases no known point mutations or deletions were found our. Further investigation should be performed to determine the type of mutations in these samples. In cases where MCV were >75 and MCH>25 .only could see a single gene deletion or point mutations(e. g. 3.7 del,4.2 del , 5Nt del or Codon19).For two gene deletions or point mutations (i.e. 20.5 and Med deletion) MCV and MCH were always less than 75, 25 respectively.

P0013. Late diagnosis of alpha-mannosidosis in four adult patients.

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Alpha-mannosidosis is a rare autosomal recessive lysosomal storage disease characterised by accumulation of oligosaccharides in various tissues. A continuum of phenotypes has been described ranging from severe infantile forms through milder forms with survival into adulthood.

We report four individuals (18- to 38-year-old) from two unrelated and non consanguineous Caucasian families.

In the first family a brother and sister share common features: mild mental retardation, late deafness, malar hypoplasia and minor radiographic skeletal abnormalities.

Although the brother and sister have similar IQs, the girl's clinical history reveals earlier and more severe onset of neurodevelopmental retardation. At the time of examination the patients do not have characteristic coarse features.

The girl of the second family has skeletal symptoms including focal lytic vertebral lesions on X-Rays, mild mental retardation and malar hypoplasia. She had early hearing loss. Her brother has severe developmental delay in addition to progressive cerebellar dysfunction. He is living in an institute and was unavailable for examination.

In all individuals the diagnosis of alpha-mannosidosis was based on reduced leucocyte alpha-mannosidase activity and confirmed by molecular study of the *LAMAN (MAN2B1)* gene in the first family.

This report brings out the wide variability in age of onset, rate of progression and severity of this storage disease. Owing to unusual clinical course or absence of striking symptom pattern, alpha-mannosidosis may be under diagnosed. Consequently, one should consider the possibility of alpha-mannosidosis, especially when confronted with progressive deafness in adults with mental deficiency.

P0014. Further evidence that D90A mutation is recessively inherited in ALS patients in Southern Italy

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Amyotrophic Lateral Sclerosis (ALS), a fatal adult-onset motor neuron degeneration is caused by mutations in the Cu/Zn superoxide dismutase (SOD1) gene. All the SOD1 mutations are autosomal dominantly inherited with the exception of D90A, very frequent in Scandinavian population, that can act as recessive. Only few cases of D90A heterozygous ALS in non-Scandinavian patients have been reported, all of them inherited as dominant trait. In Italy, only two sporadic ALS cases carrying the D90A mutation have been reported in homozygous state. The aim of this study is to investigate the presence of D90A mutation in ALS patients in Southern Italy. One hundred and fifty-four ALS patients (8 familial and 146 sporadic cases) from Southern Italy were screened for SOD1 mutations in exon 4 by standard procedures. In our study, of 154 cases investigated three ALS patients with mild phenotype showed the homozygous D90A mutation in SOD1 gene. Two out of three patients were familial cases and the remaining patient was an

apparently sporadic case. We also screened the available members of one of the FALS cases. In this family we identified an affected and an unaffected individual carrying the D90A mutation in homozygous and heterozygous state respectively. According to previous data that reported for all D90A homozygous ALS patients a phenotype characterized by slow progression of the disease, also our patients show a mild phenotype with a prolonged survival. In conclusion, our study provides further evidence that D90A is an autosomal recessively inherited mutation in ALS patients in Southern Italy.

P0015. High level of hypermetabolism in patients with familial amyotrophic lateral sclerosis

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An abnormally elevated level of resting energy expenditure (REE, measured by indirect calorimetry) has been reported in a subset of patients with amyotrophic lateral sclerosis (ALS). Hypermetabolism (measured REE/calculated REE ≥ 1.1 or 110%) was found in 42/62 ALS patients in a previous study. Interestingly, hypermetabolism has also been observed in transgenic mice harbouring ALS-causing mutations in the *SOD1* gene. We tested whether patients with familial ALS (FALS) had a REE level differing from patients with sporadic ALS (SALS). Eleven patients with FALS (from ten different families, all negative for the screening of *SOD1* mutations by direct sequencing) who had performed an indirect calorimetry in our centre during the last seven years were compared with 33 SALS patients matched for age and sex. 11/11 (100%) patients with FALS were hypermetabolic, compared with 17/33 (52%) patients with SALS (p=0.009). The mean level of hypermetabolism was significantly higher in FALS patients (124±8%) than in SALS patients (113±12%, p=0.01). Subjects with FALS are supposed to carry mutations or strong genetic risk factors predisposing to the disease, which may also be responsible for the higher REE observed in these patients. In the absence of infection, inflammation or hyperthyroidism, the observed hypermetabolism is likely to result from mitochondrial uncoupling. Several other lines of evidence support the occurrence of a mitochondrial dysfunction in the course of ALS. Our results suggest that this mitochondrial dysfunction may be genetically driven in patients with FALS and could therefore be directly involved in the pathogenesis of FALS.

P0016. Phenotypic diversity in androgen insensitivity syndrome: About two families

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The androgen insensitivity syndrome (AIS), a rare X-linked disorder caused by defects in Androgen Receptor (AR). Variable phenotypic expression has allowed the classification of AIS into complete and partial forms. Mutational screening of the AR gene have revealed over 300 mutations.

Here, we describe pedigrees of two families with three affected subjects. Pedigrees patterns were consistent with X-linked recessive inheritance. Index cases were referred to our consultation at adult age for genetic counselling.

At the first family, a 28 year old female consults because primary amenorrhea and hirsutism. She had a blind vaginal pouch without uterus. Abdominal surgery with bilateral gonadectomy was done at age 18 years but histology report was not available. Two nieces have an inguinal hernia with an apparently female phenotype. Karyotype of 3 patients reveal a 46,XY formula and diagnosis of Complete AIS was done.

At the second family, a 31 and 32 year old cousins had under masculinised external genitalia, pseudofemale hairless and breast development. Gonads were absent even after chirurgical exploration. The nephew of the first patient has ambiguous genitalia. A male karyotype was revealed for the three cases and diagnosis of incomplete AIS was done.

AR molecular investigation are conducted. Correlation between the phenotypic features and the abnormalities identified on mutational analysis of the AR gene will be discussed.

We emphasize through this report the need for accurate and early diagnosis of AIS which govern bearing on the sex of rearing, genetic counselling, and subsequent management.

P0017. Androgen receptor gene mutations in 46, XY females

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The androgen insensitivity syndrome is a heterogeneous disorder with a wide spectrum of phenotypic abnormalities, ranging from complete female to ambiguous forms that more closely resemble males. The primary abnormality is a defective androgen receptor protein due to a mutation of the androgen receptor gene. This prevents normal androgen action and thus leads to impaired virilization. A point mutation of the androgen receptor gene affecting two siblings with complete androgen insensitivity syndrome is described. On examination they both had normal external female genitalia.

Genomic DNA was extracted from EDTA-preserved blood samples and isolated according to standard procedures. The androgen receptor gene was screened for mutations using an automated sequence analyzer (ABI Prism 310). Both girls possess one substitutions (G>A at position 2086 in exon 4), leading to D695N mutation. Mother was found to be a heterozygous carrier for this mutation. GTG banded karyotype of the girls showed they both have male karyotype (46, XY). In addition, the SRY gene screening showed they both have intact SRY gene. The labioscrotal folds contained palpable gonads measuring 1.5 cm in largest diameter. Ultrasound examination of the pelvis revealed absence of the uterus.

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone values were higher than normal range.

To our knowledge this is the first confirmed instance of AIS due to an AR mutation occurring in familial cases in this country. Furthermore, the phenotype has complete association with this mutation.

P0018. Aniridia in a three-generation family

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Background: Aniridia is a rare development eye anomaly.As an isolated ocular abnormality, aniridia is an autosomal dominant disorder. Clinical phenotypes of aniridia are associated with PAX6 mutations. We report a case of aniridia in a three-generation family in which the proband has a severe ocular phenotype similar to her maternal grandmother. Objectives: to describe and compare clinical manifestations of aniridia in younger and older generations; to study the intrafamilial variability of aniridia and management implications. Patients and Methods: A three-generation Caucasian family with aniridia was investigated. Four of the family members (a one-year-old girl, her mother, her maternal aunt and her maternal grandmother) were affected and expressed variable ocular phenotypes. Evaluation included physical examination and a detailed medical history and family history. Genomic DNA was isolated from affected individuals (clinically diagnosed aniridia) and analyzed by PCR. Results: The clinical expression of aniridia was variable in this family: bilateral complete or partial aniridia, unilateral complete aniridia and iris coloboma. Aniridia was associated with other ocular defects: cataract, glaucoma, nystagmus, amblyopia and strabismus. The family pedigree showed an autosomal dominant mode of inheritance with complete penetrance and variable expressivity. The patient and her grandmother shared similar phenotype (more severe than their relatives). All cases were defined by clinical signs. All cases had the same PAX6 mutations.Conclusions: aniridia appears as a hereditary condition with clinical variation; molecular-genetic data should be integrated with the corresponding clinical findings; all patients with aniridia should be evaluated by an ophthalmologist and a geneticist.

P0019. SOX2 anophthalmia syndrome: point mutations, large deletions and a broader phenotype.

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Developmental eye anomalies including anophthalmia or microphthalmia (AM) occur in around 2-3 per 10, 000 children and are responsible for approximately 25% of childhood severe visual impairment. Heterozygous mutations in the SOX2 gene, a SOX1B-HMG box transcription factor, have been identified in around 10% of individuals with severe microphthalmia or anophthalmia associated with a range of non-ocular abnormalities, principally motor, speech and developmental delay, seizures, and hypothalamo-pituitary abnormalities. SOX2 is therefore the most common causative gene for AM identified to date. We have screened a new cohort of 95 patients with congenital eye abnormalities, mainly anophthalmia, microphthalmia and coloboma, for SOX2 mutations. Ten cases with mutations were identified, 5 with intragenic mutations creating premature translational stop codons and 5 with deletion of all or part of the SOX2 gene. The 5 deletion cases were identified by MLPA analysis of a subgroup of 47 patients with severe AM. Four were deleted for the whole SOX2 gene and one had a partial gene deletion. In two of these FISH analysis identified sub-microscopic deletions involving a minimum of 328Kb and 550Kb of DNA. The SOX2 phenotypes include a patient with anophthalmia, oesophageal abnormalities and horseshoe kidney, and a patient with a retinal dystrophy implicating SOX2 in retinal development. Our results provide further evidence that SOX2 haploinsufficiency is a common cause of severe developmental ocular malformations. Given the high incidence of whole gene deletion we recommend that all patients with severe microphthalmia or anophthalmia are screened by MLPA and FISH for SOX2 deletions.

P0020. Additional defects in congenital anorectal malformations

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Patients with congenital anorectal malformations (CAM) often have other associated congenital defects. The reported incidence and the types of associated malformations vary between different studies. The purpose of this investigation was to assess the prevalences at birth of associated malformations in patients of a geographically defined population with CAM which were collected between 1979 and 2003 in 334,262 consecutive births. Of the 169 patients with CAM during the study period, 45.0% had associated malformations.Patients with associated malformations were further classified into groups with chromosomal abnormalities,and malformation syndromes,including Townes-Brocks syndrome,Klippel Feil syndrome,Di George syndrome;sequ ences,including OEIS,Pierre Robin and Prune belly sequences;and associations including VATER(13 patients) and MURCS associations, Malformations of the urogenital system and of the skeletal system were the most common other anomalies in multiply malformed patients without recognized syndromes, followed by malformations of the cardiovascular system,the digestive system,and the central nervous system. Weight, length, and head circumference of children with CAM and multiple associated malformations were lower than in controls, as was the weight of the placenta. Prenatal diagnosis by fetal ultrasonographic examination was rarely done in isolated CAM. However, even in multiple associated malformations, prenatal diagnosis by fetal ultrasonographic examination had a low sensitivity, 33.9%.In conclusion the overall prevalence of malformations, which was close to one in two infants, emphasizes the need for a thorough investigation of patients with CAMs. A routine screening for other malformations may be considered in patients with CAM, and genetic counseling seems warranted in most of these complicated cases.

P0021. *SBDS* gene mutations predispose to acquired aplastic anemia by causing telomere shortening of leukocytes

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Aplastic anemia (AA), defined as low blood cell counts and a hypocellular bone marrow, can be either acquired or constitutional. Common to both types are short-for-age telomeres, and mutations to the telomerase complex underlie dyskeratosis congenita and some cases of acquired AA. Shwachman-Diamond syndrome (SDS), a recessive marrow failure syndrome resulting from mutation to *SBDS*, also features telomere shortening. SBDS is thought to function in fundamental, nucleolar-localized cellular processes. We therefore hypothesized that SBDS may participate in telomere repair and that its mutation predisposes to acquired AA. We report heterozygosity for the 258+2 T→C *SBDS* mutation (commonly found in SDS patients) in 4 of 91 patients with acquired AA but not in 276 ethnically-matched controls (P=0.0037, Fisher's exact test). Affected patients had reduced SBDS expression but no evidence of the pancreatic failure or skeletal abnormalities typical of SDS. Telomeres in AA *SBDS*$^{+/-}$ patients' granulocytes were significantly short in comparison to age-matched controls and even shorter in SDS (*SBDS*$^{-/-}$), inversely correlating in length with SBDS expression. Increased telomere length heterogeneity was found correlated with SBDS mutations in both acquired AA and SDS. This characteristic has previously been observed in activation of the pathway of alternative telomere lengthening. However, telomerase activity of patients' lymphocytes was comparable to controls and no physical interaction between SBDS and telomerase complex components (TERT and TERC) was indentified. These data indicate that heterozygosity for the 258+2 T→C *SBDS* mutation is a risk factor for apparently acquired AA by causing hastened telomere shortening via a telomerase-independent mechanism.

P0022. Application of HR-CGH and Chromosomal Microarray Analysis (CMA) in the Cohort of 112 Patients with Mental Retardation.

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Advances in molecular cytogenetics enable detection of small chromosomal aberrations in 5-20% of patients with mental retardation (MR). The aim of this study was to compare two genome-wide screening techniques, high-resolution CGH (HR-CGH) and targeted array CGH, termed also Chromosomal Microarray Analysis (CMA). In contrast to conventional CGH, HR-CGH enables genome-wide screening for DNA copy-number changes with 3-5 Mb resolution. Array CGH is a new powerful technology capable of identifying chromosomal imbalances with the resolution depending only on the size and distance between the arrayed interrogating probes. CMA version 5.0 (853 BAC/PAC clones) enables detection of DNA copy-number changes in more than 60 chromosomal regions of known diagnostic significance and in all subtelomeric regions in a single test. In this study, we analyzed 112 patients with unexplained MR and other features suggestive of chromosomal abnormality, with apparently normal or balanced karyotypes using HR-CGH (35 patients) and/or CMA (92 patients). HR-CGH detected seven interstitial deletions in 35 (20%) patients. CMA revealed 34,8% (32/92) abnormalities, among which 11 (11,8%) were clinically relevant, 19 (20,5%) cases were interpreted as polymorphic variants and two (2,1%) were of uncertain significance. HR-CGH and CMA findings varied in size between 0.5-12.9 Mb and were all validated by FISH. Our results show that HR-CGH and array CGH techniques have high detection rates of genomic imbalances in the tested groups. Both methods have become important components in cytogenetic diagnostics, particularly for detecting cryptic constitutional chromosome imbalances in patients with MR, in whom the underlying genetic defect is unknown.

P0023. A boy with a small deletion of chromosome 10p with incomplete Scimitar syndrome and mental retardation

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We present a 3.5 year old boy with cardiopulmonary anomalies and mental retardation. Clinical findings include broad short neck, frontal bossing, low-set ears, skinfolds behind ear helices, high muscular tonus, height 2.5-10 perc., weight 50 perc., head circumference 25-50 perc. and bilateral testis retention. He had normal hearing, but no language. There was no history of heart disease or mental retardation in the family. Pregnancy and birth was normal.
His cardiopulmonary anomalies were consistent with incomplete Scim-

itar syndrome, which is a rare anomaly of pulmonary venous return to the vena cave inferior.
Chromosome analysis on routine G-banded chromosomes was normal.
However, CGH showed a deletion on chromosome 10(p11.2p12.1). Extended G-banding (900 band-level) and array CGH confirmed the deletion, and breakpoints were suggested to be (p11.23p12.1).
Several deletions in the short arm of chromosome 10 have been published in literature and in the ECARUCA chromosome database, but to our knowledge these deletions are all larger than the deletion our patient has. In addition, all patients described have a more severe phenotype than our patient. Deletions in the short arm of chromosome 10, at or near 10p13, are associated with a DiGeorge syndrome-like phenotype, DiGeorge 2 syndrome (DGS2). Yatsenko *et.al* (2004) reviewed 19 patients with deletion in 10p and congenital heart defects (CHD) and found that atrial septum defect (ASD) is a common cardiac anomaly associated with DGS2. Thus, the short arm of chromosome 10 may comprise one ore more critical regions for normal development of the cardiopulmonary system.

P0024. Arterial tortuosity syndrome: clinical and molecular findings in 12 newly identified families

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Background: Arterial tortuosity syndrome (ATS) is a rare autosomal recessive connective tissue disease, mainly characterized by widespread arterial involvement with elongation, tortuosity and aneurysms of the large and middle-sized arteries. Recently, mutations were identified in the *SLC2A10* gene. This gene encodes the facilitative glucose transporter GLUT10 and was previously suggested as a candidate gene for diabetes mellitus type 2.
Methods: Twelve newly identified ATS families with 16 affected individuals were clinically and molecularly characterized. In addition, extensive cardiovascular imaging and glucose tolerance tests were performed in both patients and heterozygous carriers.
Results and conclusions: All 16 patients harbor bi-allelic mutations in *SLC2A10* and haplotype analysis suggests founder effects for all 5 recurrent mutations.
In this series, patients are significantly older than those previously reported in literature (p=0.04) and only one affected relative died, most likely of an unrelated cause. Although the natural history of ATS seems less severe, it does indicate a risk for ischemic events. Two patients initially presented with a stroke, respectively at age 8 months and 23 years, due to a carotid artery dissection in the former. Tortuosity of the aorta or large arteries is invariably present in all patients. Two adult probands have mild aortic root dilation and 8 patients have arterial stenoses. Heterozygous carriers do not show any vascular anomalies. In 5 unrelated families, HbA1c levels and glucose tolerance tests are normal in all 6 patients and 8 heterozygous individuals tested. As such, overt diabetes is not related to *SLC2A10* mutations associated with ATS.

P0025. An Unusual Clinical Presentation Accompanying Cardiac, Renal, Neurologic abnormalities with Asplenia

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Congenital asplenia is mostly found in association with other anomalies, particularly cardiac, hepatic, renal anomalies, and structural abnormalities of the gastrointestinal tract. It is a feature of a number of genetic disorders such as Stormorken syndrome, Kartagener syndrome and particularly asplenia [Ivemark] syndrome. Asplenia syndrome is the association of congenital absence of the spleen with a variety of visceral abnormalities, predominantly of the cardiovascular system. The incidence is estimated at 1 in 10.000 to 20.000 live births. We report a 6-month-old female presented with multiple congenital anomalies associated with asplenia and seizures with abnormal EEG findings. She had macrocephaly, frontal bossing, microphtalmia, blue sclera, long eyelashes, low set ears, broad base to nose, full cheeks, long filtrum, cleft palate, micrognathia, hypoplastic nails, sacral dimple, skin syndactyly of 2^{nd},3^{rd},4^{th} toes. She had a history of an operation for congenital umbilical hernia after birth in newborn period and seizures. Neurologic evaluation revealed motor retardation and hypertonicity. Magnetic Resonance Imaging showed the cystic changes in germinal matrix and agenesis of the corpus callosum. Atrial septal defect and ventricular septal defect were detected in echocardiography. Abdominal ultrasound showed hidronephrosis in the left side. Her karyotype and subtelomeric FISH were normal. The combination of asplenia with the anomalies detected in this case can be the variation of one of the known asplenia syndromes or a new syndrome.

P0026. The MDR1 polymorphism C3435T in bronchial asthma (BA) and steroid-resistant idiopathic fibrosing alveolitis (IFA) patients

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The objective of this study was to estimate the frequency of polymorphism C3435T of MDR1 gene occurrence and associations between this polymorphism and features of glucocorticosteroids therapy. Blood samples were taken from 55 asthmatics and 18 patients with steroid-resistant IFA. Genotypes were detected by PCR-RLFP. Frequencies of MDR1 alleles *C* and *T* were not different - 0.55 and 0.45 in BA and 0.53 and 0.47 in IFA, respectively. Distribution of MDR1 genotypes in BA were CC-36% (n=20), CT-40% (n=22), TT-24% (n=13); in IFA were CC-17% (n=3), CT-72% (n=13), TT-11% (n=2). The frequency of MDR1 CT genotype was higher in IFA (χ^2=8.00, p=0.005). Daily doses of oral glucocorticosteroids (GC) according to MDR1 genotypes in IFA were: CC-15.0$\pm$7,6mg prednisolone, CT-24.6$\pm$1.7mg, TT-30$\pm$10mg (no significant). Daily doses of inhalation glucocorticosteroids (iGC) in BA group were: CC-955$\pm$106mcg, CT-941$\pm$113mcg, TT-1057$\pm$74mcg, without significant too. But in patients with BA, which had daily dose of iGC more than 800 mcg, genotype TT was found significantly often, than in patients with smaller dose: 34%(n=13) versus 7%(n=1), χ^2=3,81, p=0,051.
Consequently, we found significant association of MDR1 CT genotype with steroid resistant IFA. We assumed that asthmatics with MDR1 TT genotype needed higher doses of iGC. It is related with possible role of genetic control mechanism in corticosteroids transport from the cell.

P0027. New genetic variants of complex V,ATPsynthase 6&ATP 6

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Five new cases of genetic variants of mutation of ATP synthase 6 gene were diagnosed with clinical and laboratory methods.The mutations were identified by PCR method sequence of mitochondrial regions in peripheral intravenose blood.The clinical symptoms,which help the diagnosis were:microbrachicephaly,muscle hypotonia,normo to areflexia, atrophy nervi optici and etc.The mental retardation was from light motor retardation to generalized mental retardation. The investigated mutation were unknown up to now and were not registrated in www.mitomap.org yet. In the first case: base changes A91355G in ATP-synthase 6 gene,C9335T in CO III gene.In second case :base changes T8538C MT ATP6gene ,transversion of aminoacid isoleucin / treonin.

In third case : mutation G8494 A in ATP-ase gene and C11674T in ND 4 gene . In fourd case G 8573A mutation of ATP -ase 6 region.In addition were found two nonsense bases changes .The PCR method for sequence of mt DNA open new possibility for optimize the diagnosis and treatment of these patients in Clinical genetics.

P0028. Partial duplications of the ATRX-gene cause the ATR-X syndrome

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ATR-X syndrome is a rare X-linked disorder characterized by profound mental retardation, a characteristic face, skeletal abnormalities and alpha thalassaemia. We show that some of the patients suspected of having ATR-X carry do not carry small mutations at the single bp level, but instead carry intragenic duplications in the ATRX gene, further expanding the spectrum of mutations found in ATRX. We identified such a duplication in two families.

In the absence of an etiological diagnosis, array CGH analysis was performed on two siblings. This showed they carry a complex chromosomal aberration: an intragenic duplication in ATRX and an additional duplication upstream of this gene. We show that these duplications lead to an absence of ATRX mRNA and of the protein.

We next extended this observation to a group of 50 patients suspected of having ATR-X but without a detected sequence alteration. Quantitative PCR screening identified one additional patient, carrying a small intragenic duplication. These findings underscore the need for including quantitative analyses to mutation analysis of the ATRX gene.

P0029. Interstitial duplication 15q11-13 in a patient with Asperger autism and seizures

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We present a boy who was diagnosed to have Asperger autism at the age of 19 years.

Furthermore the boy suffers from seizures since the age of 16 years. Intelligence is in normal range.

The patient finished non-classical secondary school with good marks. Afterwards he tried to

graduate from expert school for social sciences which could not be completed because of his special defects in social competence and communication skills, and his deficiency of empathy which, at the end of the initiated diagnostic procedure, led to the diagnosis of Asperger autism.

Since recent studies (1) were able to show a defined interstitial duplication of chromosome15q11-13 in a few patients with autism disorder (1-3%) we investigated this region with respect to this abnormality.

We were able to show a duplication of the region of interest with the proof of three alleles for the internal markers D15S122, D15S822 and D15S1234.

Furthermore we could confirm this result with FISH using the probe GABRB3 (15q11-12),which showed three signals on meta- and interphase chromosomes.

Parental investigations (FISH and molecular analysis) were inconspicuous. Microsatellite-analysis showed that the duplication did arise from the boy`s maternal chromosome 15, as described in the literature (2).

The proposed mechanism is misalignment in maternal meiotic recombination.

The influence of parental imprinting on phenotype will be discussed; the variation of the symptoms of the yet published cases will be shown.

P0030. An autosomal dominant loose anagen hair syndrome: Clinical and genetic analysis

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Loose anagen hair syndrome (LAHS: OMIM 600628) is a non-inflammatory hereditary hair disorder characterized by anagen hairs of abnormal morphology that are easily and painlessly pluckable from the scalp. The hair is usually sparse, thin, slow growing and naturally does not grow beyond the nape of the neck. The phenotype has been recently described and its prevalence is yet to be defined. It affects both the genders equally. The condition is usually isolated; however few cases associated with other genetic conditions are also reported. There are several isolated cases and families reported with LAHS. We have studied one large Indian LAHS pedigree, with an autosomal dominant mode of inheritance, containing 63 individuals including 22 affecteds (12 males and 10 females). The phenotype appears to be100% penetrance in this family since no skipping of generation was observed. All affected had typical characters of loose anagen hair syndrome. The expression of the phenotype was an early age of onset. A hair pull test of all affected individuals extracted multiple hairs easily and painless and there were no sign of scalp inflammation or scarring. Light microscopic examination was also consistent with LAS. Majority of the affected members including all females never cut their hair, however three males has the history of seldom cutting their hair. Nine patients had an additional clinical phenotype of partial woolly hair and five females had fair hair color. There were no other associated anomalies observed in this family. Cytogenetic analysis of four affected individuals did not show any abnormality. We are planning to perform a high-density genome-wide linkage analysis to identify the responsible LAHS susceptibility locus. Email: madam_fille@yahoo.com

P0031. Genetic abnormalities in patients with azoospermia in ART programs

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Azoospermia is the most severe form of male infertility. Thanks to developing of assisted reproductive techniques (ART) many patients come to reproductive centers for infertility treatment. Azoospermia may be caused by number of genetic abnormalities. In this study we investigated molecular and cytogenetic defects in 57 azoospermia patients (age 31.6±0.7). Azoospermia was defined as the total absence of spermatozoa in ejaculate even after it centrifugation. DNA was extracted from peripheral blood. We analyzed 12 mutations of *CFTR* gene and 11 STS involving the *AZFa*, *AZFb* and *AZFc* regions using PCR and PCR/RELP. The karyotype analyses were performed by GTG-banding technique for at least 12 metaphases of standard lymphocyte culture. In all cases of mosaic forms in karyotype FISH technique was used for 1000 cells. Y chromosome microdeletions were found in six cases of azoospermia men (10.5%): three with *AZFc*, one with *AZFb*, and two with both *AZFc* and *AZFb*. Mutations of *CFTR* gene (only *F508del/-*) were revealed in 4.4%. Chromosome abnormalities were observed in 21.6% cases, including 47,XXY karyotype in six cases (three of them were mosaic variants). One patient had an XX male syndrome, with presence of *SRY* gene. Two patients had combined defects: one with cytogenetically detected deletion of Y chromosome and *AZFb/c* microdeletion, and one with both *AZFb* microdeletion and mosaic Klinefelter`s syndrome. Thus, genetic abnormalities were determined in 32% of azoospermia cases. Genetic testing is necessary to determine aetiology of azoospermia and to choose ART strategies between ICSI with testicular spermatozoa, PGD, or sperm donation.

P0032. Barth syndrome associated with compound hemizygosity and heterozygosity of the *TAZ* and *LDB3* genes

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The X-linked recessive Barth syndrome is caused by the Tafazzin (*TAZ*) gene mutations and is characterized by dilated cardiomyopathy (DCM) with left ventricular non-compaction (LVNC), neutropenia, skeletal myopathy, abnormal mitochondria and 3-methylglutaconic aciduria. Autosomal dominant DCM with LVNC has also been associated with LIM-Domain-Binding-3 *(LDB3)* gene defects.

We describe a family in which the 12-year-old-proband had a past history of LVNC and DCM. His mother had five miscarriages and two postnatal deaths. The proband showed LVNC-DCM, skeletal myopathy, recurrent oral aphthae and cyclic neutropenia. The DCM progressively improved with age; medical therapy was discontinued at five years of age. At present, LV function is normal and arrhythmias are absent. Cardiac Magnetic Resonance documented LVNC. Oral aphthae recur, as does cyclic neutropenia.

In the proband we identified two novel mutations, one of maternal origin in the *TAZ* gene (p.[Glu202ValfsX15]) and one of paternal origin in the *LDB3* gene (p.[Thr350Ile]). The mother, brother and father are healthy; the latter two show prominent LV trabeculation without dysfunction.

Expression studies of *TAZ* and *LDB3* genes were performed in family members and controls. In the proband, brother and father, *LDB3* expression was similar to control cases. *TAZ* and *LDB3* expression progressively declined with age in control both blood and myocardial samples: only an endomyocardial biopsy performed in the proband at six months of age, showed significantly lower *TAZ* and *LDB3* expression than in age-matched myocardial controls.

The clinical, genetic and expression data support the hypothesis that tafazzins are essential during fetal and early post-natal life.

P0033. Complex morphological phenotype of a child with Wolf-Hirshorn syndrome and Beckwith-Wiedemann syndrome due to der(4)t(4;11)(p16.1;p15.3)pat

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Partial monosomy of the short arm of chromosome 4 has a strong effect on phenotype resulting as a rule in the Wolf-Hirschhorn syndrome (WHS #OMIM 194190) with growth delay, microcephaly, characteristic facial appearance, and distinct developmental profile and paternal trisomy of 11p can lead to Beckwith-Wiedemann syndrome (BWS, #OMIM 130650) associated with exomphalos, macroglossia, and gigantism in the neonate. We present an unusual morphological phenotype in a six months-old boy with 4p monosomy and 11p trisomy resulted from paternal t(4;11)(p16.1;p15.3). Phenotype has been described using a catalogue of 807 well-defined traits according the concept of Stengel-Rutkowski. In order to evaluate the phenotypic impact of either of the imbalanced segments we used have formulated a spectrum of phenotype traits obtained from analysis of the six children with simple monosomy 4p16.1→pter, and a spectrum traits from analysis of 11 children with BWS performed by this same method. Among 58 clinical / developmental and anthropological traits identified in our patient 6/17 (35.5%) corresponded to traits of WHS and 17/38 (44.7%) of BWS. Unexpectedly quantitative participation of set traits belongs to both syndromes were similar. In addition, methylation-sensitive Southern-Blot analyses were performed and hypermethylation in the imprinting centre region 1 (ICR1) in the telomeric imprinting domain of chromosome 11p was found confirming the diagnosis of BWS. To our knowledge the phenotype resulting from the unbalanced chromosome translocation der(4)t(4;11)(p16.1;p15.3) has not been hitherto reported .

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P0034. A case of isolated neonatal hyperinsulinism due to an atypical Beckwith-Wiedemann Syndrome

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Background: The Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome with macrosomia, visceromegaly, macroglossia and abdominal wall defects. Many of the affected patients have episodes of hypoglycemia during the first days of life. During childhood, a frightening complication of BWS is the development of specific tumors. BWS is caused by different types of anomalies at the 11p15.5 imprinted locus.

Case report: A female patient was born at 36 weeks by cesarian section because of preeclampsia. She was eumorphic, her birth occipitofrontal circumference was 33 cm (P50-P75), weight 3 140 kgs (P90), length 46.5 cm (P50). From day 3, repetitive episodes of severe hypoglycemia occurred, leading to increase the glucose intake up to 12 mg/kg/min for stabilizing glycemia. At day 15, normoglycemia was obtained by continuous enteral alimentation and diazoxide. As a diffuse form of hyperinsulinism was evidenced, a subtotal pancreatectomy was performed at 2 months of age which was followed by normoglycemia even with normal discontinuous alimentation. There was no organomegaly. At 6 years of age, neurological assessment was normal except for cognitive functions with an IQ in the lower normal range and attention deficit. At 8.5 years, FISH analysis was performed on lymphocytes using the RP11-534I22 probe, containing the IgF2 gene, and the CDKN1C probe. Using these probes a duplication in the 11p15.5 region was shown in 50% of patient's lymphocytes. At 10 years of age, follow up did not reveal any tumoral pathology.

Conclusions: Neonatal hyperinsulinism could be the only manifestation of BWS.

P0035. Distribution of beta thalassemia mutations in Northern provinces of Iran

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β-thalassemia is one of the most common autosomal recessive disorders in Iran. There are more than two million carriers of β-thalassemia and over 15,000 people affected with β-thalassemia major who live in Iran. Prevalent mutations were identified by examining genomic DNAs isolated from 392 blood samples of β-thalassemia carriers from three Northern provinces of Iran. Furthermore, 172 pregnant women were analyzed among couples with a request for PND of β-thalassemia. Allele identification was carried out using a routine Reverse Dot Blot, ARMS, and genomic sequencing. The most common mutation, IVS II-I, is followed, in order of frequency by Cd-30, FSC-8-9, FSC-22-24, IVS-I-110, IVS-I-5, IVS-II-745, IVS-I-2, FSC-8, IVS-I,3'-end;-25bp, IVS-I-1, FSC-36-37, IVS-I-6, FSC-5, -28, Cd-37, IVSII-2,3 (+11/-2), -30, -88. We have also identified seven rare mutations. The results showed a clear drift in the distribution and frequency of some mutations in Northern Iran in comparison to a significant different frequency in the Southern Iran. This is the first report of the β-thalassemia mutation presentation in three provinces of Northern Iran. These results could help with establishing a center for prenatal diagnosis, prevention, and control of thalassemia in the Northern provinces of Iran.

P0036. Dental Blaschko Lines in a boy with Triploid/Diploid mosaicism

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A 16 year old boy was seen in our clinical genetics unit for further evaluation after having been diagnosed with Triploid/Diploid mosaicism syndrome at the age of five years. He had a history of mental retarda-

tion, cardiac malformations, cranio-facial dysmorphisms and pigmentary skin anomalies following the lines of Blaschko. The chromosomal analysis of peripheral blood cells was normal, but 69,XXY/46,XY mosaicism was detected in skin fibroblast culture.A thorough examination of the patient at the age of 16 revealed the usual features typical for this type of chromosomal mosaicism (asymmetric face, prominent and wide nasal bridge, clinodactyly, mental retardation, hypotonia, patchy pigmentation and depigmentation of skin, cardiac malformations, and truncular obesity), but also the absence of one of the inferior incisors and alternating vertical bands of opaque white and translucent enamel most evident on the central and lateral incisors. These dental abnormalities represent the anatomic equivalent of cutaneous Blaschko lines. Extracutaneous analogies of Blaschko lines have been described in various organs, such as the brain, bone, lens and teeth. These lines do not follow any known nervous, vascular or lymphatic structures but do follow and represent the developmental growth pattern of the skin. The embryological basis of these lines has not up to now been elucidated.

P0037. Blepharophimosis, Ptosis, and Epicantus inversus Syndrome (BPES). Clinical study about three cases

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BPES is a rare autosomal dominant disease (less than 1:5,000). It is a complex eyelid malformation invariably characterized by four major features: blepharophimosis, ptosis, epicantus inversus and telecanthus. There are two types of BPES: type I associate the typical features with female infertility caused by premature ovarian failure and type II includes only palpebral anomalies.
We present two familial and one sporadic case of BPES. The diagnosis was based by clinical signs.
Case 1, sporadic, G.L, male, 1 years old. There are no other relatives affected. We note, normal pregnancy, newborn haemolytic disease, febrile seizures and spastic tetraparesis. Clinical and paraclinic evaluation revealed: typical BPES features, low-set ears, narrow external auditory meatus, bifid uvula, internal hydrocephaly, atrial septal defect and developmental delay.
Case 2, I.B, male, 6 years old, present clinical features of BPES type I, like his own father; his mother had sensorineural hypoacusy. Other features are tall stature, sparse hair, normal intelect. There are no other relatives affected.
Case 3, A.D, female,1 years old, present on clinical evaluation typical ocular features, low nasal bridge, low-set ears and normal intelect. The father and other 7 relatives on paternal side (males and females) have the same features of BPES type I: only males are transmitting, affected females are infertile. The karyotype was normal in all three patients.
In conclusion, we emphasize the importance of the clinical features for diagnosis, leading further to an apropiate management.

P0038. Changes in Phenotype of Bloom´s Syndrome with New Manifestations in Adult Individuals

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Bloom´s syndrome results from autosomal recessive mutations in the *BLM* gene located on human chromosome 15 at 15q26.1, encoding a DNA helicase with homology to *RecQ* in *E. coli* (MIM 210900). Its phenotype includes (i) pre- and postnatal growth retardation, (ii) facial features with dolichocephaly and a narrow face, (iii) light-sensitive facial telangiectasia in most patients, (iv) manifestations of genomic instability as revealed by a 10-fold increase of spontaneous sister chromatid exchanges, breaks and homologous exchanges between chromosomes, and an increased rate of somatic mutations. Affected individuals develop similar types of cancer as in the population, but at a much younger age (about 1 in 4). We have observed the natural history in 15 individuals with Bloom´s syndrome during the past 38 years in Germany. We found that the phenotype in adult individuals becomes less distinctive with age than it is in children. In spite of persistent feeding difficulties, such as lack of appetite or regurgitation, adult individuals tend to gain weight. A new finding is development of diabetes mellitus type 1 or type 2. This has been observed in 27 of 117 patients (23%) of individuals in the Bloom´s Syndrome Registry (J. German, M. Sanz, E. Passarge, unpublished data). The skin manifestations tend to improve with age. We conclude that the phenotype of Bloom´s syndrome is wider than recorded previously. It remains to be seen whether the molecular type of mutation present in an individual influences the phenotype.
We thank J. German, N.A. Ellis, and M. Sanz, New York, for cooperation and mutational analysis.

P0039. Implementation of a new high throughput sequencing service for BRCA1 and BRCA2 gene screening to comply with UK Government Genetics White Paper 40 day turnaround time

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In response to UK Government targets for BRCA gene screening (Genetics White Paper) the West Midlands Regional Diagnostic Genetics Laboratory (Birmingham, UK) devised a novel sequencing-based strategy to screen the large BRCA1 and BRCA2 genes. The strategy is plate-based therefore facilitating the use of highly automated processes. 63 new primer sets were designed to amplify the coding regions of both BRCA1 and BRCA2 genes simultaneously using a 2-plate system for each patient panel. Between September 2005 and January 2007 a total of 731 diagnostic BRCA reports were issued as a result of the high throughput sequencing strategy. More than 98% of these reports were issued within 40 working days (from the date of sample receipt to date of report authorisation) with an average turnaround time for all 731 samples of 24.9 days. 79% of samples were reported in less than 30 days. 128 clearly pathogenic mutations have been identified in addition to 121 missense mutations and other unclassified variants. The Birmingham lab has also performed a number of follow-up studies to investigate the pathogenicity of missense and unclassified variants as well as undertaking RNA studies to determine the effect on splicing of deep intronic variants. The high throughput service has been used by a number of diagnostic genetics service laboratories and to date 100 reports have been issued to them with an average reporting time of 23 days. This high throughput BRCA sequencing service is available upon request from the West Midlands Regional Genetics Service.

P0040. Familial Breast Cancer With Positive History In Father And Mother : A Case Report .

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Background: A family history of breast cancer in a first-degree relative is reported in 13% of women with the disease. In turn, over 87% of women with a family history will not develop breast cancer. However, only 1% of women have multiple affected relatives, a history suggestive of a highly penetrant germ-line mutation.
The probability of breast cancer associated with a mutation in BRCA1 and BRCA2 genes increases if there are affected before menopause and /or have multiple cancers, if there is a case of male, breast cancer, or if family members also develop ovarian cancer. BRCA2 is associated more frequently with male breast cancer.
Case report: We report a female case of invasive carcinoma who had 37-year-old, and positive family history with paternal and maternal involvement. She had fibrosis, inflammation, and fact necrosis in right breast that negative for malignancy and also she had invasive carcinoma in left breast. For left breast, lumpectomy was performed .
Conclusions: First, in such a cases probability of the BRCA1 mutation increases and BRCA2 mutation is possible. Then screening for these genes should be performed for them and the other members of relatives and genetic counseling is required, and as soon as clinical approach for other related cancers may be performed. Second, also other nongenetic causes in such a family may predispose cancer susceptibility .

P0041. The first evidence of a pathogenic insertion in the NOTCH3 gene causing CADASIL

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CADASIL is an autosomal dominant disorder leading to cognitive decline and dementia caused by mutations in NOTCH3 gene. These highly stereotyped mutations are located within the 22 exons, encoding for the extracellular domain of the Notch3 receptor, all mutation resulting either in a gain or loss of a cysteine residue. It has been suggested that the unpaired cysteine residue might cause aberrant interaction of the Notch3 receptor with its ligands. Here we report the case of a patient with clinical and radiological findings consistent with CADASIL having distinctive GOM deposits in her skin biopsy. We examined the exons of NOTCH3 gene in this patient and we found a novel NOTCH3 gene mutation consisting in a 3 base pair insertion in the exon 3. This mutation was not observed in 560 control chromosomes. In the subject carrying the mutations in exon 3, no mutation was found in the other exons containing EGF-like repeats (exons 2-23), examined with both DHPLC analysis and direct sequence. This insertion resulting in a gain of a cysteine residue together with the neurologic and clinical phenotype, suggests it is the causative mutation in our patient. The current findings demonstrate that this insertion of a cysteine residue in the NOTCH3 gene can cause CADASIL. This is the first evidence of a pathogenic insertion found in a CADASIL patient, and it is important to confirm the hypothesis that the change toward an unpaired reactive cysteine residue within EGF repeat domains is a very critical molecular event in CADASIL.

P0042. Association of MTHFR gene polymorphism C677T with dilated cardiomyopathy and severe LV hypertrophy

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Cardiomyopathies are heart-muscle diseases of unknown cause. There are several theories of cardiomyopathies origin, including autoimmune hypothesis. In our study we investigated three known genetic polymorphisms in MTHFR, TNFa and TNFb genes in patients with different cardiomyopathies and controls (N=200). All patients have been divided into three groups - dilated cardiomyopathy (ejection fraction 20-45%, N=83), moderate left ventricular (LV) hypertrophy (wall thickness less than 1,5 mm, N=79) and severe LV hypertrophy (wall thickness more than 1,5 mm, N=118). MTHFR, TNFa and TNFb genotypes were determined by polymerase chain reaction with subsequent restriction fragment length polymorphism technique. There were no differences in TNF (alpha and beta) allele frequencies between studied groups and controls (p>0,05). Significant differences in C677T (MTHFR) allele frequencies distribution have been found between patients with dilated cardiomyopathy and controls (chi2 test with Yets's correction =5,4; df=1, p=0,024) as well as between severe LV hypertrophy and controls (chi2 test with Yets's correction =6,6; df=1, p=0,0109). Genotype TT of MTHFR polymorphism have been associated with severe LV hypertrophy development (odds ratio = 3,15; [95% CI 1,04 - 9,86]). There was no correlation between C677T allele frequencies and patients with moderate LV hypertrophy. Despite association with other heart dysfunction diseases, neither TNFa nor TNFb are related with cardiomyopathy. MTHFR point mutation (cytosine to thymine substitution; C677T) is a known risk factor for many cardiovascular diseases, including atherosclerosis, heart attack and peripheral arterial disease. Our data shows a possible role of C677T polymorphism in development of dilated cardiomyopathy and severe LV hypertrophy.

P0043. The indications for CATCH22 phenotype FISH analysis should be widened

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The 22q11.2 microdeletion is one of the most common human microdeletion syndromes. It is usually diagnosed by FISH analysis using TUPLE1 probe at 22q11.2 and N85A3 clone control probe at 22q13.3.
This study includes 335 patients investigated for CATCH22 microdeletion by FISH analysis during 2000-2007. In 19 patients abnormal finding was found (6%). Fifteen patients had classical 22q11.2 microdeletion (79%), in 4 cases other abnormalities were found (21%). Two had 22q11.2 microduplication, one had 22q13.3 deletion and in one 22q13.3 duplication was diagnosed.
In patients with 22q11.2 deletion the indications for FISH investigation were classical: heart anomaly with different additional problems; 3 had typical facial phenotype with cleft palate or immunological problems only. In the patient with 22q13.3 deletion (developmental delay and muscular hypotonia) the main indication for FISH analysis was the cleft palate in his mother. Similarly, a patient with 22q13.3 duplication (developmental delay and abnormal face) had heart anomaly in one sister. Two patients with 22q11.2 duplication in one family had minor facial abnormalities and failure to thrive, and were investigated only because referral doctor was a cardiologist. None of four patients with other abnormalities found had cardiac anomaly.
In summary: among the cases with abnormal CATCH22 FISH results we found in 21% other abnormality than classical 22q11.2 microdeletion. The indication for FISH analysis in these cases was rather superficial. This shows that the indications should be widened for CATCH22 FISH analysis: developmental delay only (+/- dysmorphic face, muscular hypotonia or failure to thrive).

P0044. Caudal duplication syndrome with unilateral hypoplasia of the pelvis and lower limb and ventriculo-septal heart defect

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Dominguez et al. (1993) reported six cases with caudal duplication syndrome and reviewed eight reports from the literature. Kroes et al. (2002) reported another two cases, including discordant monozygotic twins. The phenotypic spectrum encompasses gastrointestinal anomalies (intestinal duplication including double appendix, anal duplication, small bowel atresia or webs, intestinal malrotation, imperforate anus, omphalocele, esophageal cysts), genitourinary tract anomalies (duplication of external genitalia, vagina, bladder, urethras, cervix and uterus, single pelvic or malrotated kidney) and abnormalities of the spinal cord. We report a 22 year old female with caudal duplication syndrome, who in addition to intestinal duplication, imperforate anus, a dydelphic uterus and a single kidney also had a VSD and hypoplasia of the left pelvis, leg, labia majora and left side of a duplicated vagina.

P0045. Forget the classic CDG phenotype; a broad spectrum of congenital anomalies in CDG type II

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CDG type Ia, the most common form of Congenital Disorders of Glycosylation presents with characteristic symptoms of hypotonia, strabismus, arachnodactyly, cerebellar hypoplasia, abnormal lipid distribution and gastrointestinal, endocrine and coagulation abnormalities.
Patients with CDG type II, diagnosed with a glycosylation disorder due to Golgi dysfunction and abnormal transferrin isoelectric focusing in blood, have very different clinical features. Mutations in *COG7*, coding for one of the 8 subunits of the Conserved Oligomeric Golgi complex, lead to a new clinical syndrome of growth retardation, severe progressive microcephaly, adducted thumbs, gastrointestinal pseudo-obstruction, cardiac anomalies, wrinkled skin and episodes of extreme hyperthermia. Features in *COG1* deficient patients include hypotonia, developmental delay, ventricular hypertrophy/cardiac dysfunction and a rhizomelic short stature. The single patient described so far with *COG8* mutation showed a phenotype similar to that in mitochondrial disease. Other defects affecting the biosynthesis of both N- and O- linked glycosylation with hyposialylation include a new subtype of autosomal recessive cutis laxa syndrome with neonatal cutis laxa, microcephaly with large fontanel, hypotonia and failure to thrive. Despite the clinical and biochemical diagnosis in an increasing number of patients with

this form of CDG type II the gene defect has not been discovered yet. Based on the recently defined clinical syndromes with N- and O-linked glycosylation defects one should consider the diagnosis of Congenital Disorders of Glycosylation in patients with a broad spectrum of different features, including pachygyria, hypotonia with adducted thumbs, cutis laxa, cardiac and cranio-skeletal anomalies.

P0046. Neurological manifestations of the Cardio-facio-cutaneous Syndrome (CFC)

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The cardio-facio-cutaneous syndrome (CFC) is a multiple congenital anomaly disorder characterized by craniofacial dysmorphia, ectodermal abnormalities, congenital heart defects, developmental delay and growth retardation. Neurological complications associated with CFC remain to be clearly defined. Recent discovery of causative mutations in the MAPK pathway now permit accurate molecular diagnosis of CFC. Objective: To characterize the neurological features of patients with molecularly-confirmed CFC. Methods: Medical records, laboratory and imaging data were reviewed for 37 mutation-positive CFC patients. Patients with a clinical diagnosis of CFC but a negative result on mutation screening of the BRAF, KRAS, MEK1 and MEK2 genes were excluded from the study. Results: Hypotonia, motor delay, speech delay and learning disability were universally present in this cohort. Macrocephaly was present in 59%, ptosis in 50%, strabismus in 64% and nystagmus in 50% of patients. Corticospinal tract findings were present in 32% of the group. Ventriculomegaly or hydrocephalus was present in 66% of patients. Other findings on MRI included prominent Virchow-Robin spaces (19%) and abnormal myelination (13%). Seizures were present in 46% of patients. No specific genotype-phenotype correlations were observed. Interpretation: Alteration of function through the Ras/MAPK cascade has traditionally been associated with oncogenesis. Germline mutations in this pathway have an adverse impact on neurodevelopment, and appear to play an important role in ocular function, structural brain anatomy and electrical activity.

P0047. BRAF gene mutation in a patient with cardio-facio-cutaneous (CFC) syndrome presenting with lipoma of corpus callosum, hepatic and renal cysts

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Introduction: Cardio-facio-cutaneous syndrome (CFC) is characterized by craniofacial features, cardiac defects, ectodermal abnormalities, and psychomotor delay. It has phenotypic similarities with Noonan and Costello syndromes.

Case presentation: a 20-month-old boy, the first child of phenotypically normal parents, who during infancy presented poor suck, and failure to thrive. At 20th month of life he presents dysmorphic features, postnatal onset growth deficiency, hypertrophic cardiomyopathy, hypotonia, and mental retardation. Dysmorphic features include relatively large head, downslanting palpebral fissures, broad nasal base, protruding nostrils, high arched palate, papilloma-lile lesion on midline of the fontal region, deep palmar and plantar creases, sparse curly hair, and hyperextensible fingers. Brain MRI reveals a lipoma of corpus callosum. Abdomen ultrasound shows hepatic and renal cysts. DNA analysis initially performed excluded Noonan and Costello syndromes. Further examination revealed a 770A-G transition in exon 6 of the BRAF gene, predicting a gln257-to-arg (Q257R) amino acid change.

Conclusion: CFC syndrome can be caused by gain of function mutations in 1 of 4 different genes: KRAS, BRAF, MEK1, and MEK2. The protein products of these genes interact in a common RAS/ERK pathway that regulates cell differentiation, proliferation, and apoptosis. The mutation found in our patient has been earlier described in 3 unrelated CFC patients (BRAF, gln257arg), by Niihori et al. (Nat Genet, 2006). Hepatomegaly and absence or hypoplasia of corpus callosum has been described in CFC syndrome. To our knowledge, our patient is the first who presents lipoma of corpus callosum, as well as hepatic and renal cystic formations.

P0048. Brain imaging in patients with *KRAS, BRAF* and *MEK* mutations

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Cardio-facio-cutaneous syndrome (CFC) is a severe developmental disorder clinically related to Noonan syndrome and Costello syndrome (CS), which has been recently linked to mutations in 4 genes of the RAS/MEK/ERK signaling pathway. Developmental delay is frequent in those diseases but abnormalities of brain imaging aren't often reported in these patients. In this study, we performed mutation analysis of *KRAS*, *BRAF*, *MEK1*, and *MEK2* in 40 patients with a CFC, 20 patients with a CS without *HRAS* mutations, and 82 patients with NS without PTPN11 mutations. We found 22 BRAF mutations, 7 KRAS mutations, 11 MEK1 mutations, and 5 MEK2 mutations. 21/45 patients with a mutation had a brain imagery. It was abnormal in 11/21 of cases (table 1). 5/14 CS with a mutation in *KRAS*, *BRAF* or *MEK* had abnormal MRI (4 patients: normal, 5 patients : unrealized). Cerebral malformations were less frequent in CFC patients (abnormalities in 3/23, 5 normal, 15 unrealized). 3 patients with NS present a ventricular dilatation associated with a cerebral atrophy.

Brain abnormalities are frequent in all gene mutated and clinical diagnosis and a brain imaging seems to be justified in medical care of these patients.

Brain RMI abnormalities in patients with KRAS, BRAF, MEK mutations

	CFC	CS	NS
KRAS (/7)		1(/1) ventricular dilatation and cerebral atrophy	2 (/3) ventricular dilatation and cerebral atrophy
BRAF (/22)	0 (/3)	4 (/5) - ventricular dilatation - periventricular grey matter heterotopia and thin corpus callosum - ventricular dilatation and gyral abnormalities	
MEK1 (/11)	1 (/2) ventricular dilatation	0 (/3)	1 (/1) ventricular dilatation and cerebral atrophy
MEK2 (/5)	2 (/3) -cerebral atrophy -tuberous sclerosis (Bourneville disease associated)		

P0049. Contiguous gene deletions involving *EFNB1, OPHN1 and PRAJA1* in patients with craniofrontonasal syndrome (CFNS) and mild developmental delay

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Craniofrontonasal syndrome (CFNS [MIM 3404110]) is an X-linked malformation syndrome characterized by craniofrontonasal dysplasia, body asymmetry, midline defects and abnormalities of the fingers, toes and hair. Unlike classical X-linked diseases, CFNS manifests in females, whereas male carriers are usually mildly affected. CFNS is caused by mutations in the *EFNB1* gene (MIM 300035) encoding the transmembrane ligand ephrinB1 of cognate ephrin (Eph) receptor tyrosine kinases. However, psychomotor delay has been observed only in a few patients. We identified three females with classical CFNS and mild developmental delay harbouring *de novo* deletions of the *EFNB1* gene. Applying haplotype analysis, Southern blot hybridisation and array-comparative genomic hybridisation (array-CGH), deletion of

EFNB1 was found to be part of contiguous gene deletions including oligophrenin-1 (*OPHN1,* [MIM 300127]), and Praja 1 (*PRAJA1,* [MIM 300420]) in two of the patients. In the third patient *EFNB1* gene deletion may include deletion of regulatory regions 5' of *OPHN1*. Previously, the *OPHN1* gene has been shown to be responsible for X-linked recessive mental retardation. Although it is too early to predict the mental performance of the two patients with contiguous gene deletion of *OPHN1 - EFNB1 - PRAJA1*, psychomotor development is slightly delayed in one. A mild learning disability has been recognized in the third patient. It is important for genetic counselling to be aware that their male offspring may be affected by mental retardation and may be carriers of CFNS, whereas half of their female offspring will be affected by CFNS and will be carriers for mental retardation.

P0050. Charcot-Marie-Tooth phenotypes are defined by the myelin protein zero (P_0) structure. An analysis of the P_0 extracellular domain

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Background. The Charcot-Marie-Tooth (CMT) phenotype caused by mutation in the myelin protein zero (MPZ) gene varies considerably, from early onset and severe forms to late onset and milder forms. The mechanism is not well understood.
Methods. We performed a computational analysis of the myelin protein zero (P_0) extracellular domain encoded by the MPZ gene. The effects of the amino acid change in two novel missense mutations as well as mutations in the same or neighboring codons were analyzed.
Results. The angle between the membrane plane and P_0 extracellular domain is crucial. Major deviation caused early onset phenotypes, while a minor angle deviation was associated with allelic heterogeneity (CMT type 1 or 2, or distal hereditary motor neuropathy). Mild and major conformational changes resulted in late and early onset phenotypes, respectively. *Conclusions.* The phenotypic variation can be explained by the structural change of the P_0 extracellular domain.

P0051. CHARGE syndrome, searching for the mild end of the phenotype

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CHARGE syndrome is an autosomal dominant condition, caused by mutations in *CHD7*. The first reported spectrum of associated features, later coined by the acronym CHARGE (coloboma, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, ear anomalies and deafness), was extended by features such as cranial nerve palsy, hypoplasia of the semicircular canals, defects in olfactory bulb development and tracheo-esophageal fistula. Following identification of the *CHD7* gene, we have studied the clinical spectrum of a cohort of 47 *CHD7* positive patients. Evaluation of this cohort confirmed the broad clinical variability among CHARGE syndrome patients. Among these first series were two familial cases; a pair of monozygotic twin sisters and a sib pair consisting of two affected brothers. Phenotypic evaluation of these patients revealed a striking intrafamilial variability, notwithstanding their identical *CHD7* mutations. These findings prompted us to study more *CHD7* positive familial cases in order to extend our genotype-phenotype studies in CHARGE syndrome. Five more families were identified. All families were characterized by an intrafamilial clinical variability, confirming our previous findings. We encountered two mutation positive parents of independent families with very mild manifestations of the syndrome, further widening our knowledge of the mild end of the CHARGE syndrome phenotype. These mildly affected persons shared the same missense mutation in *CHD7*, providing the first evidence for a genotype-phenotype correlation in CHARGE syndrome. Up till now, in two parents a somatic mosaicism in lymphocytes was identified, underscoring the importance of offering *CHD7* analysis to parents of an affected child.

P0052. Interstitial deletion 1p in a child with mental retardation and multiple anomalies diagnosed by Array-CGH.

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Less than 10 patients were reported with interstitial 1p35 or 1p34 deletion. We report the first observation with del(1)(p35.2p34.3).
The patient was the second child from non-consanguineous parents. During pregnancy ultrasound examination revealed an increased nuchal translucency, fetal bowel hyperechogenicity, oligoamnios and abnormal growth parameters. A prenatal karyotype revealed no chromosomal abnormality.
The child was born at 37 WG with birth weight : 2370g (-2SD); length : 45cm (-3SD); and OFC: 32cm (-3SD), APGAR score was 7/8/10. She showed hypogenitalia and she had a peculiar facial dysmorphism characterized by microcephaly, flat face, microstomia, hypertelorism, narrow and small palpebral fissures, small nose, a Pierre Robin sequence with high arched palate, glossopharyngeal-laryngeal respiratory obstruction, early feeding difficulties, and vagal syncope. Pyloric stenosis and severe gastro-oesophageal reflux were cured by pyloromotomy realised at 30 days-of-age, and Nissen fundoplication and gastrostomy at 4 months. Thank enteral nutrition, at 23 months, weight was 13kg (+1.5SD) ; length 83 cm (-0.5SD).
A severe axial hypotonia was present at birth and persisted, seizures appeared at 7 month-of-age, a global developmental delay was obvious at 23 months. Cerebral ultrasound observation and RMI at 1.5 month-of-age, showed abnormal cerebellum and thin corpus callosum.
A 550 R-band resolution karyotype on blood sample was normal. We used the 1Mb BAC and PAC Array-CGH (VIB Leuven-J.Vermeesch) and diagnosed an interstitial deletion in the dark R-region 1p35-p34. This result was confirmed by FISH. Parental karyotypes and FISHs were normal.
The results will be detailed and compared to the literature.
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P0053. An Interstitial Deletion del(10)(q23.32q24.1) In a Mother And Her Two Offspring

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We describe a previously unreported familial interstitial deletion in a woman and her two pregnancies. This 33-year-old G0P0 woman underwent amniocentesis after a prenatal ultrasound showed findings of increased nuchal translucency and isolated left talipes equinovarus. The fetal karyotype was found to be 46,XX,del(10)(q23.2q24.1). The fetus was subsequently miscarried and a maternal karyotype revealed the identical interstitial deletion. A subsequent pregnancy produced a son with the same unbalanced karyotype and a solitary right kidney, an atrioventricular septal defect, and a patent ductus arteriosis. Maternal medical history was significant for right talipes equinovarus, uterine didelphys, short stature, and mild developmental delay. The mother and child share dysmorphic features that includes microcephaly, a long face, short palpebral fissures, hypotelorism, a widened nasal bridge with a large bulbous nose, a long philtrum, a large mouth with micrognathia, and prominent ears. FISH analysis using a probe for the PTEN gene (10q23.31) was performed on both the patient and her son. No deletion was noted in either patient, and their karyotypes were subsequently revised to del(10)(q23.32q24.1). Interstitial deletions involving the long arm of chromosome 10 are rare and there are only three other case reports documenting specific deletions in the chromosome 10q23 segment. These patients share some features with our cases, but a consistent pattern of clinical features cannot be concluded.

P0054. Population investigation of alpha-1-antitypsin in some regions of the Azerbaijan Republic

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Alpha-1-antitrypsin (α1A) is low molecular protease inhibitor, synthesized by liver cells, which destroys neutrophil elastase of lung alveolar cells. So far, 90 mutant and normal alleles of the α1A gene are known.

In our researches we have identified α1A phenotypes in healthy persons as well as in patients with lung and liver disease. Capillary blood (0,2 ml) with anticoagulation agent-heparin was collected in eppendorf tubes. Two regions of Azerbaijan Republic (Siyazan and Kazakh) were involved in population studies. Siyazan area is located 110 km northward and Qazakh area 450 km west-northward from Baku city. Altogether 897 person's blood samples were screened.

Phenotype testing was carried out by isoelectric focusing (IEF) in thin layer polyacrylamide- ampholine gels (PAAG), pH 4-6.

Three normal phenotypes of PiM alleles were identified in homozygous as well as in compound state with frequencies of: M1M2- 32%, M1M3-20% and M2M3-12%. The frequency of PiM alleles was : M1-38%,M2-38% and M3-24%.

In six families two types of mutations were defined as with PiZ (6 persons) and PiS (4 persons).

IEF method in PAG with pH 4-6 is recommended for identifying phenotypes of alpha-1-antitrypsin in population studies.

P0055. Microstomia- cleft palate: a new syndrome?

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The unusual combination of extremely small mouth with U-cleft palate was observed in two unrelated families. While mild micrognatia was described in infancy, it was not obvious in later life. In fact, prominent and pointed chin was present in the affected adults. In the first family, a mother and her daughter had these oral anomalies and downslanting palpebral fisssures. No associated birth defects or delays were present. In the second family, the affected mother had a son and fraternal twin daughters with the same combination of oral features and brachydactyly and 5th finger clinodactyly. In addition to the oral defects, the youngest boy in this family had craniosynostosis and ureteral obstruction resulting in chronic renal failure. Whole-genome genotyping with Illumina 317K SNP Beadchip did not detect chromosomal rearrangements. The diagnosis of Pierre Robin sequence was considered unlikely based on the clinical presentation and extracranial dysmorphisms. More than 50 syndromes featuring microstomia and cleft palate were reviewed and excluded. We propose that this condition represents a new autosomal dominant dysmorphic syndrome. Linkage analysis of these and additional families with similar features is likely to pinpoint the location of a gene essential for proper development of the oral region.

P0056. Novel missense mutation: R131C in the RUNX2 gene in a case with cleidocranial dysplasia

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Cleidocranial dysplasia is a skeletal dysplasia characterized by persistently open or delayed closure of sutures, hypoplastic and/or aplastic clavicles, wide pubic symphysis, dental anomalies, and short stature. The disorder is caused by heterozygous mutations in the CBFA (core binding factor alpha1) gene, also known as RUNX2 (runt-related gene 2) on chromosome 6p21, that encodes an osteoblast-specific transcription factor. Mutations scattered throughout the entire CBFA1 gene have been related to this disorder, however, most of them affect the highly conserved Runt domain, abolishing the DNA-binding ability of the transcription factor. We report a case presenting with the classic phenotype of cleidocranial dysplasia, in which mutation analysis of the RUNX2 gene revealed a previously unreported missense mutation (391C>T), that replaces an arginine residue with a cysteine at position 131, in the Runt homology domain of the RUNX2 protein. The mutation occurred the novo, neither of the parents, who were phenotypically normal, carried the mutation identified in the patient. We describe our detailed investigation of the patient, expanding the data related to the phenotypic expression of different mutations in the RUNX2 gene.

P0057. Further Expansion of the Behavioral and Neurodevelopmental Phenotypic Prensentation of Boys with 49 XXXXY (A variant of XXY)

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This is a comprehensive study completed on a large cohort of boys with 49 XXXXY (49XY) describing intact nonverbal IQ and receptive comprehension skills with severe developmental dyspraxia. 15 boys were evaluated using standardized measures by a multi-disciplinary team including pediatric neurology, genetics, endocrinolgy, development, physical therapy and speech/language.

The mean age of the cohort was 37 months with mean birth weight of 5lbs. 7ozs. The average age at the time of diagnosis was 4 mos. Mean Nonverbal IQ was 92 with receptive comprehension standard scores of 81.7 in contrast to depressed verbal capacities with standard scores of 66.5. There was severe motor praxis deficits with global hypotonia in all children. Sensory integration dysfunction was evident.

These findings describe for the first time intact nonverbal skills and comprehension with severe motor planning deficits in boys with this rare disorder. This study expands the phenotypic presentation of 49 XY to include better nonverbal IQ capacities than appreciated previously. Speech is severely delayed secondary to verbal and oral motor dyspraxia. The presence of limb dyspraxia affects graphomotor function. Behavioral issues were evident in some but not all children. These finding support evidence of frontal lobe dysfunction as previously demonstrated in boys with XXY.

Counseling for newly diagnosed families needs to highlight variability of intellectual capabilities, the need for aggressive EI services with direct SPL and OT for motor planning deficits. Further studies are underway to further investigate phenotypic variability, MRI abnormalities and the presence of mosiacism in the higher functioning children.

P0058. Cockayne Syndrome Type II: a unique phenotype among Druze Population in Northern Israel Caused by a Highly Prevalent Novel Mutation in the *CSB* Gene.

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Cockayne Syndrome (CS) (OMIM # 216400) is a rare autosomal recessive disease characterized by severe growth and developmental retardation, progressive neurological dysfunction and symptoms of premature aging. The primary cause of the disease is a defect in the transcription-coupled DNA repair, specifically the Nucleotide Excision Repair (NER) pathway. To date, four genes along this pathway *CS-A, CS-B, XP-D* and *XP-G* have been reported to be involved in the pathogenesis of CS.

We have identified a large, highly consanguineous, Druze kindred descended from a single ancestor, with six CS patients. All patients presented with congenital severe phenotype that includes severe failure to thrive, severe mental retardation, congenital cataracts, loss of adipose tissue, joint contractures, bird-like faces with small, deep-set eyes and prominent nasal bridge, and kyphosis. All patients exhibited neither language skills nor independent sitting or walking and died by the age of 5 years.

Cellular studies in patients' fibroblasts showed significant defect in transcription-coupled DNA repair (TCR) and a marked correction of the abnormal cellular phenotype with a plasmid containing the cDNA

of the *CSB* gene. Molecular studies that followed led to the identification of a novel insertion mutation c.1034-1035insT in exon 5 of the *CS-B* gene. This mutation has been found in 1:20 healthy individuals from the same village indicating a tremendously high carrier frequency. Identification of the causative mutation might enable further characterization of the CSB protein function in this unique family, as well as prevention of this devastating disease among the population at risk from this village.

P0059. Hepatic involvement in Cockayne syndrome type A

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Cockayne syndrome (CS) is a rare autosomal recessive disorder characterized mainly by cachectic dwarfism, premature aging, mental deficiency, microcephaly, intracranial calcifications, neurological degeneration, retinal abnormalities and sensorineural hearing loss. There are two subtypes: CS-A, the less common one, whose gene responsible is called *ERCC8* and CS-B, present in 75% of the cases clinically diagnosed, whose gene responsible is *ERCC6*. These genes show a pleiotropic effect, with several different organs and systems involved. Gastrointestinal anomalies have been reported occasionally, including especially elevated plasmatic levels of liver enzymes and hepatoesplenomegaly. These abnormalities have been mild, unassociated with jaundice or other clinical symptoms and have not involved coagulation factors.

We describe the hepatic findings in a cohort of eight CS patients diagnosed by molecular analysis as subtype A. One of our patients, a 12 year-old-girl, presented with recurrent gastrointestinal bleeding, due to esophageal varices. The other seven patients, without any hepatic signs/symptoms, showed elevated liver enzymes, ranging from slightly higher than normal to very high levels. The bilirrubin levels and coagulation studies were normal.

With better management of the individuals affected by CS, the survival could be longer and problems, otherwise not recognized, may well be of importance in this syndrome. The hepatic involvement could be, sometimes, life threatening, as demonstrated in one of our patients. Therefore, we recommend that all patients affected by CS should be screened for hepatic involvement. It would be interesting to evaluate hepatic involvement in patients affected by type B, in an attempt to establish a genotype-phenotype correlation.

P0060. Clinical and molecular heterogeneity in Italian patients affected by Cohen syndrome.

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Cohen syndrome is an autosomal recessive disorder with variability in the clinical manifestations, characterized by developmental delay, visual disability, facial dysmorphisms and intermittent neutropenia. We describe a cohort of 11 patients affected by Cohen syndrome from 10 Italian families and between 5 and 52 years of age at assessment. Characteristic age related facial changes are well documented. Eyes anomalies are found in 10/11 patients (retinopathy in 10/11 and myopia in 6/11). Truncal obesity is present in 9/11 patients. DNA samples from all patients were analyzed for mutations in *COH1* by Denaturing High Performance Liquid Chromatography (DHPLC). We detected fifteen *COH1* mutations; most of them are truncating mutations, only 2 being missense changes in functional domains. Gene deletions were found in two alleles. All mutations except one are private. A single base deletion leading to p.T3708fs3769 was found, in heterozygous state, in three apparently unrelated families deriving from a restricted area of the Veneto's lowland, between Padova and Tagliamento. Given the geographical conformation of this region, a recent origin of the mutation could be hypothesized.

Table 1 Summary of the clinical findings and COH1 detected mutations

Case Clinical findings	1	2	3	4	5	6	7	8	9	10a	10b
Sex	F	M	M	M	M	F	M	M	M	F	F
Consanguineus parents	No	No	No	No	No	No	No	No	Yes	No	No
Age of assesment	10y	17y	18y	30y	5y	24y	5y	12y	6y	52y	51y
OFC at birth	5	25	<3		25	25	<<10		25		
OFC at assesment	<<3	<3	<3	<3	<3	75	<<3	<3	<3		
Height	<3	<3		<3		97	50	10	3		
Truncal Obesity	Yes	Yes	Yes	Yes		Yes	No	Yes	Yes	Yes	Yes
Neonatal Feedings		No	No	No		Yes		No	Yes		
Laryngomalacia						No		Yes	Yes		
Age sat unsupported						8m	16m	1y	12m		
Age first walked	14m	2y6m		3y		18m	2y10m	1y10m	2y		
Age first word	15m	1y		No speech		2y6m	4y	3y	2y6m		
Age spoke sentences						3y	Not yet	4y	3y		
Mental retardation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Typical Facial Gestalt	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Retinopathy	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Myopia	Yes	Yes	Yes	Yes		No	No	Yes	Yes		
Narrow Hands/ Feet and slender/ tapering fingers	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Joints Hyperextensibility	Yes	Yes	Yes	Yes		No	Yes	Yes	No	Yes	Yes
Neutropenia	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes		
Mutation	IVS41+1G>A c.1125delC	c.1125delC p.Q3772X	c.1125delC	p.R2535X	p.R1143X	p.A590T	c.2047delC p.R270X	p.S3142G	c.11564delA deletion	dele-tion	dele-tion

P0061. High frequency of submicroscopic DNA copy number changes in patients with congenital heart disease

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Congenital heart disease (CHD) is the most frequent birth defect and affects nearly 1% of newborns. The etiology of CHD is largely unknown and only a small percentage can be assigned to environmental risk factors such as maternal diseases or exposure to mutagenic agents during pregnancy. Chromosomal imbalances have been identified in many forms of syndromic CHD, but next to nothing is known about the impact of DNA copy number changes in non-syndromic CHD. Here we present a submegabase resolution array CGH screen of 105 patients with CHD as the sole abnormality at the time of diagnosis. We have detected 18 chromosomal changes that do not coincide with frequent DNA copy number variants, including 2 de novo deletions, 4 de novo duplications, 5 familial duplications and one familial deletion. Our data show that sub-microscopic deletions and duplications play an important role in the aetiology of this condition, either as direct causes or as genetic risk factors for CHD. These findings have immediate consequences for genetic counselling and should pave the way for the elucidation of the pathogenetic mechanisms underlying CHD.

P0062. Severe Autosomal Recessive Congenital Neutropenia (Kostmann disease) in an Iranian family with two affected children, and homozygous mutation in HAX1 gene.

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Severe congenital neutropenia (SCN) or Kostmann syndrome is a rare type of neutropenia. It is inherited by autosomal recessive pattern. Consanguinous marriages mostly is a predisposing factor. Patients suffer from severe and recurrent bacterial infections (pneumonia, oti-

tis media, abcesses, and…..). This disease was reported by Kostman in a large consanguineous family from Northern part of the Sweden (1956).
In this report we will present an Iranian family, with two affected children. Parents are second cousins. Both of the sibs showed neutropenia from early infancy. They have had recurrent severe bacterial infections. The older one was a boy, that showed Myelodysplastic(MDS) changes after the age of 15 and died from AML when he was 16-year-old. The younger one is a 14-year-old girl just with the similar symptoms.
The response of both of them was favorable to G-CSF.
Mutation analysis of the ELA2 gene by direct DNA sequencing of PCR-amplified genomic DNA did not identify any abnormalities.
In searching of mutations in other candidate genes a homozygous mutation found in HAX1 gene in the proband, and each of the parents was heterozygous carrier for the mutation.
The mutation was W44X, same as described by Klein & Welte in their Nature Genetics paper.

P0063. A de novo novel missense mutation in Connexin 26 in a sporadic dominant case of non-syndromic deafness.

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Mutations in the Connexin 26 gene (GJB2) can cause non-syndromic recessive or dominant hearing loss (HL) or both sensorineural hearing impairment and keratoderma.
We report here a novel missense dominant mutation of GJB2 gene associated with non-syndromic deafness, identified in a 3 year old Italian girl who has congenital profound sensorineural HL without skin disease or other clinical features.
Patient's DNA sequencing revealed an heterozygous C→G change at nucleotide 172 resulting in a proline to alanine substitution at codon 58 (P58A).
No further sequence variants were revealed in the remaining coding sequence and in the 5'UTR exon1.
We also excluded the reported deletions of Cx30, that is tightly linked to *GJB2* at 13q12, the Δ(GJB6-D13S1830) and the Δ(GJB6-D13S1854) that are the cause of deafness in patients carrying one recessive *GJB2* mutation in *trans*.
Parents were shown not to carry the P58A mutation and no other family members were reported to have a significant hearing impairment. Segregation analysis of 10 polymorphic microsatellite markers (from chromosomes 13, 18, 21 and X) confirmed the correct presence of bi-parental contribution.
This mutation was not observed among 100 healthy controls and 720 unrelated affected individuals, excluding it as a common polymorphism.
Proline at codon 58 is conserved among all connexins, suggesting that this residue is critical for the function of the protein. This mutation occurs in the first extracellular domain of the protein (EC1), which seems to be very important for connexon-connexon interaction and for the control of voltage gating of the channel.

P0064. Frequency of consanguinity and positive familial history of disability or birth defect in yazd province

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Consanguinity (family intermarriage) is commonly practiced in many Asian, African and Latin American communities. In some countries such as Iran consanguinity as in mating of first cousins is encouraged as part of social customs. This study was performed on 3957 couples that were referred to genetic counseling center of Yazd welfare organization (from 2001 to 2007). Of these, 690 couples had a child with disability or birth defect. The frequency of non-consanguineous marriages was 31.6 % (218 couples) and the frequency of consanguineous marriages was 68.4 % (472 couples). 52.9% of consanguineous couples were first cousins and 15.5% were other relationship .In fact consanguinity was so high and considerable. Furthermore, 34.5 % (238 cases) had a positive familial history of the same disability (in parents, siblings and relatives). The most common kind of disability was mental retardation (45.8%) and the least frequent problem was skin disorder (0.3%). Children with chromosomal abnormality constituted 6.4% of the cases. We conclude that the offspring of family intermarriages have a significantly increased incidence of hereditary diseases.

P0065. Deletion of 8p23.1 with Features of Cornelia de Lange Syndrome and Congenital Diaphragmatic Hernia and a Review of Deletions of 8q23.1 to 8pter. ? A Further Locus for Cornelia de Lange Syndrome

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Cornelia de Lange syndrome is characterised by facial dysmorphism; hirsutism and internal organ anomalies, including diaphragmatic hernia, and limb defects. Causative mutations in two genes have been identified: (1) NIPBL on chromosome 5q13 is dominantly inherited and accounts for approximately 50% of cases and (2) SMC1L1 (also known as SMC1) on the X chromosome, shows X-linked inheritance and accounts for an unknown proportion of cases. However, the aetiology of a significant number of cases remains unknown. A variety of chromosomal anomalies have been described in a minority of cases. We report on a child with an 8p23.1 deletion with features of CdLS and congenital diaphragmatic hernia. We review cases with cytogenetic anomalies involving 8p23.1 and discuss potential relationships between 8p23.1 deletions and CdLS or impaired cohesin complex function.

P0066. NIPBL mutational analysis in 56 individuals with Cornelia de Lange Syndrome

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Cornelia de Lange syndrome (CdLS) is characterised by facial dysmorphism, microcephaly, growth and mental retardation, hirsutism, gastroesophageal reflux and congenital anomalies including limb defects. Mutations in the gene NIPBL, the human homolog of Drosophila Nipped-B, have recently been found in approximately 50% of CdLS cases. The function of NIPBL in mammals is unknown. We present here the molecular analysis of a series of 56 children with typical features of CdLS including two-father-to child transmissions . Multiplex ligation-dependent probe amplification (MLPA) screening failed to detect either partial or whole-gene NIPBL deletions in 15 patients tested. Direct sequencing of the 47 NIPBL exons and corresponding exon-intron boundaries enabled to identify 21 heterozygous NIPBL mutations including eight missense (38%), one nonsense (4%), four frameshift (19%), one 5'UTR (4%) and seven splice site mutations (33%). These mutations were not found in 100 control chromosomes.
Our study confirms that NIPBL mutations account for about 40% of CdLS cases. We detected three SMC1L1 mutations in the same patient series. Absence of NIPBL mutation in the remaining cases further supports genetic heterogeneity of the disorder. Finally, the observation of two familial mutations in our series suggests that parent-to-child transmission may have been underestimated so far.

P0067. Incidence and clinical features of X-linked Cornelia de Lange syndrome due to *SMC1L1* mutations

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Cornelia de Lange syndrome (CdLS) is a multisystem developmental disorder characterized by facial dysmorphism, growth and mental retardation, microcephaly, and various malformations. Heterozygous mutations in the *NIPBL* gene have been detected in approximately 45% of affected individuals. Recently, a second CdLS gene, mapping to the X chromosome, has been identified: *SMC1L1* (*structural maintenance of chromosomes 1-like 1*; or *SMC1A*). In order to estimate the incidence and refine the clinical presentation of X-linked CdLS, we have screened a series of 11 CdLS boys carrying no *NIPBL* anomaly. We have identified two novel *de novo SMC1L1* missense mutations (c.587G>A [p.Arg196His] and c.3254A>G [p.Tyr1085Cys]). Our results confirm that *SMC1L1* mutations cause CdLS and support the view that *SMC1L1* accounts for a significant fraction of boys with unexplained CdLS. Furthermore, we suggest that *SMC1L1* mutations have milder effects than *NIPBL* mutations with respect to pre- and postnatal

growth retardation and associated malformations. If confirmed, these data may have important implications for directing mutation screening in CdLS.

P0068. Mutation analysis of the NIPBL and the SMC1L1 gene in German patients with suspected Cornelia de Lange syndrome.

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Cornelia-de-Lange-syndrome (CdLS) is a heterogeneous autosomal-dominant disorder characterized by typical facial features, growth retardation, developmental delay, upper-extremity malformations and a variety of other abnormalities.
The prevalence of CdLS is estimated to be around 1/10,000. Most cases are sporadic, although several familial cases are described.
Since 2004 it is known that up to 50% of CdLS cases are caused by mutations in the NIPBL gene on chromosome 5p13, the human homolog
of the Drosophila Nipped-B gene. This gene consists of 47 exons and mutations were found in the entire coding region.
Recently, Musio et al. described an X-linked form of mild CdLS caused by mutations in the gene SMC1L1 (=SMC1).
Both genes code for proteins, which are part of the cohesin complex involved in chromosome cohesion.
We investigated the prevalence of NIPBL gen mutations in 108 German patients with suspected CdLS by DGGE and/or direct sequencing.
Additionally, mutation analysis of the SMC1L1 gene was performed in all patients tested negative for mutations in the NIPBL gene. We detect_
ed NIPBL gene mutations in 29 (=27%) of our patients, although previous studies reported a higher detection rate (ca. 50%). Our lower detection rate is probably due to patient selection.
Furthermore, the prevalence of SMC1L1 mutations in our cohort will be discussed.

P0069. Genotype -phenotype analysis of cerebral dysgenesis associated with *TUBA1A* mutations

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Cortical dysgeneses are, in most cases, associated with profound neurodevelopmental disability, including severe mental retardation and epilepsy. Two major genes *DCX* and *LIS1* account for almost 40% of the cases of agyria-pachygyria, and three additional genes (*RELN*, *ARX*, *VLDL* receptor) are exceptionally mutated. Recently, mutations in *TUBA1A (NM_006009)* gene that encoded for an alpha tubulin that interacts with doublecortin, have been involved in a large spectrum of neuronal migration disorders. In order to better define the phenotype, we retrospectively studied seven unrelated patients with pathogenic *TUBA1A* mutations.
Patient and methods : Clinical and imaging assessments were performed in seven patients, aged from to 2 to 16 years with pathogenic de novo missense *TUBA1A* mutations.
Results : Patients exhibited congenital microcephaly (6/7), moderate (5/7) to severe (2/7) mental retardation, spastic diplegia (6/7). Associated clinical features were more occasional: facial diplegia (5/7), oropharyngoglossal dysfunction (4/7), and strabismus (3/7). Epilepsy was reported in 3/7 but only 2 patients have intractable epilepsy. Brain MRI revealed dysmorphic basal ganglia with balloon shape (7/7), perisylvian (5/7) to diffuse pachygyria with a posterior to anterior grading (2/7), dysmorphic (5/7) or hypotrophic (2/7) corpus callosum, mild (3/7) to severe (2/7) vermian hypoplasia.
Conclusions : Albeit each symptom observed in *TUBA1A* mutated patients is not specific, our data highlight a potentially specific distinguishable combination of developmental features that is not seen in neurodevelopmental disorders resulting from mutations in *DCX*, *LIS1* and *ARX*. We suggest that this specific neuro-clinical combination should lead the clinician to the search of *TUBA1A* mutations.

P0070. Diversity, parental germline origin and phenotypic spectrum of de novo HRAS missense changes in Costello syndrome

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Activating mutations in HRAS have recently been identified as the molecular cause underlying Costello syndrome (CS). To investigate further the phenotypic spectrum associated with germline HRAS mutations and characterize their molecular diversity, subjects with a diagnosis of CS, Noonan syndrome, cardiofaciocutaneous syndrome or with a phenotype suggestive of these conditions but without a definitive diagnosis were screened for the entire coding sequence of the gene. A de novo heterozygous HRAS change was detected in all the subjects diagnosed with CS, while no lesion was observed with any of the other phenotypes. While eight cases shared the recurrent 34G>A change, a novel 436G>A transition was observed in one individual. The latter affected residue Ala146, which contributes to GTP/GDP binding, defining a novel class of activating HRAS lesions that perturb development. Clinical characterization indicated that Gly12Ser was associated with a homogeneous phenotype. By analyzing the genomic region flanking the HRAS mutations, we traced the parental origin of lesions in nine informative families and demonstrated that de novo mutations were inherited from the father in all cases. We noted an advanced age at conception in unaffected fathers transmitting the mutation.

P0071. Crane-Heise syndrome: a further case broadening the clinical spectrum

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Crane-Heise syndrome is a rare lethal and autosomal recessive condition which has been first reported in 1981 in three siblings presenting with intrauterine growth retardation, a poorly mineralised calvarium, characteristic facial features comprising cleft lip and palate, hypertelorism, anteverted nares, low-set and posteriorly rotated ears, vertebral anomalies and absent clavicles. Since then, to our knowledge, only one isolated case and two siblings were reported with similar findings. In 2003, distal phalangeal hypoplasia, mild cardiac and gastrointestinal anomalies were suggested as additional features. We present a further case, diagnosed after a termination of pregnancy at 24 weeks' gestation, very similar to the previously reported ones, and broaden the clinical spectrum of this entity. On the 20 weeks'gestation ultrasound scan, a laparoschisis was identified, with poor fetal movements and polyhydramnios. Fetal chromosomes were normal 46XX. Pathological examination confirmed the laparoschisis but also revealed facial dysmorphic features, multiple joint contractures, a cleft palate, and severe vertebral as well as limb anomalies. Skeletal x-rays showed absent clavicles, undermineralisation of the skull, micrognathia, and abnormal phalanges. We discuss the clinical and radiological phenotype of this rare condition.To our knowledge, no molecular mechanism has been identified in Crane-Heise syndrome so far.

P0072. Cranioectodermal dysplasia (Sensenbrenner's syndrome): Report of two siblings

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Cranioectodermal dysplasia (CED, Sensenbrenner's syndrome, OMIM 218330) is a rare disorder characterized by dolichocephaly, rhizomelic dwarfism, dental and nail dysplasia, sparse hair and renal problems. Among about 20 cases reported to date, most are sporadic, but few familial cases suggest autosomal recessive inheritance. So far, the underlying genetic defect is unknown, and linkage analyses were not possible due to the limited number of patients and small family sizes. Here, we report on a 5-year-old girl and her 1-year-old brother with cranioectodermal dysplasia. Their healthy parents (16- and 25-year-old at the birth of the first child) are remotely consanguineous which supports the assumption of autosomal recessive inheritance. Clinical features included short stature with rhizomelic shortening of limbs, brachydactyly, narrow chest, craniosynostosis, dolichocephaly, full cheeks, telecanthus, broad nasal bridge, small and widely spaced teeth, dysplastic auricles and fine, sparse hair, bilateral inguinal hernia and hyperelastic skin. Psychomotor development is normal. Both children suffer from tubulointerstitial nephropathy with more severe features in the brother. We present detailed clinical features of the patients in comparison to previously reported cases, as well as prenatal features of the syndrome detected on ultrasound examinations.

P0073. Spectrum of HLXB9 gene mutations in Currarino syndrome and genotype-phenotype correlation

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Currarino syndrome (CS) (OMIM 176450) is a rare congenital disease described in 1981 as an association of three main features: typical sacral malformation (sickled-shape sacrum or total sacral agenesis below S2), hindgut anomaly and pre-sacral tumor. Moreover, neurological defects, namely tethered cord and/or lipoma of the conus, are also frequent and has to be search for as they may lead to severe complications if not treated.
CS is ascribed in half of cases to heterozygous mutations of the HLXB9 gene (OMIM 142994) located at 7q36, with an autosomal dominant mode of inheritance. HLXB9 gene encodes the HB9 protein, a 403 amino acid transcription factor that interacts with DNA through a highly evolutionarily conserved homeodomain, involved in embryological development. Thus far, 41 different heterozygous mutations have been described in clinically typical CS patients, mostly in familial cases (90 % of cases).
In this work, we describe 23 novel mutations in 26 mutated patients of a 50 index patients' series harbouring clinical CS (22 males and 28 females). Mutations concerned 19/20 familial forms and 7/30 sporadic cases. Sex predominance was not observed. Truncating mutations (frameshift or non-sens) represents 56.4 % of cases, suggesting that haploinsufficiency is the basis of CS. No obvious genotype-phenotype correlation could be identified. A genetic heterogeneity is suspected as at least 16 from the 24/50 not mutated patients harboured subtle phenotypic variations, and other genes probably account for cases with no mutation of the HLXB9 gene.

P0074. Clinical and Molecular study of 17 patients with Cutis Laxa

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Among different disorders with elastic fibres deficiencies, cutis laxa (CL) comprises a clinically and genetically heterogeneous group of acquired and genetic disorders characterized by loose, sagging and inelastic skin. Mutations in at least two genes, elastin (*ELN*) and fibulin5 (*FBLN5*), are known to be causative for CL.

Fifteen patients from four families presented: herniae (inguinal n=7, umbilical n=1), pulmonary emphysema (n=5), ankle laxity (n=2), mitral insufficiency (n=1), aorta dilatation (n=1), pulmonary arterial hypertension (n=1), vaginal prolapse (n=1), dysuria (n=3). Skin presented as inelastic on the face with inguinal folds during infancy (n=4), thick folds on the face during the third decade (n=5), and loose skin on the neck after 40 years (n=7). three deletions of 1 bp (n=13) and one deletion of 15bp (n=2) were detected in *ELN* gene.
A 11y old boy and his 8 y old sister, presented both with loose skin, bilateral inguinal hernia and hoarse voice, right ventricle dilatation (n=1), pulmonary emphysema (n=1). Homozygous missense mutation in *FBLN5* was identified.

CL is a systemic disorder with dermatologic, pulmonary and cardiovascular features. Patients with mutations in *FBNL5* all present a severe phenotype, whereas intrafamilial clinical heterogeneity is observed in families with mutations in *ELN*.
Skin involvement evolves over time: thick folds during infancy, wrinkled chin during third decade and loose skin after 40 years.
Patients affected by CL should undergo systematic exploration of clinical features, but should also take advantage of molecular analysis to confirm the genetic basis of their phenotype.

P0075. Identification of two *Alu* insertions in the *CFTR* gene

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LINE-1 (long interspersed element-1) or L1-mediated retrotransposition is a potent force in human genome evolution and an occasional cause of human genetic disease. Since the first report of two *de novo* L1 insertions in the *F8* gene causing hemophilia A, more than 50 L1-mediated retrotranspositional events have been identified as causing human genetic disease.
On the basis of the observation that both L1 elements and *Alu* sequences are abundant in the human genome, the increasing number of genomic rearrangements reported in the *CFTR* gene, and the fairly large size of the *CFTR* gene, we surmised that some previously unresolved CF chromosomes might carry hitherto undetected L1-mediated retrotranspositional insertions.
This study report the identification of two simple *Alu* insertions using quantitative high-performance liquid chromatography (QHPLC), technique previously employed to delineate the boundaries of large genomic deletions in the *CFTR* gene.
The first one, identified in a 24-year-old French girl, carrying F508del on the other chromosome, correspond to a 103 pb antisense insertion in exon 16 containing an *Alu* sequence of 46pb and a poly(A) tail of 57pb. The second one, identified in a 28-year-old Czech man, correspond to a 337pb sense insertion of an *Alu* sequence of 281 pb and a poly(A) tail of 56 pb. Both mutations are presumed to lead to aberrant splicing.
The identification of these two simple *Alu* insertions in the *CFTR* gene revealed a previously unknown mutational mechanism responsible for CF and also represents an important addition to the already diverse spectrum of *CFTR* gene mutations.

P0076. Attempts to explain why Cystic Fibrosis is of lower prevalence in the Greek-Cypriot population

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Cystic fibrosis (CF) is one of the most frequent autosomal recessive diseases in Europe. In Cyprus, the prevalence appears lower compared to the rest of European populations (carrier frequency 1/44). An exception is a village in which 1:14 individuals is carrier of mutation F508del probably because of a founder effect. We hypothesised that undiagnosed mild cases on the island may give an erroneous impression of lower prevalence while mild mutations may be responsible for cases of male sterility.Mild mutations R117C and L346P were not found in 200 Greek-Cypriot samples, concluding that their frequency is lower that 0.25%. Variant M348K was found in two individuals in heterozygosity, giving allele frequency of 0.5%. Two other polymorphisms next to each other in intron 8 are: a TG repeat with known alleles of 11, 12 or 13 repeats and a stretch of 5, 7 or 9 thymines. We designed a new approach for detecting the disease allele 5T, that includes nested

PCR with a modified primer and use of the restriction enzyme *Psil* and agarose gel electrophoresis. By testing 100 individuals of the general population we found that allele 5T has a frequency of 3.5%. In six men with azoospermia or CF patients with a single known mutation, the 5T allele was not found. Also, allele 13TG was not found in 10 random samples that were sequenced. The 5T allele does not seem to contribute to pathology in our population, unless it is found in combination with other mutations on undiagnosed patients.

P0077. Estimation of *CFTR* mutation carrier frequency in Iran based on known frequency of p.F508del

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Cystic Fibrosis (CF) is the most common life-threatening autosomal recessive disease in many Caucasian populations. Approximately one in 2500 newborns in populations of European ancestry are affected, wherein the average carrier frequency is 1:25. The disease is caused by mutations in *CFTR* gene. Among the more than 1000 mutations identified in the *CFTR* gene, the p.F508del allele is the most common worldwide. Its frequency exhibits a northwest to southeast gradient, ranging from a high of 88% in Denmark to a low of approximately 16% in Iran. It has generally been believed that the incidence of CF also follows a northwest to southeast gradient and that incidence in low in non-European populations. However, relatively high incidences of CF were predicted in Turkey and Iran based on data of frequency of individuals carrying homozygous mutations and extent of inbreeding in those populations. Specifically the carrier frequency in Iran was estimated to be 1:40, similar to that of European populations. We have now experimentally determined the frequency of p.F508del carriers among 500 randomly selected Iranians. Based on the number of carriers identified and the observation that this allele accounts for 16% of the CFTR mutated alleles of the Iranian population, the frequency of carriers of CFTR mutations was calculated to be 0.037 (95% confidence level of 0.037+/- 0.016). This figure corresponds to 1:27, close to the carrier frequency previously estimated. It is concluded that CF is a disease of considerable public health importance in Iran, and probably in other countries of the Middle East.

P0078. Description of large CFTR gene rearrangements in Italian population

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Cystic fibrosis (CF) is mainly caused by small molecular defects of the CFTR gene; despite the genotype is define in the majority of patients, a number of CF cases still remain uncharacterized. These unidentified mutations may escape detection using PCR-base techniques. The CF mutation database lists more than 25 large rearrangements.

We report here the results of our screening for CTFR gene rearrangements, performed on Italian CF patients.

A sample of 689 unrelated Italian patients (for a total of 1378 alleles), followed at CF Centre of Regione Lombardia (Milan, Italy), was collected. All patients had classical form of CF with typical pulmonary and gastrointestinal findings, and positive sweat test.

The Innogenetics assay and the DHPLC screening showed a mutation detection rate of 93%. After these analyses , 100 alleles still remain unidentified (7%).

In order to detect the rearrangements in the CFTR gene, the 27 exons were screened in these patients with the MLPA assay based on commercial Kit "SALSA P091 CFTR MALPA Kit" .

The MLPA assay was performed for 38 patients (76 alleles).

We characterized 10 different deletions for a total of 19 deleted alleles.

In conclusion, 25% (19/76) alleles had a large gene deletion. The deletion of exons 22-23 (6/76) is the most frequent in our cohort.

The analysis of CF patients performed with MLPA techniques has made possible to reach a 94.4% detection rate, improving the process of carrier detection and genetic counselling in Italian population.

P0079. Cystic Fibrosis: A frequent disease with heterogenous mutation spectrum in Iran

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Cystic fibrosis (CF) is the most common autosomal recessive disorder in European populations with about 1:25 carrier frequency and regional variation. Since the identification of the cystic fibrosis transmembrane regulator (CFTR) gene, over 1000 mutations have been reported in which ΔF508 is the most frequent. However for non European populations, the incidence of CF and the spectrum of mutations that are responsible for the disease are not critically assessed.

A few comprehensive studies are focused on the molecular basis of CF in the Iranian population. In this research we are going to present the results obtained from a 4 year study on the 110 Iranian CF patients including molecular analysis of the CFTR gene.

Our results suggest that CF is a very heterogenic disease among our population in which about 15 different mutations and polymorphisms have been detected till now. DelF508, G542X, N1303K, G551D and W1282X, the 5 most common mutations in Europeans with the overall frequency of 75%, only contain 23% of CFTR mutant alleles in Iran. The other detected mutations are rare with relative frequencies of lower than 1%.

The information provided here on the distribution of CF mutations in Iranian population may assist in the development of more appropriate diagnosis tests in Iran and also it may facilitates the mutation analysis in the neighboring countries.

P0080. 8-Years experience in cystic fibrosis neonatal screening in Castilla-Leon (Spain)

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Since the implementation of the Cystic Fibrosis (CF) Newborn Screening in our region in 1999, we have analysed 126.385 newborn samples for CF. According to the Guidelines of the European Concerted Action of Cystic Fibrosis our protocol combines the assay of immunoreactive trypsinogen (IRT) with the analysis of the most common mutations of the CFTR gene in our population . Thus, the IRT raised samples are analysed for the F508del mutation with the PAGE method, and for the exons 7, 11, 12, 13, 14b and 17b by DGGE . The coverage of this study in our population reaches the 83% of CF causing mutations. The diagnose is established when two CF causing mutations are detected, or when only one mutation is detected but the sweat test is positive. Newborns with only one mutation detected but negative sweat test are considered carriers .

RESULTS:

YEAR	NEWBORNS	Irt RAISED SAMPLES	CF	CARRIERS
1999	17.128	141	5	1
2000	17.698	97	2	8
2001	17.380	146	5	10
2002	17.835	195	5	9
2003	18.395	210	3	15
2004	18.900	150	4	10
2005	19.049	169	7	11
2006	19.431	159	2	11
TOTAL	126.385	1267	33	75

Additionally, we have extended the mutation detection to other members in some families, including:

18 siblings

11 couples of carriers

3 prenatal diagnosis

The frequency of the F508del mutation in CF alleles was 60.6% (40/66)

P0081. Cystic Fibrosis testing among Israeli Arabs

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Since the cloning of cystic fibrosis (CF) gene in 1989, more than 1,000 mutations were identified. CF is recognized in all ethnic subgroups of Israeli Arabs. Tweleve mutations were found in the CFTR gene of individuals affected with CF from Israeli Arab origin. A total of 1080 healthy Arab individuals underwent CF carrier screening, 712 (65%) individuals at high risk and 368 individuals at lower risk. All individuals were tested for the twelve "CF Arab mutations" and three mutations that were found in non Ashkenazi Jews.

A total of 55 CF carriers were identified among the 1080 individuals screened, leading to an overall observed carrier frequency of 1/15 among the group at higher risk, and 1/60 among the lower risk one.

We conclude that any screening for CF among Arab Israeli community should therefore include at least the more common 7 mutations (N1303K, deltaF508, W1282X, G85E, 4010delTATT, 3120+1Kbdel8.6Kb and 2183AA>G) found in both groups examined. We doubt the benefit of testing the other mutations which seems to be rare and confined to only a small populations, unless the counselees are known to be at higher risk for their presence based on family/village history and investigation.

P0082. Psychomotor delay, facial dysmorphism and multiple cysts of the corpus callosum: a new syndrome?

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We report on a 16 months old child presenting with psychomotor delay, facial dysmorphism and multiple cysts of the corpus callosum. Family history was negative and he was born at term (BW: 2730g, OFC: 33,5 cm). He had generalised hypotonia and could sat at age 14 months only. At age 16 months, the language was limited to sounds. Facial dysmorphism included a large forehead, bilateral epicanthic folds, rare eyebrows, anteverted ear lobes, micrognathism and glossoptosis. A micropenis was also noted. There were no seizures. Etiological investigations included normal audiograms, electroencephalogram, heart and abdomino-renal ultrasound scans, standard and high resolution chromosome analyses, plasmatic amino-acid and urinary organic-acid chromatographies. Ophthalmologic investigations revealed anisocoria and a strabism. Finally, cerebral MRI showed multiple cysts of the corpus callosum. The gyration and myelinisation were normal, and the cysts did not take the contrast. A traumatic or vascular etiology was ruled out and there was no evidence of a toxic intake or infection during pregnancy. A foetopathy cannot be ruled out but the association of facial dysmorphism and micropenis argue for a syndromic origin. A review of the literature did not permit to find similar observations, in favour of a new syndromic association.

P0083. Czech Dysplasia Metatarsal Type: Another Type II Collagen Disorder

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Background: Czech dysplasia metatarsal type is an autosomal dominant disorder characterized by an early-onset, progressive spondyloarthropathy with normal stature. Shortness of 3rd and/or 4th toes is a frequently observed clinical feature. Similarities between individuals with this dysplasia and patients with an R275C mutation in the COL2A1 gene, prompted us to analyze the COL2A1 gene in the original families reported with Czech dysplasia.

Methods: Targeted sequencing of exon 13 of the COL2A1 gene was performed, followed by sequencing of the remaining exons in case the R275C mutation was not identified.

Results: We identified the R275C substitution in two of the original patients reported with Czech dysplasia and three additional patients. All affected individuals had a similar phenotype characterized by normal height, spondyloarthropathy, short postaxial toes and absence of ocular and orofacial anomalies. The R275C mutation was excluded in a third patient reported with Czech dysplasia. However, the identification of the Y1391C mutation in this patient with disproportionate short stature made the diagnosis of spondyloperipheral dysplasia (SPD) more likely. The Y1391C mutation is located in the carboxy-propeptide of the procollagen chain and has been reported before in a patient with the Torrance type of lethal platyspondylic skeletal dysplasia (PLSD-T). Our observation of the same Y1391 mutation in an additional unrelated patient with SPD further supports the evidence that PLSD-T and SPD represent a phenotypic continuum.

Conclusions: The R275C mutation in the COL2A1 gene causes a peculiar type II collagen disorder that was recently delineated as Czech dysplasia.

P0084. Investigation on Mitochondrial DNA deletions in Iranian Dilated Cardiomyopathy patients

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Cardiomyopathies are complex disease process that can affect the heart of a person of any age. In addition, an important cause of morbidity and mortality among the world is aging population. Cardiomyopathies are conditions in which the normal muscular function of the myocardium has been altered by specific or multiple etiologies, with varying degrees of physiologic compensation for that malfunction. Persons with cardiomyopathy may have asymptomatic left ventricular systolic dysfunction, left ventricular diastolic dysfunction, or both. The reported incidence is 400,000-500,000 cases per year, with a prevalence of 2-3 million people. Recently mitochondrial DNA mutations have been associated with cardiomyopathy. Mitochondria are the major site of energy production in the cell. Thus, it is reasonable to assume that energy dependant tissues such as heart, affected by mitochondrial dysfunction. In this study, we screened 40 Iranian DCM patients for mitochondrial DNA deletions. With specific primers, four different deletions were found 37.5% 9/5 kb, 22.5% ≈7/9 kb 22.5 % ≈8 kb and one patient (2.5%) had 4977 bp "common deletion. Mitochondrial DNA deletions may occur because of aging; on the other hand, mtDNA deletions may affect myocardium and lead to secondary DCM. However, the question regarding primary or secondary role of mtDNA deletions in dilated cardiomyopathy remains unanswered

P0085. A novel 355-356 delGA framshift mutation and frequency of connexin-26 (GJB2) mutation in Iranian patients

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The commonest form of non-syndromic recessive deafness is caused by mutation in GJB2, encoding gap junction beta 2 protein on chromosome location 13q11. It is known as DFNB1 responsible for half of autosomal recessive non-syndromic deafness. The most frequent mutation 35delG accounts for about 30-63% of mutations in white people of European.

In this study we report the frequency of the GJB2 gene mutations in 51 Iranian individuals affected by hearing loss.

Eight different variants were detected in 13 individuals (25.5%) by using direct-sequencing technique in coding region of GJB2 gene. Cx26 related deafness mutations (35delG, R127H, V27I+E114G, Y155X, M163V and a novel 355-356 delGA) were identified in 9(17.6%) subjects in heterozygous form, 3(5.9%) (35delG/35delG and R143W/R143W) and 1 (2%) (R32H+35delG) were homozygous and com-

pound heterozygous respectively.
S86T polymorphism was observed in all families (100%), V153I and (F154F +F146F) polymorphisms also were detected in 5 persons.
In this population study our data showed that the rate of GJB2 mutations is high in heterozygous form so other loci and genes related to deafness must be investigated in these individuals. Moreover the most frequent mutation was 35delG because 9 out of 18(50%) mutant alleles had this mutation .This is comparable with White European population.

P0086. Absence of mutations in *GJB2* (Connexin-26) gene in an Ethnic Group of Iranian Population

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We have investigated the prevalence of mutations in the GJB2 gene in Iranian deaf population with Arabian background using direct DNA sequencing methods. A common GJB2 gene mutation (35delG) was screened in 25 families with at least two deaf members. To investigate other GJB2 mutations, we have amplified and sequenced DNA from 59 deaf patients and 25 control subjects. None of the samples studied, by sequencing, revealed any deafness-associated mutations in the coding region of the GJB2 gene. These findings differ from many reports on the GJB2 gene, describing various mutations as the cause of congenital recessive deafness.

P0087. Phenotype and molecular mapping of the breakpoints in a new case with del(4) (q33)

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The 4q deletion syndrome, comprising all microscopically visible (interstitial and terminal) is a well-recognized distinctive malformation entity, with an estimated incidence of 1: 10.000. Here we present the clinical and molecular findings in a 3 year old male with a *de novo* deletion of 4q33 [46,XY,del(4q)(q33)]. The breakpoint of the deletion was refined by 1Mb resolution array-CGH where 23 clones on chromosome 4q were found deleted. Subsequently, several FISH analyses using 3 BAC clones, subtelomeric and paint probes were performed. Based on the array and FISH results the breakpoint of the deletion is located at the beginning of 4q33 and extending to the telomere. The size of the deletion was estimated to be approximately 18.9-22.9Mb. Clinical findings of the patient include: hypertelorism, broad nasal bridge, short nose with anteverted nares, long upper philtrum, thin upper lip, micro-retrognathia, low-set and protruding ears, right pre-auricular tag unilaterally, low posterior hairline, clinodactyly of the 5th fingers, tappering fingers, hypospadias, and severe psychomotor retardation. Soon after birth he developed severe hypotonia and feeding difficulties. Echocardiography at 15 months documented aortic supravalvular membrane resulting in mild aortic stenosis and dysplasia of the pulmonary valve. At age of 18 months the cleft palate was surgically repaired. The description of additional cases with similar deletions in proximal 4q will allow a more precise prognosis and is therefore of great value for genetic counsellors, while detailed molecular characterization in any well clinically characterized patient is expected to track down individual candidate genes for specific features of the 4q-syndrome.

P0088. Detection of chromosomal imbalances in children with developmental delay/mental retardation and dismorphic features by array-CGH.

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Chromosomal aberrations are a common cause of MCA/MR syndromes. Novel high resolutions, whole genome technologies, such as array based comparative genomic hybridization (array-CGH), improve the detection rate of submicroscopic chromosomal rearrangements, allowing reinvestigation of patients in whom conventional cytogenetic techniques and subtelomeric FISH failed to detect abnormalities. We analyzed 102 children and adolescents, with developmental delay/mental retardation and dysmorphic features, using array-CGH with a resolution of approximately 0.8-1 Mb (IntegraChip, Technogenetics, Milan). Standard cytogenetics (>450 bands per haploid genome) showed no chromosome rearrangements. In addition, subtelomeric FISH analysis, carried out in most of our patients, did not detect any abnormality. Using array-CGH we detected copy number imbalances in 10 patients (9.8%). There were 4 deletions, 4 duplications, 1 interstitial deletion, and 1 deletion/duplication. Five of them were de novo, one derived from a malsegregation of a maternal reciprocal translocation, and one was inherited from the phenotypically normal mother. Array-CGH is a powerful tool for the accurate detection of genomic imbalances in clinical practice, and has the potential to identify genes involved in mental retardation.

P0089. Novel mutations in Pejvakin are associated with autosomal recessive non syndromic hearing loss in Iranian families

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Hearing loss is a very heterogenous disorder and may be due to genetic or environmental causes, or both. More than 100 genes may be involved in non syndromic hearing loss. A novel gene, DFNB59 encoding pejvakin located on chromosome 2q31.2, has been very recently shown to cause neuronal deafness in four Iranian families. We have conducted mutation analysis of the DFNB59 gene in a cohort of 30 large autosomal recessive non syndromic hearing loss (ARNSHL) families from Iran. We have identified Two polymorphisms (R265C, R265G) and three novel allelic variants (726delT, G292R, 988delG) from which two family-specific DFNB59 mutations including 726delT and 988delG were detected. Our finding revealed that DFNB59-associated deafness accounting for 6.7% of ARNSHL families examined, suggesting DNB59 mutation is of significant clinical importance in Iran.

P0090. Studies of DMD/BMD deletion carriers by microsatellite markers analysis and FISH

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Detection of carriers is very important to offer genetic counseling and prenatal diagnosis to families with Duchenne/Becker muscular dystrophy. Results of DNA and FISH studies on 54 women at risk of being carriers (only one son affected with Duchene muscular dystrophy in the family) are presented. In 27 females microsatelite markers and pedigree analysis allowed to confirm or exclude carriers. In 27 remaining females microsatellite markers analysis was not informative - homozygosity of polymorphic sites was found. In 4 of these cases FISH using exon-specifc DNA probes was applied. As a control of correct hybrydisation reaction X centromere probe was used. All 4 suspected females were identified as a deletion carriers in metaphase or interphase nucleus.

P0091. Advanced grandmaternal age is not a risk factor for the occurrence of Down syndrome (DS) in St. Petersburg, Russian Federation

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Background. Advanced maternal age is a well-established factor of DS occurrence. However the majority of DS cases are born to young couples. Despite great efforts, etiological factors different from those proposed for maternal age have not been clearly defined. Malini and Ramachandra's recent study [BMC Med Genet 2006,7:4] has revived the old idea that grandmaternal age may increase the risk of their grandchild being born with DS. Objectives. Analysis of grandparental ages in families of DS babies with regular trisomy 21 to study if ma-

ternal grandmother's age in DS families where the mother is young is higher comparing to controls. Methods. Extraction of the data on grandparental ages in DS families and in families of healthy newborns from questionnaires collected in the course of a case-control study on congenital malformations in St.Petersburg, 1990-1999. The data were analyzed according to two categories of maternal ages, <35 yr and ≥35 yr. Results. Data on 243 families of DS and 330 control families were obtained. We did not find systematic differences in grandparental age distribution between studied groups. Specifically, in 148 young couples with DS, mean maternal grandmother's age (26.6 yr) was not higher comparing to paternal grandmother's age (26.3 yr) and corresponding figures in 284 young controls (27 yr and 27.1 yr). Similarly, there was no difference in age distribution between 95 older couples with DS and 44 control couples. Conclusion. We failed to support the suggestion that advanced age of the DS grandmother is responsible for meiotic disturbance in her daughter.

P0092. Sensitivity and frequencies of Dystrophin gene mutations in Thai males as detected by multiplex PCR

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*Background .*Duchenne muscular dystrophy (DMD) is a lethal X-linked disease affecting 1 in 3500 male births. Both DMD and its more benign variant, Becker's muscular dystrophy (BMD), are caused by mutations in the dystrophin gene. It is not possible to analyse the whole gene because of its enormous size. Methods have been developed to detect the commonest mutations, namely the deletions of the exons. Although these tests are highly specific, their sensitivity is inherently less than perfect and should be quantified.

Methods. We reviewed our database for the results of Dystrophin gene mutation detection by means of multiplex PCR in Thai males, diagnosed clinically with DMD or BMD from July 1994 to November 2006. Only one index patient was chosen from each family for statistical analysis. The overall sensitity of the test, the number of exon deleted, and the deletion frequency of each exon were calculated, along with their 95% confidence intervals (C.I.).

Results. We found deletions in 99 out of the 202 index patients (49%; Agresti-Coull 95% C.I. = 42%-56%). 51% of these had deletion in one exon, while the patient with the most extensive deletions had 14 exons deleted. The mean number of deleted exons were 2.84 (bootstrap 95% C.I. = 2.37-3.48). The region spanning exons 44-52 was the most frequently deleted.

Conclusion. The multiplex PCR detected deletions only in about half of the cases, and if DMD/BMD is strongly suspected, should be confirmed by more sensitive tests.

P0093. Dystrophin gene's hotspots in Iranian patients suspected to DMD orBMD

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The dystrophinopathies_Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD)_are the most common inherited disorders of muscle. Although reliable prevalence data are lacking, the prevalence of DMD is generally estimated at 1:3,500 live male births. Both DMD and BMD are due to mutations in the dystrophin gene, located at Xp21, which comprises 79 exons and 8 tissue-specificpromoters Distributed across 2.2 Mb of genomic sequence_making dystrophin the largest gene yet described.

Dystrophin gene deletions are found in 55%of patients with BMD and 65% of patients DMD; point mutations account for 30% of mutations, and duplications account for the remainder.

Genetic testing for deletions relies on a multiplex PCR technique, with amplification of fragments containing 20 of the gene's 79 exons and with deletions detected as absent or size-shifted bands on poly Acryl amide gel analysis. Because deletions tend to occur in "hotspots" within the dystrophin gene, analysis of this limited number of exons can detect 98% of dystrophin deletions.

Hot spots are exons 3-19 and 42-60.We studied all of these exons for 23 Iranian families.

In our study most common of deletion were in exon6, exon44, exon50, exon4 respectively.

P0094. DHPLC-based whole-gene mutation scanning of the dystrophin gene in Duchenne and Becker muscular dystrophy patients

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Background: Duchenne and Becker muscular dystrophy (DMD and BMD) are caused by mutations in the dystrophin gene. Large rearrangements in the gene are found in about two-thirds of DMD/BMD patients, with ~ 60 % carrying deletions and 5-10 % carrying duplications. Most of the remaining 30-35 % of patients is expected to have small nucleotide substitutions, insertions, or deletions. Since the dystrophin gene is very large, 79 exons spanning 2.4 Mb and pathogenic changes are scattered throughout the gene, most molecular diagnostic laboratories focus only on the identification of large deletions/duplications. Objective: To detect the remaining 30-35 % of small mutations within the coding and splice site determining sequences of the dystrophin gene, we optimized denaturing high performance liquid chromatography (DHPLC) mutation screening followed by direct sequencing. Validation was performed using DNA samples harboring known dystrophin variants. Clinical inclusion criteria for extended analysis of the dystrophin gene were set. Results and discussion: DHPLC mutation scanning was applied to analyze a cohort of DMD/BMD patients without large deletion/duplication. We identified sixteen clear pathogenic changes and one unclassified variant. The inclusion criteria were used to evaluate the detection efficiency of our DHPLC-conditions.

P0095. Association of dysferlinopathy and Antley-Bixler syndrome in the same patient mimicking muscular dystrophy with early contractures

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Dysferlinopathies are autosomal recessive muscular dystrophies caused by *DYSF* mutations and are clinically heterogenous, including Miyoshi myopathy, LGMD2B and proximo-distal myopathy. Onset is usually in the young adult, and early contractures are not a characteristic feature of the disease.

We report a 40 year-old woman born to related French parents. She was referred for muscle weakness of the lower limbs starting at age 25, associated with severe contractures, affecting mostly the elbows, and at a lesser extent the knees and the ankles, and thus was suspected to have muscular dystrophy with early contractures. Of importance, she had a craniosynostosis treated by surgery at age 2. At examination, she presented with proximal weakness of the lower limbs and severe atrophy of the calves. In addition, we noted dysmorphic features related to craniosynostosis, such as frontal bossing and proptosis, and severe elbow contractures which turned out to result from radiohumeral synostosis. All those features were consistent with Antley-Bixler syndrome (ABS) (OMIM 207410). Chromosomal analysis was normal.

Creatine Kinases were massively increased and muscle biopsy showed a dysferlin deficiency consistent with the diagnosis of primary dysferlinopathy, further confirmed by genetic investigations that identified a homozygous intragenic deletion in *DYSF* predicted to produce a truncated protein. To confirm the diagnosis of Antley-Bixler syndrome, FGFR2 and POR genes, which cause autosomal dominant ABS and autosomal recessive ABS-like respectively, are under investigation. Therefore, this case associating Antley-Bixler syndrome and dysferlinopathy, illustrates the clinical diagnostic pitfalls in patients carrying fortuitously two rare genetic disorders with overlapping features.

P0096. The patient with severe dysmorphic features and chromosomes abnormality with unidentified marker

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We present a patient with chromosomal abnormality with unidentified marker and severe dysmorphic features of phenotype. Our patient is a three-year-old girl, third child of healthy non consanguineous parents from uncomplicated pregnancy. The genealogy of this family is com-

plicated: two cousins - brother and sister with symptoms of psychomotoric retardation and one child with Down syndrome from the other cousin's family. The dysmorphic features were seen from the birth of this girl. Facial dysmorphism is characterised by the triangular high forehead with expressed sagital suture, hypertrichosis, wide spaced eyes, ptosis, down-slanting palpebral fissures, strabismus, broad nasal root, very short nose with anteverted nares, short grooved philtrum, triangular mouth, thin upper lip, everted lower lip, high narrow palate, micrognathia, malformed ears with preauricular sinus on one of sides. The characteristic skin lesions are typical for chromosomal mosaicism: they involve streaked, whorled and mottled areas of hypopigmentation on trunk and limbs. The psychomotor development of our patient is with severe features of delay: she sat alone only at two years. Clinical follow-up showed these clinical findings: CT scan showed corpus callosum agenesis and hydrocephaly, X-ray: abnormal feet position - equinovarus bilaterally.
Cytogenetic analysis of peripheral blood lymphocytes revealed a mosaic karyotype 47,XX, +mar/46,XX in girl with dysmorphism of phenotype. Chromosome analysis was performed from GTG banded metaphases. The resolution level was 400-500 bands. The exact nature of the marker chromosome could not be identified in our patient. Parental karyotypes were normal.

P0097. Disproportional high frequency of CLCN1 mutations among patients with myotonic dystrophy type 2.

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Background: Myotonic dystrophy type 2 (DM2) is a multiorgan disease caused by (CCTG)n repeat expansion mutation in *ZNF9* gene. Clinical core features are myotonia, muscle weakness and cataracts. The phenotype is highly variable ranging from mild to severe forms, which makes clinical classification difficult. DM2 mutation causes aberrant splicing of different genes including *CLCN1*. Mutations in ClC1 chloride channel gene (*CLCN1*) cause recessive and rarely dominant myotonia congenita (MC), characterized by myotonia and muscle hypertrophy.
Objective: to clarify whether co-segregation of frequent recessive CLC1 mutations may have a modifier effect on the DM2 phenotype.
Methods: *CLCN1* mutations R894X, F413C and A531V were analysed in 200 Finnish and German DM2 patients and 200 controls by TaqMan Sequence Detection System (ABI) using specific primers for PCR and fluorescent oligonucleotide probes.
Results: *CLCN1* mutations R894X, F413C and A531V in DM2 patients and controls.

Cohorts	R894X hoz	R894X hez	F413C hez	A531V hez	total
Finnish DM2 patients (n=100)	0	3	2	0	5
German DM2 patients (n=100)	1	4	0	0	5
Finnish controls (n=100)	0	2	1	0	3
German controls (n=100)	0	1	0	0	1

Conclusions: In German DM2 patients the frequency of CLC1 co-segregation was 5-fold and in the Finnish DM2 patients almost twofold compared to control population. The results clearly indicate a considerable contributing effect on the clinical symptoms and the likelihood of a patient to be diagnosed with DM2. Thus recessive *CLCN1* mutations are genetic modifiers in DM2.

P0098. A novel Italian family with ectodermal dysplasia-syndactyly-mental retardation syndrome

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Ectodermal Dysplasias (EDs) are a heterogeneous group of conditions presenting with hair, tooth, nails and sweat glands abnormalities. EDs may be sub-classified into pure forms and syndromic EDs, where ectodermal signs are combined with other anomalies. We studied two sibs, a 26 year-old man and his 9 year-old sister, born to unrelated healthy parents showing a distinct phenotype. Hair, eyebrows and eyelashes were sparse, coarse and brittle with areas of progressive scalp alopecia. The older patient showed axillary and pubic hypotrichosis and mildly dystrophic nails. Oral findings included multiple frenula, small, wide-spaced teeth with peg-shaped and conical crowns. Spontaneous sweating was normal. There were also broad nasal bridge, short philtrum with anteverted nares, and small ears with thickened, overfolded helices. The older brother showed 2-3 toes syndactyly and underwent surgical correction of 2-3 and 3-4 cutaneous syndactyly at the hands, while his sister had 3-4 hands syndactyly and toes 2-3 and 4-5. An abnormal pattern of palmar flexion creases was present in both sibs, with bilateral four-finger lines in the male. The oldest patient had also mild mental retardation (MR) and unilateral conductive deafness. These subjects add to the 7 previously reported (one family with 4 affected sibs and 3 sporadic cases) presenting the association of hidrotic ectodermal dysplasia, cutaneous syndactyly and variable mental retardation and ease the delineation of an autosomal recessive (AR) ED-syndactyly-MR syndrome. Differential diagnosis, among AR syndromic EDs, include Zlotogora-Orun syndrome, consisting of ectodermal dysplasia, syndactyly, mental retardation in addition to cleft lip/palate.

P0099. Lessons from two families affected with borderline forms of Vascular Elhers-Danlos syndrome: Genetic testing is necessary!

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Vascular Elhers-Danlos syndrome (V-EDS) is a rare dominantly inherited disorder cause by mutations in the type III procollagen gene (COL3A1). The diagnosis is particularly difficult to establish and a marked intra/inter-familial phenotypic variability occurs with frequent sub-clinical manifestations revealed by genetic testing and familial investigations. In view to help to the diagnosis work-up, the Villefranche criteria define the indications of laboratory testing that in practice only provides diagnosis certainty.
We had the opportunity to study two unrelated young women with a V-EDS suspicion and a strong familial history of Familial Thoracic Aortic Aneurysm and/or Aortic Dissection (TAA/AD). Attentive familial investigation also suggested the possibility of V-EDS manifestations. Thus, we performed a first screening of the COL3A1 gene from skin cultured fibroblast. We identified heterozygous transversion in each probant: c.1835G>A and c.2357G>T. Both mutations are predicted to result in a glycine substitution (p.Gly612Asp and p.Gly786Val). Theses missenses alter an obligatory glycine residue of the Gly-X-Y tripe-helix repeated domain of the type III procollagen. The mutations were confirmed at the genomic level and mutation-based familial investigation allowed to clarify affected-status in relatives with ambiguous phenotype.
In conclusion, V-EDS must be carefully evaluated at a familial level. Suggestive familial history such TAAD/AD, sudden death but also other events may reinforce the V-EDS suspicion and genetic testing is crucial for diagnosis certainty, efficient familial screening, risk assessment and preventive follow-up.

P0100. Adult onset forms of eIF2B related disorders: diagnostic value of MRI and molecular analysis

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Mutations in the five subunits of the eIF2B translation initiation factor have been reported in a heterogeneous group of autosomal recessive leukodystrophies characterized by a CSF-like aspect of the white matter (vanishing white matter)on magnetic resonance imaging (MRI), leading to the concept of eIF2B related disorders. The key role

of eIF2B in stress response explains the episodes of rapid deterioration following febrile infection or head trauma. Severity is correlated to age at disease onset, distinguishing between early infantile (<2 years), childhood (2-5 years, CACH) and juvenile-adults (>5 years) forms. Adult onset forms have been initially individualized as ovarioleukodystrophies due to associated primary ovarian failure (POF). We report 14 eIF2B mutated patients (2 males, 12 females) with an adult onset (mean age 30). Initial neurological symptoms were motor (9) as well as cognitive/psychiatric (4), whereas two patient has no neurological signs (1 POF, 1 asymptomatic). Abnormal stress response was observed in 5 patients and POF in 50% of females. MRI showed extensive leukoencephalopathy in all cases with cavitation in only 9. Disease evolution was rapid in one case (death after 9 months) but slowly progressive (7) or stable (5) in the 13 others (mean follow-up 10 years). The eIF2B5 recurrent mutation, c.338G>A or p.Arg113His, was present in the majority of patients (11) mainly at an homozygote state (9). In adult patients, despite an heterogeneous clinical presentation, diagnosis of eIF2B related disorders could be suggested by the MRI features and rapidly confirmed by detection of the p.Arg113His mutation.

P0101. Genotype influences epilepsy outcome in patients with *CDKL5* mutations

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Mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene are responsible for a severe encephalopathy with early epilepsy. So far, the electroclinical phenotype remains mostly unknown and no clear genotype-phenotype correlation has been established.

Objective: To characterize the epilepsy associated with *CDKL5* mutations and look for a relationship between the genotype and the course of epilepsy.

Methods: We retrospectively analyzed the electroclinical phenotype of 13 *CDKL5* mutated girls and examined whether the severity of the epilepsy was linked to the type and location of mutations.

Results: Epilepsy course discloses three successive stages: early epilepsy (Stage I) (onset 1-10 weeks) with normal interictal EEG (10/13) despite frequent convulsive seizures; epileptic encephalopathy (Stage II) with infantile spasms (8/8) and hypsarrhythmia (8/8). At the age of evaluation (2.5-19 years), 7 patients were seizure free and 6 had developed refractory epilepsy (stage III) with tonic seizures and myoclonia (5/6). According to the presence of a functional catalytic domain of the protein, patients were divided in two groups: those with early truncating mutations that lead to a truncation of the catalytic domain (7/13) and those with late truncating mutations (6/13). We observed that the former developed refractory epilepsy (5/7) more frequently than the latter (1/6). Moreover, infantile spasms were more frequently controlled in patients with late truncating mutations.

Interpretation: Our data suggest that the course of the epilepsy is more severe in patients with *CDKL5* mutations that impair the catalytic domain of the protein than in patients with late truncating mutations.

P0102. Epistatic Effect of CETP and LIPC on Serum HDL Levels: The Rotterdam Study

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Objectives: Polymorphisms in the hepatic lipase (LIPC -514C>T) and cholesteryl ester transfer protein (CETP I405V) genes affect HDL levels but their relationship with cardiovascular disease, and their combined effect, is unclear. The objectives of the current study were to characterize the effect of the hepatic lipase variant, and it's interaction with the CETP variant, in terms of cholesterol levels, atherosclerosis, and risk of myocardial infarction (MI).

Design: The study was conducted in the Rotterdam Study, a large single-center prospective cohort study in people aged 55 years and older. Lipid levels were analyzed using linear regression models and risk of MI was assessed with Cox' proportional hazards models.

Results: The hepatic lipase variant was associated with an increase in serum HDL levels of 0.11 mmol/L in both genders, while an increased risk of MI was observed only in men (hazard ratio=1.32 [95% Confidence Interval (C.I.): 1.05-1.66] for CT versus CC and 1.75 [95% C.I.: 1.39-2.20] for TT versus CC). This effect was independent of serum HDL levels. LIPC -514C>T interacted with CETP I405V with respect to serum HDL levels. Those homozygous for both mutations saw a marked elevation in HDL levels (0.29 mmol/L, $p_{interaction}$ = 0.05). These increased HDL levels, however, were not inversely associated with atherosclerosis or MI risk.

Conclusions: LIPC genotype affects HDL levels, and risk of MI in males. The interaction of this variant with CETP on HDL levels helps elucidate the underlying mechanisms and suggests that the beneficial effects of CETP inhibition may vary in particular subgroups.

P0103. Female monozygotic twins with Familial juvenile nephronophthisis - a case report

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Nephronophthisis (NPHP) or Familial juvenile nephronophthisis (FJN) is an autosomal recessive condition equally distributed in males and females that almost always progresses to end-stage renal disease usually during adolescence. Smith and Graham first reported NPHP in 1945, but the first description has been attributed to Fanconi et al in 1951. A gene involved in the disease - NPHP 1 has been mapped to chromosome 2q13. It has been estimated that FJN is responsible for approximately 2.4% of the cases of end-stage renal disease in children in the United States but this it may be underestimated as studies from Europe have revealed a higher frequency of 15% (Konrad et al). We present here a case of monozygotic twins emphasizing the identical progresion of the disease and importance of examination in children with deterioration of renal function, for determining a correct diagnosis. The first symptoms developed in our twins after the age of 10 and they consist of polyuria with polydipsia and than anemia. Renal ultrasonography revealed normal-sized kidneys.

P0104. The second observation of familial congenital tetramelic oligodactyly

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Congenital limb deficiencies are rare conditions, occurring in 5-6 of every 10,000 live births. Most are sporadic, additional malformations can be detected in approximately one third of the cases. Upper limbs are involved significantly more often than lower; both are affected only in 10% of the cases. Postaxial/ulnar deficiencies are often elements of more common disorders, like the ulnar-mammary syndrome - an autosomal dominant disorder caused by a mutation of the *TBX3* gene. The *TBX2* gene considered to have similar roles in limb development as the *TBX3*. Deletion of the 4q33 region has been also described in the background of ulnar ray deficiencies.

We report on a family, an affected mother and her 2 affected children, with apparently autosomal dominant postaxial oligodactyly and/or brachydactyly affecting the 5th fingers and toes, in non-syndromic form. Postaxial deficiency occurs in a number of genetic and sporadic syndromes, but isolated inherited tetramelic, postaxial oligodactyly - exactly similar to our case - has been described to our knowledge only once (Wulfsberg et al., 1993). We performed G-banded chromosomal analysis with normal result. ArrayCGH revealed a small deletion and duplication; however, both of the mutations have been described earlier as normal variants. Linkage to the chromosomal region harbouring the TBX2 and TBX3 gene and 4q33 chromosomal region was tested with STS markers. Although the exact genetic background has remained unclear, we could exclude the most likely candidate regions' role in the development of this condition. Further investigations may reveal the cause of this new, existing syndrome.

P0105. APC gene mutations in Polish FAP patients

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Familial adenomatous polyposis (FAP) is an autosomal dominant, predisposed disorder that results in the development of numerous polyps in the colon and rectum, usually beginning in childhood or adolescence. Other extracolonic features may include polyps in the upper parts of the gastroenterological tract, desmoid tumours, ocular lesions, osteomas, dental abnormalities, and malignancies in other organs. FAP incidence is estimated at 1:10,000. FAP arises due to germ line mutations in the adenomatous polyposis coli (APC) gene, consisting of 8.529 bp open reading frame and encoding a 2.843 amino acid protein.

Seven hundreds DNA samples from persons belonging to 280 Polish FAP families were collected. 380 patients were diagnosed with FAP. The entire APC gene was screened for mutations in 240 families. The APC gene point mutations were identified in 115 FAP families. Thirty of them have not been described before. Seven mutation types recurred two or more times. The recurrent mutations were detected in 52% of diagnosed families. The large rearrangements of the APC gene were studied in 95 FAP families without the point mutation. In this group we identified 14 large APC gene rearrangements with two cases of whole APC gene deletions.

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P0106. A new familial case of Gollop-Wolfgang syndrome confirming an Autosomal Recessive mode of inheritance

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Gollop-Wolfgang syndrome is a rare condition of unknown aetiology characterized by the presence of femur bifurcation, tibial agenesis and ectrodactyly. Several additional abnormalities such as congenital heart defects, vertebral segmentation defects, ribs and CNS anomalies have been described. Based on an affected child of a consanguineous Arab family with additionally similarly diseased relatives, an autosomal recessive type of inheritance was supposed. However the presence of sporadic cases with unrelated parents as well as an association of a single case with proximal deletion of chromosome 8p suggests the existence of a dominant form.

We report on a newborn child of consanguineous Turkish family presenting unilateral bifid femur and ipsilateral ectrodactyly of the hand and foot. No additional abnormalities have been observed. Later on we were able to examine her 13 year old uncle who has similar but bilateral hand and limb anomalies. Mental development was normal, and he presented no other anomalies. The present case strongly supports an autosomal recessive type of inheritance of this condition. A possible pathway and classification of the femur bifurcation-tibial a/hypoplasia complex will be discussed.

P0107. Fetal Alcohol Spectrum Disorder - a clinical study and suggestions for an adapted questionaire concerning fetal exposure in Romania

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Fetal Alcohol Spectrum Disorder (FASD) refers to disabilities caused by prenatal exposure to alcohol - fetal alcohol syndrome(FAS), partial fetal alcohol syndrome(p-FAS) and alcohol related neurodevelopmental disorders(ARND). FAS is defined by: pre/postnatal growth retardation, facial dismorphysm and SNC dysfunction.

We have used the Canadian guidelines for FASD to analyze our patients in order to appreciate their importance in establishing the diagnosis. This involved evaluation score for upper lip, philtrum, palpebral fissures, impaired pre/postnatal growth, CNS/neurobehavioural disorders and gestational exposure to alcohol.

63 children (FASD in observation between 1994-2006) were selected for the study. Only 47 patients fulfilled the criteria for FASD (25 -definite FAS, 6 -p-FAS and 5 -ARND). The 11 children left remain in observation due to young age.

We have analyzed the frequency of defining characteristics for every category: for FAS children - microcephaly (100%), postnatal growth retardation (76%), smooth/flattened philtrum rank 5 (56%), thin upper lip rank 5 (60%), moderate mental retardation (80%), learning difficulties and attention deficit/hyperactivity disorder (100%). Prenatal alcohol exposure was confirmed in 44% of cases. Other defects recorded: heart defects, genitourinary, ocular abnormalities and hernia.

Patients diagnosed with p-FAS and ARND present similar characteristics. The screening questionaire for maternal alcohol history didn't fit to our population and we have adapted it. The detailed protocol as well as the differential diagnosis will be presented.

In conclusion, we appreciate that the guidelines are very useful for FASD diagnosis and we have adapted the protocol for our population in order to optimize FASD diagnosis.

P0108. Clinical features in patients with Opitz-Kaveggia (FG) syndrome and a recurrent mutation, p.R961W, in the *MED12* gene

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Opitz and Kaveggia (1974) reported a family of 5 affected males with mental retardation, macrocephaly, imperforate anus and hypotonia, and Risheg et al. (2007) reported an identical nucleotide substitution (c.2881C>T) in exon 21 of *MED12* causing tryptophan to replace arginine at amino acid 961 (p.R961W) in 6 families with Opitz-Kaveggia syndrome (13% of 45 clinically-diagnosed cases), including the only surviving affected male from the original Opitz-Kaveggia family. Moderately severe mental retardation with behavioral abnormalities was present in all males old enough for cognitive assessment. Partial or complete absence of the corpus callosum was noted in all six cases in which brain imaging was available, and congenital hypotonia was noted in all but one case. High prominent forehead and small, low set, simple ears were the most consistent craniofacial manifestations. Only one individual had macrocephaly (OFC >97th centile), although the occipitofrontal circumference centile was greater than the height centile in 7 of 9 cases. Imperforate anus and wide flat thumbs and great toes were present in 7 of 10 cases. Cryptorchidism, inguinal hernia, cardiac defects, and short stature were noted in less than half of affected males. Constipation was noted in 5 of 8 cases, one case had dystonia of the head and neck, one case had sagittal craniosynostosis, and one case had duplicated great toes with a split right hand. Hyperactivity, affability, and excessive talkativeness were frequent manifestations, along with socially-oriented, attention-seeking behaviors.

P0109. Fibrous Dysplasia: report of two portuguese patients

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Fibrous Dysplasia (McCune-Albright syndrome #MIM 174800), of bone is characterized by the replacement of bone by dysplastic fibrous tissue. It is classified on the basis of whether the lesions involve one bone or more than one bone. The cutaneous lesions consist of brown flat patches of pigmentation that follow an irregular contour. Sexual precocity and hyperthyroidism are common features. The condition is mostly sporadic and usually results from postzygotic activating mutations in *GNAS1.*

We present a short review of the McCune-Albright syndrome and two clinical cases of patients with the clinical diagnosis of this syndrome from our genetic clinic.

Patient 1, a female, age 17, with vaginal bleeding at age 3, and at present time with one café-au-lait spot in the dorsal region respecting the midline and polyostotic fibrous dysplasia affecting the skull causing facial asymmetry and the long bones of the four members, with more expressive lesion near the right knee. Patient 2 is a male, age 31, presented at age 25 an expansive and suggestive lesion of two left

ribs causing compression and the need for surgical treatment. He later developed some more severe lesions and there is also a café-au-lait spot in the dorsal region.
Diagnosis of McCune-Albright syndrome is established on clinical grounds. Early diagnosis allows for opportune intervention and for specific aspects of follow-up and management of skeletal and endocrine involvement that should be considered. We address the issue that this condition probably remains underdiagnosed. We emphasize the importance of early referral for genetic counselling.

P0110. The recessive form of Larsen syndrome is not due to FLNB mutations

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Larsen syndrome shows a broad spectrum of clinical manifestations ranging from a lethal form of the disorder to a mild clinical expression with absence of major diagnostic features. Autosomal recessive as well as autosomal dominant mode of inheritance have been reported.
We report on two cases of this rare condition. The first child was a dizygotic twin born at 29 weeks' gestation from non-consanguineous and healthy parents, after a normal pregnancy. She presented with multiple congenital anomalies and died soon after birth. Pathological examination and x-rays showed intrauterine growth retardation, hydrocephalus, short limbs, talipes, severe vertebral anomalies, multiple costal synostosis and dislocated knees. The diagnosis of autosomal recessive Larsen syndrome was suggested, and genetic counselling was cautious. During a further pregnancy, the first trimester ultrasound scan revealed bilateral talipes, abnormal vertebrae and dislocated knees. Fetal chromosomes were normal 46XX. Termination of pregnancy was performed at 14 weeks' gestation. Pathological examination and x-rays confirmed the talipes and revealed associated features such as cleft palate and multiple skeletal anomalies, compatible with the diagnosis of autosomal recessive Larsen syndrome.
Recently, mutations in the gene encoding filamin B have been identified in four human skeletal disorders, i.e. autosomal recessive spondylocarpotarsal syndrome, autosomal dominant Larsen syndrome and atelosteogenesis type I and III. Since the spondylocostal aspects of autosomal recessive Larsen syndrome resemble the autosomal recessive spondylocarpotarsal syndrome, we performed FLNB molecular analysis in the second case, but no mutation was identified.
Therefore, we conclude that FLNB mutations do not cause autosomal recessive Larsen syndrome.

P0111. MEFV gene mutations in armenian FMF patients

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Familial Mediterranean Fever (FMF) is an inherited inflammatory condition usually occurred in populations from Mediterranean decent with prevalence as high as 1 in 5 individuals. Identification of MEFV gene mutations have been of tremendous help for diagnosis of difficult cases. Testing is helpful for carrier screening and pregnancy planning since certain mutations have been shown to have significant correlation with renal amyloidosis, the worst possible manifestation of FMF. 12 MEFV mutations are identified in 6500 Armenian patients. Identification of MEFV mutations in FMF patients (heterozygotes, homozygotes and compound heterozygotes) in comparison with healthy individuals have revealed the most frequent mutations and genotypes, and give the information of carriers and genotype - phenotype correlation. In heterozygote carriers the most prevalent and severe cases are caused by the presence of a single M694V mutation.
We have revealed the FMF cases with following concurrent morbidity: epilepsy (M694V/M694V; V726A/M680I); Sjogren syndrome (M694V/M694V); monozygotic twins, heterozygous carriers for M680I mutation: one with FMF, and the other - non-FMF, but with epilepsy; bronchial asthma (M694V/V726A, V726A/M680I, M680I); β-thalassemia (M694V/M694V); hyperthyroidism (M694V/M680I); Tourette syndrome (M694V/M694V); ulcerative colitis (M694V/M694V); renal amyloidosis and multiple sclerosis (M680I/M680I). Neurological features are accompanied along with administration of colchicines. About 20% FMF patients (predominantly M694V homozygotes) had ankylosing spondilitis-like syndrome. For Armenian FMF children the onset of the disease presented by monoarthritis is peculiar phenomenon. In 141 patients with some FMF features no MEFV mutations were found.
In 90% of cases, Colchicine is effective to keep the inflammation under control.

P0112. Goltz syndrome or focal dermal hypoplasia: family case report with affected mother and two stillborn daughters

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INTRODUCTION. Focal dermal hypoplasia or Goltz syndrome is a rare mesoectodermal dysplasia with multisystem involvement. Patient suffers from skin, skeletal, dental, ocular and other anomalies. Although the mutated gene has not been identified, there is predominance in affected females, suggesting X -linked dominant inheritance with lethality in men who are hemizygous for the X chromosome.
PATIENTS. We describe a family in which affected mother with one apparently normal daughter was undiagnosed until the birth of severely affected female stillborn of 34 weeks gestation. The diagnosis was confirmed by the second very malformed stillborn daughter of 25 weeks gestation. Clinical features of the mother were characterized by typical ,,lobster claw" deformity of the right hand, ectrodactily of right foot, typical cutaneous lesions with rather asymmetrical distribution, and upper median incisors spacing. Stillborns had diffuse distribution of typical skin lesions, ectrodactily, exomphalos, microphthalmia and anophthalmia, dysmorphic face with malformed pinnae and micrognathia.
DISCUSSION. Reported family seems interesting because ,,mildly" affected mother with rather asymmetrical (right sided, as majority of reported cases) lesions' distribution could be somatic and germ line mosaic for an X-linked dominant mutation which would explain her less severe phenotype in comparison with two very malformed female stillborns. Unavailable mother's family study does not permit the exclusion of transmitted mutation.
In CONCLUSION. The extraordinary variable expressivity of X-linked disorders should be explained by multiple mechanisms including skewed X-inactivation, clonal expansion, cell autonomous expression and somatic mosaicism that can result in disease expression in females.

P0113. MS-MLPA as a tool to distinguish FMR1 premutations from full mutations

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The *FMR1* gene is directly associated with three distinct conditions: fragile X syndrome, fragile X-associated tremor/ataxia syndrome (FXTAS) and premature ovarian failure (POF). *FMR1* is highly conserved and contains a CGG trinucleotide repeat in the 5' UTR of exon 1. Based on the number of repeats, it is possible to distinguish four types of alleles: normal (5-50 repeats), intermediate (45-60), premutation (55-200), and full mutation (>200). Excess *FMR1* transcription occurs in the premutation setting and is associated with both FXTAS and POF, whereas *FMR1* silencing through promoter hypermethylation and/or translational suppression of *FMR1* occurs in the full mutation setting, and is strongly linked to fragile X syndrome.
Experimental determination of expansions greater than ~100-150 repeats is difficult to achieve through standard PCR, due to high GC content of the target sequence. Analysis of cases not amplifiable by PCR is normally performed using Southern blot analysis, a low-resolution, time-consuming technique that requires large amounts of patient material.
In this study, we use methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) to analyse fragile X syndrome cases not amplifiable by PCR.
Whereas results of male unaffected controls, premutants and full mutants were unequivocal, analysis of female patients was complicated by the variability of both the level of imprinting and of hypermethylation of fully mutated alleles.
We conclude that MS-MLPA can be used to correctly diagnose male patient material not amplifiable through conventional PCR, and gives

an indication of the presence of *FMR1* mRNA in female patients, which might have clinical implications regarding subsequent therapy.

P0114. Prevalence of Fragile X Premutation and Intermediate/ Grey Zone Alleles in a Basque Sample

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The Fragile X Syndrome (FXS; OMIM: 309550) is the most common inherited form of mental retardation. The molecular basis is usually the unstable expansion of a CGG trinucleotide repeat in the FMR 1 gene witch resides at chromosome position Xq27.3 and is coincident with the fragile site FRAXA.Based on the size of CGG secuence individuals are classified as having normal (6-54 CGG), premutation (55-200 CGG) and full mutation alleles (>200 CGG). In adition the term intermediate/grey zone alleles has been used to define alleles with sizes at high range of normal alleles (35-54 CGG). Our previous cytogenetic and molecular screening for Fragile X Syndrome among mental retarded people of Basque and no Basque origin showed and absence of full mutation among Basque Sample. In the present work we analized the prevalence of FMR 1 premutated and intermedite/grey zone alleles, because recent clinical and molecular studies have changed the view that premutated alleles serve only as a source for full mutation alleles in transmision of FXS and that functional and phenotyphic effects are not associated with FMR 1 repeat size in the high end of the normal range alleles. A total of 298 unrelated male individuals were included in this study. The results obtained showed that the estimated prevalence of the intermediate/grey zone alleles 1/10 in Basque males (7.32 %) was lower than that reported in Caucasian populations. The prevalence of premutation alleles is 1/298 in males. The prevalence of premutation alleles in the general populated is estimated in 1/813 males.

P0115. 47,XYY male with the fragile X syndrome: rare genetic assotiation

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We report on a 26 year-old developmentally disabled man referred to our clinic for evaluation because of pregnancy of his younger sister. Cytogenetic and molecular analysis revealed a 47, XYY karyotype and the presence of a trinucleotide repeat expansion resulting in fragile X syndrome. Direct detection of the pre- and full mutation for the affected individual and his at-risk female relatives were performed . To our knowledge, this is the third report of concurrence of XYY and fragile X syndrome in the medical literature (but one of these patients was 46,XY/47,XYY mosaic male with fragile X syndrome) . Review of sex chromosome abnormalities associated with fragile X syndrome and phenotypic considerations are presented.

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P0116. Cardiac evaluation of 34 individuals with Fragile X Syndrome

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Fragile X syndrome is the most common inherited cause of mental retardation. Patients with fragile X syndrome have cardiac defects similar to those seen in other disorders of connective tissue such as Marfan's syndrome and Ehlers-Danlos syndrome. These, and other somatic features, suggest an underlying connective tissue dysplasia. The underlying connection between Fragile X Mental Retardation Protein (FMRP) and the connective tissue dysplasia seen in FXS is still unclear. The limited numbers of studies evaluating cardiovascular aspects of FXS have yielded different results. In this study, transthoracic echocardiogram and ECG were available in 34 male participants. Mean age was 9,17 years [2-34 yrs]. There were two adult patients, one with mitral valve prolapsus (MVP), the other with aortic insufficiency. The mean age of participants under 18 years was 8,06 (the eldest 17 years old). We compared our results with the data provided in the largest study yet (n=40) by Loehr et al. (1986). The comparison suggests that the prevalence of MVP is somewhat lower 8/32 (25%) in our study, while it was 35.2% in the latter, with a similar mean age of 8.6 years, while aortic annulus dilation was higher in the current study, 5/32 (15,6%) versus (10%). There was no significant correlation between metacarpophalangeal joint hyperextensibility and MVP. This data supports previous recommendations towards routine echocardiographic evaluation in individuals with FXS. Re-evaluation of the same group especially follow-up measurements of aortic annulus diameters in our ongoing longitudinal study will reveal the natural history of cardiac complications in FXS.

P0117. Audiological evaluation of 63 males with Fragile X

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Fragile X syndrome is the most common inherited cause of mental retardation. Medical problems other than cognitive and behavioural issues have been rarely addressed in individuals with FXS.
In this study 36 individuals molecularly diagnosed with FXS were evaluated. Mean age of the participants was 8,41 yrs [1,5-27]. Median IQ score was 45 [25-103] Seventy-two percent were having occupational therapy, while 25% also attended a public school.
ENT examination revealed cerumen in 12 (33%), opaque ear drum in 4 (11%) Audiologic evaluation revealed normal hearing in 29 participants (80,5), while 7 (19,5) had minimal conductive type hearing loss. In general , infants from birth to 6 months underwent observation audiometry; children between 6-36 months of age underwent Visual Response Audiometry (VRA) in soundfield; and children 36 months of age and older underwent play audiometry in soundfield or with earphones according to the degree of the child's cooperation. Cooperation problems due to mental retardation affected this rule negatively. 19 non-cooperative patients (52,78%)(age range 7-27 yrs) were tested by VRA or play audiometry. In 7 patients(19,45%), suprathresholds responses were observed. For these patients, objective test methods were used effectively for final decision. There was no correlation between IQ scores and hearing status. Objective test methods were more feasible for diagnose in patients with Fragil X syndrome. Mean age at diagnosis was significantly lower in the group with hearing loss. This may reflect the earlier referral to a physician with recurrent infection or speech delay may have prompted earlier diagnosis.

P0118. Fragile X syndrome in Estonia

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The Fragile X syndrome is the most common human chromosomal monogenic disease associated with heritable mental retardation, and is the second most frequent cause of mental retardation after Down syndrome. Our study consists of the screening of 561 Estonian children having mental retardation, autism, delayed speech and/or behavioral problems. The patient samples were sent to the United Laboratories of Tartu University Hospital for screening of fragile X mutation from year 1997 to 2006. The molecular study for the diagnosis has been performed by two different techniques. During 1997-2001 the Southern blot analysis and later PCR with fluorescently marked primers followed by CGG repeat length detection at ABI PRISM 377 was used. Among 561 (477 boys/84 girls) patients we found 15 full mutations (14 boys/ 1 girl), and 1 premutation in a girl. The main indication for the analysis in diagnosed boys was mental retardation in 11 patients, autism in 2 patients, and characteristic phenotype in 1 patient. The girl with a full mutation was investigated due to psychomotor retardation and delayed speech. The girl with a premutation had mild mental retardation and primary ovarian failure. The birth prevalence of full mutation in FMR1 gene causing fragile X syndrome is 1 in 25,308 among individuals born during 1984-2006. We found that birth prevalence of the fragile X syndrome is significantly lower in Estonia than in other countries. The reason for that is still unknown for us.

P0119. Is she a carrier of fragile-X? Discrepancies in the results of fragile-X tests using different agarose.

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Routinely, we perform genetic and prenatal testing for the fragile-X syndrome using Gene Scan (GS) and Southern Blot (SB) analyses. About 140 women are being tested each month and the DNA extraction is being done by routine salting-out procedure for all women. When using EL-agarose (*Seakam*) for SB, about 3% of the women reveal a „smear" of the upper allele, suspected as an unstable allele. This smear starts in the normal range of the repeats and reaches into the premutation zone. This unstable allele appears stable in GS ranging between of 42-55 repeats. These women undergo a second DNA extraction from fresh blood sample and again the same pattern of instability appears in SB. This result leads us to suspect that there is a biological background for this discrepancy. When the women showing the „smear" undergo prenatal diagnosis, all fetuses revealed a stable allele. Testing the same women with other agarose gels (agarose1gel *-Amaresco*, or agarose D1 *hylabs*), the „smear" of the upper allele was not apparent and the band appears stable.
A double dilemma thus ensues. First, which of the technique reflects the biological reality? To answer this, we are currently performing sequencing of the FMR1 gene in women who show the smear in SB and in women with a stable SB allele. Second, until the reason for the discrepancy is made clear, should we consult the women with a suspected „unstable allele" to undergo a procedure of invasive prenatal diagnosis?

P0120. A case report of monozygotic twins discordant for frontonasal dysplasia: The etiology of this affection is probably not genetic

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Frontonasal dysplasia (FND), also called the median cleft face syndrome, encompasses a pattern of anomalies limited to the face and head. The main features include marked hypertelorism, lack of nasal tip, anterior cranium bifidum occultum and a "widow's peak". Associated midline defects include median clefting involving the nose, upper lip, or rarely the palate and alae nasi and an anterior encephalocoele. Intellectual development is usually normal and most of cases are sporadic. Sometimes, the cranio facial malformations of the FND are associated with extra cranial malformations such Fallot tetralogy, absent tibia or auricular abnormalities. In this syndromic form, mental retardation is a common feature and familial cases are described. The etiology of non syndromic and syndromic FND is probably not the same. In the literature several sets of monozygotic twins in which only a single twin was affected with non syndromic FND were reported.
Here we describe a patient issue from a monozygotic pair of twins with pronounced hypertelorism, short palpebral fissures, coloboma involving cranial nerves, choanal atresia, broad and bifid nasal tip, cleft palate and anterior encephalocoele corresponding to the set of craniofacial malformations of FND. The other twin is normal. This case leads us to discuss the embryologic support of these malformations with the hypothesis that FND is non-genetic in origin but a form of embryonic disturbance that can result from the twinning process itself.

P0121. Multi-infarct dementia in two brothers with a pre-mutation in the FMR1 gene : different presentation of FXTAS ?

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Fragile X syndrome is one of the most common causes of mental retardation, caused by expansion of the CGG repeat in the FMR1 gene. A full mutation (>200 repeat) leads to serious neurodevelopmental problems, especially in males, while males with a pre-mutation are generally spared the mental retardation.
Since several years however, it is known that more than one third of male pre-mutation carriers (55-200 repeats) over 50 years of age develop neurological symptoms, most frequently characterized by ataxia, tremor, dementia and Parkinsonism. The condition was called FXTAS (fragile X associated tremor/ataxia syndrome).
Typical MRI changes are seen, with cerebral atrophy and T2 hyperintensive signals in cerebellar peduncles, pons, or subcortical white matter.
Postmortem studies show eosinophilic intra-nuclear inclusions throughout the brain of affected males, and more recently it was shown that FXTAS occurred in males with elevated levels of FMR1 mRNA, leading to the suggestion that the syndrome is caused by toxic gain-of-function of mRNA.
We describe a family with Fragile X syndrome, in which two brothers with the pre-mutation show a neurological pattern, slightly distinct from the symptoms normally seen in FXTAS. It might be a variable presentation of the syndrome or a coincidental picture, not linked to the pre-mutation.

P0122. Long term outcome in 20 Iranian galactosemia patients

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During last 6 years more than on hundred and fifty cases suspected to be affected by Galactosemia were refered to the National Institute for Genetic Engineering and Biotechnology (NIGEB) for biochemical and molecular analysis.
In a retrospective study, 20 galactosemic patients, were traced during 2001-2007 and their long term outcome were evaluated. Correlation between genotype and phenotype was studied.
Although most of the patients were diagnosed on the basis of clinical symptoms in advance, Investigation of long term results of treating galactosemia by clinical, psychometric and laboratory testing has shown poor results.
Three cases with normal GALT activity but with clinical manifestations of galactosemia (cases of non classic galactosemia) were found.
All the findings emphasis on the need of a new look and a new challenge for galactosemia in Iran concerning screening tests, differential diagnosis tests and metabolites assays for Iranian newborns.

P0123. Two cases of subacute neuronopathic type of Gaucher disease

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We report two Gaucher disease (GD) patients with subacute neuronopathic type of disease with rare genotypes.
Patient 1 was an 11 years old girl. She was diagnosed at the age of 2 years as GD. The first clinical symptoms were hepatomegaly with marked splenomegaly, mild or moderate anemia, thrombocytopenia and weakness. Bone marrow showed the presence of Gaucher cells. At age 2 years old she had undergone total splenectomy. Bone lesions in the both femur were appeared when she was 3 years old. The patient suffered from bone crises. Neurological symptoms appeared at age of 9 years. There were mild disturbance of eye movements and severe involuntary movements like as choreoathetoses, which made gait impossible. She has had three episodes of generalized seizures. Now she has mild hemiparesis. MRI of the brain with contrast revealed moderately extensive changes in the capsula interna. Height and weight were below normal. The ß-glucocerebrosidase activity was 2,9 nmol/h/mg protein, genotype G377S/c999G→A.
Patient 2 was a 15 years old boy. He was diagnosed as GD after bone marrow biopsy. The initial clinical symptoms were disturbance of eye movements that appeared at the age of 2 years. At the age of 3 years mild hepatosplenomegaly with mild anemia and thrombocytopenia was noted. Height and weight were normal. The patient did not suffer from bone crises. Now his main problems are mild hyperkinesis of the head and disturbance of eye movements. The ß-glucocerebrosidase activity was 4,2 nmol/h/mg protein, genotype D409H/R120W/G202R.

P0124. Results of 5 years Genetic Counseling in Iranian Province of Hormozgan

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- Genetic diseases have high frequency in Iran because of consangious marriages.According to clerical approval of therapeutic abortion for some genetic diseases after 1997 in Iran genetic counseling has a

great importance prior pregnancy.
From 2001-2006 we performed a total of 1523 genetic counselings. Results were as follows:
- 679 couples (44.5%) came for genetic counseling because of involvement with a genetic disease and 844 couples (55.5%) came without any genetic problem and only for assurance. Consangious marriages 905 cases (59.4%) was more common than unconsangious marriages. Requesting for genetic counseling was: 678 couples (44.5%) prepregnancy, 588 couples (38.6%) prior marriage and 257 couples (16.9%) during pregnancy. The most genetic disorders were:
Hemoglobinopathies: 307 cases (45.2% includes β- thalassemia,α-thalassemia,sickle cell anemia and other hemoglobinopathies)
Mental Retardation: 228 cases (33.9%)
Genetic syndromes: 40 cases (5.9% like apert,Turner,Ehlers-Danlos,…)
Recurrent abortion: 29 cases (4.2%)
Physical disabilithies: 29 cases (4.2%)
Deafness: 17 cases (2.5%)
Infertility: 12 cases (1,7%)
Neural tube defects: 8 cases (1.2%)
Blindness: 4 cases (0.5%)
Primary amenorrhea: 4 cases (0.5%)
The main conclusion is importance of genetic counseling prior marriage and pregnancy because of high frequency of autosomal recessive diseases due to consangious marriages in Iran.

P0125. Incomplete penetrance of G61E and R390H mutations in *CYP1B1* among Iranian Primary Congenital Glaucoma patients

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Glaucoma is a heterogeneous group of optic neuropathies characterized by degeneration of the optic nerve, usually associated with elevated intraocular pressure. It is the cause of 15% of blindness worldwide. Primary congenital glaucoma (PCG), one of the three major forms of the disease, becomes apparent at birth or before the age of three and is a major cause of childhood blindness. Mutations in both alleles of the cytochrome P4501B1 (*CYP1B1*) gene, which is the only gene thus far linked to PCG, result in the disease phenotype. It has been recently shown that mutations in this gene is cause of disease in approximately 70% of Iranian PCG patients and that the common mutations in the population are G61E, R368H, R390H, and R469W. We have now done a mutation screen in apparently unaffected family members of pedigrees whose proband carried the G61E or R390H mutations. Two apparently unaffected individuals carried G61E in the homozygous state and one carried the R390H mutation in the homozygous state. This indicates incomplete penetrance for these two mutaitons. Incomplete penetrance for R390H has not been previously reported. Incomplete penetrance has implications for role of the *CYP1B1* gene in pathogenesis and for diagnosis.

P0126. Screening for GCK and HNF1α mutations in Polish women with gestational diabetes.

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Mutations in the glucokinase (*GCK*) and hepatocyte nuclear factor 1α (*HNF1α*) gene cause maturity-onset diabetes of the young (MODY) type 2 and 3, respectively. The aim of the study was to examine the prevalence of mutations in *GCK* and *HNF 1α* genes, in Polish women with gestational diabetes mellitus (GDM). SSCP analysis and/or direct sequencing of the coding regions of *GCK* and *HNF 1α* gene were done in 119 Caucasian gestational diabetic subjects, fulfilling tree from the following criteria: age <35 years, BMI before pregnant <30, an increment in glucose level during 2-h glucose tolerance test (OGTT) <3 mmol/l, and a family history of type 2 diabetes mellitus (type 2 DM) or gestational diabetes. In 11 probands (9%) we detected three *GCK* mutations: G448fs, E312Q, S383L and four intronic variants: IVS2-12C>T, IVS3-8G>A, IVS7-13A>G, IVS4+26C>A. All mutations were absent from 210 control subjects ($P< 0.05$). In families carrying *GCK* mutations the genotype- phenotype correlations were analyzed. In *HNF1α* gene we identified 11 polymorphisms and 1 rare variant sequence IVS5-27C>T. Our results indicated, that the frequency of mutations in *GCK* in Polish population of gestational diabetes patients is not high.

P0127. Gomez-Lopez-Hernandez-Syndrome in a 6-yaers old boy with behavior problems but normal intelligence

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We are reporting a boy who is the first child of healthy parents, born in time after an uneventfull pregnancy with normal birth weight, lentgh and head circumference. In the first year of his life feeding problems occurred, failure to thrive, hypotonia and strabismus were noted. Motor development was delayed. The boy started to walk at 18 month and ataxia was diagnosed. The parents observed a sleep disturbance beginning in the first weeks after birth. During the day the boy exhibited hyperactive and aggressive behavior, as well as attention deficits. An intelligence test revealed a normal IQ of 118. MRI of the brain at the age of 6 years showed fusion of cerebellar hemispheres and agenesis of the cerebellar vermis, the typical features of rhompencephalosynapsis. No further abnormalities were found. An examination at the age of 6 years showed a long face with a high forehead, mid-face hypoplasia, deeply set eyes, telecanthus, epicanthus inversus, broad nasal bridge, pointed chin, deeply set posterior rotated ears and bitemporal alopecia areata. Syndactyly of the toes II/III on both feet was present. Alopecia and rhombencephalosynapsis together with trigeminal anesthesia are the major symptoms of Gomez-Lopez-Hernandez-Syndrome, a very rare sporadic syndrome. Since the first report in 1979 ten cases of Gomez-Lopez-Hernandez-Syndrome have been reported. Additional symptomes frequently described in Gomez-Lopez-Hernandez-Syndrome are facial dysmorphism and craniosynostisis/asymmetric skull. While the intellectual impairment is common in this syndrome our patient showed normal intelligence. Difficulties in behaviour may be due to the cerebellar abnormalities.

P0128. Gorlin syndrome in seven patients

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Gorlin syndrome is an autosomal dominant disorder characterized by cutaneous basal cell carcinomas, odontogenic keratocysts and skeletal anomalies. The prevalence is estimated from 1:57000 to 1:164000. Seven patients with Gorlin syndrome are presented. First patient presented with macrocephaly, thick and prominent eyebrows, a flat maxillary region, prognathism with mandibular hypertrophy and cystic mandibular lesions, multiple hyperpigmented lesions and hypogonadotropic hypogonadism. Triventricular widening and aqueductal narrowing were detected. Chromosome analysis revealed 46,XX/46,X,t(X;11)(q24;q13). The second patient had palmar pits, maxillary hypoplasia and prognathism, odontogenic keratocysts and pigmentary skin abnormalities. Third patient had mandibular and maxillary odontogenic keratocysts and at two years of age hydrocephaly was diagnosed. He had macrocephaly, thick eyebrows, synophrys, downslanting and wide palpebral fissures, generalised skin hyperpigmentation and mild prognathism. Other two patients were a mother and her daughter. The mother had maxillary and mandibular cysts, basal cell carcinoma and scoliosis. Her father died of skin cancer. Her 5-year-old daughter had macrocephaly, coarse facies, partial agenesis of corpus callosum and café-au-lait spot. Last two patients were two brothers, both having gingival hyperplasia and odontogenic cysts. The elder brother had millimetric calcifications on the anterior part of falx cerebri, as demonstrated by cranial tomography. Both had atrophic depigmented skin lesions and the elder brother had palmar pits. The younger brother was unable to read, he had diffuse calvarial thickening in cranial CT and his chromosome analysis was 47,XXY. Gorlin

syndrome is a very heterogeneous condition and the patients should be followed for future development of clinical criteria.

P0129. Psychological manifestations in Romanian haemophilics

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Introduction. Haemophiliacs often have to adjust their aspirations, lifestyle and employment options. In Romania and in other developing countries, lack of adequate therapy generates fear and uncertainty. Patients with high complications rate may develop psychological disorders.

Aim of the study was to evaluate frequency and type of psychological manifestations in haemophiliacs.

Material and method. Our study group consisted of 234 haemophiliacs registered and treated in Haemophilia Center Timisoara. We analyzed psychological complications according to haemophilia severity and patients' age.

Results. In our patients, psychological complications appeared in 16.24% of cases. 31.58% of patients with psychological complications presented associated psychological manifestations. In 4.27% of haemophiliacs with psychological manifestations neuro-sensitive-sensorial complications were noticed.

The majority of patients with psychological complications (81.6%) had severe haemophilia. High frequency of haemartrosis in these patients, frequent and long hospitalizations, high chronic arthropathy rate which produces disability and affects body image are important factors of psychological manifestations appearance. Most patients with psychological complications (76.3%) were from other counties, major deficiencies in haemophilia care representing a permanent distress. Mean age of patients with mental complications at diagnose was 14.2 (SD=9.840. Opioid abuse (12 cases) was the main complication and was signalized only in adults, the explanation being that in this age group, most of the patients present severe chronic haemophilic arthropathy, which causes chronic pain and disability.

Discussions and conclusions. Despite all difficulties, it is essential to diagnose and treat psychological complications in haemophiliacs, presence of a psychologist in the multidisciplinary haemophilia care team being indispensable.

P0130. Role of mtDNA in Iranian patients with Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is characterized by hypertrophy of ventricles and intrventricular septum. Patients could develop serious complications including heart failure, arrhythmias and sudden death. 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, affected by mitochondrial dysfunction. Mitochondrial (mt) DNA mutations are hypothesized to be involved in the pathogenesis of HCM. In this study, 31 Iranian HCM patients were screened for mitochondrial DNA point mutations and deletions. Our result showed three patients (5.76%) with a 7.4 kb deletion and one patient (1.92%) with 4977 bp "common deletion. Mutations in G3338A (Val>Met), G9053A (Ser>Asn), G9055A (Ala>Thr) and T3285C in tRNA Leucine were found. We detected 41 polymorphisms in mtDNA that 15 polymorphisms of them have not been reported before. Our results suggest that an increased level of mitochondrial mutations may be an indicator of DNA instability. Furthermore, mtDNA mutations may play an important role in pathogenesis of cardiac arrest which has remained unexplained for long. We are investigating pathogenesis these findings

P0131. Screening of TMC1 gene mutations in DFNB7(11) locus in autosomal recessive non- syndromic hearing loss Iranian population.

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Mutations in the transmembrane channel-like gene 1 (TMC1) cause prelingual autosomal recessive (DFNB7/11) and postlingual progressive autosomal dominant (DFNA36) nonsyndromic hearing loss suggesting that this protein plays an important role in the inner ear. These loci map to the same interval on 9q13-q21. The TMC1 protein is predicted to contain 6 transmembrane domains and to have cytoplasmic orientation of N and C termini. Mutations in this gene have been reported in North America in a family with autosomal dominant inheritance, Sudan, also in our two neighbor countries Pakistan and Turkey. Therefore we decided to study this locus in our population.

Thirty nine families with autosomal recessive and one family with autosomal dominant non-syndromic hearing loss that include two or more affected children were screened for DFNB7(11) locus by linkage analysis. These families originated from different ethnic groups of Iranian population and were negative for GJB2 and GJB6 mutations in locus DFNB1 . We used D9S301, D9S175, D9S1876 and D9S1837 STR (short Tandem Repeat) markers for this study.

Three out of forty families were linked to this locus. Mutation screening of TMC1 gene in these families revealed a homozygous framshift mutation (P.N150kfrx26) in one of the recessive families and a heterozygous mutation (G417R) in dominant family. Mutation detection for the other recessive family is undergoing.

We concluded that after DFNB1 and DFNB21 mutations, TMC1 gene mutations are responsible for the most prevalent cause of non- syndromic hearing loss in Iranian population.

P0132. Screening for 15 loci in the Iranian patients with autosomal recessive non-syndromic hearing loss

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Autosomal recessive non-syndromic hearing loss is the most common form of severe inherited hearing impairment. Mutations in GJB2 gene have been reported to be the most common cause of ARNSHL in the Northern European populations. These mutations are followed closely by GJB6 deletion. Our previous studies showed that GJB2 mutations and the GJB6 deletion do not play a significant role in the etiology of deafness in Iran. In this study, we assessed the contributions made by other loci to the ARNSHL genetic load in Iran. We have been screening 50 families with normal GJB2 and GJB6 alleles for DFNB1, DFNB2, DFNB3, DFNB4, DFNB9 , DFNB21 and DFNB40 by linkage analysis using, 3 STR markers for each locus. Some of the families have been localized to above- mentioned loci. Then with exclusion of linked families, the rest of families were screened for DFNB6, DFNB7(11), DFNB8 (10) , DFNB12, DFNB16, DFNB18 , DFNB23 and DFNB29 loci. Seventeen families showed linkage to some of these loci. Three families have been localized to DFNB4 with G334V, R409H and T420I/1197DelT mutations in SLC26A4 gene , two families to DFNB21 with a 9611 bp deletion in exon 10 and 266 delT mutation in TECTA gene and one family to DFNB7(11) with P.N150kfrx26 mutation in TMC1 gene. Mutation screening of SLC26A4, MYOVIIA, TMPRS53, MYO15A, Harmonin and TMC1 genes for other 11 families is undergoing . Our results suggest that other loci may have the major causative roles in ARNSHL in Iran.

Key words: Linkage analysis, ARNSHL, DFNB.

P0133. Hidrotic ectodermal dysplasia in a Tunisian family

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Hidrotic ectodermal dysplasia (HED, Clouston syndrome, MIM 129500) was 1rst described in 1895 and later by Clouston, in families from Quebec. Although most common in French Canadians, the disorder has been identified in several ethnic groups. Clouston hidrotic ectodermal dysplasia (HED) is transmitted as an autosomal dominant condition with complete penetrance and variable expressivity. The main features of this rare form of genodermatosis are partial to complete alopecia, palmoplantar hyperkeratosis, and nail dystrophy. Sweat gland function in these patients is normal. Recently, mutations in the GJB6 gene encoding the gap junction protein connexin 30 have been shown to cause this disorder.

Here we report a girl born to consanguineous parents presenting with complete alopecia and onychodysplasia without neither oligodentia nor sweat gland function anomalies. Her father, her paternal uncle and her paternal aunt are similarly affected. The paternal grandparents are normal .The comparison with other ectodermal dysplasias is presented and discussed. The possibility of an autosomal recessive form of hidrotic ectodermal dysplasia is raised.

P0134. Mutation IVS2-2A>G in *SLC26A5 (Prestin)* gene in two Estonian families with hearing loss

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Introduction: Prestin is a multipass transmembrane protein of the outer hair cells of the mammalian cochlea and it was encoded by human gene *SLC26A5*. In humans, a single nucleotide change - IVS2-2A>G in the second intron of *SLC26A5* has been reported in association with hearing loss.

During 2005-2006 we have screened 183 individuals with hereditary impaired hearing (HIH) by arrayed primer extension method, which covers 201 mutations in 8 genes (*GJB2, Connexin-30, Connexin-31, Connexin-43, Prestin* and *Pendrin* gene, and 2 mitochondrial genes). In 3 individuals we found IVS2-2A>G mutation in one allele of *Prestin* gene (1.6%).

We report two families with mutation IVS2-2A>G in *SLC26A5 (Prestin)* gene. First family: 2 children with severe hearing loss (HL) and genotype 35delG/IVS1+1G>A. Mother has mild HL and mutations 35delG/V37I; father has normal hearing, mutation IVS1+G>A in *GJB2* gene and IVS2-2A>G in *SLC26A5* gene. Second family: mother and daughter have genotype IVS2-2A>G. Mother has severe HL and 3 years old daughter has normal hearing.

Conclusions: We found 3 persons with IVS2-2A>G mutation, only in one of them sensorineural hearing loss was found. However, hearing loss may still develop of two others. In 1.6% of investigated persons with HIH IVS2-2A>G mutation in *Prestin* gene was found. This in the correlation with previous data: 4% Caucasians and 1.3% Hispanics carry this mutation (Tang et al. 2005). Our data support the hypothesis that heterozygosity for the mutation IVS2-2A>G in *SLC26A5* gene may be not be, by itself, sufficient to cause hearing loss.

P0135. Copy number alterations in hereditary spastic paraplegia genes

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Hereditary spastic paraplegia (HSP) is a neurodegenerative condition causing progressive leg spasticity and weakness. *SPG4* (*SPAST* gene, spastin protein) is the major HSP locus; there is large heterogeneity underlying the remaining ~60% of cases. We have previously shown for *SPG4* that large genomic deletions represent a surprisingly frequent class of disease-causing alterations. We subsequently widened pertinent analyses to (i) investigate the very extend of *SPAST* deletions and (ii) screen for copy number aberrations in other HSP genes.

In 18/18 cases investigated so far, deletion of *SPAST* exon 1 is accompanied by deletion of the promotor. This finding establishes a loss of function mechanisms, i.e. haploinsufficiency, as underlying at least some cases of *SPG4* HSP. We also identified copy number aberrations in *SPG3A*, *SPG6*, *SPG7*, and *SPG31*. As not all of these are associated with an HSP phenotype, haploinsufficiency is of no relevance for the corresponding HSP genes. This information impacts on the strategies for choosing appropriate cellular and animal disease models as well as on molecular therapeutic considerations. Current efforts therefore aim at uncovering the disease-association of copy number alterations in the complete spectrum of known HSP genes.

P0136. Hermansky-Pudlak syndrome: report of two portuguese families

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Hermansky-Pudlak Syndrome (HPS) [MIM#203300] is a genetically heterogeneous autossomic recessive disorder, characterized by oculocutaneous albinism (OCA) and platelet dysfunction, caused by defects in lysosome-related organelles. Eight human subtypes of HPS have been described. Patients show hypopigmentation of hair and skin. Ocular findings include nystagmus, iris transillumination, hypopigmentation of retina and decreased visual acuity. They have prolonged bleeding with easy bruising. Severe bleeding diathesis is more frequent in subtypes 1 and 4. Patients may also have pulmonary fibrosis in the third and fourth decades. Granulomatous colitis is highly variable. Electron microscopic examination of platelets shows absent or greatly decreased number of platelet dense granules.

We present two unrelated families with a clinical and electron microscopic diagnosis of Hermansky-Pudlak syndrome. The first family, with consanguineous first cousin parents, has two affected children: the eldest daughter has clinical features of HPS along with epilepsy and mild mental retardation; the youngest son has identical clinical features but with normal development.

In the second family, parents are non-consanguineous and their only son is affected. He was initially diagnosed as having OCA. When easy bruising was noticed, the investigation of platelet function revealed abnormalities.

Patients were submitted to platelet function tests and electron microscopic evaluation of platelets. The clinical presentation of HPS is reviewed, as well as differential diagnosis. Though this condition is more frequent in Puerto Rican patients it has been described in many other parts of the world.

P0137. The syndrome comprising myopathy with excess of muscle spindles, hypertrophic cardiomyopathy, and Noonan-like phenotype is caused by mutations in *HRAS*; The severe end of Costello syndrome

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In the recent time, great progress has been achieved in the understanding of the molecular basis of Noonan syndrome (NS; OMIM 163950), Cardio-Facio-Cutaneous syndrome (CFC; OMIM 115150), and Costello syndrome (CS; OMIM 218040). These disorders represent distinct entities wich share a common pattern of congenital anomalies. They are all caused by mutations in genes involved in the

Ras-MAPK signaling cascade, leading to constitutive activation of the pathway, with specific mutations in *HRAS* being detected in the majority of individuals with CS.
We screened four unrelated patients (2 male , 2 female) previously reported with myopathy, muscle spindle excess in striated muscle, HOCM, variable features of NS and early death for mutations in *PTPN11, KRAS, NRAS* and *HRAS*. Heterozygous *de novo HRAS* mutations were found in three of the four patients examined. In the first case the 2 bp substitution c.35-36GC->TT predicting the amino acid exchange G12V was present, and in the second case another missense mutation at the same codon, G12S (c.34G->A). In the third patiënt a novel *HRAS* mutation c.187G->A (E63K)was detected in DNA derived from blood cells.
We present the clinical details and results of the molecular studies in the four patients with HOCM, myopathy with muscle spindle excess and NS features and conclude that these patients represent the severe end of CS.

P0138. HTRA1 Polymorphism in Dry and Wet Age-Related Macular Degeneration

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Objective: To investigate HTRA1 polymorphisms in unrelated Taiwan Chinese patients with age-related macular degeneration (AMD) and control subjects without AMD.
Methods: 95 unrelated Taiwan Chinese patients with AMD and 90 age- and sex-matched control subjects were enrolled in our study. Genomic DNA was prepared from peripheral blood obtained from all AMD patients and control subjects. Polymerase chain reactions were used to analyze two HTRA1 single-nucleotide polymorphisms [rs11200638 (G/A) and rs10490924 (G/T)].
Results: Of the 95 participants with AMD, dry AMD was diagnosed in 52 patients and wet AMD in 43 patients. Both rs11200638 (G/A) and rs10490924 (G/T) were significantly associated with all AMD [rs11200638: $P = 6.7 \times 10^{-7}$ for an additive allele-dosage model, OR_{het} = 1.97 (0.81, 4.81), OR_{hom} = 8.59 (3.28, 22.49), A allele: 73% in all AMD versus 47% in controls; rs10490924: $P = 9.2 \times 10^{-6}$, OR_{het} = 1.86 (0.79, 4.35), OR_{hom} = 5.08 (2.21, 11.70), T allele: 73% in all AMD versus 50% in controls]. In terms of significance of association, rs11200638 was the most significantly associated variant. Subtype analysis including dry and wet AMD also revealed the similar results. Haplotype analysis demonstrated that AT was significantly associated with wet and all AMD (*P* = 0.011 and 0.004, respectively), whereas GG was significantly associated with the control group when compared with all AMD (*P* = 0.035).
Conclusions: Our study demonstrated that both SNPs were significantly associated with dry and wet AMD and the rs11200638 was the most significantly associated variant in a Taiwan Chinese population.

P0139. Early energy deficit in Huntington disease: identification of a plasma biomarker traceable during disease progression.

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Huntington disease (HD) is a fatal neurodegenerative disorder, without effective treatment. In the absence of clear underlying mechanisms in HD, weight loss is an appealing phenotype associated with chorea and cognitive decline. We performed a multiparametric study exploring body weight and the mechanisms of its loss in 32 presymptomatic carriers and HD patients in the early stages of the disease, compared to 21 controls. We combined it to a multivariate statistical analysis, based on proton nuclear magnetic resonance (1H NMR) spectroscopy of plasma. We demonstrated an early hypermetabolic state in HD. Weight loss was observed in the HD group even in presymptomatic carriers, although their caloric intake was higher than controls. Inflammatory processes and primary hormonal dysfunction were excluded. 1H NMR spectroscopy on plasma did, however, distinguish HD patients at different stages of the disease and presymptomatic carriers from controls. This distinction was attributable to low levels of the branched chain amino acids (BCAA), valine, leucine and isoleucine. BCAA levels were correlated with weight loss and, importantly, with disease progression and abnormal triplet repeat expansion size in the HD1 gene. Levels of IGF1, which is regulated by BCAA, were also significantly lower in the HD group. Therefore, early weight loss in HD is associated with a systemic metabolic defect. BCAA levels may be used as a biomarker, indicative of disease onset and early progression. The decreased plasma BCAA levels in HD likely represent a critical need for Krebs cycle energetic substrates provided by peripheral organ metabolism for the brain.

P0140. Vitamin D dependent Rickets Type II; Report of Two Affected Siblings in a Consanguineous Iranian family with a Novel Mutation in VDR Gene

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Hereditary vitamin D resistant rickets type II(HVDDRII) is a rare autosomal recessive disorder, most often caused by mutations in the VitD receptor gene. It is usually presented with rickets not responsive to VitD treatment. Circulating levels of 1,25(OH)2 VitD3 are elevated. Alopecia of the scalp or totalis is seen in some families with HVDDRII. This is usually associated with a more severe phenotype.
In this report, we present clinical findings on a family with the typical clinical features and molecular findings in two affected siblings..
The cardinal findings in the index patient were: alopecia totalis, renal tubular acidosis, mild generalized aminoaciduria, refractory rickets, high alkaline phosphatase, and hyperparathyroidism. Other routine biochemical tests were WNL. Skin biopsy was performed and was compatible with alopecia areata. Proband has had an older brother just with the same disease he deceased at the age of 32 months. Mutation analysis of the VDR gene by direct sequencing analysis of all coding exons showed a homozygous c.122G>A(p.Cys41Tyr) variant in exon 2 with several arguments pointing to a pathogenic effect. It was a novel mutation in VDR gene has not been reported before.
Two children of a consanguineous Iranian family showed the classical clinical features of HVDRR II and a novel mutation in VDR gene. We should be aware of this very rare disease, whenever we see a patient who is suffering from refractory rickets with alopecia.

P0141. Early neurological phenotype in 4 children with biallelic *PRODH* mutations

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Hyperprolinemia type I (HPI) results from a deficiency of proline oxidase (POX), involved in the first step in the conversion of proline to glutamate. Diverse phenotypes were described in patients with HPI, prior to the identification of the POX gene (*PRODH)*: whereas various patients were asymptomatic, others had neurological and extraneurological defects. *PRODH* gene is located in the region deleted in velocardiofacial syndrome (VCFS). Heterozygous and homozygous mutations have been identified in patients with variable hyperprolinemia and various features (patients with schizophrenia, chromosome 22q11 microdeletions and/or neurological defects). A functional study has divided the *PRODH* missense mutations into three groups: those leading to mild, moderate, or severe reduction of POX activity. In this study, we report four unrelated children with HPI and a homogeneous severe neurological phenotype. We identified biallelic abnormalities in *PRODH* in these patients that led to severe reduction of POX activity. These included missense and nonsense mutations, deletions of *PRODH* and a 22q11 microdeletion. Four other children have been reported with severe biallelic *PRODH* mutations. The phenotype of these

eight patients associates early psychomotor development delay with predominant cognitive defects, autistic features and epilepsy. Their values of hyperprolinemia ranged from 400 to 2200 µmol/L. Patients with biallelic *PRODH* alterations resulting in severely impaired POX activity had an early onset and severe neurological features. Thus, children with this phenotype and those with a microdeletion in chromosome 22q11, especially those with mental retardation and autistic features, should be tested for hyperprolinemia. Hyperprolinemic patients should be screened for *PRODH* mutations

P0142. A novel mutation L324V in the fibroblast growth factor receptor 3 in familial hypochondroplasia

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Hypochondroplasia is an autosomal dominant skeletal dysplasia characterized by short-limb dwarfism with rhizomelic shortening. This phenotype is similar to achondroplasia, but the features tend to be milder, as macrocephaly with relatively normal facies. Diagnosis is made by careful physical examination and x-rays. The diagnosis is also often made later in childhood (2-4 years of age). Before three years of age, the diagnosis is difficult, as skeletal disproportion tends to be mild and many of the radiographic features are subtle during infancy. Its incidence is 1:30000 live births. Actually, about 80 percent of hypochondroplasia cases are caused by heterozygous mutations in the FGFR3 gene, unique gene known to be associated with hypochondroplasia. Two recurrent mutations in exon 13, c.1620C>G or c.1620C>A, resulting in an p.N540K substitution in the proximal tyrosine kinase domain represent ~ 60% of all cases of hypochondroplasia. Sequence analysis of others *FGFR3* exons detects rare mutations. We report a new heterozygous mutation in exon 9 of *FGFR3*, p.L324V, c. 970C>G, in a familial hypochondroplasia. The father and his son presented mild phenotype with a more severe expression in the father than in his son. This mutation, not reported as SNP, is located in the third immunoglobulin (Ig)-like domain, in a highly conserved tripeptide sequence residue within the FGFR family. This result strongly suggests that p.L324V mutation is responsible for hypochondroplasia.

P0143. Dental symptoms - clue to the diagnosis of hypophosphatasia of childhood type

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Hypophosphatasia is an inherited disorder characterized by defective bone and teeth mineralization. The disease is due to mutations in the *ALPL* gene. Variable clinical expression of the disease ranging from stillbirth to pathologic skeletal fractures in adult years reflects allelic heterogeneity in this disease.

Our presented patient is a 4 year old female with uneventful pre- and postnatal history until the age of 3 months. A head deformity was noticed at that time (turricephaly) and progressed thereafter with premature closure of cranial sutures and fontanelles. Increased intracranial pressure developed and became symptomatic (bulging fontanelle, bilateral edema of optical nerve, exophthalmos, prominent subcutaneous veins, nausea and vomiting). Skeletal symptoms appeared at about 6 months of age with the signs of rickets and waddling gait. Dental symptoms included delayed eruption of deciduous teeth (the first at 9 months of age), premature loss of both incisors and later additional 6 teeth and severe caries. These dental symptoms specifically pointed to a possible diagnosis of hypophosphatasia. Additional signs were small stature and muscular hypotony. Biochemical assays revealed low-normal serum alkaline phosphatase (37.3 UI/l, n.35-281 UI/l), significant hyperphosphaturia (fractional excretion 19.1→24.8 %), and low serum intact parathormone (1-6 pg/ml, n.10-69 pg/ml). Clearly increased phosphoetanolamine in urine was detected (152 µmol/mmol creat., n.0-21 µmol/mmol creat), plasma pyridoxal 5'-phosphate was also highly elevated (1233 nmol/l, ref.<100 nmol/l). This has confirmed the diagnosis of hypophosphatasia, childhood type.

Serum alkaline phosphatase in mother and father was investigated; it revealed 35 UI/l (n.35-104 UI/l) and 16 UI/l (n.40-129 UI/l), accordingly.

P0144. A new clinical picture with hypopigmented hair, microcephaly, motor-mental retardation and renal anomalies

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Anomalies of hair pigmentation are very important signs for the diagnosis of a number of genetic, metabolic and neurological disorders. These findings may be of considerable value in the diagnosis of genetic syndromes such as Griscelli syndrome, Menkes syndrome, Elejalde disease, Chediak-Higashi syndrome and Werner syndrome. Here we report a 4-year-old female who presented sparse, hypopigmented silvery gray hair with microcephaly, dysmorphological facial features and renal anomalies. She was born to consanguineous parents at thirty-two weeks. She was small for gestational age. Her developmental milestones were delayed. She was hospitalized due to her recurrent lung and urinary infections. Her clinical findings were microcephaly, low frontal hairline, telecanthus, long eyelashes, broad nasal bridge, micrognathia and joint laxity. She had a history of kidney stone, ectopic kidney, vesicoureteral reflux and recurrent urinary infections. Chromosome analysis by routine G-banding revealed a normal karyotype. Other laboratory investigations were normal. Her clinical and laboratory findings were not supporting any genetic syndrome which was associated with hair pigmentation anomalies. In conclusion, a new clinical picture covering silvery gray and sparse hair with urinary system anomalies and mental-motor retardation is presented

P0145. Experience of the complex genetic counselling for the patients with inherited arrhythmias in Russia

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Estimated to affect up to 1 in 3000 individuals, the inherited arrhythmias are a common reason for referral to a medical genetics unit. Since 1998, 110 families with inherited arrhythmia's have been inspected in our Center: Long QT syndrome (76%), Brugada Syndrome (11%), Idiopathic Ventricular Tachycardia (4.5%), sick sinus syndrome (4%), Short QT syndrome (2%) and mixed phenotypes (2.5%). It seems that this ratio does not reflect the true distribution of the inherited arrhythmias in Russia but it could mirror the different awareness of the cardiologists about genetic basis of these diseases, and the benefits of genetic counseling. The clinical course was dependent on the affected gene, and the type of mutation for most of these disorders. Silent mutation carriers have been detected in 12%. About 7% of probands had two mutations in responsible genes. We hypothesize that identification of more than one mutation in these genes is unfavorable and can be considered as an *independent genetic risk factor* for cardiac sudden death, and could therefore be a reason of surgical intervention. Based on complex analysis of familial, personal, clinical and molecular genetic data we developed an algorithm for genetic counseling, molecular screening and results reading, and provide recommendations for patients with the inherited arrhythmia's studied here.

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P0146. Intractable diarrhea with "phenotypic anomalies" & tricho-hepato-enteric syndrome : time to scrape up?

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Tricho-hepato-enteric syndrome and syndromic diarrhea are quite rare conditions with only 15 cases described to date. Both include severe diarrhea requiring total parenteral nutrition, facial dysmorphism, immunity defect and hair abnormalities (mostly trichorrhexis nodosa). A definite clear clinical description of the 2 syndromes lacks and the outcome is poorly known.

Here, we report 2 additional cases: one boy born with low birth weight at 32 weeks, presenting intractable diarrhea associated with facial dysmorphism, hair anomaly and immunologic defect; the second born at term presenting the same set of symptoms associated with a cirrhosis

due to iron storage resolving with time. Metabolic investigations and karyotype were normal for both.
Analysis of these observations together with a review of previously published cases suggests that patients suffering from tricho-hepato-enteric syndrome and/or syndromic diarrhea actually present the same heterogeneous disease that has been wrongly and confusingly separated into 2 differents entities. The 5 mains signs are the same (Low birth weight, Intractable diarrhea, hair anomaly, facial dysmorphism and immunological defect) and the cirrhosis is found inconsistently but in both groups.
The acknowledgment that these two syndromes represent the same disease is a crucial step toward a better description of this syndrome, of its outcome and to set studies trying to identify a putative underlying genetic defect.

P0147. Mild phenotype in a child with low-rate mosaic 11q deletion

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Monosomy of the long arm of chromosome 11 is a rare structural chromosomal aberration with facial dysmorphism and severe mental retardation. Folate-sensitive fragile site of this chromosome, located at the band 11q23.3, facilitates breakage of this part of the chromosome. Distal part of the chromosome is being lost, and causes characteristic features of Jacobsen syndrome. Mosaic deletion of the chromosome 11q has rarely been reported.
We report on a girl aged 2 years with slight developmental delay. Facial dysmorphism included bitemporal narrowing, prominent glabella, hypertelorism, up-slanted eyes, short upturned nose and wide-opened mouth. The height and head circumference were at the 3rd percentile curve. Short arms and narrow thorax were present. Neurological evaluation showed slight degree of hypotonia, but motor achievements were satisfactory for the age. Speech delay was present. Ultrasound examination of the heart showed atrial septal defect. Cerebral computed tomography, renal studies and hematological evaluation were normal. The karyotype was 46XX/46,XX,del(11)(q24-> qter) (90%/10%). The parental karyograms were normal. This finding suggests a postzygotic event resulting in a child with mild clinical findings of Jacobsen syndrome. The follow-up of the child and genetic counseling is needed since the mosaic cell line could be found in ovaries as well.

P0148. Johanson-Blizzard Syndrome - The importance of genetic counseling and multi-disciplinary approach to rare diseases and neurodevelopmental delay

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We are reporting a 11-month-old Iranian girl with Johanson-Blizzard Syndrome, third offspring of first cousin parents. Their first and second child died in the neonatal period due to midline scalp defect and secondary infection. We believe they were also affected with Johanson-Blizzard Syndrome. This syndrome is an extremely rare ectodermal dysplastic disorder considered to be an autosomal ressecive disorder. Each clinical manifestation has different differential diagnosis so it shows the importance of genetic counseling to help the family. The proband had growth and developmental delay, short stature, microcephaly, hypoplastic alae nasi, scar of a repaired scalp defect, upsweeped hair, hypothyroidism, deafness, and malabsorption, consistent with Johanson-Blizzard Syndrome.

P0149. The birth prevalence of Joubert syndrome: a population based study in the Netherlands.

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The Joubert syndrome (JBS) is a clinically variable and genetically heterogeneous developmental brain disorder with autosomal recessive inheritance. The birth prevalence of JBS, necessary for carrier frequency calculation in genetic counseling, is not clear yet. We undertook a nationwide survey of JBS in the Netherlands. Since 2002, patients were systematically ascertained prospectively and retrospectively from multiple sources.
A total of 54 patients were ascertained. The date of birth ranged from 1973 to 2005. Male:female ratio was 32:22. The number of newborn JBS patients per year ranged between 0 and 2. Numbers increased over the years, with a maximum and stable number of around 2 newborn JBS patients per year from 1998 to 2005, probably due to improved ascertainment. This renders a mean birth prevalence of JBS of 1 in 113,797 over the period 1998-2005. When also counting the uncertain cases, the birth prevalence over this period was 1 in 83,850.
We conclude that, for practical purposes, a JBS birth prevalence of 1 in 100,000 can be used. This is considerably higher than the estimate of 1 in 258,000 by Flannery & Hudson in 2004. On the basis of the Hardy- Weinberg equilibrium and the relative contribution of the different genes involved in JBS, carrier frequencies can be calculated. For the *AHI1* gene, that was estimated to be responsible for 7 to 16 percent of JBS cases, this results in a carrier frequency of 1 in 598 to 1 in 395.

P0150. BMPR1A gene mutations status in Polish JPS patients

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The Juvenile Polyposis Syndrome (JPS), OMIM 174900 is an autosomal dominant hamartoma polyposis syndrome predisposing to gastrointestinal cancer. Autosomal dominant inheritance of the disease is associated with the mutations in one of the tumor suppressor genes MADH4 (mothers against decapentaplegic, drosophila, homolog of, 4) or BMPR1A (bone morphogenetic protein receptor, type 1A). The JPS is characterized by the occurrence of juvenile polyps in colon and upper parts of the gastrointestinal tract, the average frequency of JPS is 1:100000. The polyps occurring in childhood in most are the hamartoma polyps. The solitary or not numerous and self-limited polyps are not associated with the genetic predisposition. The characteristic feature of the occurrence juvenile polyps is rectal bleeding what is an indication for further diagnostics. The JPS encompasses the cases of numerous polyps and/or a familial component. The characteristic feature of the juvenile polyps are a markedly expanded lamina propria containing dilated cystic glands, inflammatory cells, and prominent stroma with a normal overlying epithelium.
The five juvenile polyposis patients were diagnosed in specialized clinics. The recognition of the JPS syndrome in 3 cases was based on observations over 100 juvenile polyps in colon and in two cases on observation over 5 juvenile polyps in colon. The entire coding sequences of the MADH4 and BMPR1A genes were sequenced by direct PCR product sequencing. The novel BMPR1A gene mutations were detected in three JPS families.
The study was financed by the Ministry of Education and Science, Poland, grant number 2PO5E02630

P0151. A new case with homoplasmic mitohondrial mutation of MT-ATP-6 and MCAD

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The authors report a 2 year and 5 months old girl from second normal pregnancy and delivery.Family history- grandmother with cerebral stroke, with delay in psychomotor development, convulsions, hypotonia, bilateral ptosis of eyelids, more pronounced at left, divergent alternating strabismus, atrophy of nervi optici, complementary chordus in left ventricle-variant of normal, hypoplasia of right ramus communicans -variant of normal, selective metabolic screening showed MCAD and investigation of mit. DNA / PCR-SBT method, showed polymorphisms, C7028T/MT-CO1/, T 1700C/MT-RNR2, T 3197C/MT-RNR 2, A1479G/MT-CYB/, A1146G, MT ND4, G11719A/MT-ND4/, A 8860G/MT-ATP6, T9477A/MT-CO3/, T5495/MT-ND2, A16399G/D-loop/, A263G/D-loop, 309inc C/D-loop and homoplasmic mutation T8705CC in the gene of MT-ATP 6, with aminoacid change methionin with treonin, with MRI finding of delay in white matter and hiasmic atrophy.The reported child is the fifth Case in Bulgaria with mitohondrial mutation of the MT-ATP6. The mutation is not described in literature and has unknown effect on mitohondrial function.The clinical picture is common in all cases .The presence of MCAD will be further evaluated.

P0152. Kabuki syndrome - clinical study of six cases.

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Kabuki syndrome (KS) (OMIM 147920) is a syndrome, estimated at 1:32000 births. KS is a complex disorder characterized by distinctive facial features, dermatoglyphic abnormalities, short stature and mental retardation. Some cases associate congenital anomalies of heart, kidneys, cleft palate and/or lip and recurrent infections. The underlying genetic mechanism of KS remains unknown. Most cases are sporadic. KS diagnosis is based on phenotypical signs and clinical investigations.

We focus on the medical data and present clinical data of six children diagnosed Kabuki syndrome (five boys and one girl), at the age of 7-17 years. All the patients had specific ocular and auricular defects: long palpebral fissures, eversion of lower lateral eyelid, arched eyebrows, long eyelashes and large/prominent ears, fetal pads at fingertips and mental retardation. Two children were born praematurely with antenatal hypotrophy, three patients had short stature (< 2 SD) during investigation. Congenital anomalies were diagnosed in five patients-horseshore kidney (one child), thoracolumbal scoliosis (two children), thoracal kyphosis (one child), *pectus excavatum* (one child). None of our patients had congenital heart defect, none had microcephaly.

Kabuki syndrome is a complex condition, guidelines for preventive managment are given: 1.growth and development should be monitored, 2.overweight can occur in early puberty, 3.recurrent ear infections and possible hearing loss are important issues.

P0153. Benign lymphoepithelial lesion of parotid gland in a patient with Keutel syndrome and her two sibs with classic Keutel syndrome

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Keutel syndrome is a rare autosomal ressesive disorder that mainly consists of abnormal cartilage calcification, sensorineural hearing loss, peripheral pulmoner stenosis, brachiotelephalangism and maxillar hypoplasia. The molecular etiology of disorder was shown with the mutation in human matrix GLa protein gene. Defect in this gene is thoughted to be responsible for this disase. We report 3 sibs with Keutel syndrome. One of them with Keutel syndrome findings and she also has a recurrent benign lymphoepithelial lesion of the left parotid gland. In this patient we identified a new nonsense mutation in exon 2 (at c. 79C>T causing termination codon at aminoacid position 27). Other two sibs had classic Keutel syndrome whose parents were first cousins.

P0154. Molecular and clinical findings of Larsen syndrome in a large Iranian family caused by a mutation in FLNB gene.

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Larsen syndrome (LS) is an autosomal dominant osteochondrodysplasia characterised by
large joint dislocations and craniofacial anomalies such as cleft palate and midface
hypoplasia. Recently LS was shown to be caused by missense mutations or small in-frame deletions in FLNB gene, encoding the cytoskeletal protein filamin B.

We found a large Iranian family with at least 30 affected members. Clinical findings were typical. Cardinal features were: short stature, multiple joints dislocation or subluxation, prominent forehead, midface hypoplasia, hypertelorism, depressed nasal bridge, carpal, tarsal, phalyngeal bone abnormalities, and spatulate fingers. Cleft palate was detected only in one case. Most of them were suffering from deafness, but none was mentally retarded. Club feet was common. A wide range of skeletal abnormalities such as craniofacial, distal humeral hypoplasia, supernumerary carpal and tarsal ossification centers, distal phalyngeal, cervical vertebral anomalies, and thoracolumbar scoliosis all were common findings in our patients.

Patients were evaluated clinically and radiographically. After ascertainment they screened for mutations in *FLNB gene,* using a combination of dHPLC, direct sequencing and restriction endonuclease digestion.

In this large family intrafamilial phenotypic variations for LS was studied and segregation of a recurrent mutation 679G>A, leading to the substitution of E227K was detected. Recent molecular studies showed that distribution of mutations within the *FLNB* gene is non-random. Our findings in this large family collectively show an autosomal dominant Larsen syndrome and demonstrate causative mutation in *FLNB gene.* So we can provide accurate genetic counseling and prevent further cases in future pregnancies.

P0155. A novel mutation in SURF1 gene in Russian patient with Leigh syndrome

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Leigh syndrome (LS) is one of the most frequent forms of mitochondrial diseases in infancy and childhood. Typical presentation of LS is in the first year of life with failure to thrive, psychomotor regression, ataxia, signs of brainstem dysfunction, and peripheral neuropathy. LS is characterized by symmetrical bilateral lesions in the brainstem and basal ganglia. Etiologies of LS includes both mitochondrial and nuclear DNA defects. Mutations in SURF1 have been shown to be the main cause of LS with cytochrome c oxidase (COX) deficiency. The human SURF1 gene encodes a protein localized in the inner mitochondrial membrane and is thought to be involved in the assembly and biogenesis of the cytochrome *c* oxidase complex. Most of known SURF1 mutations in LS patients predict a truncated protein product. We describe a new mutation in Surf1 gene found in one Russian patient with LS. It is related to the group of complex rearrangements and represents a 114bp duplication with the 2bp deletion inside of the duplicated fragment. The duplicated fragment starts with 45bp upstream exon 8 and ends in exon 8 at nucleotide 816. This fragment except nucleotide AG at the position 749 (749delAG) is inserted after nucleotide 816. The duplication shifts the reading frame and predicts a preliminary stop-codon at the 278 amino acid position and as a consequence skipping the exon 9 of the gene. The phenotypic features of the patient are typical for Leigh syndrome.

P0156. Atypical Leigh syndrome caused by T8993C mtDNA mutation

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A case of early-onset Leigh syndrome with atypically mild course in a 11-year-old girl is presented. Family history is negative apart from ischemic stroke with complete recovery at the age of 52 years in maternal grandmother. The child was born of uncomplicated pregnancy and delivery. Since infancy she presented spasticity, generalized hyperkinesia, and ataxia with motor and speech delay but normal mental development. With age, her movement disorders and dysarthria diminished. The girl walked independently, managed some everyday activities, and was educated at home up to age. Her diagnosis was cerebral palsy, there were no somatic disorders or ocular signs. MRI showed moderate lesions of capsula externa and putamen. At the age of 9 years, epileptic seizures appeared but regressed soon with anticonvulsants. In 10 years, with no provoking factors, she developed an acute episode of somnolence, severe dysarthria, ataxia, hyperkinesias, and inability to walk. Second MRI showed no deterioration. Moderate lactic acidosis in plasma (2,33 mM) and homoplasmy for T8993C mtDNA mutation in blood cells confirmed a supposed mitochondrial disorder. With treatment, the girl's status improved and returned to initial in 5_6 weeks. Nine months later, she remains stable and seizure-free. T8993C mutation of ATP synthase 6 gene is known to be responsible for diverse phenotypes of Leigh syndrome including juvenile forms. However, a prolonged and relatively mild course with normal mental development in a very early-onset case, like ours, is uncommon. The observation contributes into phenotypic variability of Leigh syndrome.

P0157. Associated malformations in cases with limb reduction defects

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Infants with limb reduction defects(LRD)often have other associated congenital defects.The purpose of this investigation was to assess the prevalence and the types of associated malformations in a defined population.The associated malformations in infants with LRD were collected in all livebirths,stillbirths and terminations of pregnancy during 25 years in 334,262 consecutive births in the area covered by our population based registry of congenital anomalies.Of the 255 LRD infants born during this period,58.1% had associated malformations.Associated malformations were more frequent in infants who had upper limb reduction defect(63.2%) than in infants with lower limb reduction defects(48.4%).Malformations in the cardiac system and in the central nervous system were the most common other malformations,15.2% and 10.6% of the associated anomalies,respectively,followed by anomalies in the genital system(10.1%),in the renal system(6.8%),and in the digestive system(6.3%).There were 16(6.5%)cases with chromosomal abnormalities,including 8 trisomies 18,and 2 22 q 11 deletion, and 56(22.8%) nonchromosomal dysmorphic syndromes.There were no predominant dysmorphic syndromes,but VA(C)TER(L) association.However numerous dysmorphic syndromes were registered including,among them,the following : EEC,OFD,Klippel-Trenaunay-Weber,OAVS,CHARGE,Townes Brocks,Moebius,Du Pan, SLO,hypoglossia-hypodactyly,amniotic band,De Lange,Rubinstein-Taybi, Fanconi,TAR,Roberts,Holt-Oram,and fetal diethylstilbestrol. Seventy one(28.8 %)of the cases were multiply,non syndromic,non chromosomal malformed infants.Prenatal diagnosis was performed in 48.8 % of dysmorphic syndromes with LRD,whereas prenatal ultrasonographic detection was only 23.9 % in cases with isolated LRD.The overall prevalence of associated malformations,which was more than one in two infants,emphasizes the need for a thorough investigation of infants with LRD.A routine screening for other malformations especially cardiac,central nervous system,urogenital system,facial clefts,and digestive system may be considered in infants and in fetuses with LRD.

P0158. The phenotypic variability of laminopathies: a trap for the clinicians

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Laminopathies constitute a group of diseases caused by mutations of the *LMNA* gene which encodes two nuclear envelope proteins, lamin A and lamin C. Since the identification in 1999 of *LMNA* mutations in the autosomal dominant form of Emery-Dreifuss muscular dystrophy, *LMNA* mutations have been shown to result in a wide range of phenotypes including autosomal recessive form of EDMD, dilated cardiomyopathy, familial partial lipodystrophy of the Dunnigan type, mandibuloacral dysplasia, Charcot-Marie-Tooth neuropathy, Hutchinson-Gilford Progeria or Werner Syndrome, lethal restrictive dermopathy and other complex phenotypes. Here we report our clinical experience on the inter- and intra- variability of the *LMNA* mutation phenotype based on the extensive investigation of 38 patients from 13 families. Fourteen patients from 5 families showed a muscular disease associated with cardiac involvement, and 2 of these patients had clinical involvement since infancy. Eighteen patients from 3 families had isolated severe cardiac disease and these families were dramatically affected by an autosomal dominant form of sudden death. Three patients had partial lipodystrophy. Finally, 3 patients from 2 families showed an unusual complex phenotype associating cardiac involvement, mild dysmorphic facial features and acrogeria. Most of these patients had a severe cardiac disease. This series highlights that the diagnostic of laminopathies should be considered in patients with supraventricular arrhythmia and conduction system disease, even in the absence of dilated cardiomyopathy. The frequency of ventricular arrhythmia justifies to perform presymptomatic diagnosis in order to implant in mutation carriers cardioverter defibrillator which allow, in contrast to pace-maker, to prevent sudden death.

P0159. Retrospective study of a cohort of 49 french patients with Lowe syndrome. No evidence for genotype-phenotype correlation.

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Background: Oculocerebrorenal Lowe syndrome is a rare X-linked disorder, characterized by major anomalies affecting the eye, nervous system and kidneys. This syndrome is caused by mutations in *OCRL1* gene, which encodes OCRL1, an inositol polyphosphate 5-phosphatase.
Objective: To describe the clinical features of a large cohort of Lowe patients and to correlate genotypes to phenotypes.
Methods: A retrospective multicentric review of the medical, surgical and socio-educative records of patients clinically diagnosed as Lowe syndrome. Molecular investigations included sequencing analysis of the *OCRL1* gene.
Results: Forty nine patients were included (2 to 50 years).
a) Proximal tubular dysfunction was found in 100% of cases with severe acidosis in 1/3, rickets in 1/2, and among the 27 older patients, the mean age for the beginning of reduced glomerule filtration rate was 15 years.
b) Significant bilateral cataracts were diagnosed in 47/49 patients. The mean age at cataract extraction was 3.4 months. Glaucoma was detected in 63% of cases.
c) Mean age for walking alone was 48 months. Mental retardation was estimated severe in 40% of patients but moderate in 60% of the series.
d) Atypical minor features were also observed, including anemia, lipoma, hemorragic manifestations and hypercholesterolemia.
e) All the patients harboured an *OCRL1* gene abnormality. No correlation between the type or position of the mutation and the clinical phenotype was demonstrated.
Conclusion: This clinical description about the largest Lowe cohort ever published emphasizes the phenotypic heterogeneity in OCRL. The lack of genotype-phenotype correlation may result from unknown factors including modifier genes.

P0160. Clinical report: a new case of macrocephaly/autism syndrome (*MIM605309*) with a germline *PTEN* tumor suppressor gene mutation

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Macrocephaly is found in approximately 20% of patients with autism. In a subset of 18 individuals with autism spectrum disorders and extreme macrocephaly, Butler *et al*. recently reported 3 young boys with a germline missense *PTEN* mutation (*J Med Genet 2005;42:318*). Here, we report on a 6 year old girl referred for global developmental delay, language regression and pervasive developmental disorder. The association with progressive macrocephaly (+3DS at birth and +5DS at 6y) prompted us to perform a *PTEN* gene analysis in the leucocyte DNA of the patient. We identified a heterozygous G-to-A transition in exon 6 of *PTEN*, resulting in an arginine173-to-histidine (p.Arg173His) substitution. Arginine173 is a highly evolutionarily conserved residue whose subsitution by histidine has been shown to lead, in an *in vitro* assay, to a drastic reduction (95%) of the phosphatase activity of *PTEN*. Interestingly, this p.Arg173His missense mutation has already been reported by several authors but only as a somatic mutation identified in glioblastomas and astrocytomas. Initially, germline mutations of *PTEN* have been found in patients with Cowden disease or Bannayan-Riley-Ruvalcaba syndrome. However, as in the three previously reported cases, the present patient had no mucocutaneous lesions suggestive of these two hamartoma syndromes. The possibility of developing specific alterations of Cowden disease during the second decade should be considered. Our findings confirm that molecular testing for *PTEN* mutation should be performed in patients with autistic behaviour and macrocephaly.

P0161. Intrafamilial phenotypic variability in Malpuech Syndrome: Report of Three Siblings and a Review.

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We report on three sibs (2 girls, one boy) with Malpuech syndrome, born to a phenotypically normal first cousin Qatari Arab parents. This is an extremely rare autosomal recessive syndrome first described in 1983 by Malpuech et al., with few (13) patients reported so far. It is characterized by facial clefting, hypertelorism and ptosis; malar hypoplasia, urogenital anomalies, caudal appendage and sacral dimple; and growth and mental retardation. All three sibs showing the range of anomalies found in Malpuech syndrome. They have growth and mental retardation, facial dysmorphism including ptosis, thick eye brows and flat malar region; and eye abnormalities. In addition, one sister and the brother have hypertelorism, caudal appendage, and bilateral cleft lip and palate and pseudocleft respectively. While expression of the syndrome was relatively mild in the older sister, the brother and the younger sister showed the full-blown syndrome. This family further supports the autosomal recessive inheritance for this syndrome. The constellation of clinical manifestations in this sibship emphasizes the phenomenon of intrafamilial variability that may lead to difficulty in diagnosis. We will review the previously published reports and further expand the phenotype of this rare condition. Attention is drawn to the clustering of such rare syndromes in this area.

P0162. Clinical factors associated with the occurrence of an aortic dilatation within a cohort of 1013 patients with Marfan syndrome or another type I fibrillinopathy

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The cardinal features of MFS involve the ocular, cardiovascular and skeletal systems. Clinical care is complicated by variable age of onset and the wide range of severity of aortic features. Taking advantage of the data of a large collaborative study designed for a genotype-phenotype correlation study including 1013 probands with a pathogenic *FBN1* mutation, we searched for clinical factors associated with the occurrence of ascending aortic dilatation (AAD) over time. Probabilities of AAD were described using Kaplan-Meier method and compared using logrank tests according to the following clinical signs: 1) 5 or more skeletal features of the MFS spectrum (n=272); 2) ectopia lentis (n=542); 3) mitral valve prolapse (n=533); 4) striae (n=444); 5) pneumothorax (n=73) or 6) dural ectasia (n=154). Baseline was considered as the date of birth. Among the 1013 included probands, aged in median of 29 years old (IQR [15-40]) at their last follow-up, 775 (77%) had AAD. Ectopia lentis and pneumothorax were not significantly associated with the risk of AAD. Striae and dural ectasia were associated with a lower risk of developing an AAD (p=0.001 and p=0.03 respectively). Conversely, patients with 5 or more skeletal features, or a mitral valve prolapse had a higher risk of AAD (p<0.0001 and p<0.0001 respectively). In conclusion, the presence of a high number of skeletal features and cardiac features appear to be the prognostic factors of AAD in *FBN1* mutations carriers.

P0163. Matthew-Wood syndrome is caused by truncating mutations in the retinol binding protein receptor gene STRA6

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Retinoic acid (RA) is a potent teratogen in all vertebrates when tight homeostatic controls on its endogenous dose, location or timing are perturbed during early embryogenesis. STRA6 encodes an integral cell membrane protein that favors RA uptake from soluble retinol-binding protein; its transcription is directly regulated by RA levels. Molecular analysis of STRA6 was undertaken in two consanguineous human fetuses we previously described with the Matthew-Wood syndrome [MIM 601186] in a context of severe microphthalmia, pulmonary agenesis, bilateral diaphragmatic eventration, duodenal stenosis, pancreatic malformations and intrauterine growth retardation. The two fetuses had both homozygous truncating mutations predicting a premature stop codon in STRA6 transcripts. Five other fetuses presenting at least one of the two major signs of clinical anophthalmia or pulmonary hypoplasia with at least one of the two associated signs of diaphragmatic closure defect or cardiopathy had no STRA6 mutations. These findings suggest a molecular basis for the prenatal manifestations of Matthew-Wood syndrome and and that phenotypic overlap with other associations may be due to genetic heterogeneity of elements common to the RA and fibroblast growth factor signaling cascades.

P0164. Genotype-phenotype correlations at the *MKS1* and *MKS3* loci in Meckel-Gruber and Joubert syndromes

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Meckel syndrome (MKS) is a lethal autosomal recessive syndrome characterized by cystic kidneys, a brain malformation (usually occipital encephalocele), polydactyly, and bile duct proliferation of liver. Recently, two genes have been identified: *MKS1/FLJ20345* on 17q in Finnish kindreds and *MKS3/TMEM67* on 8q in families from Pakistan and Oman, encoding ciliary proteins.

In order to evaluate the involvement of *MKS1* and *MKS3* in MKS, and to determine phenotype-genotype correlations, we performed the molecular studies of *MKS1* and *MKS3* in a large multhiethnic cohort of 120 MKS and 45 Meckel-like fetuses. Our results indicate that *MKS1* and *MKS3* genes are each responsible for about 10 % of MKS cases with various mutations in different populations. A strong phenotype-genotype correlation depending on the mutated gene was observed, regarding the type of central nervous system malformation, the frequency of polydactyly, bone dysplasia and *situs inversus*. The identification of *MKS3* mutations in sibs presenting vermis agenesis and small cysts in renal medulla, lead us to hypothethise that mutations in *MKS* genes may cause Joubert syndrome (JS). Analysis of *MKS1* and *MKS3* in a series of 22 JS patients identified *MKS3* mutations in three JS cases, defining *MKS3* as the sixth JS locus (*JBTS6*). No *MKS1* mutations were identified in this series, suggesting that the allelism is restricted to *MKS3* .

These data along with our previous identification of severe prenatal forms of Bardet-Biedl syndromes among MKS - like fetuses further demonstrate that MKS is the severe end of spectrum of human ciliopathies.

P0165. MecP2 Duplication Syndrome: Description of two additional cases

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In 2005, small duplications at Xq28 including *MecP2* and *L1CAM* have been shown to cause syndromic X-linked Mental Retardation (XLMR) in 4 families. Since then, 2 other series of respectively 6 and 7 families have been reported, where affected patients exhibit a remarkably

homogeneous phenotype. The clinical picture of affected males consisted of severe mental retardation, neurological abnormalities, severe recurrent respiratory infections and death before 25 years of age. Here, we report two additional cases found by genomic Q-PCR in 35 XLMR families.
Case A is of French origin and presents with profound mental retardation, absence of speech, spasticity and recurrent respiratory infections necessitating partial pneumonectomy. Currently aged 43, he is the eldest patient reported so far, all previously reported patients being under 25 or being deceased before 25. Case B is 8 years old and is of Nepalese and Lebanese origins. He presents also profound mental retardation, absence of speech and recurrent respiratory infections. His maternal uncle died at 11 from pneumonia and presented the same clinical features. Both mothers carry the duplication and show marked skewing of chromosome X inactivation.The predisposition toward severe pulmonary infections is the hallmark of this XLMR syndrome. It is a common feature shared to some extent, with Rett syndrome in which pneumonia has been shown to be the most frequent cause of death. It remains to determine if, like in Rett syndrome, this clinical feature is associated to breathing disturbances due to dysfunction of the autonomous nervous system.

P0166. Brothers with a microduplication including the *MECP2* gene: rapid head growth in infancy and transient tendency to recurrent infections

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Males with microduplications of Xq28 encompassing the *MECP2* gene have been reported to have a phenotype characterized by severe developmental delay, infantile hypotonia, spasticity evolving in childhood, absent or limited speech, absent or limited ambulation, seizures, mild facial dysmorphism and severe, recurrent respiratory tract infections.
We report two mentally retarded brothers who are mildly dysmorphic. The older boy is 11 years old. He was shunted for communicating hydrocephalus at age 7 months. He walked at age 2 ½ years. The younger boy is 6 ½ years old. He walked at age 3 years. He was investigated in infancy because of rapid head growth, but had normal intracranial pressure. Both boys had normal development in the first few months of life. They are generally healthy but without speech.They had frequent respiratory tract infections, but only during the first few years of life. Neither boy has required mechanical ventilation because of respiratory tract infections.
The parents have no other children. The family history is otherwise non-contributory.
The mother who is cognitively normal, has a completely skewed X-inactivation pattern in blood (98:2). MLPA analysis revealed that both boys have a microduplication which includes the *MECP2* gene. Further studies are being done to delineate the extent of the duplication.
These brothers suggest that the phenotype which results from microduplications including *MECP2* may be broader than previously appreciated. Rapid head growth in infancy may be present in some individuals and persistence of serious recurrent respiratory infections may not be a mandatory feature.

P0167. Phenotypic features of males with mental retardation (MR) and mutations of the MECP2 gene

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The MECP2 gene causes Rett syndrome (RS) (OMIM: 312750), a well-defined X-linked dominant neurodevelopmental disorder affecting primarily girls. Until recently RS was considered lethal in males, although now approximately 60 cases have been reported. Males with MECP2 mutations present with a broad spectrum of phenotypes ranging from neonatal encephalopathy to non-syndromic MR.
Five males (aged 3-16 years) evaluated for MR, had normal karyotype and four of them were evaluated for molecular variation associated with the FMR1 locus, with negative results. Patient 1 presented with features of FXS, including long face, protruding ears and behavioral problems. Patient 2 had spasticity, pyramidal signs and trunk stereotypies, while his brother (Patient 3) showed slight psychomotor retardation. Patients 4 and 5 presented with autistic features. MECP2 analysis of the exons 3 and 4 was performed by Denaturant Gradient Gel Electrophoresis, sequencing and GAP-PCR, using specifically designed sets of primers.
The polymorphism p.T203M was identified in Patient 1, but not in his mother, raising questions about its contribution to the phenotype. Patient 2 and 3 had a novel deletion at the deletion-prone region of MECP2 (c.1140del86) inherited from their unaffected carrier mother. The GAP-PCR in Patient 4 showed evidence of a large rearrangement encompassing exons 3 and 4, which requires further evaluation. His mother was normal. Patient 5 presented the p.R106W classic RS mutation, although his mother was unavailable for analysis.
MECP2 mutations should be considered as a rare cause of MR in males , although great phenotypic variation hinders genotype-phenotype correlation.

P0168. MED12 (HOPA) mutation in a family overlapping Opiz-Kaveggia (FG) and Lujan-Fryns syndromes

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The MED12 (aka HOPA/TRAP230) gene located in Xq12 is a subunit of the TRAP/Mediator complex, a multi-component complex that can regulate transcription by transducing the signals between activators and RNA polymerase II. A recurrent p.R961W mutation in the MED12 was recently reported in five FG families including the original FG family (Risheg, Nature Genet 2007). A p.N1007S was then found in the original Lujan syndrome family (Schwartz, J Med Genet 2007). We report here further clinical details on the 2d family reported by Schwartz. In this family, a p.N1007S was found in a male proband and his two nephews, who were clinally diagnosed as FG syndrome. The proband, examined at age 19 (in 1989) had global developmental delay (moderate to severe), agenesis of the corpus callosum, normal OFC, long narrow face with high square forehead, bulbous nasal tip, flat malar area, short philtrum, bowed upper lip, prominent lower jaw, a hairy nevus on the right forearm and normal neurological examination. He had poor speech abilities and showed temper tantrums and aggressiveness. The two nephews showed mild to moderate developmental delay, and ectomorphic habitus. Both were tall and slender, with long face and high forehead, short philtrum, small mouth, narrow palate and crowded teeth. Brain CT was normal in both, and constipation was not a problem. One of the children had skull surgery for scaphocephaly. They showed similar behavioral problems, with hyperactivity, attention deficit, tantrums and aggressiveness, worsening with time. Nosologic overlap between FG and Lujan-Fryns syndrome will be discussed.

P0169. Megalencephaly, thick corpus callosum, dysmorphic facial features, seizures, and mental retardation in two unrelated patients: a new syndrome ?

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Macrocephaly associated with mental retardation is a part of various syndromes including overgrowth conditions, cutis marmorata telangiectatica, Cowden disease, and neuro-metabolic disorders. We report here a new association characterized by megalencephaly, thick corpus callosum, mental retardation, seizures, specific facial features in two unrelated boys. Case 1 was able to walk alone at 3 years of age, developed seizures at the age of 5 and had limited speech. The physical examination at the age of 9 revealed normal weight and length but a large head circumference (+3SD) and dysmorphic features including downslanting palpebral fissures, long and expressionless face, small mouth, enamel defect, and strabismus. Case 2 developped seizures at 3, was able to walk at 8 and had limited speech. Head circumference curve was above + 4 SD whereas length and weight curve were in the normal. He had the same dysmorphic features (long expressionless face, downslanting palpebral fissures, small mouth, enamel defect). Extensive metabolic and cytogenetic studies were normal in the two boys. In addition, molecular screening of NSD1, GPC3 and the 11p15 region was negative in case 2. In the two children, brain MRI detected bilateral megalencephaly, a thick corpus callosum, an enlarged white

matter and normal ventricles.This association of megalencephaly and thick corpus callosum has been previously reported by G. Göhlich-Ratmann in 1998 in 3 children who had in addition pachygyria and complete lack of motor development, features not observed in our cases. We hope that ongoing cytogenetic and molecular studies will further define this new entity.

P0170. Study about the family history in a population with mental deficiency

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Introduction. Mental deficiency is a very severe and common disease. There are many causes of mental deficiency. Thus, we can mention the congenital abnormalities, metabolic diseases, cranial trauma, fragile X syndrome, monogenic or polygenic syndromes and chromosomal syndromes. Also, we can mention the critical age of parents (especially of mother's), some physical, biological and chemical factors in appearance of mental deficiency.
Objectives. In this study we show the importance of hereditary factors in appearance of mental deficiency.
Material and methods. We investigated 596 children interned in Neuropsychiatry Infantile Section of Neurology and Psychiatry Clinical Hospital of Oradea, during the 1999-2001 period. Out of 596 children that were examined, 393 presented different levels of mental deficiency. We performed family studies and inspected the pedigrees.
Results. More than 65% of children with mental deficiency have one or more affected relatives in their family. The relatives may be affected by congenital abnormalities and/ or mental diseases. The frequency of similarly affected relatives is greatest in groups with mild and moderate mental deficiency. In the group with severe mental deficiency, the incidence of affected relatives is lower.
Conclusion. This result may be an argument for the hypothesis that genetic factors are very important in the inheritance of mental deficiency. The increased incidence in groups with mild and moderate mental deficiency may be partly explained by harmful factors, critical age of parents and the presence of fragile X syndrome. It seems that severe mental deficiency commonly reflects the effects of genes and chromosomal disorders.

P0171. Contributions as concerns to the incidence of cranial and facial dysmorphism in a population with mental deficiency

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Introduction. The mental deficiency is a very severe illness because it is associated in many cases with different other malformative diseases. Also, the etiology of mental deficiency is diverse, and depending on etiological cause, the number and types of traits in mentally affected person varies in large limits.
Objectives. Our study is about the incidence of cranial and facial dysmorphism in a population with mental deficiency. We decided to make this research because of different etiology of mental deficiency. Also, we wanted to establish if the cranial and facial dysmorphism is an important element in description of different types of mental deficiency.
Material and method. This research was realised on 393 children with diverse types of mental deficiency. The children were interned in Neuropsychiatry Infantile Section of Neurology and Psychiatry Clinical Hospital of Oradea, during the 1999-2001 period. The children were clinical examinated. Also, we used the methods for chromosomes analysis.
Results. We observed that the incidence of cranial and facial dysmorphism increase in accordance with the level of the mental deficiency. In the group with severe mental deficiency, we obtained an incidence over the 40% of this abnormality. The explanation for these results may be the presence of some genes and chromosomes syndromes which associate cranial and facial dysmorphism, too.
Conclusion. The cranial and facial dysmorphism is a common feature in description of mental deficiency disease. The incidence of this abnormality increase in accordance with the worsening of the mental deficiency.

P0172. Experiences of a routine diagnostic laboratory with oligo-based array CGH in the diagnosis of mental retardation

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Array CGH is a novel diagnostic tool for the detection of submicroscopic chromosomal imbalances. Such imbalances are regarded as an important cause of congenital mental retardation. In June 2006 oligo-based array CGH was diagnostically implemented in our genetic service in order to reach a resolution for screening the whole genome that extends far beyond routine cytogenetic analysis. Array CGH was performed using 44k or 105k 60mer oligonucleotide arrays (Agilent) with a theoretical resolution of about 100kb or 40kb, respectively.
First we give an overview of our cases so far examined by array CGH, followed by the representation of selected cases. These examples will demonstrate the usefulness of high resolution array CGH for different tasks: (1) further analysis of patients with syndromic mental retardation, showing initially a normal karyotype; (2) confirmation and breakpoint analysis of imbalances detected by standard cytogenetic analysis, sometimes finding additionally submicroscopic chromosomal aberrations; (3) screening of apparently balanced translocations for cryptic imbalances.
Additionally, the selected cases will show the challenges of the validation and interpretation of results revealed by array CGH analysis. Since the discovery of a rapidly increasing number of so called ,,copy number variations" (CNVs) in the genome of normal individuals, the assumption of an imbalance as casual for a specific phenotype must be carefully considered.
In conclusion, our experiences show that array CGH should be considered as an essential tool for the genetic analysis of patients with syndromic mental retardation.

P0173. Subtelomeric FISH ananlysis in 92 patients with mental retardation

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Mental retardation (MR) affects approximately 1 to 3% of the general population. Cryptic subtelomeric aberrations have been found in 5 to 7% of all cases. We performed a subtelomeric FISH analysis in 92 unrelated children with normal standard karyotype ascertained by a checklist that evaluates the grade of developmental delay, dysmorphisms, growth defect and congenital malformations. Ten cryptic chromosomal anomalies have been identified (10,8%): three de novo deletions, four unbalanced translocations of parental origin, a t(9;16)(9pter;16q24.3 qter) pat; der(20)t(16q24;20q13.3)pat; a der(6) t(6;1)(p22.3,q44) mat and a der(7)t(7;12)(q34;q24.32)mat; and three de novo unbalanced translocations: t(6pter; 6qter); a t(5;10)(pter;qter); a t(1;13)(p31.3;q22.2),ish.. Interestingly a patient presenting a dysmorphic phenotype and multiple malformations, not associated to neurodevelopment delay carried a de novo t(6pter; 6qter). Microcephaly, agenesis of corpus callosum, upslanting palpebral fissures, hypertelorism, synophris, short nose, anteverted nares and low set ears were the clinical features observed in the cases affected by 9q34-qter deletion, in agreement with the literature. In the patient carring t(9;16)(9q34qter;16q24.3qter)pat, the clinical diagnosis of Donnai-Barrow syndrome was initially proposed; in this case, subtelomeric FISH analysis allowed us to suggest the hypothesis that some o Donnai-Barrow cases observed might be ascribed to 9q terminal deletion. Clinical features of most of these patients are consistent with the corresponding new emerging chromosome phenotypes, further supporting the role of cryptic subtelomeric analysis in the work-up of children affected by MR.

P0174. Mental retardation disease among Israeli Arab: Clinical and epidemiological study in a single village

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Mental retardation (MR) is a frequently occurring disease with a major impact on the life of affected person, the family and society. MR is

defined as a disability characterized by a remarkably low intellectual functioning (IQ<70) in conjunction with significant limitations in adaptive functioning and it is estimated 1-3% of the general population. The genetic etiologies of MR are diverse and include chromosomal anomalies, recognizable malformation syndromes, monogenic syndromes, structural brain abnormalities and environmental factors. About 1,400 different conditions associated with mental retardation have been recognized to date and many more are yet to be characterized. The prevalence of genetic diseases and congenital malformation in the Israeli Arab community is relatively high, as a result of high rate of consanguinity, but their distribution is not uniform. The aim of this study was to determine the frequency of neurological manifestations with a special emphasis on the MR spectrum disorders and its association with consanguinity within a single village. Out of 4,166 children (below 18 y of age), the prevalence of neurologically related hereditary disease was 4.3% (180 children) with about 40% of these cases (73 patients) were identified with MR. About 65% of neurologically related hereditary disease cases and 80% of MR cases were outcome parental consanguineous marriages. The high prevalence of neurologically related hereditary disease that significantly associated with consanguinity prompts the necessity of implement preventive programs and design future strategies leading to avoiding the occurrence of these inherited genetic disorders.

P0175. C8002T polymorphism in endothelin-1 gene and metabolic syndrome

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BACKGROUND: Metabolic syndrome (MS) is characterized by a cluster of disorders including type 2 diabetes mellitus, obesity, glucose intolerance, dyslipidemia, prothrombotic and proinflammatory states. Insulin resistance, the main contributor to MS is associated with endothelial dysfunction. The latter represents the clue element in appearance of the vasoconstriction when the level of vasoconstrictors factors, mainly endothelin-1 (ET-1) increases. There is an important role of ET-1 in the microvascular complications of type 2 diabetes mellitus. OBJECTIVE: This study investigates a possible association of the C8002T polymorphism in ET-1 gene with MS-associated microvascular disorders including diabetic retinopathy, neuropathy and nephropathy. METHODS: We screened 208 Romanian subjects (123 MS patients and 85 control individuals). ET-1 genotypes were determined by polymerase chain reaction-restriction fragment-length polymorphism analysis. Correlations between C8002T polymorphism and clinically manifested microvascular disorders in MS patients were performed by multivariate regression analysis using SPSS Advanced Statistics 10.0. RESULTS: No significant differences were found for ET-1 gene variant between MS patients and control, or between sub-selected MS patients with or without diabetic retinopathy or nephropathy. Multivariate logistic regression analysis in MS patients showed that C8002T polymorphism was an independent risk factor (P=0.03) for diabetic neuropathy, which remained significant after adjustment for potential risk factors (age, gender, body mass index, smoking, fasting blood glucose, total-cholesterol and triglycerides). CONCLUSION: These data suggest for the first time that C8002T polymorphism of ET-1 gene may influence the development of diabetic neuropathy in MS patients.

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P0176. The dinamic observation of the adult form of metachromatic leukodystrophy in two sibs

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Metachromatic leukodystrophy (MLD) is the lysosomal storage disease with arylsulfatase A (ASA) deficiency. The adult form of MLD averages approximately 15% of all MLD cases. In contrast to other forms of MLD that start with movement problems, adult form of this disease is presented as psychiatric disturbance with schizophrenia-like disorder. There are two sibs (male and female) with adult MLD was being under our observation during five years. The diagnosis was confirmed by enzymatic assay at the age 15 for brother and at the age 20 for sister, the ASA activity was found 1% and 3% of control value accordingly. The initial sings of disease appeared at the age 14 in both patients in similar way. The major features were the teaching difficulties, memory disturbance, marked emotional lability with irrational fear, marked decrease of self-criticism and unequal behavior. The MRI of the brain revealed typical changes in the perivenricular zones. After the five years observation there are no movement disturbance in both patients. The psychiatric problems are stable in sister but progressive in brother. Generally the brother's disease progress faster than sister's one. This report illustrates the importance of differential diagnostics of MLD in cohort of psychiatric patients with behavior disturbances.

P0177. Prevalence and phenotypes associated with double *Fibrillin 1* gene heterozygosity: clinical relevance for diagnosis, prognosis and prenatal diagnosis

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Marfan syndrome type I (MFS1) is an autosomal dominant disorder of the connective tissue diagnosed following Ghent criteria and caused by mutations of the Fibrillin 1 gene (*FBN1*). Double *FBN1* compound heterozygosity seems to be exceptional and associated with neonatal severe and early lethal phenotypes.
We describe the prevalence and the clinical phenotypes of carriers of double *FBN1* gene mutations.
We identified five unrelated double *FBN1* compound heterozygotes (9 mutations) in five out of 141 consecutively genotyped families (3.5%). In three of five families, each parent carried a different mutation and one of the offspring inherited both: one mutation (major) was associated with MFS in at least one family member and one (minor) with mild, incomplete or nearly normal phenotypes not satisfying Ghent criteria for MFS. Carriers of the major mutation alone or of double mutations (compound heterozygotes) fulfilled Ghent criteria for MFS while carriers of "minor" mutations did not. In two families, the major mutation was *de novo* and the "minor" one was inherited from the mother or the father, both healthy and showing minor, non-diagnostic MFS traits.
Our data are in accord with the dominant pattern of inheritance of the MFS, however they document the double *FBN1* compound heterozygosity in a minority of MFS cases. The clinical implications must consider the contribution of the positive family history for the diagnosis, the recognition of the "major" mutation for prenatal diagnosis and the possible contribution of the "minor" mutation to the overall phenotype in double mutation carriers.

P0178. Using microarray based CGH for studying the Delleman phenotype

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Delleman syndrome (DS) or oculocerebrocutaneous syndrome has been described in a heterogeneous number of patients presenting all or some the following main syndrome's features: orbital cysts/microphthalmia, cerebral malformations, and skin lesions specifically characterized by focal dermal hypoplasia and cutaneous appendages. Array-CGH method has been successfully used to identify submicroscopic rearrangements in different series of mentally retarded patients with or without associated dysmorphic signs. The purpose of this presentation is reporting the study of three patients with the phenotype of DS by using the microarray-CGH methodology. The first patient, a sporadic case, has presented all the main clinical signs of DS. The others two patients were sibs that showed discordant phenotypes between them, but suggesting the DS. All the studied three patients had normal karyo-

type at 550 bands resolution level. No microrearrangements were found in the first patient by array-CGH method at 0.8Mb resolution. However, using the same method and also microsatellite marker studies it was possible founding a reciprocal translocation t(6;7)(q27;q34) in the father and a different segregation of the both involved chromosome to the two sibs. While one sib carried out a deletion at 6q27 band associated with a duplication of the 7q34-q36.3 region, the other sib presents the inverted microrearrangement - a partial duplication 6q plus a partial deletion 7q. We conclude that the Delleman phenotype is etiologically heterogeneous and that the involved chromosomal regions, 6q27 and 7q34 should be considered as candidate regions to looking for genes related to DS.

P0179. A study of candidate genes for dominant non-syndromic microcephaly in a family with inherited chromosomal translocations t(5q35.2;18q22.3)

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In a large pedigree a balanced chromosomal translocation t(5q35.2;18q22.3) segregates with non-syndromic microcephaly (-2 to -3.7 SD) in 7 carriers, with the exception of 1 macrocephalic (+3.2 SD) carrier. No genes were directly disrupted by the breakpoints, suggesting a positional effect to alter gene expression levels. 2 candidate genes were identified near the breakpoints of the translocation: *STC2* (stanniocalcin 2) ~72 kb downstream of the breakpoint on chr5 and *NETO1* (neuropilin-and-tolloid precursor 1) ~105kb downstream of the breakpoint on chr18.

Both candidate genes are expressed in brain and the neuropilin-domain in *NETO1* points towards a possible role in axonal guidance. Patients with microdeletions of 5q35 encompassing *STC2* present mild microcephaly.

Measurement of RNA-expression levels for the candidates was hampered by variable expression of the genes in EBV cell-lines, the only patient material available. Heterozygous transcribed SNPs to test mono-allelic expression on cDNA level were absent.

No mutations were detected in either genes in 3 other patients with non-syndromic microcephaly. Using a morpholino knock-down (KD) approach in zebrafish, severe cell death spreading from the diencephalon was observed for *stc2* KD during somitogenesis (~10-24 hpf), coinciding with the time of primary brain development. For *neto1*, one morpholino generated similar cell death, 2 other independent morpholinos only generated general developmental delay.

Confirmation of the role of one of these genes in microcephaly will be done by further mutation analysis in additional patients as well as further structural studies in the knockdown zebrafish embryo's.

P0180. Clinical, neuropathological and neuropsychological phenotype in two families with primary microcephaly due to ASPM/MCPH5 mutations

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Human autosomal recessive primary microcephaly (MCPH) is a heterogeneous disorder with at least six genetic loci (MCPH1-6). MCPH5, caused by ASPM (Abnormal Spindle-like, Microcephaly-associated - location: 1q31) gene mutations is the most commonly involved. We report new mutations in 2 families with MCPH5. In family 1 (non-consanguineous), the proband was born with an OFC of 32 cm. At age 4, OFC is -5SD and height is at -1SD. The boy shows mild psychomotor delay. A further pregnancy was terminated after echographic diagnosis of microcephaly. Neuropathology showed a small brain (biometry of 27-28 GW for 33 GW) with a mildly simplified gyral pattern for age, decreased neuronal population in cortical plate and premature depletion of the germinal zone. We found coumpound heterozygocyty for 1 nonsense and 1 frameshift (1 bp deletion) mutations: p.Gly26AlafsX42/p.Arg2078X. In family 2 (consanguineous), two brothers were affected, although with different severity. The eldest one, aged 25, had an OFC < -8SD. His IQ was 55, with homogeneous scores and preserved memory functions. The youngest, aged 10, has an OFC at -5SD. His IQ was 70 with normal memory functioning but weaknesses in executive functions. He was still in mainstream schooling. Mutation screening showed an homozygous 2 bp deletion leading to frameshift p.Lys2595fsX6. Our patients illustrate intrafamial variability of ASPM mutants, confirm surprisingly good preservation of cognition despite major reduction in brain size, and confirm the absence of specific histological anomalies in ASPM-related MCPH. Detailed neuropsychological profile of family 2 will be presented.

P0181. Familial microduplication 22q11.2 without cardiac anomaly

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The chromosomal rearrangements in region 22q11.2 lead to various types of congenital malformations and mental retardation. The most common molecular defect in region 22q11.2 is microdeletion, but recently the microduplication 22q11.2 syndrome has been identified. We describe mother and daughter with microduplication 22q11.2.

Twenty-seven-year old patient was referred for genetic evaluation by cardiologist because of facial dysmorphism. She has short stature, facial dysmorphism, velopharyngeal insufficiency, toes anomalies, and learning disabilities. She had also one seizure episode without special findings in EEG. She has also diagnosed depression and arthropathy with contractures. She had no cardiac anomaly, only some tahhycardial episodes.

Routine chromosome analysis of peripheral blood lymphocytes was performed using standard laboratory protocols and the karyotype was normal. Metaphase FISH-analysis with DiGeorge/VCFS TUPLE1 Region Probe (Cytocell, Aquarius) was performed only due to the referral doctor was a cardiologist. The analysis revealed unexpectedly two signals with different intensities on 22q11.2 (TUPLE1) regions. Further analysis of interphase nucleus revealed three signals of TUPLE1 of similar intensity and two signals of control probe located on 22q13.3 (N85A3) in each cell. The relatives of the proband were investigated. Her mother had the same cytogenetic finding - karyotype: 46,XX.ish dup(22)(q11.2q11.2). She has similar, but milder facial features. She had had some problems with fertility before her first pregnancy (now she has 5 children), she has premature menopause (39 years). She has no seizures, cardiac disturbances or psychiatric disorders. The other members of the family are under investigation.

P0182. Implications of Spontaneous Excess Expression of Fra (16)(q22): An Emerging Syndrome?

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A twelve year old girl was referred to Medical Genetics following assessment in Craniofacial Clinic for a possible cosmetic surgery. This patient had microstomia, cleft palate, micrognathia and dental crowding. Physical and mental development was normal. Her father and one of her sisters had remarkably similar physical features. Upon examination, it was thought that this family had a condition similar to what had been described previously in four other families, whom had an elevation of spontaneous 16q22.1 fragility and overlapping clinical features with our patients. Chromosome analyses were performed on metaphase lymphocytes grown in PHA-stimulated blood cultures by standard procedures. Our patient had an unusually high spontaneous expression of chromosome fragility at 16q22.1, with an otherwise normal karyotype. The fragility was expressed as either a deletion or a chromosome gap in 5 of the 30 metaphase cells (16.7%) analyzed. Likewise, the spontaneous expression of the fragile site was found to be 20% and 30% for this patient's sister and father, respectively. An unaffected sister had a normal karyotype and no expression of 16q22.1 fragility. Our observation of fragility at 16q22.1 seems to segregate with phenotypic features of microstomia, cleft palate, micrognathia and dental crowding in this family. Our findings, in conjunction with those of previous case reports, have lead us to speculate that fragility at 16q22.1 and microstomia is an emerging syndrome, possibly caused by a regulatory disturbance of genes involved especially in jaw development.

P0183. Minor physical anomalies in children and teenagers with neurological disorders

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Minor physical anomalies are defects that in themselves, have neither medical, nor cosmetic importance. They may occur even in healthy individuals. The incidence of minor anomalies has been studied in search for a relationship between different non-syndromic diseases and abnormal embryogenesis. They are used as indicators of altered embryonic differentiation. Their positive correlation with disturbances of neurological development occurring during embryogenesis has been established. Patients with mental retardation, cerebral palsy and autism were evaluated for the presence of minor anomalies. Data were compared with those obtained from a control group. The frequency of the minor anomalies was not significantly different in the two groups. When expressing the mean values as minor anomalies per child, the figures were 2.92 in affected individuals and 1.43 in the healthy ones. A positive correlation with the severity of mental retardation was noticed. 64% of the cases with cerebral palsy and 56% of the patients with autism had at least three minor anomalies. The presence of minor congenital anomalies, especially more than three, may predict the future onset of a neurological disorder.

P0184. Unilateral mirror-image polydactyly of the hand, with cutis aplasia

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Mirror-image polydactyly is rare. It is syndromic or isolated. Laurin-Sandrow syndrome (LSS) includes fibular and ulnar duplication, absent radius and tibia, polysyndactyly of the hands and feet, often described as mirror-like, and nasal/philtral anomalies. Transplantation of a zone of chick distal limb bud polarizing activity to the anterior limb margin causes mirror image duplication of limb elements. This suggests mutations in genes for limb differentiation might be associated with mirror-image polydactyly. A number of possible candidate genes contributing to pathways directing limb development have been described. Dominant inheritance has been proposed, based on a few families with vertical transmission. The features of isolated and familial cases are variable, suggesting etiologic heterogeneity is possible. The cause of isolated cases affecting only one limb is not clear and is not necessarily genetic in origin.

The right hand of this female infant had seven triphalangeal fingers arrayed around a central digit, ulnar duplication resulting in limited wrist and elbow movement, and a small area of cutis aplasia of right anterior shoulder. No other anomalies were noted. Chromosomes were normal. At 6 weeks, motor/social development was appropriate. Her parents had normal extremities and were not consanguineous.

Innis and Hedera [2004] speculated that isolated single limb mirror-image (SLMIP) polydactyly might represent natural variability of LSS but there are no reports of familial transmission of SLMIP. Postzygotic somatic mosaicism of affecting one limb bud is another possible explanation. Cutis aplasia is unique to our case and suggests disruption of limb bud development as a possible cause.

P0185. Familial aggregation of congenitally missing lower central incisors

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Background - The isolated form of congenitally missing teeth is often familial.Family studies have suggested that hypodontia may aggregate within families. Familial aggregation of a trait can suggest genetic etiology. Absence of lower central incisor is less common than the other forms of hypodontia. Objectives - To describe the clinical phenotypes, familial aggregation, inheritance and molecular diagnosis of congenitally missing incisors. Patients and Methods - Familial aggregation was investigated in 5 unrelated families with at least 2 cases with lower central incisors hypodontia. Patients were evaluated clinically and radiographically. A pedigree analysis was performed to determine familial correlations and pattern of inheritance of the hypodontia phenotype. Familial aggregation was measured by relative reccurrence risk. For DNA analysis, blood samples were collected from patients, their affected and unaffected relatives. Results - All patients and their affected family members presented hypodontia of permanent incisors as an isolated trait. Affected members within the families exhibited variable expression of hypodontia with regard to the region, symetry and number of teeth involved. Significant parent-offspring (father-son, father-daughter and mother-son) correlations in lower incisors hypodontia were observed. The type of inheritance was autosomal dominant with variable expressivity and complete penetrance. No mutations were detected in the PAX9 gene known to cause hypodontia. Conclusions - Hypodontia in one family member increased the likelihood of another family member missing a tooth. Genetic cause of congenitally missing lower incisors is attributable to a single dominant gene, but not PAX9 gene.

P0186. Mutational screening of the mitochondrial tRNA$^{Leu(UUR)}$ and tRNA^{Glu} genes in leucocytes and mucosal cells of Tunisian patients with mitochondrial Diabetes

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Mitochondrial diseases are very heterogeneous group of disorder with respiratory chain deficiency caused by either mutations in mitochondrial DNA or nuclear DNA encoding mitochondrial proteins. Several polymorphisms and mutations occurring in the mitochondrial tRNA$^{Leu\ (UUR)}$ and tRNA^{Glu} genes were found to be associated with mitochondrial diabetes.

In this study, 5 patients belonging to 5 unrelated Tunisian families with a clinical presentation highly suggestive of mitochondrial diabetes have been tested. Either mitochondrial DNA from leucocytes and from buccal mucosa has been extracted in these 5 patients. Mutational analysis in tRNA$^{Leu\ (UUR)}$ and tRNA^{Glu} genes have been performed by PCR-RFLP and direct sequencing in ABI PRISM® 3100 Avant automated DNA sequencer.

After PCR-RFLP analysis, none of the five patients were found to carry A3243G tRNA$^{Leu(UUR)}$ gene in the homoplasmic or in the heteroplasmic forms in leucocytes and in buccal mucosa. Sequencing of tRNA$^{Leu(UUR)}$ gene did not revealed any other mutations or polymorphisms in this gene.

After PCR-RFLP and direct sequencing of the mitochondrial tRNA^{Glu} gene, we revealed the T14709C mutation in this gene in heteroplasmic form in leucocytes and buccal mucosa of 4 out of the five diabetes patients. Searching of this mutation in members of the proband's families is in process.

P0187. Use of MLPA testing in subtelomeric rearrangements detection

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Genetic causes of mental retardation (MR) are very heterogeneous. Subtelomeric rearrangements play an important role in idiopatic MR determinism. Different methods have been used to identify subtelomeric rearrangements, but recently introduced MLPA (Multiplex Ligation Probe Amplification) technique seems to provide the best results. We have used MLPA to identify subtelomeric rearrangements in children with idiopatic MR. The protocol included the following sequence of steps: clinical selection based on de Vries score; karyotype; anti-FMRP test for Fragile X syndrome screening; MLPA testing using 2 independent kits in order to separate polymorphisms. We have selected cases using de Vries diagnostic score, only those with a score of 3/ more being selected. All patient data were recorded in a database that will be presented. The initial group was formed of 142 children with idiopatic MR. In 24 of them (16.9%) the karyotype revealed different abnormalities. 16 cases (11.3%) presented speech delay/ autism, but antiFMRP test was normal. 60 MLPA tests were done: 46 cases (76.7% were normal, 6 (10%) abnormal, 4 (6.7%) had polymorphism and 4 (6.7%) could not be interpreted. Clinical features of the cases identified with different subtelomeric rearrangements and polymorphisms are illustrated with photos and discussed in detail. The results

will be confirmed with FISH. In conclusion, the diagnostic score is useful in case selection for further testing and MLPA proves to be efficient in diagnosing subtelomeric rearrangements as a possible cause of idiopatic MR.

P0188. A 2-years old girl with hypoplastic glomerulocystic kidney disease caused by a novel *de novo* mutation in *HNF1- β* gene

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We report the case of a 2-years old girl with renal cysts. She initially presented with a suspicion of febrile urinary tract infection. Biological work-up revealed an increased creatinine and ultrasound showed renal cysts. Both kidneys were small for age displaying 20 small bilateral cysts. Familial history was relevant for unilateral renal agenesia in the paternal grandfather and one isolated renal cyst in the father. Personal history was uninformative, except for a neonatal pyelocalicial hypotony. She was transferred to our university hospital with a diagnosis of ADPKD. Because of the family history and the presence of tubular proteinuria, *HNF1β* gene was screened for mutation. Sequencing analysis revealed a heterozygous p.His149Gln substitution in the DNA binding region of HNF1-β factor (TCF2). This mutation not reported sofar was surprisingly undetectable in both parents, confirming a de novo transmission. There is no perturbation of liver enzymes nor cholestasis, and no arguments for glucose intolerance. In conclusion: *HNF1-β* gene mutations are often associated with renal findings that can manifest early in life. They should be included in the differential diagnosis of cystic renal diseases in children. Positive family history of various renal pictures, early deterioration of renal function, the presence of tubular proteinuria and the presence of extrarenal manifestations may distinguish the *HNF1-β*-related renal cystic disease from the others. Genetic analysis will bring definite diagnosis.

P0189. Monosomy 1p36; an atypical case with duodenal atresia and an interstitial deletion

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Monosomy 1p36 is one of the commonest chromosomal deletion syndromes affecting about 1 in 5000 newborns. Clinical features include microcephaly, developmental delay, hypotonia, seizures, hearing loss, feeding difficulty, structural heart defects and dilated cardiomyopathy. Typical facial features include deep set eyes with straight eyebrows, midface hypoplasia and small mouth with pointed chin. Most patients have subtelomeric deletions but interstitial deletions have been reported in a minority of cases.

We report a 2 year old girl with duodenal atresia, atrial septal defect, mild developmental delay and the facial gestalt of monosomy 1p36. Microcephaly, epilepsy and hearing loss were absent. Initial blood chromosome analysis and in situ hybridization using the Vysis ToTelVysion telomeric probe set were normal. However, analysis of a further G-banded chromosome preparation revealed a very subtle anomalous banding pattern in the short arm of chromosome 1. FISH using the 1p36 microdeletion probe set (Vysis), which includes a subtelomeric probe and an additional probe located 600kb more proximally, showed normal signal patterns but an interstitial 1p36.3 deletion was subsequently confirmed by FISH with a panel of tiling path BAC clones mapping to distal 1p. This report illustrates the importance of testing for interstitial deletions in patients with a phenotype suggestive of monosomy 1p36 and, to our knowledge, is the first reported case with duodenal atresia.

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P0190. Monozygotic sisters with Hurler syndrome

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In memoriam of Dr. Leonora M. Sandomirskaya.

Mucopolysaccharidose type 1 (MPS1, Hurler syndrome) is an inherited

lysosomal storage disease caused by deficiency of the lysosomal enzyme alpha-L-iduronidase (IDUA; EC 3.2.1.76) that results in undergraded glycosaminoglycans (GAGs). GAGs are either stored in the lysosome or excreted in the urine. IDUA gene maps to 4p16.3.

We report on a family with monozygotic sisters suffered from MPS1. They were born after the third properly prolonged gestation from 32 yr-old female by spontaneous vaginal delivery. Their parents deny consanguinity and hereditary diseases. At birth twins weights were 2690g and 2470g, their length were 49cm and 47cm. They have two elder healthy sisters, 11 and 9 yr. aged. The first year of twins' life was calm but their mother noted twins to have lackluster eyes. Probands came to clinical attention with umbilical hernia at the age of 14 months. By the age of 5 yr clinical symptoms conformed to MPS 1: course facial features, corneal clouding, hearing loss, short stature, joint stiffness, skeletal deformities, liver and spleen enlarged and GAGs urinary excretion elevated. In addition to these symptoms upper respiratory infections, ear infections and continuous nasal discharge were frequent. Molecular diagnosis was performed in Moscow (Centre of Medical Genetics) and the twins were found to be homozygous (Q70X, MIM 252800.0002). Now we try to find sponsors to start enzyme treatment for these girls from a large family

P0191. MPS 1H (Hurler)

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I would like to introduce a dizygotic twin (boy & girl) who are affected by hurler disease (mps 1h) a lysosomal storage disorder. They have mental retardation, coarse facies, enlarged tongue , skeletal deformities ,short stature, short and wide hands, hepatosplenomegaly, umblical hernia (only in the girl),and conductive hearing loss.

The radiologic changes of dysostosis multiplex are seen.These include a thickened calvarium, shallow orbit, enlarged j-shaped sella, anterior hypoplasia of lumbar vertebrae whith kyphosis, oar-like ribs, pelvic dysplasia, and shortened tubular bone+expanded diaphyses. The urinary excretion of dermatan sulfate and heparan sulfate is increased . Their parents have third degree of family relationship.

P0192. Facial dysmorphy, blindness and a mutation in the mtATP6 gene

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Mitochondrial ATP production via oxidative phosphorylation (OXPHOS) is essential for normal function and maintenance of human organ systems. Since OXPHOS biogenesis depends on both nuclear- and mitochondrial-encoded gene products, mutations in both genomes can result in impaired electron transport and ATP synthesis, thus causing tissue dysfunction and human disease. Over 30 mitochondrial DNA (mtDNA) point mutations and over 100 mtDNA rearrangements have now been identified as etiological factors in human disease. These mutations result in an extraordinarily broad spectrum of clinical phenotypes.

We present an 1 and ½ year old girl from first uneventful pregnancy and delivery, born on time with birth weight 2800g , length 51cm and head circumference 31 cm.. At the age of 28 days atrophia of n.opticus was suspected. A brain MRI showed severe internal and external hydrocephaly and athrophia cerebri. Dysmorphic features consisting of microcephaly, high palate, exsophtalmus and low placed ears were noted. The child is with increased deep tendon reflexes, hyper muscle tonus and profound mental retardation. She can not seat and walk and she is practically blind. An EEG suggested a locus of paroxismal activity in the left frontal region. Convulsions are not observed. Sequence-based analysis on mtDNA genom revealed G8573A-mutation in MT-ATP6 gene reflecting in amino acid change glycine-aspartic acid (second base pair of the codon). The same mutation was recently reported in Italian patient with LHON phenotype and the influence of this substitution on mitochondrion respiratory function remain to be determined.

P0193. Investigation of Polymorphisms in Non-coding Region of Human Mitochondrial DNA in 31 Persian HCM Patients

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The D-loop region is a hot spot for mitochondrial DNA (mtDNA) alterations, containing two HyperVariable Segments, HVS-I and HVS-II. In order to identify polymorphic sites and potential genetic background accounting for Hypertrophic CardioMyopathy (HCM) disease, the complete non-coding region of mtDNA from 31 unrelated HCM patients and 45 normal controls were sequenced. The sequences were aligned upon the revised Cambridge Reference Sequence (rCRS) and any incompatibilities were recorded as numerical changes in homoPolymeric C Tract (PCT), single base substitutions (SBS), insertions and deletions (Indels). Nucleotide substitutions were found to make up the majority of the mutations, rather than indels. We drew significantly high transition rate (81.8%) versus lower frequency of transversions (18.2%). 12 polymorphisms were identified in this study which had not been published in the MitoMap database. PCT changes at positions 303-309 were detected in 83% of our samples. Our results suggest that an increased level of HVS-I and HVS-II substitutions may be an indicator of mitochondrial DNA instability. Furthermore, mtDNA mutations may play an important role in pathogenesis of cardiac arrest which has remained unexplained for long.

P0194. Prevalence and Genetic Profile of Mucopolysaccharidosis Type 1 in the Irish population

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Aims: To document prevalence rates of the three clinical phenotypes of Mucopolysaccharidosis Type 1 Hurler (severe), Hurler-Scheie (intermediate) and Scheie(mild) in the Republic of Ireland using population data from the Government of Ireland census in April 2002.

Methods: Database and chart review of all live patients with MPS1 attending two specialised centres in 2002.Patient genotypes, iduronidase activity, ethnic background, province of origin, age at diagnosis and presenting clinical features were recorded.

Results: 30 patients were alive.One patient had Scheie, four Hurler - Scheie and 25 had Hurler syndrome, giving an overall prevalence of 7.66 per million for MPS 1 in Ireland. 19/25 (76%) patients from the Hurler group (age range 4 months to 14 years 10 months) were members of the "Irish Traveller" community.All had the same W402X homozygous mutation.There are 23,681 Travellers in Ireland. 10,001 are under 15 years of age giving a prevalence of 19 per 10,001 or 1 in 526 for Hurler's syndrome amongst Irish Traveller children.

The patient with the mild phenotype and all for patients with the intermediate phenotype hail from the same province.3/4 carry the same heterozygous W402X/P496L mutation.

Conclusion: A prevalence 1/526 for hurler syndrome amongst "Traveller" children is the highest recorded for this condition worldwide and equates with a carrier frequency of 1/11. Given the catastrophic morbidity of delayed treatment and availability of effective treatment (Enzyme replacement therapy by iv infusion and stem cell transplant) we recommend screening all babies from this population for Hurler syndrome

P0195. Chromosome aberration and multiple exostoses in 3 family generations

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Hereditary multiple exostoses (HME) is a genetically heterogeneous autosomal dominant disorder with near-complete penetrance that is associated with mutations in at least 3 different genes termed *EXT* ones. Three separate loci at 8q24, 11p11-12 and 19p11-13 are identified by linkage analysis. This disease is characterized with short stature, multiple osteochondromas, asymmetric growth at the knees and ankles, which may result in deformities.

We report on a family with rare coexistence HME and chromosomal aberration in three generations. For the first time we examined the proband at the age of 14 yr. He was born after the first properly prolonged gestation from 21 yr-old female by spontaneous vaginal delivery. At birth proband weight was 3250g, his length was 51cm, Apgar score was 7/8. The first bony lumps were found on tibiae and right scapula at proband's age of 4 yr. At the age of 14 yr his height was 154cm, his weight was 55kg, head circumference was 57cm. He also had hypoplastic scrotum, cryptorchidism and progressive osseous lesions. X-ray examination confirmed multiple scapular exostoses and exostoses in juxtaepiphyseal regions of long bones, genu valgum, short metacarpal, Madelung-like forearm deformities. His mother suffered from dysarthria and dyslexia, painless multiple exostoses of tibiae. His grandmother from the maternal side has short stature and multiple exostoses of the distal part of fibulae. Karyotypes of the proband, his mother and his grandmother were found to be abnormal 46XY, inv(5)(q13.1;q15) and 46XX, inv(5)(q13.1;q15)

P0196. Mulvihill-Smith progerioid syndrome: clinical evaluation and molecular characterization of a complex phenotype

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Mulvihill-Smith syndrome (MS) (McKusick 176690) is an extremely rare disorder, with only eight cases described. It is characterized by premature aging, multiple pigmented nevi, lack of facial subcutaneous adipose tissue, microcephaly, short stature and mental retardation. The proposita, a 10 years old girl, was the first child of healthy non-consanguineous parents, with a phenotype characterized by premature aging appearance, pinched nose, microcephaly, micrognatia, oligodontia, deep set-eyes and narrow nasal bridge, scarce subcutaneous adipose tissue in the face, abdomen and limbs, multiple cutaneous pigmented lesions. Reduced mobility of knee and elbow joints associated with multiple skeletal abormalities was documented by x-rays. The patient was found to have an atrial septal defect. Neurologic examination was normal, and a conductive hypoacusia and photophobia were present. Our patient presented diabetes mellitus type II associated with fatty liver and lypodistrophy, glucose levels and liver steatosis decreased after metformine therapy. Abdominal ultrasonography, CT and MRI disclosed remarkable liver steatosis with multiple hyperecogenic nodules, evolved in hepatocarcinoma within few months and treated by endoscopic radio-frequency local coagulation. G-banded karyotype was 46,XX. LAMIN A/C gene mutation screening disclosed an heterozygous C>T substitution at the codon 1698 in exon 10, previously described as a polymorphism. CGH Arrays analysis showed a 4 Mb interstitial deletion on chromosome 3 (3q26.1) inherited from the phenotypically normal mother. The genetic bases of MS remain to be established. The etiopathogenetic relevance of the large deletion on chromosome 3 in our patient and in her normal mother is open to speculations.

P0197. Expanding the phenotype of the 22q11deletion syndrome: the MURCS association.

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The MURCS association consists of Müllerian Duct aplasia or hypoplasia, unilateral renal agenesis and cervicothoracic somite dysplasia. We report a 22 year-old female with bicornuate uterus, right renal agenesis and C2-C3 vertebral fusion (MURCS association) and 22q11.2 deletion identified by array-CGH. Angio-MRI revealed aberrant origin of arch arteries with brachiocephalic vessels agenesis and hypoplastic left carotid artery originating from the aortic arch, left subclavian artery originating from descending thoracic aorta and hypoplastic left vertebral artery. Hashimoto thyroiditis, micropolycystic ovaries with a dermoid cyst in the right ovary and mild osteoporosis were also diag-

nosed. Accurate revision of Xrays permitted to identify cervical lordosis, C2-C3 fusion, lumbar scoliosis, thoracolumbar vertebral differentiation defects with lumbar ribs and S1 lumbarization. Audiometry and echocardiogram were normal.
Presently, she has short stature, obesity (BMI 30.7) and head circumference at the 3rd percentile. Long face, tubular nose with bulbous tip, low-set and small ears, high palate, nasal speech and slender hands are noted. The karyotype was normal. Unexpectedly, array-CGH analysis (75 kb resolution) revealed a 22q11.2 deletion, of about 2,5 Mb in size. MLPA analysis showed that the deletion was absent in her parents, demonstrating that the deletion was de novo. In 2006, Cheroki et al., reported another case of a 22q11 deletion in a patient with Mayer-Rokitansky-Kuster-Hauser anomaly (Müllerian Duct aplasia), Hashimoto thyroiditis and renal, cardiac and skeletal defects. We discuss whether this is a casual association or one additional syndrome due to the well known extensive phenotypic variability of the 22q11 deletion syndrome.

P0198. Screening human genes for small alterations performing an enzymatic cleavage mismatched analysis (ECMA) protocol

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Many human diseases are caused by small alterations in the genes and in the majority of cases sophisticated protocols are required for their detection. In this study we estimated the efficacy of an enzymatic protocol, which using a new mismatch-specific DNA plant endonuclease from celery (CEL family) recognizes and cleaves mismatched alleles between mutant and normal PCR products. The protocol was standardized on a variety of known mutations, in 11 patients with cystic fibrosis (CF), Fabry's disease (FD), steroid 21-hydroxylase deficiency (21-HD) and Duchenne/Becker muscular dystrophy (DMD/BMD). The results showed that the method is rapid, effective, safe, reliable and very simple, as the mutations are visualized on agarose or nusieve/agarose gels where the location of the alteration is indicated by a size marker. The method does not require special equipment, labeling or standardization for every PCR product, since conditions of heteroduplex formation and enzyme digestion are universal for all products. The protocol was furthermore evaluated in three DMD patients with the detection of three alterations, which after sequencing, were characterized as disease causative mutations (all of them nonsense on exons 21 and 44). The proposed assay, which was applied for the first time in a variety of monogenic disorders, indicates that point mutation identification is feasible in any conventional molecular lab. Alternatively it could be performed for cases where other techniques have failed.

P0199. LMX1B and Nail patella syndrome: experience in 16 families and genotype-phenotype correlation

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NPS is characterized by cardinal limb anomalies (nail dysplasia, absent or hypoplastic patellae, iliac horns, abnormality of the elbows…), in some cases nephropathy (glomerulonephritis) and/or eye involvement (glaucoma, cataract). Hearing loss and scoliosis have also been described. NPS is due to mutations in *LMX1B*, a gene located at 9q34.1. *LMX1B* codes for LIM-homeodomain transcription factor involved in normal patterning of the dorsoventral axis of the limb and early morphogenesis of the glomerular basement membrane
We sequenced LMX1B gene in 16 families (22 patients) with nail-patella syndrome (NPS).
The diagnosis of nail patella syndrome was typical in 13 index patients, due to characteristic limb anomalies. The tree remaining patients were a boy with limb features of NPS and mental retardation, but no 9q34.1deletion; a man with isolated nephropathy, and a woman with atypical limb anomalies.
We found LMX1B "stop" or "frame shift" mutations in 8/13 (62%) typical index patients and nonsense mutations in two atypical patients (the boy with NPS and mental retardation, and the woman with atypical NPS).
In all but one mutated families limb anomalies were isolated. In one sporadic patient with p.Gln37X mutation nephropathy was present and typical limb anomalies were associated with radio-ulnar synostosis.
The identified mutations were more frequent in exons 2, 4 and 5. No clear genotype-phenotype correlation appeared in this small series.
The non-mutated cases were 3 typical familial NPS with renal involvement in two instances, and one sporadic typical NPS case with proteinuria. We are searching for LMX1B deletion in these cases.

P0200. A novel type of *TPM3* mutation causing autosomal recessive nemaline myopathy in two Turkish families

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Congenital myopathies include a wide spectrum of clinically, histologically and genetically variable neuromuscular disorders defined by structural abnormalities in the muscle fibres. Nemaline (rod) myopathy (NM) is a rare congenital myopathy diagnosed on the basis of muscle weakness and nemaline bodies in the muscle fibres. The nemaline bodies are protein aggregates derived from sarcomeric Z discs and thin filaments. The six known NM genes all encode proteins for the thin filament of the muscle sarcomere: nebulin, alpha-actin1, beta- and gamma-tropomyosins, troponin T1 and cofilin 2. In order to identify the seventh NM gene we performed a genome-wide linkage study using microsatellite markers in 12 Turkish families with recessive NM. The hunt for the new gene is still ongoing, but in the context of this linkage study we identified a novel mutational mechanism for nemaline myopathy caused by alterations of the *TPM3* gene.
Two consanguineous Turkish families with two children each affected by a severe form of nemaline myopathy were found to have a homozygous deletion of the first nucleotide, an adenine, of the last exon of *TPM3*. The mutation in the last nucleotide before the STOP codon disrupts the reading frame and causes read-through across the STOP codon. The parents are healthy mutation carriers. RT-PCR predicts this to result in a TPM3 protein elongated by 75 amino acids. This is the first deletion to be identified in *TPM3,* and it may turn out to be a founder mutation among Turkish NM families. Further studies are ongoing.

P0201. Processing of Data Records on Patients with Neurofibromatosis Type 1 Using the Artificial Intelligence Methods

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250 patients with NF1 were clinically examined in detail. The patients were tested according to the NIH diagnostic criteria. The DNA bank for these patients was created containing 110 DNA samples. We have implemented direct DNA analysis of the NF1 gene using the MLPA and DHPLC methods and found *NF1* mutations in 17 patients: 15 causal mutations were detected exploring the DHPLC method. Using the MPLA method, two large deletions have been identified.12 of the above mentioned mutations were newly found.
The patient records are stored in the form of text files. Their content has to be presented in a database format to analyze them by available machine learning techniques. To support this transformation we have implemented MedAT - a tool for annotation and analysis of text files. Our program currently contains four knowledge ontologies - anamnesis, clinic symptoms, genealogy and mutations, which shape computer supported extraction of important information from patients' records. The set of ontologies can be easily modified. This approach enables dynamic processing and efficient analysis of available patients' data and it significantly contributes to achieving the main goals of this research:
• specification of diagnostic criteria for NF1 for Czech population
• genotype/phenotype correlation for patients with different types of mutations,
• determining the incidence and prevalence

Particular results will be presented.
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P0202. A clinical and molecular study in a child under 1 year of age affected by Neurofibromatosis type 2

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The aim of present study was to identify the earliest (i.e., disease onset < 1 year of age) clinical presentations of neurofibromatosis type 2 (NF2) in childhood and to investigate the presence of NF2 mutations at this young age. A NF2 child had his first disease manifestations under 1 year of age and was prospectively followed up and investigated according to our protocol for paediatric NF2. Molecular analysis of the NF2 gene was carried out by means of Denaturing High Performance Liquid Chromatography (DHPLC) and sequence analysis. The child presented at age 4 months right lens opacities and MRI scan showed colpocephaly and lesions in the posterior periventricular regions. MRI scans at age 8 months confirmed these findings and revealed bilateral vestibular schwannoma and the presence of multiple (> 40) skin NF2-plaques in the limbs. At his last head and spinal MRI the schwannoma and the periventricular lesions did not progress with any additional lesions. Molecular genetic analysis revealed a novel mutation in the exon 3 of the NF2 gene: this mutation was a small insertion of 4 bases pair at codon 94 (c.281_282 ins CCTT). This mutation was not detected in 100 control chromosomes from matched healthy individuals. This is the first time that: (1) a bilateral eight nerve tumour in a NF2 child do not show progression after a long follow-up period; and (2) a NF2 child develops large numbers of skin NF2-plaques in atypical localisations.

P0203. Evidence for high rate of gonadal failure in female patients with Nijmegen breakage syndrome - a Polish study

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Nijmegen breakage syndrome (NBS) is an autosomal recessive disease with defects in DNA repair and is characterized by microcephaly, growth retardation, immunodeficiency, radiosensitivity, and an elevated risk of cancer. The relatively frequent occurrence of NBS in Poland, which is in line with the relatively high frequency of 657del5 mutation carriership in the Polish population (0.6%), allowed us to undertake intensive follow-up studies of a large and uniform group of patients. Longitudinal observation drew our attention to lack of a pubertal growth spurt and to primary amenorrhea in all but one girl who reached pubertal age. A total of 36 female NBS patients, aged from 1 to over 20 years, observed at a single centre (CMHI) were enrolled in this study. The aim was to verify our earlier observations and to establish the frequency of ovarian failure among NBS females. The gonadotropin concentrations were found to resemble the pattern described in patients with Turner syndrome: high levels 1-3 years after birth, normal in mid-childhood, increasing to postmenopausal levels at puberty. Our findings indicate a very high rate of ovarian failure in NBS, which leads to psychological problems related to lack of acquisition of secondary sex characteristics and to reduction of bone density. Puberty must be induced or completed in such patients. Given the enhanced general predisposition for malignancy in NBS, and the additional danger of ovarian tumors arising from dysgenetic gonads, female NBS patients should be carefully monitored and diagnosed in this context.
This study was supported by grant MNiSzW 2P05E06628.

P0204. Germline KRAS mutations in Noonan syndrome: study of 4 NS patients with KRAS mutation in a cohort of 82 patients without PTPN11 mutations

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Noonan syndrome (NS) is an autosomal dominant disorder characterized by short stature, congenital heart defect, typical facial dysmorphism and mild intellectual deficit in less than half of cases. NS is genetically heterogeneous. In 40% to 50% of cases, the disease is caused by missense mutations in the PTPN11 gene, resulting in a gain of function of the phosphatase SHP2 resulting in overactivation of the Ras-MAPK signaling pathway. Recently mutations have been identified in two other genes in the same pathway: KRAS is mutated in approximatively 2.5% of NS and SOS1 in roughly 10% of cases. Most KRAS mutations result in CFC syndrome. Here, we report mutation analysis of KRAS in 82 NS patients without PTPN11 mutations. We found 4 KRAS mutations in these patients (p.Val14Ile in 2 patients, p.Thr58Ile, and p.Phe156Ile). All patients present the classic dysmorphism of NS. They appear to have a severe phenotype: short stature, developmental delay and heart defect were present in all patients. Failure to thrive (3/4 patients), sparse hair (2/4) and eyebrows (1/4) indicated a significant clinical overlap with CFC in these patients, but keratinisation defects . Dilatation of the ventricles was observed in 2 patients, confirming that abnormal CNS development could be more common with KRAS mutants than with other genetic etiologies.

P0205. Molecular, clinical and new haematological insight in Noonan Syndrome.

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Noonan syndrome (NS, OMIM 163950) is an autosomal dominant disorder, with a prevalence at birth of 1:1000-1:2500 live births, characterized by short stature, facial and skeletal dysmorphisms, cardiovascular defects and haematological anomalies including an increased risk of leukaemia. Missense mutations of PTPN11 gene (12q24) account for approximately 50% of NS cases, while molecular lesions of other genes of the Ras pathway - KRAS and SOS1 - play a minor role in the molecular pathogenesis of the disease. Twenty-two patients were enrolled in the study with a PTPN11 mutation detection rate of 35%, and no additional mutation in KRAS gene. A statistically significant association with pulmonic stenosis was found in the group positive for PTPN11 mutations whereas a statistically significant association with HCM was observed in negative ones. We heve observed a novel missense mutation, Phe285Ile, in a familial case. Cultures of peripheral blood haematopoietic progenitors were performed in the presence of decreasing concentrations of GM-CSF. Circulating haematopoietic progenitors of PTPN11- mutated NS subjects did not show the hypersensitivity to GM-CSF observed in PTPN11-mutated juvenile myelomonocytic leukaemia patients. Therefore, we propose this functional evaluation of circulating haematopoietic progenitors as a non-invasive technique useful in the follow-up of NS patients for early detection of leukaemic evolution.

P0206. The investigation of NPHS2 gene coding region in group with Hereditary Proteinuria Syndromes.

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Hereditary Proteinuria Syndromes (HPS) is a group of inherited diseases in which proteinuria is the main clinical manifestation. The courses of these diseases vary in the age of onset, in the severity of injury, and in the morphologic diagnosis. The genetic cause of these diseases is mutations in genes providing the structure and function of filtration barrier.

We have examined 48 children with biopsy proven SRNS and 15 children with isolated proteinuria syndrome (IPS) in age ranged from 2 to 17 years (SRNS group). The disease manifestation has been observed at the age ranged from 1 month to 16 years. We have investigated DNA samples for mutation in NPHS2 gene coding areas by SSCP, ACRS methods and direct sequencing. We have identified new polymorphism c.872+7A>G. The allele frequencies of this polymorphism among our patients and in population group is similar (5% and 4.76%). We have found heterozygous mutation c.328G>T (p.87Glu>Stop) in coding region of the first exon in one having been transplanted patient,

which has not been reported previously. This is SRNS patient of 2 years old, with manifestation of disease at the age of 1 month with rapid progression to the End Stage Renal Disease. In this case we can propose digenic type of inheritance. Perhaps, the most our patients` disease is caused by mutation in over genes coding proteins being involved in the structure and the function of the kidney filtration barrier.
This work was partly supported by Russian President`s grant NSh-5736.2006.7.

P0207. Mutation screening of melanocortin 4 receptor (MC4R) in a Norwegian cohort of obese patients reveals a low prevalence and four novel mutations.

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Backgound: Heterozygous mutations in the melanocortin 4 receptor (MC4R) gene are the most frequent monogenic cause of obesity. Previous studies of such mutations have given prevalences varying from 0.5 up to 5.8%.
Materials and methods: We sequenced the coding region of the MC4R gene in 703 obese Norwegian subjects. The patients were recruited from an ongoing study at Ullevål University Hospital. Adults with BMI>35 kg/m² and children whose bodyweight was above the 97.5 th percentile for height were asked to participate. All novel mutations were analyzed by a bioinformatic approach and family studies.
Results: 6 mutations were identified, two previously described and four new ones. A total of three different mutations were found among the 459 adults, giving a prevalence of 0.65%. Four patients with mutations were found among 244 children corresponding to a prevalence of 1.6%. Several known polymorphisms were also identified.
All mutations identified in the pediatric group were in second-generation immigrants, who made up more than half of this group. The same novel mutation was found in two siblings whose parents are first cousins. Both children are homozygous for a 3-bp-deletion in the MC4R-gene. The other patients were all heterozygous for their mutation.
Two previously described mutations, p.Tyr35X and p.Thr150Ile were found in female patients with adult onset obesity.
Conclusions: Mutations in the MC4R gene are not a common cause of obesity in Norway. Four of the six mutations identified have not been described previously.

P0208. Oculo-Facio-Cardio-Dental (OFCD) syndrome: Somatic mosaicism of a large *BCOR* gene deletion in 2 monozygotic twins and three novel mutations

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Oculo-Facio-Cardio-Dental (OFCD) syndrome is a rare disorder associating congenital cataract, microphthalmia, characteristic dysmorphia, congenital heart defects, oligodontia, and radiculomegaly. OFCD syndrome results from mutations in the *BCOR* gene, located on Xp11.4, encoding a key transcriptional regulator during early embryogenesis. X-linked dominant inheritance is suggested, although a singular *BCOR* missense mutation, whose relevance remains controversial, was described in a male presenting with Lenz microphtalmia syndrome (microphtalmia, mental retardation, and multiple congenital abnormalities).
To further delineate the clinical spectrum of these disorders, we studied seven females with OFCD syndrome and four males with Lenz microphtalmia, from six unrelated families. *BCOR* mutations were screened by direct sequencing, QM-PSF, and deletions were confirmed by FISH analysis.
Somatic mosaicism for a large deletion of *BCOR* (45 % in peripheral leukocytes) was identified in two monozygotic twins presenting with typical OFCD syndrome. One twin transmitted this large deletion homogeneously to her daughter. In addition, three novel mutations were identified in three unrelated females: two frameshift (p.Pro288ArgfsX90 and p.Pro190ProfsX26), and one nonsense (p.Arg1480X) mutation. No mutation of *BCOR* was found in the male patients with Lenz microphtalmia.
In conclusion, we report four novel *BCOR* mutations in females with OFCD syndrome. These results broaden the clinical spectrum of OFCD syndrome, as three patients did not present heart defects, and one patient had mild mental retardation, thus suggesting that the condition is underdiagnosed. The first description of somatic mosaicism in OFCD syndrome is of particular relevance to genetic counselling.

P0209. A natural history of a six years-old girl with Ohdo syndrome

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Ohdo blepharophimosis syndrome (OBS) (249620 #OMIM) is a rare genetic entity characterized by blepharophimosis, blepharoptosis, dental hypoplasia, heart disease, short stature and intellectual disability. The phenotypic variability in OBS has been reported . We present a natural history of a girl observed during six years from the birth. OBS has been recognized on the basis of following features: short stature, blepharophimosis, ptosis of eyelids, epicanthic folds, microphtalmia, flat, wide nasal bridge, narrow mouth fissure, long philtrum, thin upper lip, high palate, hypoplastic teeth, dysplastic, low-set ears, joint laxity, scoliosis, sacral dimple, congenital heart anomaly (pulmonary stenosis, VSD, PDA, PFO), severe hypotonia and distinct developmental profile. Morphological phenotype has been described according to the catalogue elaborated by Stengel Rutkowski et al. and evolution of some traits has been found. Interestingly, some features as: a very soft skin, curly, fragile hair , a lack of nose cartilage, small maxilla and, a radiological abnormalities in knees resembling Blout disease were not previously described in relation to this syndrome.
We suggest that a better knowledge of the clinical and anthropological spectrum of OBS can be helpful for setting a diagnosis as molecular basis of this disorder remains still unknown.

P0210. Ophthalmic status of patients with Goldenhar syndrome

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Aim of the work: investigation of ophthalmic status of patients with Goldenhar (facio-auriculo-vertebral) syndrome.
Material and methods. There were 5 patients (10 eyes, 3 males and 2 females aged from 6 to 27 yr) with Goldenhar syndrome under our observation. Routine basic ophthalmic investigation was performed for all patients.
Results. Ophthalmic status of all patients included sclera-corneal lipodermoid (6eyes) followed by reduced vision and demanding partial fibered keratoplasty (1 case) and optic correction picking up of all the patients because of astigmatism (5 patients). Two clients had petite lipodermoid of sclera without surgical correction. One client had inborn anomaly in the form of the upper lid coloboma removed with surgical intervention. All clients were operated on in early childhood apropos of a cleft lip and a palate. A 10 year-old boy had atresia of the auditory passage and the malformed ear followed by unilateral inborn hearing disorder. Mental development of all clients was intact. Our clients' relatives denied congenital and inherited pathology. Correction of refractional amblyopia with spectacles resulted in stable and high visual function. Development of xerotic cornea of the eye with the upper lid coloboma was prevented.
Conclusion. To make diagnosis at proper time and correct face, eye and ear dysplastic features using surgical intervention by ophthalmologists, orthodontists and specialists of aesthetic surgery are sine qua non for successful life and disease prognosis.

P0211. Orofacial clefts with associated anomalies in Lithuania

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Lithuania.
The aim was to investigate the incidence and type of orofacial clefts (OCs) associated with congenital defects in Lithuanian population.
Patients and methods. There were included 235 cases of OCs with one or more major congenital anomalies investigated from 1993 to 2005 in the study. OCs were subdivided into three groups: cleft palate alone (CP), cleft lip alone (CL) and cleft lip with cleft palate (CLP). Each case was assigned to one of categories: nonchromosomal syndromes, OCs with chromosomal anomalies and unidentified syndromic OCs with one or more associated major anomalies, which were grouped according British Pediatric Association Classification of Diseases.
Results. There were 70 cases of nonchromosomal syndromes (20 different syndromes), 26 cases of OCs with different chromosomal abnormalities and 141 non-syndromic patients with OCs and one or more associated major anomalies of total 235 persons with OCs and additional anomalies. There were 420 different anomalies in patients with unidentified syndromic OCs, a mean of 2,9/proband. The commonest organ system affected was the musculoskeletal system (133 anomalies), followed by cardiovascular (90) and anomalies of face (including eye and ear) and neck (64).
Conclusions: This study expanded the phenotype of patients with OCs. According to the results of this study we can propose that all patients with OCs should be examined by the team of specialists such as pediatricians, plastic surgeons, orthodontists, cardiologists and others with closer collaboration of clinical geneticists. A routine screening for other associated malformations, especially skeletal, central nervous system, and cardiac defects, is required.

P0212. Tunisian family with ocular, squelettal and abdominal malformations

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We report on two sisters who had a unique association of facial, ocular , skelettal defects and abdominal muscle hypoplasia. They are the fourth and the fifth sibs, born to healthy first cousin parents originating from Tunisia. The family history is unremarkable. The phenotypic findings are compared with the previously reported patients referred to as Carnevale, OSA, Michels and Malpuech syndromes. All are autosomal recessive. We conclude that the present patients resemble most patients with OSA syndrome previousely reported in 1996 by Mingnarelli et al .
Despite the presence of apparently distinctive key features, it appears that these four entities share multiple similarities in the facial and the pattern of malformations.

P0213. Osteopetrosis mutation study among an Iranian family; TCIRG1 gene

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Autosomal-recessive osteopetrosis is a severe genetically heterogeneous disorder due to osteoclast failure. Dense bones prone to fracture, severe hematological failure, and neurological impairment are among the prominent features of the disease.
It has been shown that mutations in the TCIRG1 gene may result in three disaese phenotypes characterized as autosomal recessive, infantile malignant and also in association with bone mass forms associated with 38, 10 and 1 mutations respectively.
Previous studies showed that 19 out of total 50 known mutations (38%) were splicing mutations, which may also be expected to be the most common mutations in Iran.
In our study a family of 37 members was the subject of investigation for TCIRG1 gene mutations for 3 cases of severe osteopetrosis after the clinical diagnosis of the proband who have been involved with the disorder through the first seven years of his life using the standard PCR method for all 20 exons and then sequencing to detect the risk of next pregnancies involvements in the parents of the dead proband.

P0214. Epidemiological study of some familial cases with primary osteoporosis.

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Osteoporosis is a multifactorial disease characterized by a decrease in bone mass and deterioration of bone architecture. Genetic factors are determinants of peak bone mass and may influence age-related decreases of bone mass. WHO has established an operational criterion based on bone density measuring, the T-score. 122 cases were studied, 51 diagnosed with primary osteoporosis. Mean age was 57.6 years. The main inclusion criterion was the acceptance of affected individuals to participate in the study. Accurate family history was taken. Daughters of affected persons were evaluated by DEXA technique. 67.2% of them had T-score values that indicated osteopenia or osteoporosis (mean value -2.3SD). Molecular testing was not available. We are interested in future collaborations. In conclusion, descendents of affected parents are at a high risk for osteoporosis, important aspect for primary prevention.

P0215. Otopalatodigital type I syndrome: report of a familial case

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Otopalatodigital syndrome spectrum disorders include four phenotypically related conditions: otopalatodigital syndrome (OPD) type I and II, frontometaphyseal dysplasia and Melnick-Needles syndrome . Mutations in the *FLNA* gene have been reported in the majority of the patients. Most pedigrees are consistent with X-linked inheritance. We report on a typical OPD type I familial case, a 7y7mo-old boy presenting characteristic dysmorphisms with prominent supraorbital ridges, hypertelorism, submucous cleft palate, deafness, short broad thumbs, spatulate finger tips, partial syndactily of the fingers, overlengthening of the second toes and foreshortening of the great toes, broad terminal phalanges of the other digits. His mother was considered normal with normal stature (1.55m), without facial dysmorphism, exhibiting only bifid uvula and longitudinal darkish lines striates on nails of the 1th, 2 th and 3 th fingers, similar to the ones found in craniofrontonasal dysplasia. They showed unimpaired intellect. A mutation in the *FLNA* gene was detected in both mother and son. Our case reinforces the phenotypic variability in OPD syndrome type I, and also the importance of molecular analysis in mild phenotypes and in females to confirm a carrier status, in order to perform a more precise genetic counseling.

P0216. Parkinsonism and essential tremor in a family with pseudodominant inheritance of PARK2

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The *Parkin* gene *(park2)* plays an important role in the early onset forms of Parkinson's disease (PD). Mutations in this gene have been described in approximately 50% of familiar cases and in the 10-20% of sporadic cases with an onset age ≤ 45 years. This study shows the results of a *parkin* gene analysis in a family from Southern Italy.This family included five affected individuals showing either essential tremor (ET), parkinsonism, or both, consistent with pseudo-dominant inheritance of *PARK2*. The affected patients were screened for mutations in the *parkin* gene by a combination of gene dosage and sequencing of entire coding region. We identified a 226 C>G homozygous missense mutation leading to the amino acid substitution R42P in two parkinsonian subjects and in 2 other not affected members of the family. Four subjects carried the R42P substitution in heterozygotic form. Exon rearrangements were excluded. Striatal dopamine transporter density was reduced in accordance with phenotype and number of mutated alleles. Apparently, this family did not show consanguinity, but haplotype analysis revealed a common chromosomic region shared by some members of both branches of the kindred, suggesting a single founder. Whether postural and kinetic tremor in this family really represents ET or is a first manifestation of parkinsonism requires further follow-up. Nevertheless, we suggest that *parkin* mutations may have a role in families with occurrence of both diseases.

P0217. Partial mosaic duplication (X)(q21.3q24) in a girl with clinical signs of Pelizaeus-Merzbacher disease

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We report on the first child of a healthy non consanguineous German couple. During pregnancy prenatal diagnosis was performed because of advanced maternal age. An interstitial duplication Xq in about 40 percent of amniotic cells was detected. The girl was born after 41 weeks of gestation with normal birth measurements (weight 2820g = 3rd-10th percentile, length 50cm = 10th-25th percentile) and normal APGAR score (10/10). Feeding difficulties and muscular hypotonia were noted after birth. Motor delay became obvious between six and twelve months (no sitting, no crawling, no turning), but good social communication was observed. Clinical investigation at the age of 12 months showed no facial dysmorphisms and normal body measurements (length 73cm = 25th percentile, weight 7.7 kg = 10th percentile, OFC 45cm = 25th percentile). As a clinical sign of chromosomal mosaicism streaky and patchy hypopigmentation of the right leg was noted. Cytogenetic investigations of blood lymphocytes showed a similar distribution of normal and duplicated cells compared to amniocytes (mos 46,XX[17]/46,X,dup(X)(q21.3q24)[13]). Parents had normal karyotypes. MRI of the brain of the girl with 10 months showed no myelination like in Pelizaeus-Merzbacher disease. FISH analysis with probes specific for the *PLP1* region confirmed the suspected duplication in 40% of cells. A duplication of the *PLP1* gene is the cause of Pelizaeus-Merzbacher disease in most male patients with this X-chromosomal recessive disorder and was described as cause of clinical symptoms in several female heterozygote carriers, so we speculated that the Xq duplication might explain the girl's developmental delay.

P0218. Genetic variants of the phosphodiesterase (PDE) gene superfamily in patients with bilateral adrenocortical hyperplasias

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Several types of adrenocortical tumors that lead to Cushing syndrome may be caused by aberrant cyclic AMP (cAMP) signaling. In search for cAMP-signaling related genetic factors involved in adrenocortical tumorigenesis we have performed association and loss-of-heterozygosity analysis based on genome-wide 10K SNP mapping of both tumor and germline DNA from patients with bilateral adrenocortical hyperplasia (BACH). We found inactivating mutations in 2q32-located phosphodiesterase (PDE) 11A (*PDE11A*) gene to be associated with the disease in a subset of our BACH patients. We further selected several other genes from the *PDE* superfamily including *PDE3A*, *PDE4A*, *PDE4B*, *PDE7A*, *PDE8A*, *PDE8B*, and *PDE9A* that were favored by our analysis and have shown relatively high expression in the adrenal gland. We identified a number of synonymous and non-synonymous substitutions to be present in our BACH patients and not in normal control individuals from a variety of sources. We further investigated the effect of these variations on cAMP and cGMP degradation *in vitro*. We speculate that variations in genes coding for PDEs may be involved in adrenocortical tumorigenesis by affecting the efficiency of cyclic nucleotide degradation and other means that lead to increased cAMP signaling.

P0219. Germline and somatic mosaicism at the PHOX2B locus in Late-Onset Central Hypoventilation syndrome.

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Idiopathic late-onset central hypoventilation syndrome (LO-CHS) is a rare disorder occurring from early childhood to adulthood. The physiopathological relationships between LO-CHS and congenital central hypoventilation syndrome (CCHS) have been debated and it has now been shown that they are allelic disorders.

Here we report a series of 26 patients with LO-CHS referred from 5 weeks of age to adulthood. We identified a heterozygous PHOX2B gene mutation in 14/26 patients. The most frequent mutant allele results in a +5 alanine expansion of the series of 20 alanines C-terminal to the homeodomain of the protein (10 cases). We observed also one +8 alanine, twos truncating mutations and one missense within the homeodomain. Semi quantitative PCR showed somatic mosaicism in one case only. This has major consequences in terms of genetic counselling. In this series, one adult with LO-CHS had a child with CCHS. These data raise also the question of the follow-up of apparently healthy parents of a CCHS child who were found to harbour a somatic mosaicism for a PHOX2B gene mutation (5% of the parents in our series). Among the 12/26 patients with no PHOX2B mutation 3 presented hypothalamic-related endocrinopathies and behavioral problems, suggesting genetic heterogeneity of idiopathic LO-CHS.

Altogether, these data demonstrate a genetic link between CCHS and, at least, a subgroup of LO-CHS. Furthermore, combined genetic and environmental factors may explain the variability of disease onset ranging from neonatal period to adulthood for an identical PHOX2B gene mutation (+5 alanine expansion).

P0220. Clinic and genetic study in a family with a clinical picture of pantothenate kinase-associated neurodegeneration

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Pantothenate kinase-associated neurodegeneration (PKAN) is a disorder characterized by dystonia, parkinsonism, and iron accumulation in the brain. Many patients with PKAN have mutations in the gene encoding pantothenate kinase2 (PANK2) and a specific MRI pattern of hyperintensity within the hypointense medial globus pallidus (eyes of the tiger). Abnormal accumulation of iron in the brain is detected also in other neurodegenerative diseases such as neuroferritinopathy associated to mutations in the ferritin light polypeptide (FTL) and in the ferritin heavy polypeptide (FTH1) genes. We performed a molecular study in a family with a PKAN classic phenotype. The patient, a 21-year-old woman, originated from Southern Italy. Her parents were healthy and consanguineous. Brain MRI study showed the eyes of the tiger pattern. Serum ferritin was 16 ng/mL (N= 20 to 300). On the basis of the clinical and neuroimaging features, a diagnosis of classic PKAN was made but the sequencing analysis of *PANK2* gene revealed absence of mutations and microsatellite analysis with 7 microsatellite markers from the PANK2 region (20p12.3-p13) excluded linkage with PANK2 locus. Considering the abnormally low levels of ferritin in the serum of the patient, we supposed a ferritin-related neurodegeneration but the sequences of the *FTH1* and *FTL* genes were found to be normal. These findings suggest a probable involvement of other iron regulatory proteins. Recently, a locus for neurodegeneration with brain iron accumulation was mapped to chromosome 22q12-q13 and mutations in *PLA2G6*, encoding a calcium-indipendent phospholipase, was identified. Sequencing of *PLA2G6* gene is being carried out.

P0221. Familial occurrence of Poland's Syndrome: further evidence of the genetic component.

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Poland's syndrome consists of variable clinical features, but always includes unilateral aplasia of the chest wall muscles and ipsilateral anomalies of upper extremities. The incidence of Poland's syndrome, reported by different authors, ranges from 1:10,000 to 1:100,000. It is observed more frequently in males than in females, with the right side of the body affected more often than the left. The aetiology of the Poland's syndrome is still discussed and different etiologic factors are taken into account: genetic, embriogenetic but also teratogenetic effects of environmental xenobiotics .

However most of described cases were sporadic, rare familial incidence of Poland's syndrome was also reported, suggesting a possible autosomal dominant transmission.

We present 5 familial cases in which absence of the right pectoralis major muscle (RPMM) and aplasia of the ipsilateral breast was observed in 4 males and hypoplasia of the RPMM in 1 female. The absence was confirmed by muscle ultrasound and MRI. Although none of 5 patients had congenital upper limb abnormalities, we believe that they still qualify as having a Poland's syndrome.

Mild forms of Poland's syndrome are in fact more frequent than severe

forms, and may go undiagnosed, especially in females. Hypoplasia of one breast or a horizontal anterior axillary fold may be the sole clinical manifestation of this syndrome.
Data here reported while adding further evidence of the genetic component of the Poland's syndrome and of the AD pattern of inheritance, in the same time suggest a reduced penetrance and a variable phenotypic expression of the disease.

P0222. Paternal deletion 15q11-q13 versus maternal disomy - how different are these two Prader-Willi syndrome phenotypes in a Polish cohort?

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We present the results of a study whose aim was to determine the impact of a different genetic background on the phenotype of individuals with Prader-Willi syndrome (PWS).
The molecular screening was performed in patients referred to our centre with a clinical diagnosis of PWS. The patients were divided into following groups: 25 with maternal disomy, 62 with *de novo* deletion 15q11-q13, 2 with deletion resulting from a balanced paternal translocation and 3 with a microdeletion in the imprinting center (IC). Two affected sibs inherited the microdeletion from their father and their paternal grandmother was the carrier. The third child's father was mosaic for microdeletion which occured *de novo*. Forty seven patients with normal methylation pattern at 15q served as a control group. Detailed clinical investigations and data collected from the original questionnaire enabled us to characterise each group.
Although there were many statistically significant differences between patients from the control group and patients with confirmed PWS, comparison of individuals with deletion and disomy showed only slight differences. The children with disomy were taller, had longer trunk, broader chest, milder dysmorphic traits, less severe behavioral problems and started to walk earlier than those with deletion. The outcomes of the study are discussed in view of a current literature. The characteristics of cases with unbalanced translocations and IC microdeletion will also be shown.
The work was supported in part by grant No 2PO5E06228 from the State Cometee for Scientific Research of the Republic of Poland.

P0223. Genotype and Phenotype Analyses of Prader-Willi Syndrome Patients in Taiwan

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Aim: To compare the genotype and phenotype correlation of patients with Prader-Willi syndrome (PWS) in Taiwan.
Methods: We performed a retrospective analysis of 67 molecularly confirmed diagnoses of PWS from January 1980 through July 2006 in five medical centers in Taiwan. Clinical manifestations were compared between the deletion and maternal uniparental disomy (UPD) groups.
Results: The genetic analysis identified deletion in 56 (84%), UPD in 10 (15%), and a probable imprinting center deletion or imprinting defect in 1 (1%). Compared with UPD type, PWS patients with deletion type were more likely to have the phenotypes of hypogonadism ($p < 0.001$), small hands and feet ($p < 0.001$), and hypopigmentation ($p < 0.002$). We also found a higher maternal age ($p = 0.015$) and a higher paternal age ($p = 0.021$) in the UPD group. No other clinical features were found to be significantly different between the two groups.
Conclusion: Our study in Taiwan is in contrast to most previous reports in Western populations that indicated a higher incidence of UPD in PWS. The possible subtle phenotypic differences between patients with PWS on the basis of deletion type versus UPD type would be useful both in clinical diagnosis and in understanding the genetic basis of the subtypes of PWS.

P0224. Lack of Evidence of Monosomy 1p36 in Patients with Prader-Willi-Like Phenotype

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Some researchers suggested the existence of two possible phenotypes for the 1p36 Monosomy. We would like to describe our experience about the possible correlation between 1p36 microdeletion and the Prader-Willi phenotype.
22 patients aged 8-23 years-old who tested negative for Prader-Willi syndrome by Souther blot.
Clinical characterization of the patients was performed using a protocol with the characteristics of 1p36 microdeletion Prader-Willi-Like syndrome. Hight Resolution Cytogenetic Studies performed at a 550-850 bands level, and eleven polymorphic markers of 1p36 region (D1S243, D1S468, D1S2660, D1S2795, D1S2870, D1S2145, D1S214, D1S2663, D1S450, D1S244, D1S2667) were analyzed.
After cytogenetic analysis, no subtelomeric deletion was observed over 40 metaphases by patients, and all the polymorphic markers for 1p36 were negative for microdeletions too.
According to the results, our sample is consistent with the Prader-Willi phenotype (mental retardation/obesity 100% hyperphagia 86.4% developmental delay 59% hypotonia 68.2% infant feeding problems 79% abusive behavior 63%).
It also can be suggested that our methodology is adequate, 98% of the 1p36 microdeletions could be detected by high resolution cytogenetic and, the number and position of the markers used which are located in all six intervals suggested by Wu et al. 1999 observed clearly by Heilstedt et al. 2003, even more we used 2 polymorphic markers inside the obesity/hyperphagia chromosomal segment 1p36.33-36.32 (D'Angelo et al. 2006).
The phenotype defined by Heilstedt et al. 2003 excluded hyperphagia/obesity. Our results do not confirm the suggestion of D'Angelo et al. 2006 about a specific hyperphagia/obesity chromosomal region in 1p36.

P0225. Detection of the G646C polymorphism of *DYT1* gene in patients with primary focal dystonia.

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Introduction: Early-onset generalized primary dystonia is a dominantly inherited movement disorder, mostly caused by a 3-bp (GAG) deletion in exon 5 of *DYT1* (*TOR1A*) gene encoding torsinA. It's penetrance is estimated to be 30-40%. Moreover, in some families, members carrying the same mutation could be either asymptomatic or display dystonia, which may be focal, segmental, multifocal, or generalized in distribution, suggesting the role of other genetics modifiers. It was shown that cells expressing the G646C polymorphism in exon 4 of *DYT1* gene (replacement of aspartic acid with histidine at residue 216) developed inclusions similar to those associated with GAG-deleted torsinA. The aim of this study is to determine the putative role for this polymorphism in primary focal dystonia.
Patients and Methods: Genomic DNA from 50 patients with focal primary dystonia, in whom no GAG deletion in *DYT1* gene had been identified, was purified by „salting-out" method. The detection of the polymorphism was performed in an ABI PRISM 7500 Real-Time PCR system using a validated TaqMan SNP Genotyping Assay from Applied Biosystems. Data were analyzed with ABI PRISM Sequence Detection Software.
Results and Discussion: 7 patients were heterozygotes G/C for G646C polymorphism. The rest of the samples were homozygotes G/G and no homozygotes C/C were identified. No relationship between the polymorphism and the severity and evolution of the disease was found. In order to complete this work, G and C allele frequencies will be studied in healthy Basque Country population.

P0226. "Wiedemann-Rautenstrauch syndrome": new case report

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We presented a clinical data of new case of "Wiedemann-Rautenstrauch syndrome" (WRs) - a rare autosomal recessive disorder, mani-

fested progeroid features from infancy (OMIM 264090).
Proposita - 6 month age male is a single child of young healthy non-consanguineous couple (G1;P1). He was born at 38 weeks gestation, labor was unremarkable (BW=2540; BL=47cm; OFC=34cm). Prenatal hypoplasia and aged signs was present at birth. At age of 6 months old progeroid appearance became more pronounced, patient showed growth delay (W=4.6kg; L=60cm,; OFC=41cm all<3rd centile), muscle hypotrophy, mental retardation, dystonia, prominent veins on scalp, aged face, wide forehead, sparse eyebrows, eyelashes and scalp hair, posteriorly rotated dysmorphic ears, shallow orbits, protruding eyes, nystagmus, angioretinopathy, thin lips, arched palate, severe micrognathia, small narrow beaked nose with hypoplastic alae, subcutaneous tissue loss, abnormally visible subcutaneouse veins, thin limbs, camptodactyly II-V, adducted thumbs, heart defect (small ASD), large abdomen, scleroderma-like changes of skin on the buttocks. Sonography of brain and abdomen was normal. Chromosomal, biochemical analyses were normal.
Clinical features of our patient were compared with published data of WRs, other premature aging syndromes, nonclassified progeroid conditions. We have established “neonatal progeroid Wiedemann-Rautenstrauch syndrome” based on association of characteristic aging appearance present at birth, failure to thrive, mental delay, hypotrichosis, signs of generalized lypodystrophy and paradoxal fat accumulation.
Because of high genetic risk early diagnostics is important for prognoses in affected families. New cases of progeroid disorders must be collected for further delineation of phenotype and counseling improving.

P0227. Pseudoxanthoma Elasticum - a four-generation family with typical features but apparent absence of the ocular phenotype

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Pseudoxanthoma elasticum (PXE) is a progressive disorder of elastic fibres with ocular, dermatological and cardiovascular manifestations. Mutations in the ATP binding cassette transporter gene (ABCC6) have been identified and most familial cases show autosomal recessive inheritance. Autosomal dominant (AD) inheritance is rare. Plomp et al (Am J Med Genet 2004;126A:403-412) found only 3 families with definite PXE in 2 successive generations and no families with definite PXE in 3 or more generations.
We report a large, four-generation family showing typical skin signs of PXE and evidence of a major vascular phenotype with cardiac complications, strokes and claudication. However, the ocular phenotype appears to be absent, and ABCC6 screening in one affected individual did not identify a pathogenic mutation. Linkage analysis at the ABCC6 locus is planned for this family, with the possibility of progressing to genome-wide scanning.
This family has >60 individuals of whom 15 are believed to be affected. Fifty-three individuals are available for study, of whom 10 are affected. Clinical evaluation has been completed for 17 individuals, of whom eight are affected. Cutaneous signs were present in all affected individuals and elastorrhexia was confirmed on lesional skin biopsy in one. Most affected individuals have suffered cardiovascular complications but there is no evidence of angioid streaks in these cases.
The evidence for AD inheritance in this family is overwhelming. They may represent a variant of PXE without an ocular phenotype, and are likely to provide evidence for genetic heterogeneity in this condition.

P0228. Association of PSORS1 genes with psoriasis in Russian populations

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Background: Psoriasis is a chronic inflammatory dermatosis affecting approximately 0,3-5% world-wide. Numerous population-, family- and twin-based studies point to a very strong genetic component of this disease. Knowledge of the genetic factors leading to this disease will lead to an understanding of the genetic, immune and pathogenetic aspects of psoriasis. So far 9 psoriasis susceptibility loci have been identified (PSORS1-9). The strongest genetic association has been found with the HLA-C region (PSORS1) on chromosome 6p21. Altogether, eigth genes are characterized in this completely sequenced region: HLA-C, OTF3, TCF19, HCR, CDSN, SEEK, SPR1, STG. Aims: To assess the genetic contribution of HCR single nucleotide polymorphisms (SNPs) and HLA-C in the pathogenesis of psoriasis.
Methods: A case-control study with 400 psoriasis patients and 410 controls in Russian populations was conducted. All individuals were genotyped for SNPs of HCR-305, 325, 477, 2327 and HLA-C association.
Results: Significant increase of the HLA-Cw6 allele was found in psoriasis patients (44% vs.18%, OR=3.46, 95% CI 2.70-4.45, p=0.0005). The frequencies of the HCR-325*T, HCR-2327*G alleles were significantly increased in psoriasis patients compared with controls (OR=3.61, p=0.005 and OR=2.45, p=0.0005, respectively). In subset analysis there were no other significant differences in allelic frequencies for the HCR-305G/A, HCR-477T/C polymorphisms.
Conclusions: Our results suggest that HLA-Cw6 remains the major risk allele in Russian psoriatics, and that the HCR gene may play a role in the development of psoriasis.

P0229. Radial hypoplasia in an infant with trisomy 18

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Trisomy 18 is a rather common autosomal trisomy with well-known pattern of malformations. These include prenatal growth deficiency, craniofacial dysmorphism, congenital heart disease, and overriding fingers. A five-months old baby girl, referred for multiple congenital anomalies including right radius hypoplasia and facial dysmorphic findings, is presented. She was born at 34th gestational week with a birth weight of 1900 gr (25th-50th percentile) as the second child of healthy consanguineous parents. During the first month of life, she had feeding problems. On physical examination, she was 54 cm, 3800 gr with a head circumference of 37 cm (all below 3rd percentile). She had thick eyebrows, wide and flat nasal bridge, bilateral epicanthic folds, strabismus, a prominent philtrum and low-set ears. Facial asymmetry with right facial microsomia was evident. Hypoplasia of the right ala nasi and posterior rotation of the right ear were noted. She had right-sided radial hypoplasia, hypoplastic right thumb, bilateral club feet and overriding toes. Right side of the body appeared hyperpigmented as compared to the other side. A systolic murmur of 2nd degree was present and echocardiography revealed ventricular septal defect, patent ductus arteriosus and pulmonary hypertension. Cranial MRI showed mega cisterna magna. The karyotype was 47,XX,+18. The patient represents an interesting presentation of trisomy 18, as radial aplasia and other preaxial limb deficiencies are seen rarely in patients with trisomy 18.

P0230. Establishment of Rare Disease Services in the West Midlands: Translation of Research into Routine Molecular Diagnosis.

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The West Midlands Regional Genetics laboratory has recently set up routine services for a number or rare genetic disorders. These include Wolfram syndrome (DIDMOAD), Combined Pituitary Hormone Deficiency (CPHD), Sotos syndrome, Alstrom syndrome and CHARGE syndrome. This is mainly due to the establishment of local clinical expertise within these medical sub-specialities which has driven research into these areas. Furthermore, guidelines set out by the UK Department of Health with regard to test rationalization, reporting times and increased automation have resulted in the production of a very successful rapid high-throughput screening strategy, which is now applied as a model for many new disorders within this laboratory.
The recent identification of genes involved in Micro syndrome, ARC syndrome and Infantile Neuroaxonal Dystrophy by the closely linked Medical Genetics Department has resulted in several requests for prenatal diagnosis in advance of the establishment of a routine diagnostic service.Results from interesting cases will be discussed together with the challenges faced in the provision of rare disease services.

P0231. Renin-angiotensin system gene polymorphisms and arterial hypertension in children.

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The I/D polymorphism of the angiotensin converting enzyme gene (*ACE*), the M235T polymorphism of the angiotensinogen gene(*AGT*), and the A1166C polymorphism of the angiotensin II type 1 receptor gene (*AGTR1*) were identified in 85 children with arterial hypertension (aged 7-17), 146 controls (aged 7-17).

Arterial hypertension was defined as systolic/diastolic blood pressure measurements higher than 95 age-gender-height percentile of the adopted reference values.

DNA was extracted from blood samples according to standard protocols and analyzed by the PCR technique.

Table 1 shows the genotype distribution and allele frequency in two groups. The distribution of *ACE* genotypes and allele frequency did not differ significantly between patients with arterial hypertension and control subjects. The frequency of the *AGT* TT genotype and T allele were significantly higher in hypertensive patients than in controls. There were significant increase in the CC genotype frequency and C allele of *AGTR1* in children with arterial hypertension compared with control subjects.

These results suggest an association of the M235T polymorphism *AGT* and A1166C polymorphism *AGTR1* with essential hypertension in children.

Table1. The allelic and genotypic frequencies of the renin-angiotensin system polymorphisms in hypertensive and control subjects.

ACE	Hypertensive (n=85)	Control (n=146)	*p*
II	24 (28,2%)	44 (30,2%)	0,861
ID	41 (48,2%)	72 (49,3%)	
DD	20 (23,6%)	30 (20,5%)	
I	89 (52,3%)	160 (54,8%)	0,681
D	81 (47,7%)	132 (45,2%)	
AGT	Hypertensive (n=85)	Control (n=96)	
MM	16 (18,8%)	52 (54,2%)	0,001
TM	41 (48,2%)	37 (38,5%)	
TT	28 (33%)	7 (7,3%)	
M	73 (42%)	141 (73,4%)	0,001
T	97 (58%)	51 (26,6%)	
AGTR1	Hypertensive (n=85)	Control (n=56)	
AA	21 (24,7%)	31 (55,4%)	0,001
AC	39 (45,9%)	19 (33,9%)	
CC	25 (29,4%)	6 (10,7%)	
A	81 (47,6%)	81 (72,3%)	0,001
C	89 (52,4%)	31 (27,7%)	

P0232. Molecular genetic analysis of Rb1 gene - differentiation of hereditary and non-hereditary forms of retinoblastoma

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Retinoblastoma is an uncommon malignant tumour of the eye in children(1:18,000 - 30,000 in live born). It is a model example of genetic disposition for tumour disorder. The fundamental part in genesis of retinoblastoma represents the tumour suppressor gene Rb1. The presence of at least one functional alela of the gene leads to normal production of protein pRB. The exact analysis of Rb1 gene in children with retinoblastoma influences the way of treatment received by the patients with retinoblastoma and their families. The hereditary form of retinoblastoma is in about half of the patients and is due to mutations in Rb1 gene. The differentiation of hereditary and non-hereditary forms has a fundamental influence on the treatment, prognosis and genetic counselling in the family. We perform an analysis of Rb1 gene in all children with retinoblastoma.

With conventional cytogenetics we can detect 8% of changes in Rb1 gene, with FISH 10%, with Southern blot hybridisation 16%, with PCR and sequence analysis about 75%. At University Hospital Brno we use a combination of PCR amplification of all 27 exons of Rb1 gene and subsequent sequence analysis in DNA from peripheral blood. Up to the present time we have found a mutation in Rb1gene in 6 patients with hereditary form of retinoblastoma and in one foetus.

In the future we will introduce RNA and subsequent DNA analysis, MLPA and methylation analysis of the promoter, in cooperation with other oncological centres a population study to verify the causality of the found mutations.

P0233. Determination of direction of skewing demonstrates effect of X chromosome inactivation on phenotype of Rett syndrome associated with mutation in the X chromosome gene, *MECP2*

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Previous attempts to demonstrate a genotype-phenotype correlation among patients with Rett syndrome (RTT) have shown some effect of the type of mutation (e.g. nonsense or frameshift versus missense) on severity of phenotype. It has been clear that the pattern of X chromosome inactivation has been important - as with the recognition of some clinically unaffected mutation carriers with highly skewed XCI - but it has been difficult to demonstrate the effect of XCI in lymphocytes on the severity of phenotype among affected individuals because only the extent of skewing but not its direction was known. This led us to examine the relationship between XCI in lymphocytes and disease severity in a group of UK and Australian patients in whom the direction as well as degree of skewing of XCI could be determined. Although limited by lack of informativeness for SNPs within the MECP2 gene, we have been able to show that the clinical severity in RTT patients with a p.R168X or p.T158M mutation is statistically related to the direction and degree of skewing of XCI in lymphocytes but that this is not likely to be helpful in establishing the prognosis in an individual case.

P0234. Novel mutation in the cellular retinaldehyde-binding protein gene *RLBP1* associated with severe juvenile flecked retinal dystrophy

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Retinitis punctata albescens (RPA) is a rare form of autosomal recessive (and rarely dominant) retinal dystrophy characterized by early-onset severe night blindness, and aggregation of irregular white flecks throughout the fundus. RPA is progressive and evolves to generalized atrophy of the retina.

A distantly similar but distinct clinical entity, Fundus albipunctatus (FA) is also characterized by white dots of the fundus but is apparently a rare form of stationary night blindness. RPA is caused mostly by mutations in the cellular retinaldehyde-binding protein (*RLBP1*), and occasionally in rhodopsin (*RHO*), retinal degeneration gene (slow) (*RDS*), and retinol dehydrogenase 5 (*RDH5*). Three patients from a consanguineous family displaying a flecked retinal dystrophy were examined for best-corrected visual acuity, and by visual field and color-vision testing, electroretinography, dilated fundus examination, and fundus photography. We analyzed *RLBP1*, *RDH5* and *RHO* by both linkage analysis and direct sequencing in this family. While an initial clinical diagnosis of FA was made in these patients, follow-up investigations revealed a significant progression of the disorder within a few years, which let to a reclassification of RPA. *RDH5* and *RHO* sequencing did not reveal any variants, but in *RLBP1* we identified a novel homozygous 12-bp in-frame deletion (c.666-677del12). We conclude that mutation analysis of *RLBP1* is suited to help differentiate between FA and RPA, which is important for counselling of concerned families, and *RLBP1* is a major gene for RPA.

P0235. The first report of two cases of spino cerebellar ataxia (SCA) in Hamadan province, Iran

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Two cases with ataxia and gait disturbance with first diagnosis as MS was referred to our Genetic counseling clinic in Hamadan, Explained as below:

Case 1:

A 29 years old man with problem in walking and diagnosis of ataxia which was began from 5 years before with alternating dizziness, ataxia, tremor, vision disturbance and chocking.
Normal mental state, cranial nerve, autonomic function, motor exam in inspection with mild hypotonia and normal sensory were seen in examination.
In ophthalmologic exam all was normal but nystagmous with low speed and range.
All cerebellar exam was disturbed including, ataxic gait, scanning speech, intention tremor, hyperreflexia, dysphagia, dysmetria and dysdiadokinesis. Brain MRI was normal.
There was history of such a problem in his mother, his mother's 3 sibling and also grandfather which was presented in higher age and in their children in lower age.
Case 2:
A 36 years old man with progressive ataxia and tremor which was began from 6 years before with one apparently normal child, normal mental state, disturbed cerebellar exam including progressive ataxic gait, dysartheria, intention and postural tremor, dysmetria, dysdiadokinesis was seen in exam. Brain MRI showed cerebellar atrophy.
There was some history of such a problem in his father, sib line and also in pedigree
Diagnosis:
Extracted DNA was investigated for the GAA expansion repeat in the FRDA gene and SCA types including 1,2,3,6,7.
The two probands showed abnormal pattern for SCA 7 and SCA 2 respectively.

P0236. Juvenile and young adults SCA1 patients in Poland - genetic and clinical features.

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Spinocerebellar ataxia type 1 (SCA1) is the most common form of ADCA in Poland. The phenotype and age at disease onset are highly variable. The aim of the study was to investigate the influence of the transmission pattern on genetic and clinical features of juvenile SCA1 patients.
Methods: 125 SCA1 patients from 101 families were analysed retrospectively. The mean age at onset was 35.6 +/- 9.6 and the average CAG repeats in affected family members was 50.7 +/- 5.5, ranging from 42 to 72. Paternal and maternal transmission was equal. In 21 SCA1 families with 25 juvenile patients, the relation of clinical severity, sex, CAG repeats number and age at disease onset of transmitting parents was analysed. The International Cooperative Ataxia Rating Scale (ICARS), electrophysiological and psychological examinations were compared in regard to transmission pattern.
Results: In 14 cases with paternal and in 11 patients with maternal transmission the mean age at onset 20.5 +/-3.6 y. (range 12 - 25 y.), disease duration 6.3 +/-3.7 y. and the CAG repeats number, mean 58.0 +/- 5.7 were not different. However, mean age at onset of fathers was 33.0 y. and of mothers 29.1y. Severity of non-cerebellar signs and ataxia were more pronounced in patients with paternal than with maternal transmission: ICARS 34.8 and 32.8, Intelligence Quotient 92.9 and 107, respectively.
Conclusions: In our SCA1 patients the anticipation was more prominent in subjects with paternal than with maternal transmission.
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P0237. A case of spinocerebellar ataxia type 17 (SCA17) associated with homozygous 46/47 repeats of the *TBP* gene

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Spinocerebellar ataxia type 17 (SCA17) is a dominant progressive neurodegenerative disorder, caused by a triplet repeat expansion within the TATA-binding protein gene (*TBP*); normal expansions range from 29 to 42 repeats, whereas abnormal expansions range from 43 to 63 repeats. A reduced penetrance is associated to 43-48 repeats. The disease is characterized by progressive limb and gait ataxia, dysarthria, motor, cognitive and psychiatric abnormalities.
In this study, we describe a SCA patient from Southern Italy with ataxia, pyramidal and extrapyramidal signs and peripheral neuropathy.
We investigated this patient for CAG repeat expansions in the genes of the spinocerebellar ataxias SCA1, SCA2, SCA3 SCA6, SCA7, SCA8, SCA12, SCA17 and DRPLA.
Genomic DNA was amplified with fluorescent primers spanning the SCA expansions. PCR products were separated onto a capillary sequencer (3130XL genetic analyzer-Applied Biosystems) and the length of specific SCA fragments was calculated referring to a size standard and to related SCA controls. We identified an abnormal CAG/CAA repeat expansion of 46/47 size, within the *TBP* gene, confirmed by direct sequencing of the expanded SCA17 alleles showing the following structure: (CAG)3 (CAA)3(CAG)9CAACAGCAA(CAG)26/27CAACAG.
This is the second case of homozygous expansion reported in a patient with SCA17. Currently, genetic and clinical analysis of other family members are ongoing to evaluate the genotype-phenotype correlation in this family.

P0238. Identification of genomic dosage variation of CNTNAP2 in patients with schizophrenia and epilepsy

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Contactin-associated protein-like 2 (Caspr2) clusters voltage-gated potassium channels (K v1.1) at the nodes of Ranvier and is encoded by CNTNAP2 - the largest gene in the human genome. Homozygous stop mutations in exon 22 of this gene were recently identified in Old Order Amish children with cortical dysplasia, focal epilepsy, relative macrocephaly, and diminished deep-tendon reflexes. We identified deletions affecting (part of) CNTNAP2 in three patients by routine chromosome analysis and microarray-based genomic copy number analysis methods.
A deletion covering the chromosomal region 7q34-7q36.1 was revealed in one severely mentally retarded epilepsy patient by routine chromosome analysis, and shown to be de novo by Fluorescent In-Situ Hybridisation (FISH) analysis. The deletion was 10.7 Mb in size, affecting 58 genes including CNTNAP2. A second deletion was identified by array-based Comparative Genomic Hybridisation (arrayCGH) in a patient suffering from both epilepsy and schizophrenia. This deletion of 1.5 Mb was confirmed by Multiplex Logation-dependent Probe Amplification (MLPA), and also affected CNTNAP2. To study the link between schizophrenia and CNTNAP2 further, we developed a targeted Single Nucleotide Polymorphism (SNP) array for this gene and its surrounding genomic region. Out of 315 patients and an equal number of controls, we identified a deletion in intron 3 of CNTNAP2 in a third schizophrenic patient, which also has a history of epilepsy.
Together these results confirm that CNTNAP2 is associated with epilepsy, but they also show that genomic rearrangements resulting in haploinsufficiency of CNTNAP2 may lead to a more complex phenotype that also includes schizophrenia.

P0239. A Case of Seckel Syndrome conceived by Assisted Reproductive Technology

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Seckel syndrome is a rare autosomal recessive disorder characterized by severe pre- and postnatal growth retardation, microcephaly, mental retardation and typical facial appearance. It has also been called "bird-headed dwarfism". Related with Seckel syndrome, three loci have been mapped and to date the only causative mutation has been observed as a hypomorphic mutation in Ataxia telengiectasia and Rad3-related (ATR) gene. Although assisted reproductive technologies (ART) including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are generally considered safe, some studies have suggested an excess occurrence of birth defects and low birth-weight. Recently, various syndromes involving epigenetic alterations have been reported to occur in individuals following ART. Here we report a patient with Seckel Syndrome conceived by ICSI. To the best of our knowledge, Seckel syndrome has not been reported in patients conceived by ART and we think that epigenetic mechanisms may be related with this syndrome in our patient.

P0240. Extended pedigree with multiple cases of XX sex reversal in the absence of *SRY* and of a mutation at the *SOX9* and RSPO1 loci

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It is well established that testicular differentiation of the human embryonic gonad depends on the action of the Y-chromosomal gene *SRY*. However, exceptional cases such as *SRY*-negative cases of 46,XX testicular disorder of sexual development (DSD) (previously known as 46,XX males) and of 46,XX ovotesticular DSD (previously known as 46,XX true hermaphrodites) document that testicular tissue can develop in the absence of the *SRY* gene. These *SRY*-negative XX sex reversal cases are very rare and usually sporadic, but a few familial cases have been reported. We present a large, consanguineous family with nine affected individuals with phenotypes ranging from 46,XX testicular DSD to 46,XX ovotesticular DSD, with predominance of male characteristics. Absence of *SRY* in peripheral blood was documented by fluoresence in situ hybridization (FISH) and PCR analysis in all nine affected individuals, and by FISH analysis on gonadal sections with testicular tissue in four affected individuals. By quantitative PCR, a duplication of the *SOX9* gene was excluded. In addition, as linkage analysis showed that the nine affected members of the family do not share a common *SOX9* haplotype, any mutation at the *SOX9* locus could be ruled out. Furthermore, no mutation within the *RSPO1* gene that was recently implicated in XX sex reversal was found by sequence analysis. Together, these findings implicate a mutation at a sex-determining locus other than *SRY, SOX9 and RSPO1* as the cause for the XX sex reversal trait in this family.

P0241. Unique phenotype associated with SHOX gene deletion

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SHOX (short stature homeobox-containing) gene haploinsufficiency has been implicated in Leri-Weill dyschondrosteosis (LWD) and SHOX-related short stature, while SHOX nullizygosity is associated with Langer mesomelic dysplasia (LMD). The classic clinical findings in SHOX-related disorders include short stature, Madelung deformity and mesomelia. We present a 10-month old male with short stature, height of 62 cm. (< -2 SD), arm span/height ratio of 0.95, with prominent forehead, rhizomelic shortening of the extremities, bowing of the lower extremities, increased soft tissue folds and lordosis of the back. The skeletal survey revealed decreased interpedicular distances of the lower lumbar vertebrae, squaring of the iliac bones, small sciatic notches and flattened acetabular angles; shortening of the tubular bones especially proximally with metaphyseal flaring; and an increased skull size. These findings were clinically and radiographically consistent with achondroplasia. Molecular analyses for FGFR3 1138G>A and 1138G>C mutations, which account for over 99% of individuals with achondroplasia, were negative. Similarly, analysis for hypochondroplasia, specifically FGFR3, N540K mutation was also negative. However, analysis of the SHOX gene coding region showed a whole gene deletion. To our knowledge, the above clinical and radiographic findings have not been described as part of SHOX gene deletion/mutation syndromes in the literature. This case report describes a unique phenotypic expression of SHOX gene deletion and indicates a need for further investigation of the SHOX gene and its modifiers.

P0242. Should Silver-Russell patients with UPD7(mat) develop myoclonic dystonia ?

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Uniparental disomy (UPD) describes the inheritance of a chromosome pair from only one parent. This anomaly can result in human diseases when it affects chromosomes with imprinted genes. Silver-Russell syndrome (SRS) and myoclonus dystonia syndrome (MDS) can be both related to imprinting defects on chromosome 7. Approximately 10% of SRS have a maternal UPD7, and 20% of MDS have a mutation on the paternal allele of the maternally imprinted gene epsilon sarcoglycan (*SGCE*) located on 7q21.3.

We report here on a 36 six-year-old man with both SRS and MDS. His features consistent with SRS included severe intrauterine and postnatal growth retardation with normal OFC, feeding difficulties in infancy, triangular face, prominent forehead, prominent low-set posteriorly rotated ears, micrognathia and brachydactily. At the age of 17, he developed movement disorder with progressive worsening. On examination, he had shock-like myoclonic jerks of upper limbs, trunk and face. In addition he had mild cervicofacial dystonia with retrocollis and increased blinking. Neurophysiological study showed sub-cortical myoclonus.

Mutation screening of *SGCE* was negative. Karyotype showed that 75% of cells had a supernumerary small ring chromosome which hybridized with the chromosome 7 centromeric probe. Supernumerary marker chromosomes are known to be associated with UPD, therefore, we would like to propose that our patient carry an UPD7(mat) that could explain both SRS and MDS. Validation of this hypothesis is under process.

P0243. Angulated Femurs and the Skeletal Dysplasias: Experience of the International Skeletal Dysplasia Registry (1988-2006)

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Angulated or bent femur (isolated or associated with other long bone bowing) in the fetus or newborn is relatively common when evaluating patients with skeletal dysplasias. To determine the extent and heterogeneity of disorders associated with angulated or bent femurs, we analyzed cases in the radiographic database (1998-2006) of the International Skeletal Dysplasia Registry (ISDR) and determined which established skeletal dysplasias and genetic syndromes are associated wtih this finding. The results show that more than 40 distinct disorders with varying frequency (very rare to more commonly-occuring disorders) can be associated with bowed/bent/angulated femurs. Sixty-six percent of the cases with angulated femurs belonged to three well described groups of disorders; campomelic disorders (24.4 %), thanatophoric dysplasia (23.9 %) and finally osteogenesis imperfecta (OI) (18.1 %). With specific emphasis on these, this cross-sectional cohort provides discussion of data on other rare disorders associated with angulated femurs and the importance of the finding relative to its occurrence within a diagnostic group. This study aims to provide differential diagnosis of entities to be considered when a fetus or newborn is found to have congenital bowing/angulation of the femur.

P0244. Identification of SLC26A4 gene mutations in Iranian Families with hereditary Hearing Impairment

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Mutations in the SLC26A4 gene in DFNB4 locus is responsible for syndromic (Pendred syndrome) and non-syndromic hereditary hear-

ing loss (HHL). In many populations mutations in this gene have been reported as a second cause of HHL. We investigated the prevalence of SLC26A4 mutations in our HHL consanguineous families. After completing clinical investigation the signed consent form was taken from each family. We included 80 families with two or more affected individuals, who have been referred to GRC. All families who had previously been tested negative for the DFNB1 locus, were considered as candidates for homozygosity mapping using STR (Short tandem repeat)s linked to DFNB4 locus. Families localized to this region were subjected to complete DNA sequencing for SLC26A4 gene. Ten out of eighty families were mapped to DFNB4. Sequence analysis of ten linked families revealed eight mutations in seven families (T420I, 1197delT, G334Y, R409H, T721M, R79X, S448L, L445W) and
The T420I , G334V and R79X were novel mutations. We have been able to localize total of 10 families (12.5%) from non-DFNB1 families to the DFNB4 locus. We detected in all ten families some degrees of diffuse or nodular goiter, eight out of 10 families showed normal thyroid function and in six of ten families we found positive prechlorate discharge test. All of affected had normal temporal bone scan.
This investigation, demonstrated that the SLC26A4 gene mutation is the most prevalent syndromic hereditary hearing loss in Iran.This result is in accordance with reports from other countries.
Key words:, SLC26A4, hearing loss,pendred

P0245. Molecular analysis of SMN1 gene common deletions in some Iranian Spinal muscular Atrophy patients

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Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the SMN1 gene, mainly intragenic deletions, the commonest of which are deletion in exons 7 and 8. The disorder is subdivided into three clinical groups (type I - III). The molecular basis for variation in clinical manifestation depends on the copy number of SMN2 gene in each patient. In this study we present fifteen families who had at least one live affected SMA patient, selected for molecular characterization. They fulfilled criteria for inclusion by demonstration of the characteristic clinical features of SMA phenotype. These clinical diagnoses were corroborated with EMG and NCV investigations as well as CPK measurement for the majority of the cases. The patients DNA samples were prepared from whole blood by standard salting out method. Characterization of deletions in exons 7 and 8 of SMN 1 gene was carried out by utilizing a pair of mismatched primers which differentiate between SMN1 and SMN2 genes. We found deletion in seven patients, about 50%. Five patients had both exons 7 and 8 deleted and two patients had just exon 7 deletion. Although the present sample size is small, it may be concluded that the observed deletions has lower frequency compared to European populations which is 95%. Therefore other SMN1 gene defects should be looked in Iranian patients.

P0246. Case study: "A family with several cases of SMA type 1 due to multiple consanguinous marriages in three consecutive generations, in the pedigree."

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The propand is a three months old floppy boy who is the result of third degree consanguinous marriage (the first sibling).
At birth his mother had hard NVD and the baby was born with cyanosis.
He had apnea and poor feeding for the first days of his life.
He was hospitalized for 12 days.
His grandparents from both sides have third degree relationships.
He has the typical clinical features and characteristics of SMA type 1.His molecular test for SMA type 1was positive and he has deletion of exons 7 & 8 of SMN1 gene.
The special point in this family is the existence of several cases of SMA type 1 due to multiple consanguinous marriages in three consecutive generations, in the pedigree.

P0247. Clinical forms of Smith-Lemli-Opitz syndrome and their relation to mutations in the *DHCR7* gene _ classification based on a group of 50 Polish patients

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Smith-Lemli-Opitz syndrome (SLOS) is a metabolic malformation disorder, caused by impaired activity of 7-dehydrocholesterol reductase, the last enzyme in cholesterol biosynthesis. Its best known dysmorphic features include: microcephaly, ptosis, short and up-turned nose, 2/3 toe syndactyly, and external male genital malformations. Nevertheless, as the spectrum of observed features is very broad, a question about phenotype-genotype correlations still emerges.
Poland represents a unique distribution of mutations in the *DHCR7* gene. Moreover, one of them is thought to be spreading in our country, hence our effort to delineate the clinical variability of SLOS in relation to the causative molecular defect.
The study was based on a group of 50 patients with proven diagnosis (13 f and 27 m), classified into 3 severity forms: mild (13 cases), moderate/classical (20 cases) and severe (17 cases). We analyzed: facial dysmorphy, congenital malformations (expressed as a Severity Score: 0 - 100 points), biochemical chromatographic data (cholesterol and 7-, 8DHC) and molecular data (mutations in *DHCR7* gene).
While chromatographic data shown its significant correlation with clinical forms of SLOS, the genotype-phenotype correlation was no so clear and we found as below:

Class of mutation	Number of patients	Severity Score	Congenital anomalies
0/0	3	55 (n=2), 60	microcephaly, heart and renal defect, polidactyly, abnormal male external genitals; CNS - dilated ventricles (1)
0/TM7	15	20, 30 (n=3), 35 (n=2), 40, 50, 55 (n=4), 60 (n=2), 65	microcephaly (13); heart defect (11); cleft palate (6); renal anomaly (5); GI tract defect (4); CNS malformation (3); cataract (1); polidactyly (10); syndactyly other than 2/3 toe (1); abnormal external genitals: male (9/11), female (2/4)
0/4L	3	40, 50, 65	microcephaly, hart defect, 2/3 and 4/5 toe syndactyly, cleft palate, enlarged clitoris (2/2)
0/TM3	3	40, 55, 60	microcephaly, cleft palate, hart defect, polidactyly, abnormal male external genitals
0/TM8	5	20 (n=2), 30 (n=2), 40	microcephaly, abnormal male external genitals (4); pyloric stenosis (2); cleft palate, heart defect, cataract (1)
TM1/CT	2	5, 5	microcephaly, slight 2/3 toe syndactyly
TM7/1L	2	10, 15	microcephaly, cryptorchidism, hypospadias (2); 2/3 toe syndactyly (1)
TM7/TM7	3	15, 20, 30	microcephaly, cleft palate (2); heart and renal defect, cryptorchidism, CNS - dilated ventricles (1)

We hope that our study will give new insights into the genetics of Smith-Lemli-Opitz syndrome.
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P0248. Smith-Lemli-Opitz syndrome - novel mutation with a mild phenotype

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis, caused by mutations in *DHCR7*gene. Although its phenotype is well characterized, SLOS is recognized

rarely with one of the reasons being underdiagnosis of mild variants. Our extensive 3-year prospective study (based on the structure of the Polish Register of Birth Defects) aimed at identifying all new cases of SLOS in Poland allowed us to detect a four year old boy with a mild phenotype of SLOS, in whom molecular study of *DHCR7* gene demonstrated a novel mutation.
He was born at 41 weeks of gestation with birth weight 3070 g, OFC - 33,5 cm. Infancy was marked by poor weight gain and recurrent pneumonia. He walked at 24 months.
At the age of diagnosis he was able to communicate with only few words and had psychomotor hyperactivity. Weight was ~ 10 centile, length > 50 centile and OFC < 3 centile. On physical examination subtle dysmorphic features were noted: bilateral epicanthal folds, blepharoptosis, short nose with anteverted nares. There was unilateral partial 2nd and 3rd toe syndactyly and - within external genitalia - hypospadias and cryptorchidism. CT of the head revealed hypoplasia of corpus callosum.
Laboratory tests confirmed the clinical diagnosis of SLOS. We found elevated serum 7-dehydrocholesterol and two missense mutations of the *DHCR7* gene: a novel one mutation c.655T>G (p.Y219D) and a previously described mutation: c.461C>T (p.T154M), responsible in a compound heterozygous state for a mild form of SLOS.
The work was supported by grant PBZ-KBN-122/P05/01-10.

P0249. Mutation spectrum and clinical features of SPG4 HSP

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Mutations in SPAST/*SPG4*, the gene encoding spastin, are the most frequent cause of autosomal dominant hereditary spastic paraplegia (AD-HSP). In the context of a systematic routine diagnosis, we investigated the spectrum of *SPG4* mutations and the associated clinical phenotypes. A large series of 543 patients with either pure or complex spastic paraplegia, was screneed by denaturing high performance liquid chromatography (DHPLC) and/or multiplex ligation-dependent probe amplification (MLPA). We identified 164 (30%) positive families, 133 (25%) of which have substitutions or small deletions/insertions detected by DHPLC and 31 (6%) which have larger exonic deletions ranging from one exon to the whole gene, detected by MLPA. The great majority of the mutations were associated with pure HSP in the families. However, few families also showed additional features including cognitive impairment, dementia, mental retardation, peripheral neuropathy or extra-pyramidal signs. A high proportion of mutations, especially of missense type, were found in sporadic patients. Phenotype-genotype correlation showed reduced and age-dependant penetrance, and we also identified a *de novo* mutation associated with mosaicism in one patient. Our findings support the hypothesis of several mechanisms underlying the penetrance and age at onset variability including genetic modifiers.

P0250. Delayed speech development with facial asymmetry and transverse earlobe creases

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Delayed speech is one of the most frequent features in patients with MCA/MR phenotype. In 1993, Mehes described a Hungarian family with three patients, a mother and her son and daughter, presenting delayed speech development, facial asymmetry, strabismus and transverse earlobe creases (TEC). In 2005, four new unrelated Hungarian children were reported with similar features.
We describe a new patient, of Spanish and French origins, who presents a similar phenotype.
The girl was the eutrophic product of a 36 week gestation. No motor delay was observed but delayed speech marked the development of the girl. At 4 years autistic behaviour was suspected and psychiatric therapy was instored. The proposita began to speak at 5 years and her course was marked by mild mental retardation with anxious behaviour. At 20 years, weight, height and OFC are in the normal range. The patient presents facial asymmetry, narrow down-slanting palpebral fissures, low-set ears with TEC. She has umbilical hernia and hypertrophic small labia. Skeletal X-rays were normal, cerebral MRI shows abnormal signal of right putamen. Chromosome studies : metaphase karyotype, subtelomeric analysis (MLPA), and FISH studies (22q11.2, 11p15) showed no anomaly. Molecular analysis for Beckwith-Wiedemann syndrome was also normal. This new observation of a patient with delayed speech development combined with facial asymmetry and transverse earlobe creases confirms the existence of this new syndrome. Transverse earlobe creases are a cardinal feature which is easily found on physical examination. To date, inheritance is not defined.

P0251. Analysis of the SMN and NAIP genes in Brazilian Spinal Muscular Atrophy Patients

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Spinal muscular atrophies (SMAs) are inherited motor neuron degenerative disorders that cause progressive muscular weakness, with high mortality. Three types of SMA were studied, type I, which is the most severe form, type II or intermediate form, and type III. There are two genes linked to SMA: the survival motor neuron (SMN) and neuronal apoptosis inhibitory protein (NAIP). This work has the objective of showing it is possible to make an efficient molecular diagnosis of the SMAs by studying genotypic composition of SMN and NAIP genes in individuals suspected clinically as SMA patients. Nested PCR was used for both exons 7 and 8 of SMN gene, frequently deleted in SMA patients, followed by enzymatic digestion and 12% poliacrilamide gel electrophoresis. For exons 5 and 6 of NAIP gene, PCR was performed followed by 2% agarose gel. In all groups but SMA patients, no deletions were observed neither in exons 7 and 8 of SMN gene, nor in exons 5 and 6 of NAIP gene. This study showed a high frequency of deletions in SMA patients (88%), and 32% in NAIP gene. It was not possible to avoid SMA diagnosis, even in those patients without deletions. DNA study represents an efficient confirmatory test for SMA patients, demanding a non-invasive sample.

P0252. A rare autosomal recessive skeletal dysplasia: Spondylo-meta-epiphyseal dysplasia, short limb- abnormal calcification type

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A boy 1-month-old boy was referred to our department because of considered achondroplasia on prenatal USG. The parents are first cousins. He had one healthy older brother. His weigth and heigth were 3. centile and his head circumference was between 2 and 50 centile. Clinical findings consist on short limbs with small hands, narrow chest, flat face, hypertelorism, broad nasal bridge with wide nostils, hypertelorism, microretrognathia and wide open anterior fontanelle (6X5 cm). Radiological investigations showed severe platyspondyly, very short ribs, short tubular bones with irregular and flared metaphyses, flared iliac wings with convex inferior iliac magrin. The patient was followed-up until he was 2 years old. His height is very short (61 cm) and he had language and social development were compatible with 2 years of age but gross motor skill was delayed at that age. Re-evaluation of radiologic finding revealed premature calcification on costal cartilages and femoral epiphyses . We diagnosed Spondylo-meta-epiphyseal dysplasia (SMED), short limb-abnormal calcification type. This condition is a very rare autosomal recessive inheritance disorders characreristic by platyspondyly, short limbs with short hands, short ribs, meta-epiphyseal irregularity and cartilage calcification. Shorting of the tubuler bones, platyspondyly, meta-epiphyseal irregularity and cartilage calcification are also seen in chondrodysplasia punctata, recessive type but severe platyspondyly, short hands and ribs associated with SMED, short limb- abnormal calcification type.

P0253. Mutations in *TBX1* genocopy the 22q11.2 deletion and duplication syndromes: a new susceptibility factor for mental retardation

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dació Son Llatzer, Palma de Mallorca, Spain.
A screen for *TBX1* gene mutations identified two mutations in patients with some features compatible with the 22q11.2 deletion syndrome but with no deletions. One is a *de novo* missense mutation and the other is a 5' UTR C>T change that affects a nucleotide with a remarkable trans-species conservation. Computer modelling shows that the 5'UTR change is likely to affect the mRNA structure, and *in vitro* translation experiments demonstrate that it produces a two fold increase in translation efficiency. Recently, duplications in the 22q11.2 region were reported in patients referred for fragile-X determination because of cognitive and behavioural problems. Because the 5'UTR nucleotide change may be a functional equivalent of a duplication of the *TBX1* gene, we decided to screen 200 patients who had been referred for fragile-X determination and 400 healthy control individuals. As a result, we found the 5'UTR mutation to be present in three patients with mental retardation or behavioural problems and absent in control individuals of the same ethnic background. This observation suggests that it may be reasonable to screen for such mutation among patients with unspecific cognitive deficits and we provide an easy and quick way to do it with an Amplification Refractory Mutation System (ARMS) approach. To our knowledge this is the first human mutation showing that *TBX1* is a candidate to cause mental retardation associated with the 22q11.2 duplication syndrome.

P0254. Association between Telomerase Complex Mutations and Autoimmune Diseases

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Some mutations in *TERT* or *TERC* impair telomerase activity by haploinsufficiency, and heterozygosity of these mutations is a risk factor for acquired aplastic anemia (AA), a disease in which the hematopoietic tissue is the target of an autoimmune process. We investigated whether loss-of-function mutations in the telomerase components *TERT* and *TERC* predispose their carriers to the development of other autoimmune diseases that are associated with short telomeres. Among 91 patients with multiple sclerosis (MS), 49 patients with ulcerative colitis (UC), and 96 patients with insulin-dependent diabetes mellitus (IDDM), we found three novel *TERT* mutations; MS and UC patients were also found to carry a mutation that encodes TERT H412Y, which has been described previously in AA patients. Notably, TERT H412Y has only 50% the telomerase activity of wild-type TERT. The overall mutant allele frequency was significantly higher for patients with MS (1.6%; Fisher's exact test, P=0.0343) and UC (2.0%, P=0.0424) as well as all patients with autoimmune disease (1.5%, P=0.0143) than in 188 healthy controls. Additionally, the TERT A1062T-encoding polymorphism, which may be associated with reduced telomerase activity, was more commonly found in patients with autoimmune disease (allele frequency=1.5%) than in 528 controls (0.7%) though the difference was not statistically significant (P=0.0781). Functional studies are underway to determine whether the novel mutations affect telomerase activity. Our findings suggest that abnormal telomere maintenance is a risk factor for multiple autoimmune diseases.

P0255. Observation of Hb Ernz [β(123)Thr-->Asn] for the first time in IRAN

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Betha - thalassemia is one of the major genetic problem world wide in Iran. We have been running a national program for prevention of thalassemia since 1997.
Families come to our center for prenatal diagnosis. When a person has MCV<80 Fl , MCH<27 Pg and A2>3.5% . we regard them as carriers and start molecular analysis. Sometimes, due to different reasons, we may notice near normal values for MCV or MCH or normal A2 level.
A family had came to our center for prenatal diagnosis (PND). The husband had MCV 76/3 Fl, MCH 24/9 Pg, A2 2/8% and the wife had MCV 62/3 Fl, MCH 20/2 Pg and normal A2 5%.
We sequenced their DNA. and the husband only showed Hb Ernz [β(123)> Asn] (ACC > AAC) and the wife had Hb Ernz as above and also codon 22 /24 (-AAGTTGG) → (GAA GTT GGT > G T).Based on our finding Hb Ernz is harmless and is not the cause of raised A2 level or low MCV or MCH levels.
This is the first case of Hb Ernz reported in Iran and Second in the literature.

P0256. Partial trisomy 10q in a boy due to a paternal translocation t(7,10)(q36;q25.2)

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We report a 2 years and 8 months old boy with a moderate psychomotor retardation and characteristic dysmorfic features.He is the first child of unrelated,healthy and yong parents.The pregnancy has a normal evolution.He was born at term by caesearian section.The infant presented: micropthalmia,epicanthus inversus,hypertelorism,blepharophimosis,palpebral ptosis,low-set ears,congenital heart disease,undescendend testis and anomalies of hands(bilateral simian creases,clinodactyly of the fifth finger) and feet(syndactyly).The weight and the length were at 3-rd centile correspondingly.Conventional chromosome analysis(GTG-banding) revealed additional material on chromosome 7q.The father has a balanced translocation t(7;10)(q36;q25.2).The additional material on chromosome 7q was characterized by FISH using chromosome paints as part of chromosome 10.

P0257. Patau Syndrome - Clinical and gentical discussions over two cases

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Patau syndrome, also known as trisomy 13, represents a chromosomal disorder with an incidence of 1 case per 8,000 live births. Its clinical features consist of multiple malformations that involve the central nervous system, the internal organs, the upper and lower extremities and the cranio-facial extremity.
Our paper presents two cases (patient S.A. aged 2 months and patient S.A. aged 20 days), which were suspected of chromosomal aneuploidy (trisomy 13 or 18) based on clinical features. For an accurate diagnosis, the karyotype was determined in both cases using the classical cytogenetic technique with a 72-hour lymphocyte culture, followed by the G-banding of the chromosome preparations. The chromosomal formulas obtained were 46,XX/47,XX, 13+ for patient S.A. and 47,XX, 13+ for patient P.A. In the case of patient S.A., clinical features correlate very well with the chromosomal formula determined by karyotyping; in the case of patient P.A., however, clinical features and child evolution at the moment of diagnosis would have pleaded for a somatic cellular mosaicism. Anyway, in cases of trisomy 13, patient's evolution is difficult to evaluate and in most cases is unfavorable, being strongly related to the number and severity of malformations.

P0258. One female patient with trisomy 18 and bilateral congenital corneal opacity

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Introduction: Trisomy 18 was first described by Edwards et al. in 1960. It occurs in 1/8,000 live births. The principal features are clenched hands, rocker bottom feet, low set or malformed ears and ventricular septal defect. Low birth weight has been reported. We present one patient with prenatal diagnosis by kariotype of amniotic fluid of trisomy 18 and bilateral congenital corneal opacity. Case report: Propositus is a female of 2 months of age, product of the III full term pregnancy, characterized by polyhydramnios, intrauterine growth delay and mother, with gestational diabetes during the pregnancy, of 42 (Gravida:3; abortion:2; caesarean:1) and the father 39 years-old at the time of conception. Bilateral congenital corneal opacity and dysmosphism was detected. Actually somatometry under 3rd centile, dolichocephaly, microphthalmia, hypertelorism, bilateral corneal opacity, ears with overlapping of helix over antehelix and down rotate, hands show 1st and 2nd fingers with bilateral ulna deviation, overlapping of 2nd over 3rd and 4th over 5th fingers, feet with hypoplasia of nails, 1st trigger toe and

brachydactyly and generalized hypotonia. Discussion: Trisomy 18, is the second most common autosomal trisomy in newborns. The frequency of the associated findings are: heart (38.1%), gastrointestinal (25.4%), limb (24.6%), head (20.3%), eye (11%) and genital anomalies (9.3%). Conclusion: We present a case with trisomy 18, showing unusual clinical findings. This case should make us to be careful at the time to establish the diagnosis and to suspect a chromosomopaty. By the wide clinical spectrum of this syndrome the karyotype give the final diagnosis.

P0259. A novel mutation in SLC19A2 gene in an Iranian patient with TRMA syndrome.

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Thiamine - Responsive Megaloblatic Anemia (TRMA) or Roger syndrome is a rare autosomal recessive disorder with childhood onset. This disorder is characterized by the occurrence of multiple clinical manifestations including megaloblastic anemia, diabetes mellitus and sensorineural deafness, responding in varying degrees to thiamine treatment. The gene SLC19A2 which codes for a thiamine transporter is responsible for this syndrome and it is located at chromosome 1q. To date 15 different mutations have been reported in 28 families worldwide. Here we present a new case with a novel mutation, a 20 years old male patient with characteristic features of TRMA with good response to thiamine therapy. His SLC19A2 gene was screened by direct sequencing and a single nucleotide base substitution in homozygous form was found. This mutation leads to a premature stop codon (W233X), and can be considered diseases causing because of its nature. Considering the limited number of affected cases reported so far in the literature, it is evident that TRMA is a very rare condition with heterogenous molecular basis. As far as its distribution is concerned, out of 28 reported TRMA families worldwide, five families including this present case are from Iran who had different mutations. The rest of the patients were from Indian subcontinent (India, Kashmir and Pakistan) suggestive of higher frequency in Asian populations.

P0260. Six pregnancies in a woman with Turner syndrome, including a case of holoprosencephaly

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Ovarian failure is a typical feature of Turner syndrome (TS). Only 2% of these patients (pure 45,X or mosaic) have natural pregnancies. Furthermore, these pregnancies have been plagued not only by chromosome anomalies and fetal malformations, but also by spontaneous abortions. We report an unusual case of a woman with TS and chromosome mosaicism (karyotype 45,X[2]/46,X,r(X)[4]/46,XX[94]) and normal fertility. At 12 years and 6 months of age, her height was 127.5cm and she was forwarded to a clinical evaluation due to short stature. Menarche was at age 13 years and her menstrual cycle had varied from 25 to 30 days. At 24 years of age, she has had six documented pregnancies, including one child with holoprosencephaly with normal karyotype. For patients with TS and spontaneous puberty, genetic counseling and prenatal diagnosis are indicated.

P0261. Turner Syndrome mosaicism: an unusual case with a large dicentric marker chromosome: 45,X/46,X,der(X), ter rea(X;X), de novo.

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Turner's Syndrome(TS) is characterized by the total or partial absence of one normal second X chromosome. TS occurs in 1/2500-3000 liveborn females. The phenotype is variable and include short stature and gonadal dysgenesis. Approximately 50 percent of the patients has a 45,X0 karyotype with no second sex chromosome, and 5 to 10 percent have a duplication (isochromosome) of the long arm of one X. Most of the remaining cases involves mosaic karyotypes in which only a proportion of cells is 45,X, with one or more additional cell lineages.
X/X translocations are quite rare in man. The effect of this anomaly on the phenotype depends on the amount of deleted material and whether the chromosomes are joined by their long or short arm.
We report an unusual case of Turner's Syndrome mosaicism in a sixteen year old girl, who was referred to our Institution for primary amenorrhea and short stature. Endocrine evaluation revealed hypergonadotropic hypogonadism, which required a study of the karyotype. Cytogenetic analysis, performed on peripheral blood leucocytes, showed a 45,X/46,X,der(X),ter rea (X;X)de novo karyotype. The prevalent cell line was 45,X(90% cells). A second cell line(10% cells) showed a very large marker chromosome, similar to a large metacentric chromosome. FISH and molecular analysis revealed that the marker chromosome was dicentric and totally derived from the paternal X chromosome. To the best of our knowledge, this is the first reported case of TS mosaicism with a marker 45,X/46,X,der(X), de novo, that has been characterized by molecular and FISH analysis.

P0262. Molecular investigation of Angelman and Prader Willi syndromes. Screening for UBE3A mutations: preliminary results

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Molecular defects within chromosomal region 15q11- q13, which contains genes regulated by the imprinting centre (IC), are responsible for Angelman (AS) and Prader Willi (PWS) syndromes. Differentially methylated regions of the IC play a major role in the establishment of the methylation pattern and genomic imprinting of the locus. PWS is considered a "contiguous gene syndrome", while UBE3A imprinted gene has been implicated as the AS gene, since genomic mutations of which have been identified as the sole molecular defect in AS patients.
Routine molecular analysis with Methylation Specific PCR and Dinoucleotide Repeat Polymorphism analysis was performed in 185 patients referred for AS (87) and PWS (98).The diagnosis was confirmed in 11 AS and 16 PWS patients. 30 patients referred for AS were further analyzed by mutation screening of the UBE3A gene by automated sequencing of exons 9,12, 15 and 16.
Large deletions were excluded by the presence of the expected PCR product and no mutations were identified.
4- 6% of AS patients are predicted to have mutations in UBE3A gene, most of which are located in exons 9, 12, 15, 16. Since 75- 80% of these mutations are familial characterization of the defect and genetic counselling are essential. Although this study, has not as yet detected any mutations, further screening of all exons of UBE3A, as well as other genes, related to AS like phenotypes (MECP2 gene) will probably identify mutations, confirming the clinical diagnosis and providing information about the molecular mechanisms.

P0263. The "characteristic" clinical phenotype of maternal UPD(14): does it really exist? A clinical, cytogenetic, and molecular study of three new unrelated subjects

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Maternal uniparental disomy for chromosome 14 [UPD(14)mat] is firmly associated with abnormal phenotypes, including pre- and postnatal growth retardation, features overlapping PWS, and other abnormal clinical aspects. The presence, in a diploid genome, of a chromosome pair (or of a chromosome fragment) derived from one genitor, carries two main types of developmental risk: the occurrence of an imprinting disorder, or the inheritance of a duplicated mutant of a recessive trait leading to the homozygosity (Engel E., 2006).

These preliminary remarks well support the hard comparison of the clinical phenotype and natural history of three new italian patients, two females and one male, with complete maternal isodisomy (two cases) or heterodisomy of chromosome 14. Growth parameters, including pre- and postnatal length, weight, and OFC, heavily differ from borderline values to a hypochondroplasia-like phenotype. Similar variability was observed for the psychomotor development, completely normal in a subject, respectively mildly or seriously retarded in the two others. Feeding problems and obesity also differentiate the natural history of the three subjects. Precocious puberty was observed only in one of the two girls. In conclusion, we confirm the advisability of considering upd(14)mat in patients with low birth weight, growth retardation, neonatal feeding problems, muscular hypotonia, motor delay, precocious puberty and truncal obesity, as well as in patients with PWS like phenotype, negative for SNRPN mutation. The rapid testing, by using the methylation status of the imprinted MEG3 locus represents a new useful diagnostic tool.

P0264. Molecular Analysis of Usher Syndrome Types 1 and 2 in Iranian Usher Patients

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Usher syndrome (USH) is the most frequent cause of combined deaf-blindness. It is clinically and genetically heterogeneous and mainly inherited as an autosomal recessive trait. A total of 12 loci have been reported to be associated with the usher syndrome, assigned to the USH types (USH1A-G, USH2A-C and USH3A). From the three types are known for usher syndrome, type 1 and 2 are the most frequent forms and type 3 is the modest form, so the objective of this study was to investigate USH1 loci and USH2B between USH2 loci.

We have performed linkage analysis for 30 families using STR polymorphic markers, for DFNB2, DFNB12, DFNB18, DFNB23 and DFNB6 which mutation in the gene located in these loci cause ARNSHL as the same as USH1B, USH1C, USH1D, USH1F and USH2B. Our data up to now showed that in three families 3 STR markers (D10S1432, D10S537 and D10S535), used in this investigation linked to DFNB12, in which results conclude usher syndrome 1D in these families and in one family 3 STR markers (D10S1226, D10S546 and D10S1762) linked to DFNB23, which is related to usher syndrome 1F in this family. We found a mutation in MYO7A (USH1B) and a mutation in VLGR1(USH2C).

Based on our results we suggest that the gene CDH23 causing non syndromic autosomal recessive deafness (DFNB12) and deafness associated with retinitis pigmentosa and vestibular dysfunction (USH1D) has a higher prevalence in our population in comparison with other Usher loci.

P0265. The development of uterine leiomyoma is associated with Val158Met polymorphism in COMT gene and the 3´-untranslated ApaI-IGF2 gene polymorphism in Russian population (Bashkortostan)

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Leiomyoma, the most common benign uterine neoplasm, occurs in around one-forth of women during their lifetimes. Leiomyoma is caused by a complex interaction between multiple genes, hormone, growth factor, cytokines, and the environment. Cytochrome P4501A1 (CYP1A1), catechol-O-methyltransferase (COMT) and glutation-s-transferase (GSTM1) are key enzymes in the estrogen metabolism pathway that result in the hydroxylation, methylation and conjugation. Estrogen (ESR1) and progesterone (PR) receptors are mediators of hormonal bioeffects. The IGF-II growth factor is known to support myoblasts differentiation among other cell types. P53 is a tumor suppressor gene.

We evaluated the association between the GSTM1 null-allele, CYP1A1 Ile462Val, COMT Val158Met, ESR1 PvuI and XbaII, p53 Arg72Pro, PR Progins and 3´-untranslated ApaI- IGF2 gene polymorphisms and uterine leiomyoma risk in a case-control study: 200 women with and 200 without uterine leiomyoma living in the Bashkortostan (South Ural region of Russia).

The frequency of the low-activity COMT Met/Met genotype was significantly higher in patients than in the healthy control group (44 % and 15%, respectively: x^2= 29.96, p=0.0005; OR= 4.46, 95% CI 2.51-7.96).

The frequency of AA homozygous (mutant allele) was significantly higher in women with leiomyomas than without (32 % and 16%, respectively: x^2= 11.0014, p=0.0017; OR=2.32, 95% CI 1.39-3.89).

There was no significant difference between the two groups regarding the distribution of the GSTM1, CYP1A1, ESR1, PR and p53 genes polymorphisms frequencies.

Our results suggest that the Val158Met polymorphism in COMT gene and the 3´-untranslated ApaI-IGF2 gene polymorphism are associated with increased risk of uterine leiomyoma in Bashkortostan population.

P0266. Severe kaposiform hemangioendothelioma associated with Kasabach-Merritt Syndrome - genes need to be identified

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Background: Vascular anomalies are localized errors of vascular development. Vascular tumors include: hemangioma, kaposiform hemangioendothelioma (KH) and tufted angioma. Genetic studies led to the identification of a number of genes that cause vascular malformations.

KH is a rare vascular tumor usually presenting at birth or shortly thereafter. The tumor is locally aggressive and produces major complications.

Aim: To present a rare case of severe KH in a 5yo girl, diagnosed at 7 months, unresponsive to medical treatment. She developed major complications: Kassabach-Merritt-Syndrome and CHF.

Matherial: At 7 months, the infant was admitted with a large vascular lesion involving the left side of the abdomen, left vulva and left leg. A large, rapidly growing, vascular tumor, warm, non pulsatile, red purple, tense, with significant distortion of anatomy was palpable in the abdomen. Clinical examination, Doppler ultrasound, MRI, biopsy and laboratory tests were performed.

Results: KH was the diagnosis. Kassabach-Merritt-Syndrome was the first severe complication, manifested as thrombocytopenia and DIC. Parents refused chemotherapy; hence, treatment was: Prednisone, α-2a-IFN, blood products. Surgery was considered too dangerous given the size and location of the tumor. Despite all efforts, the tumor became giant, invalidating the child and life-threatening.

Conclusions: Kasabach-Merritt-Syndrome and heart failure in unresponsive hemangioendothelioma to medical therapy raise the mortality risk from 30-40% to 50-55%. Gene therapy could be life-saving and despite the rarity of the disease no efforts should be spared to identify the genes involved in KH. In such cases gene therapy could be the only chance for survival.

P0267. Reverse-hybridization-based genetic testing for the prediction of anticoagulant dose requirement

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Coumarin derivatives (Warfarin, Phenprocoumon, Acenocoumarol) are the most widespread oral anticoagulant drugs for the prevention and treatment of arterial and venous thromboembolic disorders. However, these vitamin K antagonists have a narrow therapeutic range and a wide inter-individual variability in dose requirement. Despite adjustment for clinical variables, adverse events, such as delay in achieving a stable maintenance dose or bleeding complications, are frequently encountered during the initial phase of therapy. Genetic polymorphisms in the drug-targeted vitamin K epoxide reductase complex 1

(VKORC1) and in the drug metabolizing cytochrome P450 isozyme CYP2C9 have been reported to account for the majority of variations in the therapeutic response to warfarin.
We have developed a genetic test (StripAssay) for the detection of -1639G>A and 3730G>A in the VKORC1 gene, and 430C>T and 1075A>C in the CYP2C9 gene. The StripAssay is based on multiplex PCR, followed by reverse-hybridization of biotin-labeled amplification products to a parallel array of allele-specific oligonucleotides on membrane teststrips. Genotyping for VKORC1 polymorphisms and the functionally defective CYP2C9 variants *2 and *3 allowed the classification of patients into high, intermediate and low dose responders to phenprocoumon (Marcumar), the most commonly used oral anticoagulant in Central and North European countries. Favourable properties, such as the rapid DNA extraction protocol, ready-to-use reagents and teststrips, as well as the potential for automation of the hybridization/detection step, make the StripAssay convenient and easy to perform within less than 6 hours. The results obtained in our study will assist clinicians to achieve a more individualized anticoagulant therapy. (oberkanins@viennalab.co.at)

P0268. ‚De novo' *SOX10* mutation in a 2-year-old child with Type 4 Waardenburg syndrome: case report and literature review

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Type 4 Waardenburg syndrome (WS4) is part of neurocristopathy diseases resulting in absence of melanocytes in inner ear cells, on skin and variable degree of parasympathetic enteric neurons absence. *SOX 10* gene acts as a key regulator for peripheral glial development. *SOX10* gene haploinsufficiency was associated with a distinctive phenotype combining WS type 4, mental retardation and dysautonomic features. (1)
We report the natural history of a 2 year-old child with congenital deafness, delayed psychomotor development. Language retardation and autistic-like behaviour were noted. The cerebral MRI showed bilateral absence of the semi-circular canals and delayed myelinisation. The ophthalmological exam identified deep blue iris along with an albinotic-like retina. These clinical features authorize the screening for *SOX10* gene mutation encountered in WS4 spectrum. The patient was identified with a 698-2 A→C *SOX 10* gene substitution, responsible for a premature STOP codon. This mutation occurred 'de novo' since both parents did not carried this substitution. Among 12 patients, Touraine et al. identified 3 children with *SOX 10* mutation who developed WS 4 phenotype with additional neurological symptoms: mental retardation, cerebellar ataxia, nystagmus, spasticity. They also had dysautonomic features (alacrima(2/3), asialia(1/3) or reduced sweating(1/3)). Noticeably, our patient so far did not show any digestive anormaly nor any dysautonomic sign. The precise phenotype features in this patient lead to specific molecular investigation. The genetic counseling is now accurate to the patient and for his family.
Reference (1)- *R. Touraine et al. Am J Hum Genet 2000;66:1496-1503*

P0269. A novel point mutation (Met387Ile) in the *RECQL2* gene in a patient with Werner syndrome

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Werner syndrome is autosomal recessive disease, which characterized by symptoms of the adult-onset premature aging. The features of Werner syndrome are scleroderma-like skin changes, especially in the extremities, cataract, subcutaneous calcification, premature arteriosclerosis, diabetes mellitus, and a wizened and prematurely aged face. The *RECQL2* gene, also known as *WRN*, is in charge of this disease and encodes a helicase.
We have investigated 4 Russian patients with Werner syndrome. We found out one novel mutation (ATG→ATA) in exon 9 in heterozygous state in this gene which leads to substitution of Met by Ile at position 387 (p.M387I). Also we found out single nucleotide substitution c.355+4G>C and didn't find it in 100 control DNA samples.
Also we investigated by SSCP-analysis LMNA gene, which encoded two protein products lamin A and C, and take part in beginnings of atypical Werner syndrome. Here we didn't find any mutation in the patients.
This work was partly supported by Russian President's grant NSh-5736.2006.7.

P0270. Neonatal progeroid Wiedemann-Rautenstrauch syndrome: follow-up of new case in Belarus

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Neonatal progeroid syndrome, or Wiedemann-Rautenstrauch (WRS) is a rare autosomal recessive disorder, with few published case reports. WRS is characterized by progeroid appearance at birth, lipoatrophy, slow growth (MIM 26409). We present results of 3 years observation of female patient, the first child of a young healthy nonconsanguineous Belarusian couple. Pregnancy and labor were unremarkable. The newborn (BW 1890 g, BL 44 cm) showed typical progeroid features (aged triangular face, beak-shaped nose, telecanthus, microstomia, pseudohydrocephalus, widened fontanelles and sutures, hypotrichosis, wrinkled skin, prominent veins and lipoatrophy). The infant had feeding problems and small weight gain. Initial follow-up at 4 months demonstrated growth and mental retardation; myopia and hip joints dysplasia were revealed. Ultrasound examination of brain and heart did not reveal any abnormalities. At 12 months dentition was delayed, contractures at knees and elbows and paradoxical caudal fat accumulation occurred. At age of 3 years progeroid signs became more pronounced and psychomotor retardation was preserved. Chromosomal and biochemical analyses were normal. Differential diagnosis was done with other similar genetic syndromes (Cockayne syndrome, cutis laxa-growth defect, lipodystrophy syndrome, oculo-mandibulo-facial syndrome, progeria including progeroid syndrome with Ehlers-Danlos features). We have established WRS on the basis of association of neonatal progeroid appearance, lipoatrophy with consequent caudal fat accumulation, growth and mental retardation. Clinical features of the patient were compared with published data, which vary in expression and severity. The case presented differs in absence of natal teeth. The patient is alive; the parents are informed of the diagnosis and recurrence risk of 25%.

P0271. Intracranial space occupying lesions could be the forth component of Wildervanck (cervicoocculoacustic) syndrome

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Cervicooculoacoustic (Wildervanck) syndrome (MIM 314600) is characterized by the triad of Klippel-Feil anomaly, bilateral abdusence palsy with retractio bulbs (Duane's syndrome) and hearing loss. Since it is commonly observed in female patients, X-linked dominant mode of inheritance with increased chance of lethality in males has been suggested. We have observed a total of 10 Wildervanck cases (8 females, 2 males) within a range of 7 months to 36 years old. All of them had cervical vertebral fusions and hemivertebrae, two of them had diastometamyelia, three of them had dermal sinus into intradural space. Along with these typical manifestations seven of them had occipital bone defects and mid-line masses. Closure defect examples such as, dermoid cysts, hamartomatous cyst, meningeal cyst, meningomyelocelle and encephalocelle located in the posterior fossa were documented histopathologically in different cases with cranial masses. One female patient was suffer from three times meningitis due to dermal sinus. Postmortem examination of this patient demonstrated that a chronic infected dermoid cyst. Cleft palate malformation was observed in another female patient associated with typical symptoms. Those closure defects could be more frequent than it was previously anticipated in Wildervanck syndrome. MR examinations should be added in the diagnostic procedure of this syndrome in order to identify additional posterior fossa abnormalities and/or other intracranial space occupying lessions.

P0272. Williams Syndrome in young child - case report

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Williams syndrome is a rare genetic disorder characterized by mild mental retardation, distinctive facial appearance, abnormalities in calcium balance and blood vessel disease. It is present at birth and affects males and females equally.The syndrome is caused by a deletion in the locus of the elastin gene localized at 7q11.23.

We present a 24 months old male admitted in our clinic for fever and vomiting. He is the second child of a healthy couple, the pregnancy was not followed-up and the birth weight was low. Feeding problems and slow weight gain were noticed.

By clinical examination we noticed failure to thrive (weight of 9400 g), short stature, characteristic facial features (microcephaly, broad forehead, short palpebral fisures, left eye strabismus, periorbital fullness, flattened nasal bridge, long philtrum, wide mouth with full lips, low placed proeminent ears). There were present musculoskeletal anomalies (hyperextensible joints and lumbar scoliosis), anxiety, a friendly behaviour and mild delay in intellectual, motor and language skills. Calcium blood level was elevated and echocardiography was normal.

Conclusions: We diagnosed the syndrome by clinical features and hypercalcemia. Variable expression in the phenotype makes the absence of one of the main anomalies possible. FISH analysis may certify the diagnosis.

The child requires a comprehensive multidisciplinary approach to his care and periodic cardiovascular evaluations.

P0273. Presenting phenotype and clinical evaluation in a cohort of 22 Williams-Beuren syndrome patients

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Williams-Beuren Syndrome is a rare multi-system genomic disorder, caused by 7q11.23 microdeletion with a prevalence of 1/10000-1/20000 live births. Clinical phenotype includes typical facial dysmorphism (elf-in face), mental retardation associated with a peculiar neuropsychological profile and congenital heart defects. We investigated 22 WS patients (mean age of 9.7 years, range 1 day-39 years) with a multispecialistic follow-up protocol comprehensive of neuropsychological, cardiologic, nephrologic, ophthalmologic, endocrinologic, gastoenterologic, odontostomatologic and orthopaedic evaluations. The mean age at diagnosis was 5.38 years, being 1.02y when genetic evaluation was requested for congenital heart defects (CHD) and 10.68y in case of mental retardation/abnormal neuropsychological profile without an evident CHD. All patients showed facial dysmorphisms, with supravalvular aortic stenosis (SVAS) as the most common cardiovascular anomaly (12/22) associated, followed by peripheral pulmonary stenosis (9/22); interestingly, we detected in one patient a total anomalous pulmonary venous return (TAPVR), confirming the possible association of this rare CHD with WS. Hypertension was detected by 24 hours ambulatory blood pressure monitoring in 7/22 cases. Mental retardation was observed in 9/22 patients, mostly (7/9) in a mild form and Chiari malformation type 1 was found in 3 patients. Our study underlines a remarkable diagnostic delay in patients who present to genetic evaluation because of mental retardation/neuropsychological profile lacking an evident cardiopathy and confirms the multi-systemic nature of WS leading to a high clinical presentation's variability and complex follow-up strategies.

P0274. Monitoring minimal residual disease in pediatric ALL using real-time quantitative PCR for Wilms tumor gene (WT1): Prognostic significance, and correlation with disease status

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Continuous Wilms' tumor gene (WT1) expression is a typical feature of leukemic blasts in AML, ALL, and blast crisis CML patients. WT1 is only transiently expressed in normal hemopoiesis. levels of leukemic cells (MRD) in peripheral blood samples from children with ALL using WT1 as a marker should have clinical importance. We aimed to develop a quantitative method based on the RQ-PCR in order to monitor WT1 level in children with ALL and assess its prognostic importance. Furthermore, we wished to compare the sensitivity, efficiency, and reliability of this method with the conventional semi-quantitative RT-PCR .

Fourteen newly diagnosed ALL children were included in this study. The peripheral blood samples were collected before induction, at the second week of induction, at the start of consolidation and beginning of the maintenance phase of chemotherapy. The last blood sample was collected from each patient at random 2 to 6 month after the maintenance phase started.

The proportion of patients with detectable WT1 was 92% at diagnosis and 42% at the second week of therapy, 38% at the start of consolidation, and 0% at the beginning of maintenance. The absence of WT1 showed accordance with the clinical course of patients. CQ PCR and RQ PCR results were correlated together.

results indicate that real time PCR can be employed to monitor MRD in the leukemic patients during chemotherapy. Successful development of RQ PCR by SYBR Green I resulted to an easier method with lower cost in comparison to probe based methods.

P0275. Clinical manifestation and molecular-genetic diagnosis of Wilson's disease in Republic of Moldova

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Hepatolenticular degeneration is an inherited disorder of copper metabolism in an autosomal recessive manner. The gene is located on chromosome 13 and encodes a copper-transporting P-type ATPase protein, which controls the insertion of copper into apoceruloplasmin in the trans-Golgi network and the biliary excretion of excess copper through a vesicular-lysosomal-apical membrane route.

Our patients from 30 Wilson's disease(WD) families had presented with predominantly hepatic, neurological or psychiatric manifestations. The varied neurological abnormalities displayed by patients include any combination of dyskinesia, rigidity, resting and intention tremor, dysarthria, dysphagia, abnormal gait and psychological disturbances.

The search was effectuated on two ATP7B exones(14, 15) using single strand conformation polymorphism method (SSCP) of DNA molecule. In the 14th exone was determined His1069Glu missense - mutation, which corresponds to the Thomas's G. data recorded in 38% of Europeans. Analysing 60 chromosomes of WD patients, missense - mutation determined in 13,3%. In 2 patients the mutation was detected the homozygote form, but in 4 patients was detected the heterozygous form. The presences in patients with certain clinical manifestations the heterozygous occurrence of missense - mutation can presumably take part a compound phenomenon.

During the SSCP analysis of the 15th exone in our trials was not detected the frames with abnormal electrophoretic activity. Thereby, the search of deletion in the 15th exone(C3400delCgene) in studied patients hadn't any results. Searching Prp1134Pro-fs mutation in 60 chromosomes showed its absence.

Conclusion: The missense mutation His1069Glu has been established in 13.3% cases, but the deletion Prp1134Pro-fs has not been determined.

P0276. Epilepsy in adults with Wolf-Hirschhorn syndrome

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Wolf-Hirschhorn syndrome (WHS) is a well recognised malformation syndrome resulting from a partial 4p deletion. Features include pre- and post-natal growth delay, microcephaly, characteristic facies,

midline defects and epilepsy. The cause of WHS is heterogeneous; some individuals have deletions which may be large or small whereas others have unbalanced translocations. The epilepsy may be of early onset and difficult to control but there have been some reports of the seizures ceasing after a period of years. However, relatively limited information exists detailing the seizure activity in adults with WHS.
We investigated seizure onset and frequency in 21 young adults over the age of 16 years diagnosed with WHS. They had a mean age of 24 years; range 17-34 years. Data were obtained by contacting parents using a telephone interview.
Epilepsy was found to occur in all 21 cases, with one exception onset of seizures was between 1 and 2 years of age. In 8 cases (mean age 26 years; range 17 - 34 years), a seizure had occurred within the last 3 years. Whereas in 13 cases (mean age 23 years; range 17 - 33 years), the most recent seizure had occurred more than 3 years previously (mean number of years 15; range 6 - 28 years). For those 13 cases the last year of seizure occurred at a mean age of 9 (age range 2 - 15 years).
These data may be valuable in counselling families of a newly diagnosed child.

P0277. Detection of genomic copy number changes in patients with idiopathic mental retardation by high-resolution X-array-CGH: frequent increased gene dosage of known XLMR genes

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A tiling X-chromosome-specific genomic array with a theoretical resolution of 80 kb was developed to screen mental retardation (MR) patients for submicroscopic copy number differences. We first validated the X-array using 4 MR patients with aberrations previously detected at lower resolution. This allowed for delineation of the location and extent of the aberration at high resolution and subsequently, more precise genotype-phenotype analyses. Next, we screened a cohort of 108 patients with idiopathic MR consisting of 57 patients suspected of X-linked mental retardation (XLMR), 27 probands of brother pairs, and 24 sporadic patients. We identified 15 copy number changes in 14 patients (13%). These include 2 deletions and 13 duplications ranging from 0.1 - 2.7 Mb. The aberration is considered to be associated with the phenotype in 5 patients (4.6%) based on the following criteria: *de novo* aberration; involvement of a known or candidate MRX(S) gene; segregation with the disease in the family; absence in control individuals, and skewed X-inactivation in carrier females. These include deletions that contain the MRX(S) genes *CDKL5, OPHN1* and *CASK*, and duplications harboring *CDKL5, NXF5, MECP2* and *GDI1*. In addition, seven unbalances are apparent novel polymorphic regions because they do not follow the proposed criteria. Taken together, our data strongly suggest that not only deletions but also duplications on the X chromosome might contribute to the phenotype more often than expected, supporting the increased gene dosage mechanism for deregulation of normal cognitive development.

P0278. Female phenotype with karyotype 47,XYY

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Our patient is a 7 year old girl with blood karyotype 47,XYY. Her development has been normal with the exception of growth retardation. On examination at age 5 years height was 5cm below the 2.5th centile, weight at the 50th centile for height and head circumference at the 10-25th centile. Her neck was broad and her posterior hairline somewhat low. She had minimal cubitus valgus. A striking feature was a hypertrichosis which was congenital and had increased over time. She had long, coarse, fair, hairs along her entire spine, most pronounced in the lumbar region, as well as hypertrichosis of her forearms.
Her external genitalia were normal. A normal sized uterus, but no gonads, were identified on ultrasound. At laparoscopy she was found to have small gonads which were excised. Histological examination revealed ovarian stromal cells and bilateral gonadoblastoma. No testicular differentiation was seen.
The karyotype of all 34 analysed lymphocytes was 47,XYY. The karyotype in cultured fibroblasts was 45,X in 7 and 47,XYY in 14 of 21 metaphases. FISH analysis of 199 interphases also revealed mosaicism: XYY in 68, X in 114, XY in 4 and XX in 13 cells. Gonadal mosiacism was present with 45,X in 37 and 47,XYY in 13 of 50 cell. Sequencing of the *SRY* gene revealed no mutation.
A 47,XYY karyotype in lymphocytes is usually associated with a male phenotype. The presence of cells without a Y chromosome in the gonads may explain the female phenotype in this patient.

P0279. Mutation screening of ARX gene in male patients with non-specific mental retardation from Russia

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Mutations of the ARX gene, considered to be the second most common cause of X-linked mental retardation (XLMR), give rise to a wide variety of distinguishable syndromes (Partington syndrome, X-linked infantile spasm, etc.), and they are also an important cause of non-syndromic XLMR. The striking finding concerning ARX is the phenotypic heterogeneity associated with the most frequent and recurrent mutation (identified in >5% of XLMR families): the in-frame 24-bp duplication (c.428_451dup24) expanding the 16 polyalanine tract to 23 (amino acids 100-115). In order to evaluate the frequency of ARX mutation in mentally retarded (MR) males from Volga-Ural region of Russia, we performed mutational analysis of ARX gene in 214 unrelated MR (diagnosis based on the ICD-10) male patients (Russians N=72, Tatars N=56 and Bashkirs N=34) with average age of 13.02±3.47 years (range, 5-18 years) negative for mutations in the FMR1 and MECP2 genes. The recurrent c.428-451dup24 mutation was identified by sequencing in three (1.4%) MR males (Russians N=1, Tatars N=1 and Bashkirs N=1). The subsequent genotype-phenotype analyses confirmed that polyalanine tract expansion can result in a highly variable disease phenotype, even with respect to mental retardation a great deal of variability, with intellectual impairment ranging from mild to severe, which mitigates against early clinical diagnosis. These data, together with those reported in the literature, imply that screening for c.428_451dup24 mutation should be recommended in males with non-specific MR, especially in population Volga-Ural region of Russia. The work was supported by grant of RFH, project (No.05-06-06168a).

Po02. Cytogenetics

P0280. Further delineation of the 13q deletion syndrome: clinical report and molecular characterization of an interstitial 13q31 deletion.

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The 13q deletion syndrome usually comprises a wide variety of clinical manifestations including developmental delay, mental and growth retardation, hypotonia, microcephaly, trigonocephaly, dysmorphy, ocular anomalies, and diverse malformations of limbs, brain, heart, and kidney. This variability of clinical expression depends on the size and location of the deleted segment. So a classification was proposed pointing band 13q32 as the ''minimal critical region'' of the 13q- syndrome. Deletions involving this band are associated with major malformations, those limited to proximal bands q13-q31 share no major anomalies but mainly growth retardation and, distal deletions are rather complicated by severe retardation but minor abnormalities.Here, we report the case of an 11 years old boy who presented mental and growth retardation, dysmorphic features, hand and foot abnormalities, hyperactive behaviour and an atypical West syndrome. Chromosome analysis identified a *de novo* interstitial deletion on the long arm of a chromosome 13. Further characterization of this deletion was conducted by FISH with use of BAC probes extending from 13q21.33 to 13q32.3. We found a deletion of 11 Mb long limited to band 13q31. The observed phenotype in the proband is supposed to be related to haplo-insufficiency of the genes contained in the deleted region. Interestingly, abnormal behaviour and west syndrome are two clinical entities never described in the 13q- syndrome. This could be explained by the fact that deletions concerning only band 13q31 have never been reported. This observation underlies the importance of an accurate characterization of deletions in order to better establish a karyotype/phenotype correlation.

P0281. Chromosme 18q paracentric inversion in a family: Report of a case

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Paracentic inversion is a rare cytogenetic abberation, especialy paracentric inversion of chromosome 18q. We have found only a few case reports on that subject.

We report a married couple that was send to a Genetic laboratory because of a problem with infertility (3 missed abortions). Both, man and woman, are healthy normal people, with normal gynecological status. He has also normospermia. Cytogenetic analysis, G band, and additional YUNIS analysis confirmed the karyotype of the probant 46,XY,inv18(q21q22.3). Analysis of the karyotype of the probants family confirmed that the probants mother has the same chromosomal abberation.

The family of the probant has no mentaly retarded members or pregnancy losses. The probants sister has two normal children but she is not available for further investigation.

The paper will discuss different effect of the same chromosomal abnormality on fertility. Also our results will be compaired with the results of other authors.

P0282. A duplication of the distal segment of 22q in a patient with mental retardation, microcephaly and mild facial dysmorphism.

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We present a patient with a terminal 22q duplication due to an unbalanced translocation 46, XX, der(22)(qter->q13.31::p11->qter). She presented at the age of 4.5 years with a mild to moderate mental retardation, microcephaly and mild dysmorphic facial features including a large forehead, a broad nasal bridge, a thin upper lip and a large mouth. A high resolution karyotype was apparently normal. Because of nasal speech, FISH analysis for the DiGeorge/VCFS region was performed with a commercial probe mix containing TUPLE1 (22q11.2) and a control probe LSI ARSA (22q13). Normal signals were seen for TUPLE1 and LSI ARSA on both chromosomes 22q. A third signal for LSI ARSA was present in all cells on the short arm of one of the chromosomes 22. Therefore the diagnosis of terminal duplication 22qter was made. By means of 1Mb array-CGH the duplication was confirmed and further characterized as a 5.5 Mb region: 46, XX, dup(22)(q13.31qter)(CTA-268H5->CTB-99K24)x3. FISH analysis for LSI ARSA was normal in both parents.

Duplications of the distal segment of 22q seem to be very rare. Twenty four patients have been described with only 3 of them carrying a very small duplication (22q13.3 to 22qter) like in our case. Two of these 3 patients are a father and his son with very similar facial features compared to the patient we describe. They also have mild to moderate mental retardation and microcephaly. Terminal duplications of 22qter may be more common than generally assumed and may be detected more frequently by the application of array-CGH.

P0283. Screening of 22q11.2 Microdeletion and Microduplications in 110 Patients With Clinical Findings of Digeorge/Velocardiofacial Syndrome

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22q11.2 microdeletion associated with Di-George Syndrome/ Velocardiofacial Syndrome (DG/VCFS) is the most common chromosomal deletion in humans affecting 1 in 4000 live births. Main clinical features of this syndrome includes conotruncal cardiac anomaly, velopharyngeal insufficiency, nonvisible/hypoplastic thymus, immunodeficiency, characteristic facial dysmorphism, palatal anomalies, language impairment, developmental delay/ learning difficulties and immunologic anomalies. Recently microduplication 22q11.2 sydrome which presents overlap with DG/VCFS has been identified. In this study we screened 22q11.2 microdeletions and microduplications in 110 patients (60 female and 50 male) with clinical findings of DG/VCFS by Fluorescence in Situ Hybridization (FISH) using dual color TUBLE1 and N25 probes and conventional cytogenetics. We detected 22q11.2 microdeletion for both of the TUBLE1 and N25 region probes in 14 of 110 (13%) cases (5 male and 9 female). Clinical findings of our cases with 22q11.2 deletion will be discussed according to literature. We could not detect microduplication of the analysed region showing that microduplication is a rare event in patients with clinical findings of DG/VCFS.

P0284. Distal 22q11.2 deletion: a new microdeletion syndrome with predispostion to rhabdoid tumors.

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Few cases of 22q11.23 deletions, distal to the 3Mb VCFS/DGS region have been reported. We report two new patients. Patient 1 was diagnosed at birth with a severe CHD. He was hypotonic with delayed motor skills. At 14 months, an atypical teratoid/rhabdoid intracranial tumour was diagnosed. Patient 2 was investigated at age 4 for developmental delay. He developed a rhabdoid tumour of the kidney at age 7. Patient 1 had a cytogenetically visible inversion of chromosome 22. FISH studies, demonstrated inversion of genes proximal to 22q11.23, associated with a 0.9-1.8 Mb deletion encompassing BCR locus. Patient 2 had a pure deletion extending from clones RP11-379N11 and RP11-20P18 suggesting that the recombination event occurred between LCRs 4 and 7. We compared the phenotype of our patients with other patients reported in the literature. Most of the patients reported to date had CHD but none had velopharyngeal insufficiency, clefting or hypocalcemia. All these patients had a similar facial appearance, rather different from the gestalt of VCFS/DGS patients, including low-set and posteriorly rotated ears, anteverted nares, thin lips and full cheeks. All patients with deletions encompassing the *SMARCB1* gene, developed rhabdoid tumors. Since the 22q11.2 region contains several LCR, different recombination events can give rise to non-overlapping deletions. Patients with a distal 22q11.2 deletion must not be considered as VCFS variants and require different clinical assessment and follow-up. It is important to determine precisely the status of the *SMARCB1* locus, since patients hemizygous at this locus are at risk of developing rhabdoid tumors.

P0285. Chromosome 22q13.3 deletion syndrome with a *de novo* interstitial 22q13.3 cryptic deletion retaining *SHANK3*

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The 22q13.3 deletion syndrome (MIM 606232) is characterized by developmental delay, absent or severely delayed speech, autistic behavior, normal to accelerated growth, and minor dysmorphic facial features. Among the three genes of the minimal critical region (from centromere to telomere: *SHANK3*, *ACR* and *RABL2B*), *SHANK3* is considered to be at the origin of the neurobehavioral symptoms.

We report a child with a *de novo* interstitial 22q13.3 deletion detected by MLPA and confirmed by FISH.

She demonstrates mental retardation, severe speech delay, epicanthic fold, and protruding ears with normal growth. This non specific phenotype is concordant with the classical presentation of 22q13.3 deletion syndrome.

Subtelomeres analysis by MLPA showed a discrepancy between the P036B and P070 kits (MCR Holland): P070 MLPA probe (targeting *ARSA* gene) showed a deletion but P036B (targeting *RABL2B* gene) showed a normal result. FISH analysis using LSI *TUPLE1*/LSI *ARSA* (Vysis) probes confirmed deletion of *ARSA*, whereas FISH with N25/N85A3 (Cytocell) probes, targeting *SHANK3* locus was normal. This was confirmed by quantitative real time PCR of exons 23 and 24 of *SHANK3*. Supplemented FISH analysis using BAC clones were performed to delineate both breakpoint regions of the interstitial deletion.

These data highlight the difficulty of performing an appropriate test in order to search for cryptic 22q13.3 deletion. Furthermore the molecular characterization of this interstitial 22q13.3 deletion contributes to clinical and genetic delineation of the 22q13.3 deletion syndrome.

P0286. Double trisomy involving chromosome 21 and the sex chromosome (48,XXX,+21) - a case report and literature review

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Occurrence of double trisomy in the same individual is a relatively rare phenomenon. In most cases double nondisjunction leads to an inevitable prenatal lethality. Multiple aneuploidy, observed in live born children usually involves sex chromosomes together with trisomy 13, 18 or 21. Multiple aneuploidy occurs as a consequence of a minimum of two errors during meiosis. The zygote carrying a double aneuploidy usually results from a double nondisjunction in a single germ cell however the coincidence of a single nondisjunction occurring in both gametes was also observed. More than 90 cases of nonmosaic trisomy 21 and numerical sex chromosome aberrations were presented by Kovaleva and Mutton, but among them only 14 cases were diagnosed with 48,XXX,+21. We report a case of double trisomy involving chromosomes 21 and X in a female infant (karyotype 48,XXX,+21). The child presented dysmorphic features such as hypotrophy, microcephaly with a flat occiput, midface hypoplasia, up-slanting palpebral fissures, epicanthic folds, low set ears, open mouth with macroglossia and protruding tongue, short neck, small hands and feet, simian creases, wide space between first and second toes, a umbilical hernia, dislocated hips, congenital heart defect, a mild hearing loss and hypothyroidism. The observed dysmorphic features as well as other deformities are consistent with those characteristic for trisomy 21. However, genetic counselling in such cases is difficult. Neither data concerning the clinical outcome of the double trisomy children nor any information concerning the recurrence risk for their parents exist to date.

P0287. Delineation of the deletion breakpoints in two unrelated patients with 4q terminal deletion syndrome

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INTRODUCTION: Deletion of the terminal region of the long arm of chromosome 4 results in a series of clinic features including developmental delay, cleft palate and limb defects. Due to the similarities of the clinical symptoms a 4q-deletion syndrome has been suggested. This study aims to find the critical region responsible for the 4q-deletion syndrome through fine mapping the terminal 4q deletions in two unrelated patients.

METHODS: The patients were analysed using array CGH and FISH. Array CGH was carried out using a submegabase resolution whole genome tiling path BAC array and the results were verified by FISH analysis, using standard protocols.

RESULTS:

The 4q terminal deletions of two patients with cleft palate, limb defects and developmental delay were fine mapped using array-CGH. Both deletions were positioned at 4q33-4qter. Furthermore, one of the patients displayed a cryptic unbalanced translocation between chromosome 4 and chromosome 20, resulting in the loss of 4q33-4qter and trisomy of the 20p13-20pter region. The chromosomal aberrations were *de novo* in both patients.

CONCLUSION: In the present study we investigated two patients with deletion of the terminal long arm of chromosome 4. Our data support that 4q33 may be the critical region for the 4q-syndrome. This study also underlines the power of array-CGH in identifying cryptic unbalanced translocations.

P0288. Clinical aspects of the deletion 9p syndrome - two new cases

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Nearly 100 cases of deletion 9p has been published. Often, the deletion occurs de novo. We reported two unrelated children who share particular clinical findings including trigonocephaly, microstoma, hypotelorism, broad nasal root, upslanted fissures, motor retardation. One patient has a congenital cardiac anomaly (VSD and PDA). Family history did not reveal any other family members with similar findings or different congenital anomalies.

The similar findings of these cases suggested a chromosomal disorder. Cytogenetic investigation demonstrated del(9)(p22->pter) in both cases. The chromosomal analysis for parents are in progress.

The first patient has a number of clinical findings invariably found in the 9p- syndrome. However, the other patient has a partial optic nerve atrophy which has not been reported before.

P0289. Cytogenetic, morphologic and immunophenotypic pattern in omani patients with *de novo* acute myeloid leukemia

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Objective: To characterize the cytogenetic, morphologic and immunophenotypic pattern in ethnic Omani patients with *de novo* Acute Myeloid Leukemia (AML).

Material and Methods: Successful cytogenetic results were obtained from 63 *de novo* AML patients. Forty-one were male and 22 female with a median age of 25 years. Chromosomes were obtained by short-term cultures and interpreted after G-banding. The diagnosis of AML was based on French-American-British (FAB) cytomorphology criteria & the immunophenotyping of bone marrow.

Results: Karyotypic abnormalities were present in 39 patients(61.9%) with 44.2% in adults and 17.7% in children. Karyotypes with sole abnormalities amounted to 31.7%(n=20). Chromosomal abnormalities were more common in patients with FAB-M2 subtype (n=22;68.2%). The most frequent subtype observed was M2 (n=22;34.9%). Among the normal karyotypes (n=24;38.1%) M2 -subtype was again the most frequent (n=7;29.2%) followed by M4(n=4;16.67%). Among balanced translocations t(8;21) and t(15;17) were observed in 11.1% and 9.5% respectively. Inv(16) was seen in 3.2%. Trisomy 8 was the most frequent numerical anomaly found in 11.1%. Monosomy 7 was seen 4.7%.

Discussion: This is the first systematic cytogenetic report from the ethnic Omani population [1.78 million] studied over the last 5 years from a single institution. We observed that findings in our study were similar

to reports from Saudi Arabia and Kuwait but the frequency of abnormalities varied with a mixture of +8, t(8;21) & t(15;17). Morphologically our patients differed with M2 subtype as the commonest, whereas, their reports showed M4 and M3 subtypes as most frequently occurring subtype in their patient population respectively.

P0290. Screening of subtle copy number changes in Aicardi Syndrome Patients with a high resolution X-chromosome array-CGH

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Aicardi syndrome is a very uncommon neurodevelopmental disorder affecting almost exclusively females. Chief features include infantile spasms, corpus callosal agenesis, and chorioretinal abnormalities. Aicardi syndrome is a sporadic disorder and hypothesized to be caused by heterozygous mutations in an X linked-gene but up to now without any defined candidate region on the X chromosome. Array based comparative genomic hybridisation has become the method of choice for the detection of microdeletions and microduplications at high resolution. In this study, for the first time, 18 Aicardi syndrome patients were analyzed with a full-coverage X-chromosomal BAC arrays at a theoretical resolution of 82 kb. Copy number changes were validated by real time quantitation. No disease-associated aberrations were identified. For such conditions as Aicardi syndrome, in which there are no familial cases, additional patients should be studied in order to identify rare cases with submicroscopic abnormalities, and to pursue a positional candidate gene approach.

P0291. The role of chromosomal abnormalities in primary amenorrhoea

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Amenorrhoea is absence or cessation of menses. If menstruation does not begin by the age of 16 years in the presence of female secondary sexual maturation, or does not begin by 14 years in the absence of secondary sexual maturation, the condition is classified as primary amenorrhoea. This paper deals with investigation of how primary amenorrhoea and chromosomal abnormalities are related. For the period of last ten years we have analyzed 62 patients with primary amenorrhoea. Karyotype from the peripheral blood lymphocytes and G banding were performed according to standard protocols. Chromosomal abnormalities were detected in 16 cases (25,8%). Male karyotype (46,XY) was found in seven cases. In three cases monosomy X (45,X) was found. In two cases isochromosome X (46,XiXq) was detected, as well as a case of X chromosome trisomy (47,XXX). Mosaic karyotypes 45,X/46,XiXq, 46,XX/47,XXY and 45,X/46,XX were found in one case each. These findings suggest that a consistent number of cases with primary amenorrhoea (about 25%) can be associated with genetic anomalies.

P0292. Array-CGH analysis: new deletion syndromes and atypical phenotype in old deletion syndromes

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A group of 28 unrelated MCA/MR patients with normal karyotype, selected from those attending the Medical Genetics Unit of the University of Siena has been analyzed by oligo array-CGH with an average resolution of 75Kb (Agilent Human Genome CGH Microarray Kit 44B). A new “de novo” deletion has been identified in 3 patients (table 1). During the screening, we have also identified, 3 known deletions in atypical patients, 1 duplication reciprocal of the deletion responsible for Smith-Magenis and 2 polymorphisms (table 2).

In conclusion, in this highly selected population we identified pathogenic segmental deletions/duplications in 7 out of 28 patients (about 25%). We compared the clinical features of patients with new deletions with other rare cases described in the literature in order to define clinical diagnostic criteria useful in the clinical practice. In particular, for the 6q25 deletion the emerging phenotype is characterized by short palpebral fissures, smooth philtrum, thin upper lip, asymmetric dysplastic protruding ears, heart septal defect, and slightly delayed psychomotor development. These characteristics should be taken into account in order to identify other patients.

Table 1. New deletions

P.	Clinical features	Chr. Deletion size Breakpoints	Reference
1	4y2m girl with postnatal growth retardation, microcephaly, ptosis, down-slanting palpebral fissures, long eyelashes and micrognathia. Halluces are long, broad and medially deviated, while the other toes are laterally deviated and remarkably short with hypoplastic phalanges. She shows developmental delay, seizures, lack of eye contact, hand stereotypies and sleep disturbances with breath holding	2q24.3-31.1 10.4 Mb 165.580-175.871 Mb	Pescucci et al, Eur J Med Genet, 2007, 50:21-32
2	13y8m boy with severe mental retardation, absence of speech, sleep disturbances, behavioural problems. He presents macrocephaly, high forehead, thick and coarse hair, thick eyebrows, synophris, increased inner and outer canthal distance, bifid nasal tip, high palate, micrognathia, dysmorphic, right ear, bilateral sandal gap and long and tapering fingers.	2q31.2-32.3 13 Mb 180.13-192.88 Mb	Mencarelli et al, in press, Am J Med Genet
3	7y6m female with neurodevelopmental delay, postnatal short stature and atrial septal defect. Patent ductus arteriosus and tricuspid insufficiency were also noted at birth. Medial flare eyebrows, dysmorphic helix of the right ear, cupshaped left ear, anteverted nares, long and smooth philtrum, thin upper lip, high vaulted palate are noted.	6q24.3-25.1 2,6 Mb 148.785-151.289 Mb	Caselli et al, in press, Eur J Med Genet

Table 2. Other results.

Clinical features	Chr. Position (approximate size)	Type of rearrangement	Conclusions
Case 1. Hypotonia, seizures, ataxia, sleep disturbances, gastroesophageal reflux, dolichocolon, hypospadia, monolateral inguinal hernia, bilateral 2-3 syndactyly of toes.	15q11-13.1 165.8-175.8 (5 Mb)	deletion	Known deletion in atypical patient
Case 2. Bilateral corneal leukoma, iris and retinal coloboma, cleft lip and palate, VSD, postaxial polydactyly of hands and feet.	22q11.21 17.2-19.7 2.5 Mb	deletion	
Case 3. MURCS association , short stature, delayed bone age, long faces, tubular nose with bulbous tip, high palate and nasal speech, small and low-set ears, left carotid artery hypoplasia.	22q11.21 17.2-19.7 2.5 Mb.	deletion	
Case 4. Psycomotor delay, obesity, round facies, macroglossia and macrostomia, prognathism, synophris, thick eyebrows, strabism, depressed nasal bridge, bulbous nasal tip, small feet.	17p11.2 16.5-20.1 3.6 Mb	deletion	
Case 5. Neonatal hypotonia, mild mental retardation, visuo-spatial deficit, sociable attitude, hoarse voice, strabismus, dysmorphic features such as telecantus, epicantus, down-slanting palpebral fessures, medial flared eyebrows.	17p11.2 16.5-20.1 3.6 Mb	duplication	Reciprocal of the Smith-Magenis syndrome deletion
Case 6. Truncal obesity, tall stature, hypoacusya, hypothyroidism.	Xq25.1 124.7-127.5 2.8 Mb	deletion	Polymorphism
Case 7. Mental retardation, obesity, thick lips, large prominent teeth, tapering fingers, wave shaped eyelids.	2q37.3-qter 242.4-ter 0.5 Mb	deletion	

P0293. Array-CGH screening in 100 patients with mental retardation

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Mental retardation (MR) is a major problem affecting 3% of the population. Chromosome imbalances larger than 5 Megabases are detected by 550 band resolution R or G band karyotype. The Array-CGH is able to detect interstitial smaller deletions or duplications.

We report on the results obtained by molecular karyotyping with 1Mb BAC and PAC array-CGH screening in a population of 100 patients affected by MR with or without multiple congenital anomaly (MCA). For

25 patients of this study, an abnormal karyotype had been previously identified.
We used the 1Mb BAC and PAC Array (VIB Leuven), six patients were analysed by experiment using a triangle testing. The data were analysed with the arrayCGHbase software provided by J.Vermeesch.
Results : Copy number variants (CNV) were found in 45% of patients : 24 known and 7 not yet described.
Array-CGH confirmed and mapped the unbalanced karyotype rearrangements in all the 25 patients with known cytogenetic abnormality. For two of these patients, additional deletions and duplications improved the cytogenetic analysis and therefore modified the genetic counselling.
Among the 75 left MCA/MR patients : Two had large deletions and one had a large duplication involving homogeneously R-stained regions regarding 1p (3.2Mb), 10q (2.2Mb) and 11q (1.5Mb) loci. Nineteen patients had rearrangements involving a single BAC or PAC clone.
For three cases, the mother became pregnant during the study and a prenatal diagnosis could be proposed. These results will be reported and discussed with the clinical data.
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P0294. Array-CGH characterization of familial and *de novo* "apparently balanced" translocations in patients with abnormal phenotype

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We have applied 1Mb resolution array-CGH to investigate 12 cases of "apparently balanced" translocations in patients with mental retardation and congenital malformations. Six cases were *de novo* and six familial. In the familial cases the patients had an abnormal phenotype but their karyotype appeared identical to other phenotypically normal translocation carriers of the family. Chromosomal and various FISH analyses suggested that the rearrangements were "truly balanced" in all patients. Array-CGH however, revealed cryptic genomic imbalances in three cases (25%), two *de novo* and one familial. All array-CGH findings were confirmed by FISH. The nature and type of abnormalities differed among the three cases. In the first case a *de novo* t(9;15)(q31;q26), a complex rearrangement was identified involving a ~6.1Mb duplication on chromosome 9, a ~10Mb deletion and an inversion on chromosome 15. These imbalances were near but not directly associated with the translocation breakpoints. In the second case a *de novo* t(4;9)(q26;p24), a ~6.6Mb deletion was identified on chromosome 7 which is unrelated to the translocation. In the third case (t(4;7)(q21;p15)-familial), a ~4.3Mb and a ~2.3Mb deletions were found at the translocation breakpoints. In the remaining cases the translocations appeared balanced at 1Mb resolution. This study provides additional evidence that cryptic genomic imbalances are common in patients with abnormal phenotype and "apparently balanced" translocations not only in *de novo* but also in familial cases. The use of microarrays with higher resolution such as high-density oligo-arrays may reveal that the frequency of cryptic genomic imbalances among these patients is even higher.

P0295. Effect of borax to human chromosome abnormalities

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The effect of borax to human chromosome abnormalities was analysed in this study. Borax is a form of boron compound. The chemical name of borax is sodium borate. Borax is toxic for the somatic cells and may cause abnormal human genetic materials. Venous blood from 30 male students in Thammasat University (age 18 - 25 years) were collected to do lymphocyte cell culture. This experiment was divided into two groups, the first group was the control group and the second group was the experimental group. The lymphocyte cells in the control group were cultured without borax. The experimental group was divided into four sub-groups. The lymphocyte cells in each experimental sub-groups were cultured with different borax concentration (0.1 mg/ml, 0.15 mg/ml, 0.2 mg/ml and 0.3 mg/ml respectively). Human chromosomes were studied for abnormalities through Giemsa-staining and G-banding. The results show that the number of metaphase chromosomes are reduced when lymphocyte cells are cultured with 0.15 mg/ml (57.22 %), 0.2 mg/ml (50.84%) and 0.3 mg/ml (42.27%) borax concentration. The results show statistically significant difference between control and experimental sub-groups ($P<0.05$). The sister chromatid separation is found in the 0.3 mg/ml borax concentration experimental subgroup. It shows that borax (0.15, 0.2 and 0.3 mg/ml) effect to cell and human chromosome abnormalities (both numerical and structural abnormalities). Borax may cause human chromosome abnormalities and lead to genetic defect.

P0296. Significance of Her-2/neu, c-myc amplification and p53 inactivation detected by FISH in Egyptian patients with breast cancer

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Breast cancer is a leading cause of cancer-related deaths in women worldwide. The clinical course of this disease is highly variable and clinicians continuously search for prognostic parameters that can accurately predict prognosis, and indicate a suitable adjuvant therapy for each patient. Amplification of the two oncogenes: Her-2/neu and c-myc and inactivation of the tumor suppressor gene p53 are frequently encountered in breast carcinomas. The purpose of this study was to use the fluorescence *in situ* hybridization (FISH) for the assessment of Her-2/neu and c-myc amplification and p53 inactivation and to relate these molecular markers with the clinicopathological factors. The study was conducted on 34 samples obtained from 33 females and 1 male with breast carcinomas and 17 samples obtained from 16 females and 1 male with benign breast lesions. Results revealed that the level of her-2/neu, c-myc and p53 in the malignant group was significantly increased as compared to the benign group. On relating to clinicopathological factors, p53 was significantly associated with increased patient's age. The sensitivity of the investigated markers significantly increased with larger tumor size. Her-2/neu and p53 showed a significant increase in low-grade tumors whereas c-myc showed a highly significant increase in high-grade tumors. Her-2/neu and c-myc showed significant increase at late stages of disease. p53 and her-2/neu were significantly associated with positive lymph nodal status. A significant correlation was obtained between the levels of the three biomarkers to each other. Conclusively, the combination of Her-2/neu, c-myc and p53 can stratify patients into different risk groups.

P0297. Mosaic Down syndrome with i(21q) associated with der(22)t(21;22)(q11.2;q11.2) derived from the carrier mother with mosaic duplication of cat eye critical region.

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Rearrangements of 22q11.2 region represent several genomic disorders, including the 22q11.2 deletion syndrome (VCFS/DGS), cat eye syndrome (CES) and der(22) syndrome. The rearrangements are thought to be caused by nonallelic homologous recombination between low-copy repeats (LCR22s). The 22q11 rearrangements disorders have a wide variety of clinical features.
We report a boy with mosaic Down syndrome with 46,XY,i(21)(q10) and 46,XY,der(22)t(21;22)(q11.2q11.2), who has mild developmental delay and dysmorphic appearance. His mother, phenotypically normal female, also had the 46,XX,der(22)t(21;22)(q11.2;q11.2) clone with normal female karyotype with the ratio of 2:8. FISH analysis using BAC clones mapped 22q11 region (RP11-100K2, RP11-71J20, RP11-357H16) showed that the breakpoint was proximal to the LCR-2. The duplicated region was correspondence with the CES type I region. The mechanism of mosaic i(21q) is likely to be associated with the rearrangements and division of chromosomes in mitosis with unbalanced translocation between the acrocentric chromosomes. This is the first case report of duplication of CES type I region with mosaic Down syndrome. We present phenotypic variation among the 22q11 rearrangements disorders including the boy and his mother.

P0298. Female-specific instability of pericentromeric regions in human embryo development: the earliest clinically significant manifestation of difference between the sexes

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Recent studies on the sex ratio (SR, male to female ratio) among chromosome mosaicism carriers suggested female-specific chromosome loss and centromere instability during early human development [1]. Mosaicism results from later gestation postzygotic formation, while formation of a chromosome rearrangement in the first zygotic cleavage results in nonmosaics. Purpose: Nonmosaic homologous acrocentric rearrangements (Rea), both balanced (blRea) and unbalanced (unblRea) mostly result from post-fertilization events [2]. Therefore, the study on the SR in carriers of a de novo nonmosaic Rea might elucidate when sex-specific centromere instability becomes manifest. Method: Review of balanced and unbalanced homologous Rea cases of known sex and mode of ascertainment identified from the literature. Results: (1) Increased proportion of females in prenatally detected carriers of both blRea (5M/8F) and unblRea (10M/13F). (2) Female prevalence among postnatally ill-defined carriers of unblRea (25M/39F). (3) These data cannot be explained by a strong intrauterine selection against male carriers of unblRea since a female preponderance was found among miscarried fetuses (8M/13F). (4) Female prevalence among patients with PWS due to 45,der(15q;15q) (6M/14F). (5) Overall, there were 54 males and 87 females in the study group (SR=0.62), significantly different from the expected ratio of 1.06 (p=0.0023). Conclusion: These observations indicate sex-specific centromere instability at the earliest stage of human embryo development. It is suggested that reactivation of the paternal X chromosome occurring in the female zygote, might interfere with the chromatin remodeling process, thus contributing to the increased fragility of pericentromeric regions.

[1]Kovaleva NV. AJMG 136A:401-13
[2]Robinson et al. AJHG 54:290-302

P0299. High resolution oligo array CGH analysis of challenging samples.

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In recent years, array-based Comparative Genomic Hybridization (aCGH) has been refined to determine chromosomal changes at progressively higher resolutions. This evolving technology is, however, somewhat hampered by the large amounts of input DNA required - a minimum of 150,000 copies of a human genome, or 0.5 µg, are generally needed to process one aCGH microarray. The GenomePlex Whole Genome Amplification (WGA) kit provides a rapid method for processing biological samples of limited quantity, expanding the application of aCGH technology to analysis of nanogram quantities of DNA. Furthermore, this global exponential amplification method enables researchers to representatively amplify genomic DNA from samples that have been fragmented to an average size of less than 1 kb. This feature may enable CGH analysis of FFPE samples that were previously thought to be unusable due to their limited amounts of DNA and high level of degradation. The data presented in this poster demonstrate the high quality data that can be generated using Agilent's high density oligo aCGH microarrays in combination with Sigma's GenomePlex WGA kit. Firstly, WGA lowers the required minimum amount of starting DNA to as little as 10 ng. In addition, the ability to amplify low molecular weight DNA, less than 0.5-1 kb in size, is demonstrated. Finally, we compare the performance of the GenomePlex WGA method to that of a Phi29-based isothermal amplification method in generating high quality aCGH data on Agilent microarrays. All aCGH testing has shown GenomePlex amplified material to behave comparably to larger quantities of purified genomic DNA.

P0300. Comparison of subtelomeric FISH, Multi-FISH and CGH for the screening of chromosomal rearrangements in 90 patients with unexplained mental retardation and dysmorphic features

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Mental retardation affect 1-3% of the general population and the aetiology remains unknown in many cases. Conventional cytogenetics detected rearrangements of more than 10 Mb in size. The development of molecular cytogenetic techniques permits to identify cryptic rearrangements, but their frequency is uncertain in patients with unexplained mental retardation. Only few studies have been performed to compare the potential of molecular cytogenetic techniques to detect chromosomal rearrangements. Here we present a study including 90 patients with unexplained mental retardation and dysmorphic features with normal high-resolution karyotypes, investigated by subtelomeric FISH, CGH and Multi-FISH.

A total of 6/90 abnormalities were found (6.6%), including three de novo deletions (1pter, 4pter and 3q21.1-q21.3), two de novo unbalanced translocations [der(8)t(6;8)(p25;p23] and [der(22)t(10;22)(q26;q13)], and one unbalanced translocation inherited from the father [der(2)t(2;10)(q37;q26)pat]. Subtelomeric FISH evidenced 5/6 chromosomal unbalances but could not detect the interstitial 3q21.1-q21.3 deletion. CGH revealed 6/6 chromosomal rearrangements but did not detected the 10qter duplication of the de novo unbalanced [der(2)t(2;10)(q37;q26)pat] translocation. Multi-FISH did not permit to detect any chromosomal abnormalities. Molecular cytogenetics studies with specific BACs probes of the rearrangements are in progress to better define the size of the chromosomal abnormalities. We compared our results with previously published studies. This study confirmed the significant frequency of subtelomeric rearrangements in patient with unexplained mental retardation, dysmorphic features and demonstrates the importance of CGH technology to detect interstitial and subtelomeric rearrangements.

P0301. Identification of prognostic chromosomal aberrations in childhood acute lymphoblastic leukemia

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Cytogenetic abnormalities emerge as a very important characteristic of chilhood acute lymphoblastic leukemia (ALL) with major diagnostic and prognostic impact.

We report bone marrow cytogenetic analysis (CTG) at the time of diagnosis 85 children (36 females and 49 males) with de novo ALL aged from 2 months to 15 years old. Uninformative investigations were 2.1%. Normal karyotype was detected in 40.0% (3 with constitutial +21) cases. The analysis was performed in the period of the last five years.

Clonal chromosomal abnormalities were present in 60% of the patients. Changes in ploidy were found in 65.3% of abnormal cases. The distribution of ploidy groups was: hyperdiploidy with >50 chromosomes in 34.7% (seven of them were associated with structural chromosomal changes), hyperdiploidy (47-50 chromosomes) in 14.2%, pseudodiploidy in 4.1%, hypodiploidy (35-45 chromosomes) 12.2% of the patients. The most frequently acquired numerical abnormalities were: +4, +6, +8, +14, +17, +18, +21 and +X. Structural aberrations were found in 34.7% of the patients. The most frequent structural aberrations were: del(6q), del(9p), t(9;22), rearrangments of chromosome 14q, 5q and 12p (mostly like a part of complex karyotype), and t(12;21)(p13;q22.3).

The stuctural and numerical aberrations observed in 69.5% patients were correiated by FISH. Translocations were: t(12;21), t(9;22), t(1;12), and one marker chromosome was identified as der (21)t(21;?). For residual disease, interphase-FISH in combination with cell enrichment or RT-PCR is extremely useful.

Cytogenetic investigations at diagnosis and during follow up documented unique chromosomal aberrations which yielded diagnostic and/or prognostic significance for each relevant patient.

P0302. Cytogenetic analysis of the human genome reactivity to the potentially mutagen environment represented by ionizing radiation

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One of the most important mutagen agents in developing different types of cancer is the ionizing radiation. So, in this study, we are focusing our analysis on persons who are working in a potentially mutagen environment (near an accelerator for ionizing radiation).

We've analyzed twelve persons (one control and ten workers) by classical cytogenetic technique and by FISH.

After the cytogenetic analysis we've determined that there were not any important chromosomal modifications, the only one present being PCDs (premature centromere division) with a frequency of 5 -11%. Counting on the fact that the PCDs was present even in the control in 2 % frequency and in the field literature being considered normal to have 7-8% of PCDs, we have concluded that the percentage we've obtained for that subjects is normal and it is not due to the effect of the working environment.

The most important targets for ionizing radiation being the telomeres, we've tested from this point of view on our subjects the eventual effect of radiation, presumptive to be in the environment, by FISH technique.

Comparing the frequency of the telomeric signals obtained from the control (98%-100 %/metaphase) to those from that subjects (91%-96%/metaphase), we found that the differences can not be considered significant.

Corroborating the obtained data, we can conclude that for our eleven analyzed subjects there are not obvious chromosomal modification determined by fact that they are working with a radiation source.

P0303. Frequency of mosaic aneuploidy in children with idiopathic autism

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It has been repeatedly suggested that numerous human diseases including major psychiatric disorders could be associated with mosaic aneuploidy. Here, we have attempted to test this hypothesis concerning autism, a common childhood psychiatric disorder. About 5-10% of autism cases are known to be caused by chromosomal abnormalities or gene mutations. However, mosaic aneuploidy in autism has not ever been studied. We surveyed stochastic aneuploidy in children with/without idiopathic autism by interphase multiprobe fluorescence in situ hybridization (mFISH). The rate of chromosome loss and gain involving six arbitrarily selected autosomes (chromosomes 1, 9, 15, 16, 17, 18) and sex chromosomes was assessed in peripheral blood cells of 60 unaffected male children and 116 male children with idiopathic autism. Studying over 420,000 cells, we have determined the mean frequency of stochastic aneuploidy in control and autism:

Control	Chromosome loss	Chromosome gain
Autosomes Sex chromosomes	0.58% (95% CI 0.42-0.75%) _	0.15% (95% CI 0.09-0.21%) 1.11% (95% CI 0.90-1.31%)
Autism		
Autosomes Sex chromosomes	0.60% (95% CI 0.37-0.83%) _	0.22% (95% CI 0.14-0.30%) 1.01% (95% CI 0.85-1.17%)

CI - confidence interval

The difference of stochastic aneuploidy rate was insignificant. However, the frequency of mosaic aneuploidy over the background level was found in 19 (16%) of 116 children with idiopathic autism, while outlier values were not found in controls. These data identify mosaic aneuploidy as a new autism genetic risk factor. Therefore, molecular cytogenetic analysis of somatic mosaicism is warranted in children with idiopathic autism. Supported by INTAS and RGNF (060600639a).

P0304. Cytogenetic examination of healthy individuals for the assessment of their individual sensitivity to ionizing radiation

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The increased level of chromosomal abnormalities in somatic cells is proposed as the marker of increased cancer risk. Recent reports have suggested that elevated chromosome aberration yields observed following irradiation of peripheral lymphocytes in G_2 phase of cell cycle caused by the impaired DNA repair and hereditary predisposition to cancer.

G_2 assay is based on high chromosomal sensitivity of human peripheral blood lymphocytes to radiation. Examination of 103 relatively healthy individuals was carried out by means of the adopted G_2-assay consisting in the analysis of radiation induced cytogenetic effects in peripheral blood lymphocytes in the most radiosensitive post-synthetic phase of cell cycle. The dose of test gamma-irradiation of cell cultures was 1,5 Gy.

The obtained cytogenetic parameters induced by the test irradiation in G_2-phase of cell cycle revealed high interindividual variability: 0,18 - 1,24 aberrations/metaphase, with mean value - 0,410,10; coefficient of variation (CV) - 24%. Chromatid breaks prevailed in aberration spectra up to 98% with mean 0,370,097, CV = 27%. Analysis of the character of chromatid aberrations distribution made it possible to reveal 12% (12/103) individuals with increased chromosomal radiosensitivity. Indications, which reflect the prime contingent for cytogenetic assessment of individual radiosensitivity, were carried out.

Taking into account connection between the radiation induced high levels of chromosomal aberrations, genome instability and the tendency toward increased risk of cancer, individuals with high values of chromosomal radiosensitivity can be related to the group of potentially increased radiation and cancerogenic risk who require thorough medical and cytogenetical monitoring.

P0305. Clinical findings in a patient with interstitial deletion 1q31 and two patients with terminal deletion 1q syndrome

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We report 3 cases of de novo 1q deletion. The first case is 3 years 6/12 months old child that has a story of IUGR. His language and social developmant was delayed but gross motor skill was normal and he had microcephaly, fine and sparse hair, sparseness on medial parts of eyebrows, flat-high forehead, broad nasal root, short nose, low-posterior set ears, simian crease on his left hand, clinodactyly and micropenis. His weight, height and head circumference were under 3. centile. An accessory spleen found in abdomen USG. Karyotype analysis showed 46,XY, del (1)(q25.3-q31.3). Second and thirth cases are 3 years and 4 years 7/12 months old of males. They both have a story of SGA and clinical features of hyperactivity, severe neuromotor and mental retardation, fine-sparse hair, microcephaly, epicanthus, hypertelorism, depressed nose root, hipospadias and seizures The second case also has prominent glabella, pseudostrabismus, big ears, cryptorchidism and his MRI shows agenesia of corpus callosum. The thirth case was referred us with the complaint of frequent pulmoner enfections with cellular immune deficiency and atypic face that consists of full cheek, microretrognathia apart from the second. These cases karyotype analysis revelaed 46,XY, del (1) (q42.2-qter). In conclusion; our first case has similar features with two other cases of 1q31 deletion reported in the literature, however, motor development was normal. Second and thirth cases each have the characteristic features of del 1(q42-qter) patients reported before as hipospadias, severe motor-mental retardation, seizures, depressed nose root, fine-sparse hair and agenesia of corpus callosum.

P0306. Lipoatrophic panniculitis and developmental delay associated with an interstitial deletion of chromosome 10q

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A three year-old girl presented with a history of an erythematous rash on the lower limbs that was followed by widespread atrophy of fat all over the body, but sparing the head. Skin biopsy revealed findings in keeping with Weber-Christian panniculitis (WCP), but no other signs or laboratory findings of autoimmune disease. She later developed a patch of morphoea in the epigastric region. She had had a history of an operation to remove an area of fibroepithelial hyperplasia on the hard palate as a baby, and she had a known diagnosis of mild pulmonary valve stenosis and a small ventricular septal defect (now closed). She had a broad face but was not strikingly dysmorphic. Speech development was very significantly delayed, and other aspects of development were only mildly delayed. Chromosome analysis revealed an interstitial deletion of chromosome 10q, ISCN: 46,XX,del(10)(q21.2q22.1). Further analysis to refine the breakpoints is ongoing. WCP is a rare autoimmune condition resulting in the inflammatory destruction of fat cells and localised or generalised lipoatrophy. Deletions of this region of chromosome 10q are rare, although WCP has been reported in a case of an unbalanced translocation of 10p material onto 10q26. We postulate that a gene (or genes) within our patient's deleted region is involved in WCP (and potentially similar autoimmune disorders).

P0307. Prenatal diagnosis of a *de novo* partial duplication of the long arm of chromosome 17 in a foetus with polydactyly, cardiopathy and hydrocephaly

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We report a female dysmorphic infant born at 37 weeks of gestation who presented polydactyly, hydrocephaly, short limbs, a VSD, and hypotonia. Fetal ultrasounds performed at 29 weeks of gestation showed IUGR, short limbs, cardiopathy, and hydrocephaly. The clinical diagnosis of Ellis-van Creveld syndrome was evoked. Amniocentesis was performed and revealed a partial duplication of chromosome 17, confirmed by FISH analyses. Parental karyotyping was normal, indicating a de novo anomaly in the child. Parents decided to continue the pregnancy. At birth, the baby had dysmorphic features, including small and low-set ears, long philtrum, micrognathia, midface hypoplasia, down-turned corners of the mouth with thin lips, low-set and wide-spaced nipples, short neck, pre- and postaxial hexadactyly of upper and lower limbs, single palmar crease, rhizomelia, wide anterior fontanel, hyperlaxity of knees, and hirsutism. She was hypotonic, had feeding difficulties, and died after ten days of life from a necrotizing enterocolitis. Postnatal karyotyping confirmed the partial duplication of chromosome 17q. Microarray CGH confirmed the duplication of chromosome 17q for the region localized between q21.31 and q23.2. A pure partial duplication of the long arm of chromosome 17 for this region has only rarely been reported so far. Clinical findings present in our case are compared to those of other reported cases with dup(17q).

P0308. Phenotype-genotype correlation in Egyptian patients with chromosome 18 aberrations

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The purpose of the present study was to provide an insight into the understanding of the relationships between the clinical phenotypes and chromosome 18 abnormalities.

Fifteen patients were recruited with chromosome 18 abnormalities (8 males 7 females).

Their age ranged from 2 months to 28 years. Positive consanguinity was present in 9 patients. Similarly affected sibs in the family were found in 5 patients. There was delayed milestones and broad forehead in 9 patients (60%), ear abnormalities in 12 (80%), short stature was present in 3 patients (20%), epilepsy in 4 (26.7%), eye anomalies in 7 patients (46.7%) and recurrent abortion in 2 patients, brain demyelination was present in 9 patients accompanied with hypgenesis of corpus callosum in 3 patients and mental retardation was present in 7 patients. Chromosomal studies showed 18 deletion in 7 patients (deletion of 18p in 5 patients and 18q in 2 patients), translocation in 3, and duplication in 2 patients, inversion in 2 and ring in one patient. The collection of the cytogenetic and clinical data will help to delineate the phenotype associated with various chromosome 18 aberrations these findings will be informative for the families at risk in genetic counseling and prenatal diagnosis for subsequent pregnancies. In this study there was bilateral knee dislocation and hyperpigmentation in a case of 18(q11.2) duplication a finding to our knowledge has not been described before, which indicate a possible relation ship between chromosome 18 and knee dislocation.

P0309. Case report. A newborn with ring chromosome 21.

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A newborn boy with congenital heart and cleft palate malformations and with hypotrophy -2SD was hospitalized. The chromosome analysis from cultured lymphocytes was performed using trypsine-Giemsa method. The chromosome abnormality was established: 46,XY,-21,+mar. There was very little dark part from chromosome left.

The FISH analysis was performed and revealed a ring chromosome 21:

46,XY,r(21)(::?-q22.13::q22.3->qter).

Usually such abnormalities originate *de novo* .

The parents refused to give material for chromosome analysis.

Several abnormalities was founded in the clinical evaluation of the patient: large bilateral cleft palate, microrethrognathia, deep-set eyes, short palpebral fissures, big nouse, bicuspidal aortic valve, pulmonar stenosis.

The patient died about 12 month age.

P0310. Five cases with chromosome anomalies involving chromosome 5 - genotype-phenotype correlation

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We present 5 cases with different abnormalities involving chromosome 5 in order to illustrate suggestive features for the diagnosis, but also to discuss management directions (investigation protocol and follow-up). The cases were diagnosed between 2002-2006 in Iasi Medical Genetics Center.

Patient 1: (3-year-old female): pregnancy- uneventful; birth- term, Apgar score 5, Wt 2,700 g; clinical features- failure to thrive, microcephaly, typical cri-du-chat face and cry, stridor, hyperextensible joints, hypertrichosis, severe mental retardation; karyotype- 46,XX,del(5p). Normal echocardiography and ophtalmologic examination.

Patient 2 (3-year-old female): pregnancy- polyhydramnios; birth- term, intrauterine growth retardation, Apgar score 8; clinical findings- growth retardation and microcephaly, dysmorphic face, typical cry, bilateral clynodactyly 5, moderate mental retardation. Echocardiography- subaortic VSD, tricuspid insufficiency; karyotype- 46,XX,r(5)(p21;q35)/45,XX,-5/47,XX,r(5)(p21;q35),r(5)(p21;q35). Normal parental karyotypes. Detailed phenotypical analysis is presented.

Patient 3 (5-year-old male): pregnancy- uneventful; birth- term, Wt 2,600 g; clinical findings- growth retardation, microcephaly, dysmorphic face, hypertrichosis, irregular toe insertion, severe mental retardation. Echocardiography- incomplete atrioventricular canal. Karyotype: 46,XY,add(5)(p15).

Patient 4 (male, aged 3 years): pregnancy- uneventful; birth- preterm, Wt 1,900 g; clinical features- failure to thrive, microcephaly, seizures, mild dysmorphic face, moderate mental retardation; karyotype: 46,X Y,ins(8;5)(p23;p11p13). Even if the chromosome formula looked balanced, we appreciate that the phenotype is related to the breakage points.

Patient 5: young, unrelated, apparently normal couple with repeated miscarriages. Female karyotype: normal; male karyotype: 46,XY,ins(5;4)(q31;q27q31).

Genotype-phenotype correlation and management protocol will be

provided for every case.
In conclusion, we present 5 different chromosomal abnormalities involving chromosome 5 in order to discuss variability of clinical features and management protocol.

P0311. Structural chromosome abnormalities and male fertility

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The aim of this study is to evaluate the prevalence of structural chromosome abnormalities in infertile men and to assess their effect on the spermatogenesis and male fertility.
The examined cohort consisted of 550 men from infertile couples. The semen analysis has been performed according to the WHO recommendations (1999). According to the sperm tests all patients were subdivided into five groups: azoospermia (n=177), oligozoospermia (n=203), asthenozoospermia (n=81), teratozoospermia (n=39), and normozoospermia (n=50). Chromosome analysis has been carried out on peripheral lymphocytes using standard cytogenetic techniques with GTG- and C- staining.
In 3.1% examined men structural chromosome abnormalities have been found. The translocations have been found in 16 patients, and pericentric inversion of chromosome 5 - in one azoospermic patient. In 53% cases the chromosome rearrangements are Robertson translocations. Most commonly chromosome translocations between chromosomes 13 and 14 were found.
Chromosome aberrations have been found in 1.7% azoospermic, 4.4% oligozoospermic, 2.6% teratozoospermic, 3.7% asthenozoospermic, and 2.0% normozoospermic men. Between patients with revealed chromosome abnormalities the azoospermia has been diagnosed in 17.6%, oligozoospermia - 52.9%, asthenozoospermia - 17.6%, teratozoospermia - 5.9%, and normozoospermia - in 5.9% cases.
Our results have demonstrated obvious polymorphism of status of sperm parameters in men with structural chromosome abnormalities. In most infertile men the chromosome translocations associated with oligozoospermia.

P0312. Molecular characterization of terminal Xp deletions by m-Banding and Array-CGH in four families

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Xp deletions are diagnosed in about 6% of Turner stigmata. The phenotype is probably associated with haploinsufficiency of genes within the deleted regions but the correlation between the length of the deletion and the phenotype is still unknown.
We studied the length of the terminal Xp deletion through four families. We report here the phenotypic and laboratory findings in 8 female members affected by the Xp deletion. The deletions were diagnosed cytogenetically using 400 band R and G-Banding, X specific mBanding and were further delineated by array comparative genomic hybridisation (array-CGH). We used a 1 Mb BAC and PAC array (VIB Leuven), DNAs were tested by experiment using a triangle testing with control DNAs. Data were analysed with the arrayCGHbase software (J.Vermeesch).
Results:
Cytogenetic studies showed the following breakpoints: del(X)(p22.2), del(X)(p21.3), del(X)(p11.4), der(X)(qter->p22.2::q22->qter) in families 1,2,3 and 4 respectively.
Array-CGH established the length of the Xp deletions: 7.5 Mb, 20.3 Mb, 24.2 Mb, 9.5 Mb in families 1,2,3 and 4 respectively.
None of the 8 female patients had mental retardation. Their common feature was short stature when present (height: 1.40m to 1.64m in adults). Interestingly SHOX region was deleted in all of them. Array-CGH enabled a comparison of each genotype-phenotype association. An important interindividual and intrafamilial variability was observed, caused by parameters such as X inactivation bias, or specific gene deletion or disruption. Our data showed no correlation between the adult height and the deletion length and may contribute to the evaluation of GH treatment when proposed.
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P0313. Molecular characterization of chromosome Y structural abnormalities in two cases of ambiguous genitalia

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Malformations of the external and/or internal genitalia may be caused by structural abnormalities of sex chromosomes.
With the aim to understand the underlying cause of ambiguous genitalia, and to contribute at management of the patients with abnormal phenotypic sex, we investigated two cases using GTG, CBG banding, accomplished by FISH and PCR techniques.
FISH included: DAPI stain, Y-specific painting probe XCP-Y (MetaSystems), Ycen DYZ3, Yq12 DYZ1, Yp11.3 SRY, Xcen DXZ1 (Vysis), subtelomeric probes for X- and Y-chromosome, locus DXYS130 and DXYS224 (Q-Biogene). PCR method was performed with a short DNA sequence from Amelogenine gene on the short arm of both X and Y chromosomes (Xp22.31-p22.1, Yp11.2), plus DYS392 STR marker (Yq11.2).
GTG banding showed mosaic karyotypes in both patients: 45,X[84]/46,X idic(Y)(qter--pter::pter--qter)
[16], and 45,X[65]/46,X idic?(Yp)[35], respectively. By CBG banding the provenience of idic(Y)(pter--qter) it was clear in the first case, but difficult in the second one. By FISH, the karyotype was:
1).46,X,psu idic(Y)(p11?.2).ish psuidic(Y)(qter--pter::pter--qter)(wcpY+,DXYS130++,SRY++,DYZ3++.
DYZ1++,DYS224++). PCR revealed both Amelogenine gene sequence and DSY392 marker.
So, it was confirmed that abnormal Y is an isodicentric with breakpoint distal from subtelomeric locus DXY130 in Yp.
2). FISH karyotype was: 46,X,?idic(Y)(q11).ish idic(Y)(pter--q11::q11--pter)(SRY++). PCR revealed Amelogenine gene sequence, but not DSY392 marker so, the fusion point is in the long arm Yq11.
By our results we can conclude that, various shapes of structural abnormalities in Y chromosome may result in disorders of sex determination pathway which are poorly understood. Only the presence of SRY gene is not enough for male phenotypic normality.

P0314. Cytogenetic diagnosis of chromosomal pathology in various stages of pregnancy

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The detection of chromosomal anomalies is one of the main tasks in prenatal diagnostics. It is known, that the majority chromsome abnormalities result fetal death or the birth of children with handicaps. We studied fetal karyotypes in a group of high-risk pregnancies.
Materials and methods of research. In total we analyzed chorion villus and fetal blood of 879 pregnant women in various terms of pregnancy.
The preparations of chromosomes were prepared from chorion villus by direct culture. For preparation of preparations from fetal blood used standard methods with addition PHA.
Results of research and discussion. The analysis cytogenetically of results has shown, that in among chromosomal abnormalities, numerical anomalies prevailed. In 53,3 % cases Down's syndrome was diagnosed - 47, XY +21, both complete and mosaic forms. In 3,3 % cases we diagnosed Patau syndrome - (47, XY+13). In 25,9 % cases we detected marker chromosomes. Besides numerical changes we detected structural changes in 10 % cases: Robertsonian translocation - 45, t(15;21), 46,isoX(q) and a reciprocal translocation - 45, t(3;Y).

P0315. Detection of mixed chimerism and minimal residual disease after bone marrow transplantation in chronic myeloid leukemia by conventional and molecular cytogenetics in tunisian patients

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Chronic Myeloid Leukemia (CML) is an hematopoietic malignancy characterized by the proliferation of myeloid precursors. The hallmark of CML is the presence of Philadelphia (Ph[1]) chromosome that results from balanced reciprocal translocation between chromosomes 9 and

22 leading to the formation of *bcr/abl* fusion gene.
Disease relapse still represents the major cause of treatment failure in patients with CML, following bone marrow transplantation. Thus, the early detection of minimal residual disease (MRD) has relevant therapeutic implications.
Sequential cytogenetic analysis was done using standard methods in bone marrow samples from10 patients with CML. Further dual colour Fluorescence In Situ Hybridisation (FISH) analysis was done to assess molecular response using *bcr/abl*. The chimerism pattern and bcr-abl status were studied. The results of present study show that bone marrow transplantation induce both cytogenetic and molecular response in a significant proportion of CML thereby improving their prognosis and survival. The importance of using FISH analysis on interphase nuclei and poor-spread metaphases that cannot be analyzed using conventional cytogenetics was also highlighted. Thus the present study stresses on the need for sequential cytogenetic and molecular analysis in diagnosis and disease management of myeloid leukemias.

P0316. Molecular cytogenetic characterization of der (9) deletions in a case of Philadelphia-negative chronic myeloid leukemia

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About 95% of the patients with chronic myeloid leukemia(CML) have a Philadelphia chromosome resulting from a reciprocal translocation between bands 9q34 and 22q11.2 that juxtaposes the 3'*ABL* gene to the 5'*BCR* gene. Recent studies using fluorescence in situ hybridization(FISH) have reported deletions due to the loss of sequences proximal to chromosome 9 breakpoint or distal to chromosome 22 breakpoint. In this paper we report on a detailed molecular cytogenetic characterization carrying der(9) deletions in an apparently Philadelphia negative CML patient.
FISH experiments were carried out in bone marrow sample from CML patient at diagnosis. A detailed study using conventional cytogenetic and FISH analysis was done using ES-FISHprobes, Whole chromosomes painting and BAC in order to elucidate the mechanism of 9q deletion.
ES-FISH probes disclosed the *BCR/ABL* fusion gene on der(22)chromosome and deletion of signal on der(9) chromosome.
Whole chromosomes painting revealed deletion of *ABL/BCR* fusion gene on der(9).
Using FISH with an appropriate set of BAC probes located proximally to BCR and ABL genes we have characterized the deleted region on both chromosomes 9 and 22.
Our study shows that the location of the deleted sequences was downstream of the *BCR* breakpoint and upstream of *ABL* gene and that genomic microdeletions were concomitant with t(9;22) rearrangements.
FISH analysis allowed not only the identification of chromosome rearrangements that could not otherwise be detected by conventional banding procedures, but also the recognition of 5'*ABL* and 3'*BCR* deletions which could carry with it a poor prognosis that indicates rapid disease progression in CML.

P0317. Prenatal diagnosis of an unexpected *de novo* complex chromosomal rearrangement.

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We report a prenatal diagnosis of de novo apparently balanced reciprocal translocation t(2;10)(q?24;p?15.1) which turned out to be a complex event after molecular cytogenetic analysis. Fetal conventional R-banded karyotype was performed on amniocytes at 32 weeks gestation because of sonographic findings: IUGR, single umbilical artery and arachnoid cyst of the posterior fossa. To precise the breakpoint on chromosome 10, molecular cytogenetic analysis by FISH was used with a 10p14 band-specific probe: unexpectedly, the signal wasn't localized on the derivative chromosome 2 but on a chromosome 5. Then, hybridization of whole chromosome painting probes WCP 2, 5 and 10 showed an increasing complexity of the rearrangement leading to complex derivative chromosomes: der(2) and der(10) were found to have segments from the three chromosomes as a consequence of terminal exchanges and insertions. Subtelomeric probes of chromosomes 2, 5 and 10 revealed a terminal deletion of the long arm of chromosome 2 (2qter). Finally, this complex chromosomal rearrangement seemed to involve 3 chromosomes and 5 breakpoints. The pregnancy was terminated and the fetopathological examination confirmed prenatal ultrasound report and showed additional congenital anomalies: facial dysmorphism, heart defects, corpus callosum and cerebellum abnormalities. 2qter deletions are associated with variable clinical features and in our case, the 2qter microdeletion should be responsible for the phenotype. Array-CGH will be performed in order to precise the size of the 2qter deletion and to screen for additional cryptic genomic imbalances near the breakpoints or elsewhere.

P0318. Cytogenetic and Molecular studies in Egyptian patients with congenital heart defects, A pilot study.

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Objective: To analyze the frequency of chromosomal anomalies and molecular defects in Egyptian patients with congenital heart defects (CHD). Patients and Methods: The study included 34 patients with congenital heart defects; confirmation of the heart defect was accomplished by echocardiography and/ or cardiac catheterization. Chromosomal analysis was done using GTG-banding and FISH techniques. Mutation detection in transcription factor GATA4 and NKX2.5 was done using PCR/ SSCP and DNA sequencing. Results: Patients were classified into isolated heart defects (n=21) and CHD associated with other congenital malformations (n-13). Associated malformations were: (Down syndrome (2), Aarskog Scott S. (1), velocardiofacial S. (2 sibs), Russel Silver (1), hand anomaly (2), trisomy 18 (1); and dysmorphic (4). Chromosomal anomalies were found in 9/30 patients (30 %). They were: Trisomy 21 [47, XY,+21 (1); 46, XX, t(21;21) (1)]; 47, XX+13 (1); 47,XX,+18 (1); 46, XY, t (14; 18) (q11.2; p11.2) (1); 47, XY + mar (mat.) (1); 46, XX del 11q 23.3- qter (2 sibs); 46, XX, dup13q33-34 (1). DNA sequencing of NKX2.5 gene in 15 patients with isolated ASD revealed normal sequence in all cases. Sequencing of GATA4 gene in 12 patients with Fallot's tetralogy has shown polymorphism in exon 6 at nucleotide 53423 (A-G) in 2 patients. Conclusion: Chromosomal analysis of patients with heart defects and other congenital anomalies has a great impact on diagnosis, prognosis and genetic counseling of patients. The mutations in isolated defects might be mainly of somatic origin that could not be observed in peripheral blood lymphocytes.

P0319. A case with Prader-Willi phenotype and a de novo t(2;15)(q31;p11)

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In recent years, the spectrum of available methods for the characterization of chromosomal rearrangement has dramatically increased. Thus, the correlation between phenotype and genotype has been done accurately. In the present report, we describe the clinical and cytogenetic findings of a patient with 46,XX,t(2;15)(q31;p11). A 2-year-old girl with a clinical feature show Prader-Willi phenotype. The patient and her family were studied by the conventional (GTG, NOR and C-bandings) and molecular cytogenetic (for PW region) techniques. The results showed the proband with a balanced *de novo* karyotype 46,XX,t(2;15)(q31;p11) by using GTG-banding. Clinical and genetic findings were compared to the other patients published in the literature.

P0320. Balanced (9;11) translocation in a patient with mental retardation and dysmorphic features disrupts the protein tyrosine phosphatase delta (PTPRD) gene

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Here we report on an 11-year-old girl presenting with mental retardation and various dysmorphic features such as hypertelorism, depressed nasal bridge, short nose and wide mouth with plump leaps. The patient is friendly and able to smile but does not reply to any questions and demonstrates severe speech delay. She cannot write or do elementary mathematical calculations. Computed tomography revealed commissural alterations in the cerebellum and cerebral hemispheres as well as a subarachnoid spacand liquor cistern substantial narrowing.

G-banding of patient metaphase chromosomes prepared from peripheral blood leukocytes revealed a de novo balanced translocation t(9;11)(p24;q12). We have mapped the breakpoints on both derivative chromosomes by FISH with selected YAC and BAC clones. On chromosome 11 the breakpoint region contains several genes including the brain-expressed reticulon 4 receptor-like 2 (RTN4RL2) gene. The much better candidate disease gene is on chromosome 9. This breakpoint disrupts the receptor type protein tyrosine phosphatase delta (PTPRD) gene. PTPRD is highly expressed in the developing mammalian nervous system and Ptprd-deficient mice showed impaired learning with enhanced hippocampal long-term potentiation. The present case reveals novel autosomal candidate genes for mental retardation.

P0321. Idiopatic mental retardation - importance of clinical diagnostic scores for case selection

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We present a retrospective study aimed to identify the correlation between de Vries clinical score and the detection of chromosomal abnormalities in mentally retarded (MR) children. We have used the score to identify patients who should be tested by karyotiping and subsequently MLPA for subtelomeric rearrangements.

Our group is formed of 36 children (21 females and 15 males) with variable MR associated with other anomalies. 18 children had chromosomal defects, whereas 18 had normal karyotypes. In the group with abnormal karyotype, total scores varied between 3 and 7. Chromosomal anomalies identified were: numerical (4) and structural (14). Chromosomes involved were: 1,4,5,7,8,9,17,X. Deletions were the most common and correlate with a greater score (>/=4). Common items were: short stature (66.7%), microcephaly (72.3%), nasal (72.3%), ear (66.7%) and hand anomalies (83.4%). In the group with normal karyotype, 44.4% of cases had a low score, whereas 55.6% had a high score. Most frequent item is hand anomalies (61.2%). The detailed analysis of the cases will be provided. The score proved very useful for the identification of major chromosomal abnormalities. In the second group, cases with a high score have to be further tested (e.g. using MLPA) in order to identify minor defects. In our opinion a high score indicates the karyotype and then a MLPA testing.In conclusion, we present a retrospective study that proves the use of de Vries diagnostic score in the identification of chromosomal abnormalities in MR children.

P0322. Prader-Willi Syndrome due to paternal deletion of chromosome 15q11-q13 in a girl with (14;22) Robertsonian translocation

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We report a case presenting a combination of deletion (15)(q11-q13) and der(14;22), both of the paternal origin. The proposita was a second child of unrelated, healthy and young parents. Prenatal history was complicated by threatened abortion. The girl was born at 39 weeks of gestation through vaginal delivery. She had severe hypotonia and feeding difficulties in early infancy, increased appetite and rapid weight gain after 12 months. Clinical examination at the age of 25 months showed short stature (< 3rd centile), overweight (90-97th centile), facial dysmorphism (bitemporal narrowing, small mouth with down-turned corners, prominent chin), fair hair, small hands with tapering fingers and small feets. Clinical impression was that the patient had features consistent with Prader-Willi syndrome (PWS). The karyotype of the patient was 45,XX,der(14;22)pat. The karyotype of the mother and proband's sister was normal. Methylation analysis showed abnormal methylation pattern. Paternal deletion (15)(q11-q13) was identified confirming the diagnosis of PWS (molecular investigations were performed in the Institute of Medical Genetics, University of Zurich). The derivate chromosome was found to be inherited from the phenotypically normal father [45,XY,der(14;22)].

We present a new case of PWS with additional cytogenetic aberration. Our report confirms a statement about an increased risk for the parents with Robertsonian translocations to have a child with unbalanced karyotype.

P0323. Phenotypic characteristics of 9p deletion syndrome: a case report

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Background Deletion 9p syndrome is a rare congenital syndrome, originally described in 1973. Approximately 100 reported cases have been published worldwide. We present the first case of deletion 9p syndrome diagnosed in Lithuania.

Case report Our presented patient is a 3 years old girl, the second child of healthy non consanguineous parents, born on time with complicated delivery due to craniostenosis. Major complains were irritability, psychomotor retardation, hypersalivation, unstable gait. Characteristic findings included trigonocephaly, upslanted palpebral fissures, epicanthal folds, arched eyebrows, depressed nasal bridge, poorly formed ears with hypoplastic helix, an extra flexion creases on 3-4 phalanges, excess whorls on the fingers with short nails, corrected bilateral inguinal hernias and moderate mental retardation. Typical phenotype characteristics lead to the suspection of 9p deletion what was confirmed cytogeneticaly. Chromosome analysis of peripheral blood lymphocytes revealed 46,XX,del(9)(p22) karyotype. Cytogenetic analysis was performed from GTG banded metaphases. The resolution level was 400-500 bands.

Conclusion Karyotyping of both parents and detailed molecular assessment of genes flanking the breakage site would be desirable.

Key words: deletion 9p syndrome, trigonocephaly, hypersalivation.

P0324. DNA fragmentation and meiotic segregation in spermatozoa of male carriers of a chromosomal structural rearrangement.

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Frequency of somatic chromosomal abnormalities, mostly balanced reciprocal or robertsonian translocations and pericentric inversions, is increased in infertile males. Different hypotheses, such as an increase of spermatozoa with DNA fragmentation and / or with unbalanced chromosomal equipment, could explain their infertility. This study investigated the relationship between DNA fragmentation and meiotic segregation in the spermatozoa among carriers of a structural chromosomal abnormality.

DNA fragmentation of ejaculated spermatozoa from 23 carriers (12 reciprocal and 5 robertsonian translocations and 6 pericentric inversions) and 24 fertile men were analyzed using TUNEL (terminal deoxynucleotidyl transferase mediated dUTP nick end label). The frequency of unbalanced chromosomal spermatozoa among carriers was estimated using FISH with appropriate probes.

A higher sperm DNA fragmentation rate was found among carriers of a structural chromosomal abnormality (6.64% ± 5.59) compared to the controls (1.20% ± 0.95) ($p<0.001$).

The frequencies of unbalanced spermatozoa were estimated between 45.32% and 65.60% among reciprocal translocation patients, between 8.78% and 21.62% among roberstonian translocation carriers and between 0.33% and 39.06% among pericentric inversion patients. No correlation was found between the frequency of unbalanced chromo-

somal sperm and that of apoptotic sperm (p>0.05), suggesting that there is no relationship between the apoptotic and meiotic mechanisms in spermatozoa from these patients.
In conclusion, based on the increase in DNA fragmentation and the elevated rate of unbalanced chromosomal spermatozoa, this study indicates that these exams should be integrated in the genetic exploration of male carriers of a structural chromosomal abnormality and used to predict the outcome of ICSI.

P0325. DNA methylation patterns in human adult and embryonic metaphase chromosomes: similarities and differences

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DNA methylation plays key role in gene silencing and maintenance of chromatin structure. In our previous study we have shown band-specific distribution of 5-methylcytosine-rich DNA in human metaphase chromosomes from adult and fetal lymphocytes by immunofluorescence with anti-5-methylcytosine antibodies(Eurogentec). This raised question about reproducibility of 5-methylcytosine-rich DNA distribution along chromosomes from other tissues at different developmental stages. The present research was focused on study of 5-methylcytosine distribution in metaphase chromosomes from cells of different human embryonic tissues: liver, lung, brain and cytotrophoblast at 5-12weeks of gestation, as well as from adult mesenchymal stem cells. Pattern of 5-methylcytosine-rich DNA distribution was similar in all analyzed cell types: 5-methylcytosine-rich sites corresponded to all T-, majority of R-bands, to short arms of acrocentrics and heterochromatin of chromosomes 1,9,16. Specificity of signal distribution along chromosomes allowed identification of specific landmarks for each chromosome pair. These landmarks could be easily detected in any metaphase. In spite of overall DNA methylation pattern similarity, local tissue-specific peculiarities were detected. Heterochromatin of chromosomes 1,9,16 was hypomethylated in cytotrophoblast, as well as number of other bands, resulting in decreased overall methylation level, if compared to lymphocyte chromosomes. Overall decrease in methylation is also typical for mesenchymal stem cells, whereas chromosomes from embryonic tissues demonstrated either decreased or increased methylation level in number of bands. Thus, due to high reproducibility, clearly distinguishable landmarks and minor tissue-specific differences, we suggested that distribution of 5-methylcytosine-rich DNA can be considered as specific banding pattern of human metaphase chromosomes - MeC-banding. Supported by CRDF&RFBR.

P0326. An unusual transmission of a supernumerary marker chromosome from a mother to a baby presenting congenital malformation

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In humans, the presence of supernumerary chromosomes is an unusual phenomenon, which is often associated with developmental abnormalities and malformations. Among supernumerary chromosomes double minute (dms) are extensively associated with cancer cells. There are few reports of their presence in the peripheral cells of normal individuals. Double minutes (dmin) are small chromatin particles that represent a form of extrachromosomal gene amplification.
We describe a 5-month-old boy who had dysmorphological features with a karyotype presenting supernumerary marker chromosomes as minutes and double minutes in a mosaic pattern. There was no history of any teratogen exposure during the pregnancy. His developmental milestones were delayed. He was brought to hospital for the first time at 2 months of age because of dysmorphological features and hypotonia. He was noted to have depressed nasal root, blue sclera, hypertelorism, epicanthus, micrognathia, retrognathia, high arched palate, dysmorphic ears, hypopigmented skin lesion on thoracic wall, umblical hernia and hypoplastic nails. Laboratory tests including complete blood count, urinalysis, thyroid function tests were normal. Cranial magnetic resonance imaging and skeletal X-ray survey were normal. Abdominal ultrasonography revealed hepatomegaly. Visual Evoked Potentials and Brainstem Auditory Evoked Potentials revealed delayed latencies. His karyotype revealed 47,XY,dmin+[7]/47,XY,min[2]/46,XY[16]. 47,XX,+min[2]/46,XX[18] was detected in his mother's cytogenetic analysis. To the best of our knowledge this unusual transmission and presence of minute and double minute chromosomes have not been reported in the literature. This unusual existence may contribute to the literature regarding the possible association between extrachromosomal gene amplification and congenital abnormalities.

P0327. Xp duplication in two brothers: phenotypic and molecular cytogenetic characterization

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Duplications within the short arm of the X-chromosome are rare in males. Surviving males with Xp duplications are nearly all invariable mentally retarded. Here we report a detailed characterization of a partial Xp duplication in two brothers with severe mental impairment.
Conventional chromosome analysis illustrated a structurally abnormal X chromosome in both brothers. The phenotypically normal mother carried the same abnormal X chromosome, which is preferentially inactivated. The sister of the brothers had a normal karyotype. Further characterization of the aberrant X chromosome by ±1 Mb spaced large clone insert array-CGH revealed a duplication of approximately 13 Mb with the proximal and distal breakpoints located to Xp21.3 and Xp22.2. The exact karyotype of the brothers was defined as 46,Y,dup(X)(p21.3p22.2).

P0328. Molecular cytogenetic analysis of a *de novo* interstitial duplication at 1p36 in a female patient.

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Chromosomal imbalances are a frequent cause of mental retardation and congenital malformations. Although aneuploidy accounts for the majority of imbalances, structural aberrations contribute to a significant fraction of recognized chromosomal anomalies.
The female patient reported here was the first child of non-consanguineous parents. She had a healthy younger brother. The family history was unremarkable. The patient had congenital bilateral ptosis and swallowing difficulties, necessitating tube feeding during some months. Examination at the age of 7 months showed severe ptosis, epicanthus, telecanthus, strabismus convergens, short upturned nose and open mouth appearance. Examination at the age of 11,5 years showed in addition a very narrow palate, bifid uvula, narrow entrance to the pharynx, dental crowding with braces and micrognatia. She has a delayed development and mild mental retardation.
Conventional karyotyping based on G-banding revealed a duplication at the terminal end of the short arm of chromosome 1. Analysis of parental chromosomes revealed normal karyotypes suggesting a *de novo* duplication in our patient. The duplicated region was further characterized using a 250 k SNP array (Affimetrix). The duplication was 3.3 Mb in size with the breakpoints in band 1p36.33 and 1p36.32. Therefore, the karyotype was defined as 46,XX,dup(1)(p36.33p36.32).
Although monosomy 1p36 is very common, duplications of 1p36 are extremely rare. The genotype-phenotype correlation of this case will contribute to a better understanding of the effect of copy number variation of genes within the 1p36 region.

P0329. Epigenetic background of chromosomal mosaicism in human miscarriages

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Chromosomal mosaicism is a feature of abnormal embryo development. It results from mitotic non-disjunction in aneuploid or in primary euploid cells. However, the ratio of both mechanisms as well as inducing factors of mitotic instability are poorly investigated. It is interesting that increasing of mosaicism frequency is coincided with the wave of epigenetic reprogramming. We have suggested that alterations in the

methylation status of the cell-cycle checkpoints genes may be one of the factors leading to genomic instability and mosaicism. To test this hypothesis methylation analysis of the *RB1* and *P14ARF* genes in 50 miscarriages with different pattern of chromosomal mosaicism was performed. A model of tissue-specific cell lines compartmentalization was developed after meta-analysis of available literature data about mosaicism in human embryos. According to this model miscarriages with chromosomal mosaicism confirmed by interphase FISH-analysis in our study were classified into 3 groups. Mitotic origin of aneuploidy was suggested for 19% of embryos. Meiotic or mitotic errors were related with aneuploidy in 27% of mosaics. Meiotic non-disjunction was suggested for 54% of miscarriages. For the first time aberrant methylation of the promoters of the cell-cycle genes was observed. Moreover, the total frequency of epimutations was significantly reduced from 32.5×10^{-2} per allele in the group1 to 7.0×10^{-2} and 2.6×10^{-2} in the groups 2 and 3, respectively. Our finding indicates that accumulation of epimutations may increase genomic instability and mosaicism in the embryos with primary normal karyotype in the zygote. This study was supported by RFBR (N 05-04-48129) and FASI (N 02.512.11.2055).

P0330. Investigation of cytogenetic changes in late stage endometriosis by fluorescence in situ hybridisation (FISH) technique

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In the present study, the incidence of somatic chromosomal numerical alterations in chromosomes 11 and 18 in late stage endometriosis cases were investigated in comparison with the endometrium and peripheral blood samples of the same patients and the same tissues of normal healty women.
The study group was composed of six endometriosis samples (n:6). Control group included; normal endometrium samples (n:6, control I) and peripheral blood lymphocytes (n:6, control II) of the same patients with normal endometrium samples (n:5, control III) and peripheral blood lymphocytes (n:5, control IV) of the healty women.
Multicolor Fluorescence in situ hybridization (FISH) technique was used in the study and the results were statisitically analysed by Khi squared test.
For chromosome 11; the frequencies of trisomy and monosomy were 3.6 % and 0.4 % respectively the endometriosis samples and control group I the frequency of trisomy was 1.3 %. These values were statistically higher when compared with the other control groups.
For chromosome 18; in the endometriosis samples, the frequency of monosomy was 1.3 % while, in the first control group the frequencies of monosomy and trisomy were 2.7 % and 1.0 % respectively and the frquency of trisomy of the Control group II was 0.9 %. These values were statistically significant in comparison with the other groups.
Finally, the genetic alterations in chromosomes 11 and 18 were found to be significantly different ($p<0.0001$) in the endometriosis specimens than in normal endometrial cells and peripheral blood lymphocytes of the same patients and healty control group.

P0331. Whole genome array CGH (Comparative Genome Hybridization) of epilepsy patients with congenital abnormalities

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INTRODUCION: The search for chromosomal abnormalities in patients with severe epilepsy and congenital abnormalities plays an important role in neurology and neuropediatrics. Disclosure of the etiology of the seizures may be of psychological value for the family, and it is essential for proper genetic counselling. The aim of this study was to estimate the significance of whole genome array CGH for patients with epilepsy and congenital abnormalities.
METHODS: We recruited 75 epilepsy patients with different congenital abnormalities from The Danish Epilepsy Centre. All patients, except for three, had been reported to have normal G-banded karyotypes. Array CGH was carried out using a submegabase whole genome tiling path BAC array.
RESULTS: In total 39 DNA copy number changes were detected, which did not coincide with a known variant and also were not repeatedly identified in a reference set of more than 700 samples analysed on the same platform. Up to now, the hereditary basis has been investigated for 23 of the aberrations. Eight were *de novo*, two segregate with epilepsy in the family and 13 were inherited from an apparently normal parent.
CONCLUSION: In the present study we investigated 75 patients with epilepsy and congenital abnormalities. We identified copy number variations that were likely to be pathogenic in 38% of the patients. This study suggests 25 new loci to the current list of candidate loci involved in epilepsy. In conclusion, array CGH is the method of choice in identifying genetic changes in epilepsy patients with congenital abnormalities of unknown cause.

P0332. Cytogenetic contribution in diagnosis of Bloom Syndrome and Fanconi Anemia in Tunisian patients

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Fanconi Anemia (FA) and Bloom's syndrome (BS) are rare autosomal genetic diseases that predispose to cancer and are associated with genomic instability. Cytogenetic diagnosis can, in the most cases, confirm the clinical diagnosis.
In this study we report the cytogenetic data of 4 BS patients and 147 patients suspected with FA.
Spontaneous and induced chromosome damage was analyzed in cultures of PBLs from patients with FA, with BS and healthy controls.
The results show that the spontaneous frequency of chromosomal breakage was significantly higher in lymphocytes from all the patients than in the control cells.
Sensitivity of FA cells to alkylating agents such MMC is much more increased than controls cells, but sensitivity of BS cells to the action of MMC did not differ from that of control cells.
Cells from patients with BS cultured in the presence of BudR exhibited a striking increase in the number of sister chromatid exchanges (SCEs) in comparison to that in cells of a healthy patient. Thus, it appears that an increased incidence of SCE in BS constitutes the syndrome's most characteristic cytogenetic feature.
In conclusion, although, the genetic instability syndromes of FA, XP, AT, are associated, respectively, with hypersensitivity to DNA cross-linking agents, UV and ionizing irradiation, the sensitivity of BS cells to DNA-damaging agents has been controversial.The basis of their instability is totally different.

P0333. Going deep into Fanconi Anemia chromosomal instability

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We studied a cohort of 136 Fanconi Anemia (FA) patients, diagnosed by DEB test in our laboratory. The chromosomal instability (CI) has been scored, the tests having been performed by the same cytogeneticist and all revised in blind by two others. The CI rates were subjected to statistical routine analyses in Statistica© 5.1 software (level of significance $\alpha = 0.05$) revealing a significant gender difference.
This result increases the knowledge of the cellular mechanisms underlying the FA CI, seeming show that the CI can be an epiphenomenon also depending on factors different from the specific genetic defect. This evidence lead a strong implication on clinical management of FA patients which must change if the patient is a "mosaic", but "mosaicism" is now diagnosed only based on FA patient's CI, rated independently from the gender.
Moreover, we studied chromosomal rearrangements by the molecular cytogenetic technique named "Spectral Karyotyping" (SKY - ASI - Israel) obtaining evidence of a random chromosome involvement in rearrangements, while some chromosomes seem to be preferentially involved in breakages.

P0334. Radiosensitivity of Fanconi anemia lymphocytes *in vitro* measured by CBMN assay

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Fanconi anemia (FA) is an autosomal recessive disorder characterized by bone-marrow failure and cancer susceptibility. Early studies pointed to the specific sensitivity of FA cells to MMC and DEB which became the tools for the current diagnostic tests for FA. Although there is the long-standing clinical impression of radiosensitivity, in vitro studies have yielded conflicting results. We exposed peripheral blood mononuclear cells of FA patients (10 subjects) and carriers (20 subjects) to γ-rays (^{60}Co), dose of 2 Gy *in vitro* with aim to determine their radiosensitivity using CB micronucleus (MN) test. Incidence of spontaneous occurring chromosomal aberrations and MN in unirradiated-control samples also was examined. Mean incidence of chromosomal aberrations in FA patients was 0.088±0.08; which is 3.5 fold higher than in carriers, baseline level of MN was 11.66±6.7 whereas average incidence of radiation-induced micronuclei was 122.63±95.6. Baseline level of micronuclei find in parents lymphocytes was 16.29±8.4 (fathers); 14.04±11.17 (mothers), which is 2.3 fold higher compared to common population. Radioresponse of FA lymphocyte in vitro in most cases corresponds to resistant in vitro response, (with exception of one case where radiosensitive in vitro response was observed). Incidence of spontaneously occurring chromosomal aberration highly correlates with baseline incidence of MN, number of cell carrying aberrations and radiosensitivity (r=0.81, p<0.05) Mild radioresistant in vitro response was observed in mothers lymphocytes, whereas fathers response could be described normal-as in the common population. The authors will discuss the possible explanations for resistant response of FA lymphocytes *in vitro*.

P0335. Conventional and FISH analysis of deletion and three-way complex bcr-abl rearrangement in chronic myeloid leukemia

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Chromosomal analysis was carried out in bone marrow sample of an 11years old girl suspected myeloproliferative disorder. Conventional G-banding study detected a complex three-way translocation involving 7, 9 and 22, which has resulted in the formation of a variant Philadelphia chromosome causing rearrangement of *abl* and *bcr* genes in 87% cells. Fluorescence in situ hybridization (FISH) confirmed the fusion of *bcr-abl* oncogene. Thus the bone marrow karyotype was observed as 46,XX (13%) / 46,XX,t(7;9;22)(q11;q34;q11) (87%). Metaphase painting with whole chromosome probes (WCP) for 9 and 22 didn't show transfer of chromosomal material from 9 to 22, due possibly to a cryptic deletion of derivative 9. Hyperdiploidy was present in two cells. In this study, both conventional cytogenetic and FISH diagnosis proved to be significant to identify the variant nature of the Philadelphia chromosome, cryptic deletion in derivative 9 and hyperdiploid condition for introduction of a suitable treatment regimen and estimation of life expectancy of the young girl.

P0336. Molecular cytogenetic analyses in 142 patients with brain gliomas.

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Diffuse gliomas form a heterogeneous group of primary tumours which represent approximately 25% of all brain tumours in adults. New diagnostic and prognostic indicators must be sought to enable stratification of treatment. One possibility is subclassification of patients according to specific chromosomal aberrations in tumour cells.

For detection of deletions of tumour-suppressor genes *TP53*, *CDKN2A* and *RB1*, deletion of 1p36 and/or 19q13.3, amplification of *EGFR* gene, trisomy 7 and monosomy 10 we used I-FISH with locus-specific and/or α-satellite DNA probes (Abbott Vysis). We examined fresh non-fixed tissue specimens in 142 patients with different types of gliomas (25x low-grade astrocytoma, 17x anaplastic astrocytoma, 75x glioblastoma, 25x oligodendroglioma/oligoastrocytoma). Results of I-FISH were correlated with morphological and clinical findings. Molecular cytogenetic analyses were successful in 137 patients (96.5%) and due to non-adequate tissue specimen were not informative in five patients only. I-FISH in the most cases corresponded well with histological findings, in 58 patients I-FISH contributed to more precise diagnosis and/or prognosis. The most significant was finding of combined deletion 1p36/19q13.3 in patients with oligodendroglial tumours, which is considered to be a predictor of better prognosis. Deletion 1p36/19q13.3 was proved in 17 patients, in 12 cases as sole abnormality (progression in 33.3% of them) and in five cases in combination with other aberrations typical for high-grade astrocytomas (progression and/or exitus in 80% of them). A systematic molecular cytogenetic analyses by means of I-FISH showed in our cohort advancement of diagnosis, grading and classification of brain tumours.

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P0337. Conventional and molecular-cytogenetic diagnosis of patients with sex chromosome abnormalities

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We report on 5 male cases who presented with male infertility and sex chromosome abnormalities and 5 females referresd for suspicion of Turner syndrome who have a mosaic karyotype.

We performed RHG banding, FISH using whole chromosome painting probe (WCP) of X chromosome, specific locus identifier (LSI) for SRY gene, and X and Y cocktail centromeric probe.

The main problem with conventional karyotyping is that routinely 20 metaphases are analyzed and counted, this may miss low-grade mosaicism.

The obtained results demonstrate the importance of FISH for the improvement of conventional cytogenetic diagnosis and further correct and timely medical care for the patients with sex chromosome abnormalities.

P0338. Evaluation of fragile X syndrome in moderate mental retardations in Rafsanjan city

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Background: Fragile X syndrome (FXS) is the most common cause of inherited mental retardation. Patients with this syndrome show variable mental disabilities, long and narrow facial appearance with large ears and prominent fontanelle and frequent macro-orchidism. It is generally associated with a fragile site at Xq27.3, which can be observed in the metaphase chromosome following selective condition. Prevalence rates of FXS have been estimated in different ethnic groups one per 1500 in male individuals and one per 2500 in females the aim of this study was to determine of FXS prevalence in moderate mental retarded individuals of Zohreh Shamsaei in Rafsangan city.

Materials and Methods:52 moderate mental retardations (IQ=55-75) with clinical suspicion of FXS were screened for fragile X chromosome by cytogenetic methods. Blood samples were collected and cultured in selective condition. G-Banding method were used for karyotype preparation.

Results: Patients include, 37 males (71.2%) and 15 females (28.8%) with mean age 12.7 (7-17) years and mean IQ 65.3 (55-74). From males 8.1% and from females, 6.6% were found to have fragile X site at Xq27.3 (total 7.7%). The frequencies of fragile X-positive cells in males and female were between 8-52% and 12-27%, respectively.

Conclusion: The frequency of fragile X positive cases found in this study is similar to other reports of fragile X syndrome in preselected patients.

P0339. Determination of Heterozygosity at the FMR1 gene by an Enhanced Polymerase Chain Reaction Assay

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The fragile X syndrome (FXS) is a common cause of X-linked mental retardation. It is caused by a dynamic expansion mutation and subsequent hypermethylation of CGG repeats in the 5'-untranslated region

of *FMR1* gene. Population screening is highly justifiable but no cost-effective technical approach has been developed. Normal individuals show a range of allele sizes from 8 to 40 repeats with 20-22 and 29-31 being the most common allele lengths. Accurate measurement of allele size is crucial for diagnostic purposes and uses a combination of Polymerase Chain Reaction (PCR) and Southern Blot (SB) techniques. The latter is required to identify large alleles but it is expensive, time-consuming and requires a large quantity of DNA. One particular use of SB is distinction of normal homozygous alleles in females, which are found in approximately 30% of cases, from pre-mutation and full mutations. We developed a more sensitive PCR assay, which distinguishes alleles differing by only one repeat. Reanalysis of female DNA samples alleletyped as homozygous using a previous PCR assay has shown that 50% are actually heterozygous and therefore do not need to be verified using SB. This method has considerable advantages compared with other diagnostic tests and greatly facilitates testing of large numbers of samples.

P0340. Loss of methylation in imprinting control region *KVLQT1OT* in first-trimester human spontaneous abortions

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Genomic imprinting plays a significant role in regulation of early embryo development. The search of imprinted genes mutations in human miscarriages is a way to determine its effects. However, there are little evidences about high level of imprinting alterations in spontaneous abortions, except of hydatidiform mole and single instances of uniparental disomy. We have suggested that expected negative effect of imprinting disruption could be realized through epimutations of imprinted loci rather than rare events of uniparental inheritance. The global changes in genome methylation during preimplantation development as well as a growing number of reports about association of assisted reproductive technologies and imprinting disorders reinforce our suggestion. In the present study we have performed methylation analysis of three imprinting control regions (*SNURF-SNRPN, H19, KVLQT1OT*) and imprinted gene *CDKN1C* in 84 first-trimester spontaneous abortions by methyl-specific or methyl-sensitive PCR. Two tissues (extraembryonic mesoderm and cytotrophoblast) with different patterns of epigenetic reprogramming were investigated. Twenty four induced abortions were studied as a control group. Differential DNA methylation of *SNURF-SNRPN, H19* and *CDKN1C* was registered in all cases. As to *KVLQT1OT*, loss of methylation (LOM) in maternal allele was found in 8 miscarriages (9.5%). Surprisingly, LOM was confined by extraembryonic mesoderm or cytotrophoblast in 5 and 4 embryos, respectively. To our knowledge this is a first report about epimutations of imprinting control region in human miscarriages. Moreover, tissue-specific restriction of LOM allows suggesting independent sporadic epigenetic aberrations in different embryonic germ layers. This study was supported by RFBR (N 05-04-48129) and FASI (N 02.512.11.2055).

P0341. CGH-array study of 40 holoprosencephaly patients

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Holoprosencephaly (HPE) is the most common developmental brain anomaly in humans, usually associated with facial features. Our group focuses on patients with HPE and normal karyotype. We previously reported our results for point mutations screening and gene dosage in HPE genes (SHH, SIX3, ZIC2 and TGIF), describing genomic defects in up to 27% fetuses and 30% newborns. Recently, we screened subtelomeres by MLPA and found alterations in known HPE candidate loci but also in new regions, including many unbalanced translocations. Therefore, we decided to screen HPE patients using CGHarray to assess the frequency, location and size of such disorders.
We used Agilent CGH-array 44K technology. Out of 40 patients, we screened 10 DNA samples with known quantitative anomalies and 30 samples with no alterations identified to date. First we localized the breakpoints of the 10 DNA with known alterations (loss and/or gain in specific loci) and showed no correlation between the size and severity of the defect. Out of the 30 DNA with no genetic aetiology, we found 5 new rearrangements involving new potential HPE loci located on chromosomes 21q, 20p, 15q, 6q, 5q, 17q and 18q. We observed both isolated or associated genomic loss and gain, the latter suggesting an unbalanced translocation from parental origin. Detected alterations ranged from less than 1Mb to 16 Mb. Our data strongly reinforce the multigenic and multihit origin in HPE and participate in the explanation for the wide phenotypic spectrum described in this developmental disorder.

P0342. High Resolution CGH (HR-CGH) in the diagnosis of patients with mental retardation, dysmorphic features and normal karyotype

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Introduction: Mental retardation (MR) is a common disorder, but etiologic factors are not revealed in about half of the cases. CGH (Comparative Genomic Hybridisation) techniques have been proved useful not only in cancer genetics, but also in the diagnosis of MR . HR-CGH is a whole-genome screening technique which detects cryptic subtelomeric or interstitial chromosomal aberrations, not visible by conventional cytogenetic studies.
Subjects-Methods: Five patients, (3 boys-2 girls, aged 3 months-4 years) were evaluated for MR. Although they presented multiple dysmorphic features and two of them had congenital anomalies (heart-renal), they could not be connected to any known genetic syndrome in all cases. Karyotype by G-banding was normal. HR-CGH was then performed in DNA samples labelled with FITC dUTPs (nick translation). Normal DNA, labelled with Texas Red, was used as reference DNA. The results were analysed with the Applied Imaging software.
Results: Four of the five patients had redundant chromosomal material: 1) 46,XX, ish enh (1)(p31p34), enh(13)(q22), 2) 46,XX, ish enh (12p13.3), 3) 46,XX, ish cgh enh (1)(q24) and 4) 46,XX, rev ish enh (16p12p13.1) and 5) the fifth patient had a subtelomeric deletion [ish cgh 46,XX, del (18)(q21.1qter)]. For the confirmation of the results, the patients' parents were analysed with the same technique and were found normal.
Conclusions: HR-CGH contributes to the detection of chromosomal aberrations along with conventional karyotype, FISH analyses and quantative molecular methods are a useful adjunct and are well suited in the diagnosis of mentally retarded and dysmorphic patients.

P0343. Stage-by-stage study of genome-wide DNA methylation patterns in metaphase chromosomes of human preimplantation embryos

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We aimed to study DNA methylation patterns of metaphase chromosomes in human preimplantation embryos, which allows establishment of differences between parental genomes and DNA remethylation timing for individual chromosome loci. 83 metaphases from 54 IVF triploid and abnormal diploid human embryos from zygote to blastocyst stage have been analyzed by immunofluorescence with monoclonal anti-5-methylcytosine antibodies(Eurogentec). At the first cleavage one homologue was more or less methylated than another one(s), indicating different degree of methylation of parental genomes. In two-cell embryos all chromosomes were hemimethylated - one chromatid was more methylated than another one. Homologues differed only in methylation degree of old chromatids. Newly synthesized chromatids were hypomethylated in all homologues, showing that differential DNA methylation of parental genomes is not maintained any more. Hypomethylation affected all regions along chromosome arms with no band-specificity. Hemimethylated chromosomes were typical up to blastocyst stage, but their number decreased during subsequent divisions. Chromosomes with both hypomethylated chromatids appeared at four-cell stage and increased in number up to morula stage. Surprisingly, since four-cell stage proportion of hemimethylated and hypomethylated chromosomes varied from blastomere to blastomere, with no preference of certain chromosome being hemi- or hypomethylated.

Within the same metaphase hemimethylated and hypomethylated homologues were represented in different combinations, which may result from random segregation of hypomethylated, undermethylated and methylated chromatids in daughter blastomeres. Band-specific DNA methylation pattern, typical for fetal tissues and adult lymphocytes, was obvious in all metaphases since blastocyst stage. This is a pioneer study in human preimplantation metaphase cytogenetics. Supported by CRDF&RFBR.

P0344. Late cytogenetic effects of radiation expressed in human cells following Chernobyl accident

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We performed an evaluation of late cytogenetic consequences following the Chernobyl accident of human radiation exposure. These include hidden, delayed, and transmissible chromosome instability and "bystander effect". For the investigation of radiation-induced chromosome instability two methods had been applied - provocative mutagenesis assay (G_1-dimatyph or G_2-bleomycin tests used for persons differed on absorbed radiation doses*)* and two-termed (during 48 and 144 hours) cultivation of peripheral blood lymphocytes received from children born to irradiated parents. In children that lived in a region contaminated by ^{137}Cs as well as in clean-up workers with low radiation doses «adaptive response» had been detected; in high-doses patients recovered from acute radiation sickness increased chromosome sensitivity (hidden chromosome instability?) to additional mutagenic exposure was revealed. In progeny of irradiated parents an increased frequency of chromosome aberrations (single fragments and abnormal monocentrics) was established especially in long-termed cultures. Induction of chromatid breaks confirmed possibility of expression of delayed chromosome instability in subsequent mitosis; appearance of stable aberrations can be considered as a biomarker of transmissible chromosome instability. In the model system proposed by us - "mixed human lymphocytes culture" consisted of cells differed by cytogenetic sex markers - interaction between X-irradiated *in vitro* (in doses 250 and 1000 mGy) and intact cells was discovered. A difference between the spectrum of aberrations in exposed and intact cells was established - in targeted cells specific cytogenetic markers of irradiation dominated, in "bystander" cells chromatid types of aberrations (chromatid breaks and terminal chromosome deletions - cytogenetic indicators of chromosome instability) were mainly induced.

P0345. An apparently balanced complex chromosome rearrangement involving chromosomes 1, 10, and 14 in a man with infertility

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In study investigation was on Congenital complex chromosomal rearrangement compatible (CCR) with life is rare in man. We describe a complex and unique apparently balanced translocation involving chromosomes 1, 10, and 14 with 3 breakpoints, in a patient who was referred to our clinic because of infertility. Conventional karyotyping identified a complex rearrangement involving 3 breakpoints: chromosome 1q21.2, 10q21.1, 14q22.

The relationship between this apparently balanced and complex rearrangements and possibly produced unbalanced gametes responsible for high reproductive failure is discussed. The relationship between this apparently balanced and complex rearrangements and possibly produced unbalanced gametes responsible for high reproductive failure is discussed.

P0346. The shape, length and banded structure of chromosome 5 in interphase of HeLa cells

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In contrast to metaphase chromosomes, little is known about the shape, length and the banding pattern of human interphase chromosomes mainly due to technical problems in visualizing interphase chromosomes. We analyzed the structure of chromosome 5 in interphase nuclei using high-resolution multicolor banding (MCB), which paints the total shape of chromosomes and creates a DNA-mediated, chromosome region-specific, pseudo-colored banding pattern and allows the identification of telomeres and the measurement of the length of chromosomes. The investigations were performed on HeLa cells arrested at different phases of the cell cycle. The results show that the shape, length and banding pattern of interphase chromosomes of HeLa cells are similar to those of the corresponding metaphase chromosomes at all stages of the cell cycle. The length of the chromosome axis of flattened interphase chromosomes is 13.9µm (+/- 4.1) and comparable to that of a metaphase chromosomes. The MCB pattern also allows the detection and characterization of chromosome aberrations which may be of fundamental importance in establishing chromosome analyses in non-dividing cells. Consequently, we strongly recommend to reassess the concept of chromosome condensation during mitosis and to replace it by the new concept of a hierarchically organized chromosome region specific protein swelling based on protonation and hydration thereafter.

P0347. Cytogenetic, molecular and clinical characterization on an interstitial deletion of chromosome region 18q21 - a case report

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The 18q- syndrome (MIM 601808) originally described in 1984 is a relatively frequent chromosomal cytogenetic event normally resulting from a terminal deletion that may include bands from 18q21.1→qter and has a characteristic phenotype (midfacial hypoplasia, prominent antihelix and whorl digital pattern). Interstitial deletions involving the same region are rare events and include variable phenotypes, from moderate development delay and craniofacial asymmetry (Engelen *et. al* , 2003) to profound mental retardation but no life-threatening, unspecific malformations (Wilson *et al.,* 1979).

The authors present the clinical description, cytogenetic studies and molecular findings of a male patient age 34 with severe mental retardation and marked dysmorphic features: plagiocephaly; asymetric face, with midfacial hypoplasia and prominent antihelix. High resolution GTG banding karyotypes of the proband and parents revealed a "de novo" del(18q21.2→18q21.3) in the proband. Cytogenetic molecular techniques (FISH) excluded the involvement of any other chromosome and MLPA probes within bands 18q21 and 18q23 (kit P095 Aneuploidy) allowed us to redefine the bands involved and will hopefully be useful to improve the phenotype/genotype correlation in patients with interstitial 18q deletions.

The authors compare the present case with the ones previously described in the literature and emphasize the importance of high resolution GTG banding in the characterization of dysmorphic/psychomotor delay syndromes and of making accurate clinical descriptions of the patients to contribute to syndrome clarifications.

P0348. A patient with Williams Beuren syndrome and inv dup(15)

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In the literature, very few reports describe the concomitant presence of 2 anomalies leading to 2 different genetic syndromes. The contribution of each anomaly to the phenotype is still unclear. This report describes a case presenting contemporarily a Williams-Beuren Syndrome (WBS) and an inv dup(15) syndrome.

WBS is a microdeletion syndrome characterized by typical cardiovascular defects, facial dysmorphisms, connective tissue abnormalities, mild mental retardation, specific cognitive profile, endocrine abnor-

malities and feeding difficulties. Hypotonia and hyperextensible joints can result in delayed attainment of motor milestones. Inv dup(15) syndrome presents peculiar physical findings (muscle hypotonia, minor facial dysmorphisms), mental retardation, seizure, autism.
An 11 years old boy with mild mental retardation showing some of these anomalies was referred to our examination. He was born at term with eutocic delivery with normal auxologic parameters. Phisycal examination: talus valgus pronate feet, gastroesophageal reflux, hypotonia and feeding difficulties. Later, he manifested systemic hypertension, growth (weight/height < 3rdcentile) and psychomotor delay. Echocardiography showed a mild supravalvular aortic stenosis, hypothyroidism was excluded. Calcium blood profile was normal. Cytogenetic analysis revealed this karyotype: 47,XY,+inv dup(15)(q12)de novo.
Although some features of the patient such as cortex anomalies, short and stubby hands and feet were specific of the iso dic(15), the peculiar facies and cardiac defect suggested a WBS.
Molecular cytogenetic analysis (FISH) was performed with WBSCR-specific probe, the presence of specific microdeletion in 7q11.2 was confirmed. In this work, we compare the phenotype of patient with both syndromes to evaluate the different contribution of concomitant chromosomal anomalies.

P0349. Clinical findings in pericentric inversion of chromosome 9 - three years experience

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Pericentric inversion of chromosome 9 is one of the most frequent structural balanced chromosomal aberrations. Commonly it is considered as a normal variant of karyotype.
We reported 6 cases analyses over a three years period. Age of diagnosis was under 18 years (2 cases) and over 18 years (4 cases). The motivation for genetic consultation was repeated miscarriage (3 cases), multiple malformations in a new-born who died in the neonatal period (1 case) and mental retardation, dysmorphic face (2 cases). The karyotype showed pericentric inversion of chromosome 9 in all cases. In a single case we detected a complex anomaly - 45,X,inv(9)/46,XX,inv(9) - Turner syndrome.
It is difficult to assess the correlation between clinical signs and pericentric inversion of chromosome 9. Genetic counselling and prenatal diagnosis is helpful for most families.

P0350. Breakpoint cloning and haplotype analysis of a novel cytogenetic variant consisting of a 12 Mb inversion on chromosome 10, inv(10)(q11.22;q21.1)

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We have identified an inherited paracentric inversion of chromosome 1 0[inv(10)(q11.22;q21.1)] revealed by high resolution karyotyping. A detailed mapping of the inversion was done through fluorescence *in situ* hybridization (FISH) and Southern blot hybridization in three non-related Swedish individuals. Cloning and sequencing of the breakpoints revealed that the inversion was identical in the three individuals. The inversion spans 12 Mb and it is almost perfectly balanced at the nucleotide level. No predicted transcripts or known genes are disrupted by the inversion breakpoints. The 10q11.22 breakpoint is located within a long terminal repeat (LTR) and the 10q21.1 breakpoint is 900 bp away from a short interspersed nuclear element (SINE). Haplotype analysis of the three individuals and their parents indicated that a shared haplotype was inherited with the chromosome 10 inversion which favours a single event for the inversion. A retrospective study of amniocenteses and blood analyses performed in Sweden showed presence of the inversion in 7 out of 8,896 amniocenteses and in 3 out of 2362 blood analyses. In all cases the inversion was inherited from one of the parents. From our results, we suggest that the inv(10)(q11.22;q21.1) is a rare variant and that it is identical by descent.

P0351. Molecular and cytogenetic analysis in IVF failure cases

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Chromosomal abnormalities in infertile couples results in spermatogenic arrest, premature ovarian failure, implantation failure and consequently failure of *InVitro fertilization* (IVF). The aim of the study was to determine genetic basis for recurrent ART/IVF failure. Thirty eight infertile couples with IVF failure having poor blastocyst development and implantation were analyzed cytogenetically and for molecular analysis of AZF loci in the men. Two females with recurrent IVF failure showed partial deletion of Xq and the other female had 10% cell line showing deletion of pericenteromeric region of long arm of chromosome number 1. Of these couples microdeletion analysis of 30 cytogenetically normal infertile men, only two cases showed deletion; one with AZFc loci and the other case had deletion of AZFb loci. The couples where female partner had deletion of long arm of X chromosome(Xq-) resulted in repeated failure of blastocyt development, in 4 IVF cycles. The case with AZFb microdeletion had maturation arrest and case with AZFc deletion had hypospermatogenesis. In these cases sperms could be retrieved from the testis and to be used for IVF or Intracytoplasmic sperm injection. (ICSI). In cases with sex chromosomal and autosomal aberrations there is probability of poor embryo development and consequently poor implantation, which may be a result of high segregation abnormalities and may negatively affect the outcome of assisted reproductive techniques. ART is a very expensive technique and recurrent ART/IVF failure results in severe financial stress coupled with emotional stress, thus all couples opting for ART must undergo genetic analysis.

P0352. An unusual 11q deletion derived from a complex maternal karyotype in a Jacobsen-like patient

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We present a family with multiple cytogenetic abnormalities, identified through a newborn girl with several dysmorphic features and cardiac problems, suspected for Jacobsen syndrome. Cytogenetic analysis showed a 46,XX,del(11)(qter) karyotype, which was confirmed by fluorescence *in situ* hybridization (FISH). Cytogenetic investigation of the parents showed a chromosome aberration in both: the father presented a t(11;12)(p13;q22) and the mother was carrier of an ins(4;11)(p14;q24q25). Additional FISH analysis with BAC probes from chromosome 11q23.3-q25 showed that the maternal ins(4;11)(p14;q24q25) was in fact the result of two subsequent events: first, a small inversion in the long arm of one chromosome 11, and second, an insertion of a small distal part of the long arm of the inverted chromosome 11 into the short arm of one chromosome 4. Therefore, the maternal karyotype was revised into 46,XX,der(4)(4pter→4p14::11qter::11q24.1→11q25::4p14→4qter),der(11)(pter→q23.3::q25→q25:). The karyotype of the newborn girl was accordingly rewritten as 46,XX,der(11)(pter→q23.3::q25→q25:)mat.
The aberrant karyotypes in both parents implicated an increased risk of unbalanced fetal chromosome composition, and thus a high risk for a child with multiple congenital abnormalities. Therefore, during the next pregnancy, the couple opted for invasive prenatal diagnosis by amniocentesis. To obtain an early indication of the expected fetal karyotype, an interphase FISH strategy for uncultured amniotic fluid cells was designed. Karyotyping of cultured amniotic cells confirmed one of the two predicted cytogenetic options, demonstrating the importance of an interphase strategy for couples with both parents being carrier of a chromosomal abnormality.

P0353. Jumping translocation and Prader-Willi syndrome: about one case

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Jumping translocations are rare chromosomal events and are defined as chromosome rearrangements involving a donor chromosome segment which is translocated to various receptor chromosomes.
The majority of jumping translocations have been observed in haematological malignancies.

Less than 10 cases of Prader-Willi syndrome associated with a jumping translocation have been reported in the litterature.
Cytogenetic analysis performed on a 13 year old boy suspected of Prader-Willi syndrome showed a constitutionnal mosaicism with different cell lines :
45,X,der(Y)t(15;Y)(Ypter→Yqter::15q13→15qter),-15 [27] /
45,X,der(Y)t(15;Y)(Yqter→Ypter::15q13→15qter),-15 [9] /
45,XY,der(15)t(15;15)(15pter→15qter::15q13→15qter),-15 [1] /
45,XY,der(13)t(13;15)(13pter→13qter::15q13→15qter),-15 [1] /
45,XY,der(17)t(15;17)(17qter→17pter::15q13→15qter),-15 [1] /
45,XY,der(22)t(15;22)(22pter→22qter::15q13→15qter),-15 [1].
In all cases, the majority of the long arm of one chromosome 15 is translocated on different receptor chromosomes and the centromere of the derived chromosome seems to belong to the receptor chromosome.
FISH studies show deletion of the region 15p11.2, the centromere and the region 15q11-q13.
The deletion of the region 15q11-q13 confirms Prader-Willi syndrome. Hypothesis of the mechanism leading to this jumping translocation is discussed.

P0354. A report of a case with partial trisomy 13q with 46,XY,der(14)t(13;14) (q13;p11.1),+13q13 Karyotype

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This has been shown that the trisomy of the distal part of chromosome 13 is related to different clinic findings than cases with classic trisomy 13. The different trisomic segments of the long arm of chromosome 13 have been reported which might be either translocated or inserted in different chromosomes.
Our case was a baby boy and the first child of an unrelated family. He was born at term following a normal pregnancy. He referred to our clinic at the age of 4 months. He had postaxial polydactyly and syndactyly of the left hand. He was deaf and legally blind.
He was generally presented with developmental delay, microcephaly, trinogocephaly, hypotelorism, and with feeding problem.
Cytogenetic analysis carried out using Tripsin Giemsa G banding (GTG), the karyotype 46,XY,der(14)t(13;14) (q13p11.1)+13q13 was determined in our patient . His mother and father were investigated and found to have normal karyotypes. To our best knowledge, this is the first report of a case where the trisomic segment of chromosome 13 is translocated on to chromosome 14.

P0355. Is routine karyotyping necessary in the evaluation of hypospadias cryptorchidism and micropenis?

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The incidence of intersex states has been reported to be 27% to 100% in patients with hypospadias and cryptorchidism, and routinely determining karyotypes has been recommended. This incidence seems much higher than in our experience. We reviewed the records of patients with hypospadias, cryptorchidism and/or micropenis as well as those referred with ambiguous genitalia to determine whether these findings were associated with a high incidence of chromosomal abnormalities and whether they warrant routine karyotype screening. We reviewed the records of patients with undescended testis, hypospadias and/or micropenis, and those with ambiguous genitalia who presented between 2004 and 2006. Patients without karyotype determination, and those with iatrogenic cryptorchidism, retractile testes, congenital adrenal hyperplasia or female-appearing external genitalia were excluded from study. Of the 32 patients whose records matched study inclusion criteria, two patients (6,25%) had chromosomal abnormalities.Ambiguous genitalia was associated with sex chromosomal abnormalities in the 2 children and no case with autosomal chromosomal abnormalities. Most patients who present for the evaluation of hypospadias, micrpenis and undescended testis have a normal karyotype. Routine karyotype investigation of all patients with hypospadias, cryptorchidism and micropenis does not seem warranted. If karyotypic intersex abnormalities are identified, those patients are more likely to have ambiguous genitalia, especially those with perineal hypospadias and cryptorchidism.

P0356. Sex chromosome abnormalities in asoospermic males

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Cytogenetical analysis has been carried out on 32 patiens diagnosed with azoospermia or hypogonadism. Conventional cytogenetical G - banding method and Flurescent in situ hybridization technique were considered in cultured peripheral blood.
22 cases showed karyotypes pure 47,XXY - Klinefelter syndrome, 2 cases 47,XXY/46,XY - mosaic Klinefelter sy., 1 case 47,XXY/48,XXXY, 2 cases 48,XXY,+ mar, 3 cases 46,XX male, 1 case 47,XYY, 1 case 45,X,psu dic(Y,13).
FISH identified two marker chromosomes, detected SRY gene translocation on X chromosome in cases with karyotyp 46,XX male, also in karyotyp 45,X,psu dic Y,13. Cytogenetical research is very important to detect the cause of male infertility.

P0357. Cytogenetic Investigation in 945 Iranian Leukaemic Patients

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We analyzed bone marrow and peripheral blood samples of more than 550 Iranian Patients referred from Major hematology-oncology centers at Tehran and provincial capitals. They were either suspected of leukemia at presentation or being monitored for their response to medication. Bone marrow or/and blood cells were cultured, harvested and G-banded according to the standard protocols. Chromosome analysis was performed following ISCN guidelines. The patients were divided into six major groupings as far as the leukemia subtypes were concerned: CML, AML, ALL, MIDS, Lymphoma and others. They were more male patients than female (1.32: 1 ratio). In terms of sample type most cases had bone marrow aspiration whereas peripheral blood was utilized only in fraction of cases. The common typical chromosomal abnormalities as well as rare and complex forms were observed. The overall chromosomal abnormality rate obtained was around 50%. The breakdown figures for different categories were roughly as follows: 80% in CML, 40% in AML, 25% in ALL, 30% in MDS and 50% in other types. Compared to published data, the observed chromosomal abnormality rate in the present study is considered average

P0358. Cytogenetic characterization of a diffuse large B-cell lymphoma case using multi-color fluorescence in situ hybridization

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The diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy of mature B-lymphocytes, and accounts for approximately 40% of all NHL cases. This entity shows great clinical and genetic heterogeneity. Conventional cytogenetic techniques (CCT) has provided a useful, but incomplete analysis of the chromosomal changes observed in this disease due to the complexity and poor morphology of chromosomes that characterize the karyotypes obtained in this malignancy. This results in partially characterized karyotypes containing many chromosomal alterations unresolved in a large number of cases. The multi-color fluorescence in situ hybridization (M-FISH) analysis has helped to identify and characterize previously unidentified chromosomal abnormalities.
M-FISH was performed on lymph node from one DLBCL case in order to deliniate the complex chromosomal alterations that remained unre-

solved after CCT analysis.
M-FISH analysis resolved 100% chromosomal aberrations that had been misclassified by CCT analysis and masked as additional material (adds) on unbalanced derivate chromosomes and marker chromosomes.
Chromosomes preferentially involved in karyotipic aberrations were 4, 8, 3, 5, 6 and 7 (in decreasing order). Breakpoints included 4q11, 4q31, 8q12~q13, 3q27, 5q23, 6q14 and 7q31.
In conclusion, M-FISH represent an essential tool for clarifying karyotypes with abnormalities that are not completely defined by CCT. In this sense, accurate cytogenetic characterization using a combination of M-FISH and CCT in DLBCL cases will facilitate comprehensive correlation with clinical features.

P0359. Three patients with primary macroglobulinamia, strongly seropositive to *Hp* infection and t(11;18)(q21;q21)

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Introduction: Primary macroglobulinemia (PMG) is a proliferative disorder of an IgM producing B-cell clone with bone marrow infiltration by small lymphoid cells, plasma cells, anemia, hepatosplenomegaly, and hyperviscocity syndrome. On the other side *Hp* chronic infection can produce a MALT type lymphoma of the stomach, B-cell origin in which commonly the MALT1 gene(18q21) is fused to the AP12 gene(11q21).
Aim of the study: We report 3 cases of PMG with t(11;18)(q21;q21) who were strongly seropositive to *Hp*. Our report demonstrates that part of PMG is the result of chronic *Hp* infection.
Patients: 1st patient: A man 47y with diplopia.
2nd patient: A man78y with mild anemia.
3rd patient: A woman 80ywith severe bone pain.
By immunoelectrophoresis all had IgMk paraproteinemia.
Their bone marrow was infiltrated by 10% abmormal lymphoid cells CD5- and CD10-.
Upper gastrointestinal endoscopy with biopsy showed small Bcells[L26+,CD3-] and Tcells[CD3+,L26-]
Serum anti*Hp* IgG and IgA titers were increased.
Methods: Bone marrow specimens and PB lymphocytes were cultured using standard techniques.Thirty GTG banded metaphases were analyzed (ISCN1995).
FISH was performed using the LSIAP12/MALT1 t(11;18)(q21;q21) dual color, dual fusion translocation probe (VYSIS).
Results: The karyotypes looked normal.With FISH we evaluated as a translocation a one orange(MALT),one green(AP12) and two fusion (AP12/MALT) signal pattern.
Two hundrend interphase nuclei were counted.Up to 15% of the cells carryied the translocation.
Conclusions: 1) PMG cases with t(11;18)(q21;q21) are clinically distinct from other B-cell origin cases with this translocation. 2) Since PMG and MALT lymphomas are of B-cell type and share the same t(11;18)(q21;q21) it is suggestive that the chronic *Hp* infection can result to an abnormal proliferation of lymphoplasmacytic cells and to participate in the machanism of lymphomagenesis.

P0360. Cytogenetic abnormalities in male infertility

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Infertility is the inability to get pregnant after trying for at least one year without using birth control methods. About 25 percent of couples experience infertility at some point in their lifes. The incidence of infertility increases with age. The male partner contributes to about 40 percent of cases with infertility. This is a serious problem which concerns families in respect of economical and spiritual aspects. Recently the medical developments in the diagnostic procedure of those cases with infertility provided opportunities for the families. There are several reasons that cause male infertility. The most common reason is varicosele in male infertility which is followed by sex chromosome abnormalities.
In this study we aimed to evaluate the postnatally screened karyotype results in the individuals who were referred to our department due to primary infertility between 2000-2006 years in Izmir. A total of 179 cases with infertility were evaluated retrospectively in our study. The mean age was 35.00±6.06 years. Twenty out of 179 (11.17%) cases showed chromosomal abnormality. Thirteen (7.3 %) were 47,XXY, 3 (1.68%) were 46,XY,inv(9) (p11;q13), one (0.56%) 46,XY/45,XO, one (0.56%) 46,XY/47,XXY/48,XXXY, one (0,56%) 46,XY,t(X;1) and one (0,56%) 46,XX. In conclusion we point out the importance of cytogenetic evaluation in those cases with infertility.

P0361. Y chromosome abnormalities and male infertility

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Genetic component is responsible for about 15% of male infertility. Among them, constitutional chromosomal abnormalities are found in about 5% of oligozoospermic men and 15% of azoospermic men. Males attending the infertility clinic and seeking for assisted reproductive techniques (ART) are routinely referred to our center for genetic testing. During the year 2006, 7/33 males (21.2%) with infertility were found to have chromosome abnormalities; four with 47,XXY, three with structural abnormalities of the Y chromosome and none with Y microdeletion for SRY, AZF and DAZ regions by PCR. Routine G-banding and appropriate FISH tests were carried out, and the karyotypes of the three cases with Y chromosome abnormalities were confirmed as :
Case 1 : 45,X,der(18)t(Y;18)(pter-p10::q10-qter).ish der(18)idic t(Y;18)(p10;q10)(SRY+,DYZ3+;D18Z1+)[80]/ 46,XY,i(18)(q10).ish Y(SRY+),i(18)(D18Z1+)[20]
Case 2 : 46,X,idic(Y)(qter-p11.32::p11.32-qter).ish idic(Y)(CEPY++,SRY++)
Case 3 : 47,XYY[10]/46,XY[40]
Dicentric Y is the most common rearrangement of the Y chromosome, with varying break point regions, and is mostly present as part of a mosaic karyotype. However, Only 5 cases of isodicentric Y with breakpoint at Yp11.32 are reported so far. We found a similar karyotype in an azoospermic male (case 2) but surprisingly non-mosaic. This could possibly be a germinal or a very early mitotic event. Since the entire Y chromosomes are present in this rearrangement, it is genetically similar to the XYY individuals. Case 1 was predominantly monosomic for 18p and loss of Yq, and presented with mild dysmorphic features and rudimentary gonads. The genetic findings, genotype-phenotype correlation and possible reproductive options in the three cases are discussed.

P0362. Supernumerary marker chromosomes derived from chromosome 15

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Supernumerary chromosomes markers (SMCs) of chromosome 15 are the most common SMCs in human accounting for 60% of all those observed. Molecular cytogenetics methods are necessary to identify these additional chromosomal markers. Conventional cytogenetics techniques are limited in terms of providing the necessary information about their origin. We present two cases, initially studied with standard GTG banding technique followed by complementary C-banding and NOR-staining.
Case 1: 47, XY, +mar Clinical features: Infertility
The marker is dicentric and with the application of M-FISH we identify that the marker was der (14) or der(15). Using specific centromeric FISH probes we were able to identify that it was derived from chromosome 15.
The patient is infertile.
Case 2: 47, XY, +mar Clinical features: autism
The marker is dicentric and using PW/AS FISH probe we have detected that the marker was duplicated for this region, then the patient presents parcial tetrasomy for chromosome 15. Microsatellite analysis of the 15q11-q13 region is undertaken.
The comparison of the clinical data of our patients with those with simi-

lar markers taken from the literature allowed us to establish phenotype-karyotype correlations.

P0363. Chromosomal findings in 8727 Iranian patients with mental retardation

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The identification of a de novo chromosomal imbalance in a patient with mental retardation (MR) is usually considered causal for the phenotype. Most of imbalances in patients with MR are inherited from a healthy parent.
Here we review our cytogenetic investigations on 8727 Iranian patients referred to IBTO due to MR. Among these MR patients, 31 cases diagnosed with Fragile X and 112 with Down syndrome.
In 721 patients the following chromosomal abnormalities could be detected:
46,XY, rec(10)dup(10p),inv(10)(p11.2q26.3)mat
46,XX,del5p
46,XY,del22q(11.2)
46,XX,add 15p ?
46,XX,del18 (21.3)[18]/46,XX[33]
49XXXXY/48,XXXX
46,XY,t(16 ;17)(q22 ;p13)
46, XXadd(22)(p13),
mos47,XY,+ mar[8]/46,XY[12]
4 7 , X X , d e l (2) (q 2 2 q 3 2 . 2) + r (2) (q 2 2 q 3 2 . 2) [8 1] /
46,XX,del(2)(q22q32.20[9]
46,XX,del(6)(p25)[7]/46,XX46]
46,XY,del(18)(q23)
47,XX,+der(22),t(11,22)(q23;q11.2)
45,Xx,t(7,22)(q36.2;q11.1~11.21)
45,X
46,XXinv6(p23q21)
47,XY,+ 21, dic(21;21)(q22.1;q22.3)
$5,XXder(13,14)(q10;q10)
48,XXYY
46, XX,inv6(q22.1;q25.1)
47,XXY
45,XY,der (13,14))q10;q10)
46,XX,del5(p15.2)
46,XX,t(2,3)(q23;25)
45,X
47,XY,+21,invY
48,XXYY
46,XXr(18)(q21.2qter)
46,XY,del(4)(p15.31)
46,XX,del(11)(q23.2
46,XY,add15p
48,XXX,+mar[2]/ 47,XXX[22]/46,X,+mar[8]/45,X[2]
47,XX,+13
46,Xy,del50p15.2)
In the remaining 45 MR patients no chromosomal abnormalities could be identifies. Our findings support the view that screening for chromosomal rearrangements has a great positive role. If cost and resources permit, conventional karyotyping should be the next diagnostic test of choice in a child with unexplained MR.

P0364. XYY in mentally retarded boy with tall stature, prognathism, hypoplastic toe nails and malformations of the hands

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Here we report a 25-year-old man referred to our laboratory for evaluation of possible Fragile X syndrome on the basis of mild mental retardation. He was tall (194cm). His face was mildly dysmorphic. Prognatism was detected. He often showed excess negative mood and aggressiveness. He could finish his primary school. He worked as a mechanic. He suffered from spasm and muscle cramp. He had hypoplastic toe nails and short hands. Although he had a hydrocoele repaired, his genitalia were otherwise normal. The patients found to be negative for Fragile X. However, He was found to be 47 XYY from chromosomal examinations.Our case is the second reported case of a XYY boy with malformations of the hands. We could find just one previous article concerning XYY males with hands malformation. This combination of XYY male and nails and hands deformities may be coincidental. However, we think it is important to report the patient.
In summary, it is apparent that the mental and physical characteristics of the average XYY boy are yet to be determined. This is very important to help the parents who are seeking prenatal diagnosis and whom found to have a fetus with XYY karyotype to make the right decision.

P0365. Chromosomal aetiology of congenital conotruncal heart malformations

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Important advances in the diagnosis and treatment of congenital heart malformations have been made in the past 50 years. Nowadays while echocardiogram plays an important role in the diagnosis, etiologic assessment at the genetic level becomes mandatory. In order to identify the frequency of chromosomal rearrangements and 22q11 microdeletions in patients with conotruncal heart defects, we studied by karyotyping and FISH procedures 44 patients. Each patient was investigated by echocardiography. Anamnestic and clinical informations with regard to developmental milestones and dysmorphic features were recorded. Conotruncal heart defects, included tetralogy of Fallot (TF; n = 27), pulmonary atresia/ventricular septal defect (APSO; n = 7), double-outlet right ventricle (VDDI; n = 4), transposition of the great arteries (TGV; n = 1), truncus arteriosus (TA; n = 1), subaortic ventricular septal defect (SACIV; n = 3), and interrupted aortic arch (IAAo; n = 3). Phenotypic features and associated abnormalities were examined in these patients. One patient (2.27%) who had a TF had a de novo cytogenetic chromosomal abnormalities: 46,XY,der(16)t(16;?)(q24;?) and four (9.09%) had 22q11 microdeletions, including 33.33% of IAAo, 16.67% of APSO and 7.40% of TF.
In patients with chromosomal abnormalities or 22q11 microdeletions, abnormal phenotypic features and associated abnormalities were observed.
We conclude that children with congenital conotruncal heart malformations, should undergo karyotyping and microdeletion testing mainly when heart defects are associated with other congenital anomalies. Detection of chromosomal anomalies has a significant impact on prognosis and follow-up of patients, as well as on genetic counseling of families.

P0366. Whole genome amplification from microdissected chromosomes

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The uniform whole genome amplification (WGA) of smallest amounts of DNA is a great challenge not only for single cell analysis in preimplantation diagnostics or oncogenetic research but also for investigation of the parental origin of de novo balanced chromosome aberrations. In the latter it is not possible to differentiate marker alleles of the derivative chromosomes from alleles of their normal homolog by analysis of genomic DNA. For such investigations the derivative chromosomes and their normal homolog must be separated. For this purpose single chromosomes from lymphocyte metaphase spreads were dissected with a glass needle, and collected in a glass capillary filled with collection buffer. Then, the DNA of the microdissected chromosomes was amplified by different types of WGA strategies. I-PEP (improved primer extension preamplification), a PCR based WGA using random priming, Genomiphi® and Repli-g® which are based on multiple displacement amplification, and GenomePlex® single cell kit, which is based on library preparation and amplification using adaptor primers were compared according to their efficiency and practical application for subsequent microsatellite analysis. First preliminary data show a more efficient WGA by the Genomiphi®/Repli-g® kit and the Genome-

Plex® single cell kit than by the I-PEP approach. Further comparative investigations are in process and will be presented on the meeting. Practical applicability will be demonstrated in some cases with rare chromosomal rearrangements. In conclusion, WGA of single microdissected chromosomes will be a new and powerful tool to improve our knowledge on meiotic and mitotic chromosomal behaviour.

P0367. Microdeletion in 17p13.3 telomeric to LIS1 and monosomy 21petr 21q21 in a man presenting azoospermia

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Monosomy for the segment 17(p13.3 pter) was first associated with the Miller-Dieker syndrome: recognized as a contiguous gene-deletion syndrome (MDS). It includes lissencephaly, significant facial dysmorphism and occasionally other congenital anomalies. These disorders result from a deletion of a common critical region including gene *LIS1*. However some MDS cases have been previously described to been associated with more distal deletions to *LIS1* suggesting the implications of others genes in the brain development and neuronal migration. Here we describe a 24 years man consulting for azoospermia. Clinical findings associate moderate mental retardation and non specific dysmorphic features. R banded chromosome analysis revealed 17p13.3 monosomy associated to a 21(pter,q21) monosomy as a result of an unbalanced segregation of a reciprocal maternal translocation (45,XY,der17t(17;21)mat,-der21). Fluorescent in situ hybridisation studies on the mother and the proposita confirmed the reciprocal translocation in the mother and a terminal deletion in the patient, which resulted in the retention of *LIS1* and a deletion of the 17p telomere**.** The study show also that the proximal breakpoint in chromosome 21 is proximal to the gene APP: the proximal boundary of the “monosomy 21” critical region implicated in the “monosomy 21” phenotype: arthrogriposis-like syndrome.Interstingly, telomeric 17p deletions without *LIS1* deletions have been previously described frequently with severe mental retardation, hypotonia and abnormal brain imaging. However in our case the discrete clinical features contributes to illustrate the relationship between 17p13.3 genes deletions, the spectrum of the associated phenotypes and their implications in the brain development and the neuronal migration.

P0368. High recombination density and low interference in the domestic cat

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During meiotic prophase hundreds of double-strand DNA breaks (DSB) are generated. Majority of them are repaired in non-crossover way, while the minority are resulted in crossovers. In most organisms, neighbouring crossover sites interfere with each other. The strength of the interference is estimated by the distance between adjacent crossover sites and by the shape parameter of gamma distribution. This parameter estimates a ratio of DSB randomly placed along the bivalent to the number of them resolved in crossover way. We analyzed the chromosome-wide patterns of meiotic recombination and interference in the domestic cat (*Felis domesticus* L.). We prepared synaptonemal complex (SC) spreads of meiotic chromosomes from the testes of three male cats and mapped 2633 sites of recombination along 1098 individual autosomes, using immunolocalization of MLH1, a mismatch repair protein marking recombination sites. We estimated a total recombination length of the male cat genome as 2175.5 centimorgans. The cats showed the highest recombination density per unit of SC and lowest interference among mammals so far studied. Many recombination sites were located in close vicinity to each other and to the centromeres. Average distance between neighbouring sites was 22% SC length, while in humans and other mammals studied it usually exceeds 50-60%. For the chromosomes of comparable size the shape parameter in the cats was equal to 4.1 while in mice and shrews was about 14-18, , i.e. as much as one quarter of the precursors were converted into crossover sites in the cats against only 5-7% in other mammals.

P0369. MLPA in detailed characterisation of chromosomal rearrangements: experience gathered from cases with psychomotoric retaradation, dysmorphic features and congenital anomalies

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Exact characterisation of chromosomal rearrangements is important for the identification of genes involved in different instances of psychomotoric retardation (PMR), in patients with dysmorphic features and/or congenital anomalies. Commercial MLPA SALSA kits (MRC-Holland; P036B, P070, P019, P020) were used in 26 cases that in parallel underwent cytogenetic and/or FISH examinations. MLPA datasets were analyzed by our ,,in house“ software and subtelomeric rearrangements (del./dupl., or their combinations) were found in 5 / 26 probands (19.2%): 1/ del. 3q29, ΔT 745kb (distance from the telomere) was associated with a progressive neurodegenerative disorder, psychomotoric retardation, including disintegration of white / gray brain matter. However, this rearrangement was also revealed in the unaffected mother and maternal grandmother, who also bears a duplication at 21q22.3, ΔT 80kb, thus causal relationship was excluded; 2/ in the case of Caudal regression syndrome with bilateral optical optical atrophy FISH revealed del./dupl. at 4p16.3. MLPA revealed the del. with ΔT 346-874kb; 3/ subtelomeric dupl. 15q26.3 ΔT 334-337kb was associated with microcephaly and PMR; 4/ del. 9q34.3, ΔT 706 kb and dupl. 12p24.33 ΔT 176-316kb was detected in a patient with PMR and dysmorphic features and 5/ del. 18q23, ΔT 220-539 and dupl. 4q35, ΔT 3872kb was associated with PMR, mutliple congenital anomalies and axial hypotony. In conclusion, MLPA supplements the FISH examination by more exact localization and characterisation of the range of del./dupl. in heterogeneous clinical cases. Differentiation between causal rearragements and variants is only possible via family analysis. Supported by VZFNM 000064203.

P0370. Molecular neurocytogenetic survey of intercellular genomic variations manifesting as aneuploidy in the human brain

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Classical genetic views suggest all the cells forming the human brain to possess identical genomes. However, recent molecular cytogenetic studies of the brain have refute these experimentally unproven assumptions and proposed a new biomedical direction, molecular neurocytogenetics. Regardless previous molecular neurocytogenetic studies showing the brain populated by aneuploid cells, we are still far from knowing in what extent aneuploid cells affect the human brain. Using state-of-art interphase molecular cytogenetic techniques (multiprobe FISH with quantitative FISH, multicolor banding (MCB), PRINS, and PNA) and scoring about 1 million nuclei, we were able to establish the rate of mosaic aneuploidy of chromosomes 1, 7, 9, 14, 16, 18, 21, X, and Y in the unaffected, schizophrenia, and ataxia-telangiectasia (AT) brain (cerebral cortex):

Brain	Autosomes (mean per autosome pair)	Sex chromosomes
Unaffected	Losses Range: 0.1-0.7% Mean: 0.57% Gains Range: 0.1-0.2% Mean: 0.12%	Chromosome X loss (females): 2% Chromosome X gain (males): 0.4% Chromosome Y gain: 0.1%
Schizophrenia	Losses Range: 0.8-1.7% Mean:1.2% Gains Range: 0.2-0.6 Mean:0.43%	Chromosome X loss (females): 2.2% Chromosome X gain (males): 0.6% Chromosome Y gain: 0.2%
AT	Losses Range:1.3-3% Mean: 2% Gains Range:0.5% Mean:0.4%	Chromosome X loss (females): 3.5% Chromosome X gain (males): 2.8% Chromosome Y gain: 0.6%

Thus, the data show that aneuploidy do present in the human brain. However, the rate of stochastic aneuploidy differs between unaffected individuals and those affected by psychiatric and neurodegenerative diseases. We observed the schizophrenia brain to demonstrate significantly increased aneuploidy rate as to control. Furthermore, in AT (neurodegenerative disease featured by chromosome instability), the brain exhibited even more significant increase of aneuploidy as to both control and schizophrenia. We conclude that aneuploidy in the human brain possesses the potential not only to produce neuronal diversity but also to be a mechanism for neuropsychiatric disorders.
Supported by INTAS, AT Children's project, and DFG.

P0371. A direct comparison between whole genome oligonucleotide microarrays and whole genome BAC microarrays and an evaluation of the use of whole genome oligonucleotide microarrays in a prenatal diagnosis setting.

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Array comparative genomic hybridization (aCGH) allows a high resolution whole genome analysis of copy number changes and can reveal submicroscopic duplications and deletions in individuals with normal G-band karyotypes.
Two genomic DNA samples from patients with known chromosome abnormalities were tested using both BAC and 60mer oligonucleotide array-CGH platforms.
The dye swap experiment using BAC arrays produced the clearest results, whereas the oligonucleotide arrays picked up more regions of possible copy number change for the same samples. Two of the detected regions of copy number change were followed up by FISH: one of was confirmed, and one was found to be a genomic variant.
The Oligonucleotide array platform was also used to test two samples with low DNA concentrations (a clump of 3-5 normal female buccal cells and fibroblast DNA that had trisomy 21) by amplifying their DNA using multiple displacement amplification (MDA). The results of these experiments showed that biased whole genome amplification resulted in some regions being over- or under-represented in the final mix. The large amount of copy number variation in the human genome complicates the distinction between normal variant and pathogenic copy number changes, and in addition background noise from experimental artefacts such as those found with the MDA amplified samples can further complicate data interpretation.
There is a need to standardize this technology and the methods of data analysis as well as a need to take into account variant regions in order to ensure proper data interpretation.

P0372. Monosomy 7q3 in a patient with karyotype 47,XXX,del(7)(q3436)/46,XXdel(7)(q3436)

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Structural anomalies associated with a mosaic form of aneuploidy are rare events.
Partial monosomy 7q is a rare chromosomal disorder, which results in multiple congenital anomalies syndrome with severe mental retardation and growth failure.
We report a case of two month old female infant with a deletion of 7q3 chromosome region. The infant presented hypotonia and poor growth of prenatal onset, microcephaly, cranial asymmetry with broad forehead, upslanted palpebral fissures, strabismus, bulbous nose with anteverted nares, short wide philtrum, short neck, enlarged and low - set malformed auricles, broad and short fingers, sacral dimple. CT scan was normal, but cardiac ultrasonography showed congenital heart defect.
Traditional R banding revealed a 7q terminal deletion involving segment between cytogenetic bands 7q34 and 7q36 and mosaic form of 47,XXX syndrome. The girl's karyotype was 47,XXX,del(7)(q34q36)(16%)/46,XX,del(7)(q3436)(84%).
Two important genes have been described to the 7q3 region: the homeobox HLXB9, for dominantly inherited sacral agenesis, and Sonic hedgehog, the major gene causing holoprosencephaly. Interestingly our patient had sacral dimple but not clinical features or CNS malformations of holoprosencephaly suggesting that the holoprosencephaly spectrum with 7qter deletion is extremely variable.
For the prevention the genetic counselling was indicated. The cytogenetic examination of the parents was recommended. Parental karyotypes were normal. The deletion has occurred de novo. The risk of recurrence of monosomy 7q3 for patient's sib was not increased.
The 47,XXX chromosome constitution is not associated with an obviously abnormal phenotype, and females with this karyotype cannot be distinguished from normal 46,XX females.

P0373. Molecular and cytogenetic analysis on multiple displacement amplification (MDA) products from single cells for Preimplantation Genetic Diagnosis (PGD).

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Preimplantation Genetic Diagnosis (PGD) is a technique where only a single cell is available for diagnosis. This limitation of the technique may be overcome with the use of MDA which amplifies the entire genome in few hours.
Buccal cells, lymphocytes and fibroblasts were used as a source of single cell DNA. Three cell lysis methods were used (Proteinase K, alkaline lysis, and modified alkaline lysis). DTT was excluded in the modified lysis. DNA from single cells was amplified either by MDA or by DOP. Two multiplex fluorescent PCR reactions were applied to the MDA products to test for MDA accuracy. MDA and DOP products were also used for metaphase-CGH and array-CGH analysis to check whether known aneuploidies could be detected.
Molecular analysis showed that the modified lysis protocol provided more accurate results. Different accuracy rates were obtained from different cell types for the loci tested (buccal cells: 44.3%, lymphocytes: 74.3% fibroblasts: 73.4%). Allele dropout (ADO) ranged 18.8-24.5% in MDA products. In metaphase-CGH, DOP products from single cells produced clearer results compared to MDA products from the same starting material. 50% of MDA products from single cells could not be interpreted because of high background noise. In array-CGH, two constitutional slides were tested with gDNA and MDA products with the latter giving unreliable results with high background noise.
In molecular analysis results seem promising for wider use of MDA for PGD. Further optimisation is needed for cytogenetic analysis especially in reducing the background noise.

P0374. Cytogenetic and Fluorescence *in situ* Hybridization Analysis of 229 Chinese Myelodysplastic Syndrome Patients Diagnosed Using the WHO Classification

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Two hundred and twenty-nine Chinese myelodysplastic syndrome (MDS) patients were diagnosed prospectively according to the WHO classification. There were 151 (66%) RCMD, 60 (26.2%) RAEB, 11 (4.8%) RA, 4 (1.7%) MDS-u, 2 (0.9%) RARS, and 1 (0.4%) 5q- syndrome. Conventional banded chromosome analysis revealed 37.4% of the cases with clonal aberrations while FISH using the probes for +8, 20q-, -7/7q-, -5/5q- and 11q23- showed the abnormalities in 33.3%

of the cases. The common chromosomal aberrations were +8 (15%), 20q- (10.1%), -7/7q- (5.8%), and -5/5q- (4.3%). The majority of WHO MDS cases had karyotypic features that are considered as either low or intermediate risk according to the International Prognosis Scoring System. Relatively higher percentages of poor risk karyotypes were observed among MDS subtypes RCMD and RAEB. Our study also demonstrated that banded chromosome analysis is the method of choice for MDS cytogenetic study and FISH is a useful adjunctive tool.

P0375. The cytogenetic analysis of 79 prenatally detected neural tube defects among 8370 prenatal samples

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Open neural tube defects (NTD) with an incidence of 0.1-0.15 % in West Europe and USA, are among the most common congenital anomalies. Prenatal detection of these birth defects has been increased by improved ultrasound techniques and maternal serum alpha-fetoprotein screening. It has been reported that 2-10 % of fetuses with neural tube defects have chromosomal abnormalities; however, there is no consensus on whether to offer karyotype analysis to patients with isolated neural tube defects detected by ultrasound examination. The aim of this study is to evaluate the chromosomal results of 79 prenatally diagnosed fetuses with NTD in 8370 (0.94 %) prenatal samples during the 7-year period from 2000 to 2007. Among those cases, open spina bifida was detected in 37 fetuses (46.8 %), anencephaly in 24 (30.3 %), encephalocele in 7 (8.9 %), meningomyelocele in 5 (6.3 %), holoprocencephaly in 4 (5.0 %), and acrania in 3 (3.8 %). Cytogenetic analysis was performed in all fetuses with NTD detected by USG examination. Of the 75 fetuses who were successfully karyotyped, 69 were found to have normal karyotype, whereas one had 69,XXX, 2 inv(9)(p11;q13), one 46,XX,t(5;7) and one 46,XX,-13,+der13. The overall rate of consanguinity between the couples who had prenatally detected fetuses with NTD was 21.5 % (17/79). We point out the importance of prenatal cytogenetic analysis in the patients with NTD

P0376. DNA secondary structure-forming propensity dictates translocation frequency

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There is evidence accumulating to suggest that non-B DNA structures have a potential for genomic instability that induces genomic rearrangements including translocations and deletions. One of the best studied examples in humans is the recurrent t(11;22)(q23;q11) constitutional translocation. The t(11;22) translocations have breakpoints on both chromosomes within specific sequences named palindromic AT-rich repeats (PATRRs). We suggest that the PATRRs form adopt a cruciform structure that is the source of genomic instability. Previously, we demonstrated that the existence of de novo t(11;22)s in sperm samples from normal healthy males using translocation-specific PCR. In this study, we show that polymorphism of the PATRRs affects the frequency of de novo translocation events. Symmetrical and longer alleles preferentially generate translocations, while asymmetrical and shorter alleles produce them at lower frequency. Further, we have validated the secondary structure-forming propensity of various PATRRs using in silico, in vitro, and in vivo analyses. As a result, symmetrical and longer alleles were more likely to form cruciform configuration. The secondary structure-forming propensity correlated well with the translocation frequency. Our results provide indirect but strong support to the hypothesis that the PATRR adopts a cruciform conformation in living cells that induces genomic instability leading to the translocation.

P0377. Orientation of tri- and polysomic Chromosomes reflect Intranuclear Order

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Ordered arrangement of chromosomes has been reported from many plants and animals including murine and human specimens [1, 2]. Genetic observations also indicated similar order [3, 4]. We see many indications exhibiting the order, e.g., in deviations in copy number of chromosomes. Tri- or polysomy is generated by mitotic nondysjunction and by asynchronous replication of a chromosome. One of the two chromosomes of a homologue is generally involved in polysomy. Orientation of these chromosomes conform to the basic order of homologues, which lie opposite each other. Here we shall present evidences of intranuclear order from human cells at meta- and interphase following GTG banding and fluorescence in situ hybridization.

P0378. Pericentric inversion in chromosome 9 in Indian population

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Pericentric inversion in chromosome 9 (p 11q 13) has been detected in 150 Indian individuals who had some clinical complications. Conventional peripheral blood lymphocyte culture and G-banding employed for individuals suspected carrying some chromosomal abnormalities where other investigational parameters were inconclusive. Unstimulated bone marrow culture and G-banding study was carried out in ten (10) teenage patients suffering from hematological malignancy. Individuals carrying inversion in chromosome 9 detected in peripheral blood had some kind of clinical problems related to either delayed puberty or infertility. Adults had the primary or secondary infertility as the major clinical expression. Pericentric inversion in 9 has earlier been reported to cause infertility, however its association with adolescent development has been found in teenage girls who have not attained menarche. Since pubertal delay in girls is a serious concern among parents, they have been found predominant. However, at adult stage mostly male carriers are the contributors to infertility. Apparently there is no phenotypic anomaly among the carriers. In another group of teenage whose bone marrow was tested for cytogenetic diagnosis pericentric inversion was additional abnormality, which was subsequently confirmed as constitutive through peripheral blood culture. In this subset of population, the inversion the inversion could be responsible for early onset of malignancy. Though human genome project (HGP) mapped the genes located in chromosome 9 and there is no phenotypic abnormality in carriers, clinical correlation of expression of this intra-chromosomal alteration needs to be investigated and establish to bring in some solution to its infertility related problem.

P0379. Pallister-Killian syndrome in a child with rare karyotype - a diagnostic problem

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Pallister-Killian syndrome (PKS) is a rare disorder characterised by a specific combination of anatomic anomalies, mental retardation and dysmorphic features. PKS results from a mosaic tetrasomy 12p and was first described, independently by two groups. PKS is one of the chromosome aberration syndrome in which clinical diagnosis is an important starting point in the cytogenetic diagnosis involving into the analysis not only lymphocytes, but also fibroblasts. An additional 12p in PKS patients are usually observed in only 2% of analysed lymphocytes, in 50-100% of analysed fibroblasts while in 100% of amniocytes and bone marrow cells. We report a case of PKS presenting an unusual karyotype: mosaicism 12p - tetrasomy/trisomy/disomy in fibroblasts and trisomy/disomy in lymphocytes. Marker chromosomes were investigated with conventional cytogenetic techniques followed by FISH. Only several reports of PKS presenting similar mosaic karyotype to this observed in our case were published. At the beginning of diagnostic process we considered the diagnosis of 12p trisomy. Thus, in combination with clinical manifestations and the fibroblasts karyotype, the Pallister-Killian syndrome was diagnosed. In our proband we observed a characteristic facial dysmorphism, severe intellectual dis-

ability with no speech ability, but we detected neither severe structural anomalies nor skin pigment abnormalities. Luebe et al. speculated that PKS patients with rare, mosaic tetrasomy/trisomy/disomy have milder phenotype and better prognosis than patients with the classic i(12p) mosaic in fibroblasts. However, our case does not confirm this speculation. Each additional case of PKS presented in literature will contribute to better understanding of this rare disorder.

P0380. Male with delusional disorder, minor anomalies and apparently balanced chromosomal rearrangement 46,Y,inv(X)(p11.23q23)

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We report on a 35 years old male patient with pericentric inversion of the X chromosome: 46,Y,inv(X)(p11.23q23) who was referred for cytogenetic analysis because of delusional disorder and minor anomalies. At his first visit he denied the presence of any psychiatric symptoms, and complained only about the somatic difficulties. He was convinced of having a uterus, ovaries and enlarged breasts and he wanted a confirmation of being a female. During the psychiatric interview he was calm, very talkative, with good eye contact, spontaneous and he presented his life experiences in an interesting way. He stressed that he had a menstrual bleeding once in the past. Somatic delusions, delusions about bodily functions were bizarre in the content and unarguable for the patient. They were central to his concern and his life. He was in paranoid position towards family and his social circle. Psycho diagnostic tests revealed a borderline intelligence and no cognitive dysfunctions.
In somatic examination pectus excavatum, kyphoscolyosis thoracolumbalis, gothic palate, convergent strabismus and the right eye amblyopia were found.
Cytogenetic investigations revealed an inverted X chromosome. Fluorescent in situ hybridization (FISH) using subtelomeric DNA probes for Xp and Xq confirm the pericentric inversion of the X chromosome. Further FISH analysis using a panel of BAC clones, allowed the identification of BAC's spanning both the Xp11.23 and Xq23 breakpoints.
No microdeletions or microduplications were found but array CGH of X chromosome should be performed in the future.
Regarding genotype-phenotype correlations no such a case was described in the literature.

P0381. Possible implication of *NPAS3* gene in a boy with psychiatric disorder and interstitial deletion of chromosome 14 revealed by microarray analysis.

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We report the case of a 19-year-old boy, second born of three children, who presents psychomotor delay, moderate mental deficiency, selective progressively worsening mutism and increasingly variable performances. Clinical evaluation did not fit a specific psychiatric disorder and the suggested diagnosis was pervasive developmental disorder not otherwise specified. Chromosome studies on blood lymphocytes showed proximal chromosome 16p duplication inherited through a phenotypically normal mother. DNA microarray studies (Affymetrix) showed a 1.68 Mb deletion in the chromosome 14q12-q13.1 region which was subsequently confirmed by FISH and molecular biology. Database analysis of the deleted region revealed at least 5 genes. Among these genes, Neuronal PAS domain-containing protein 3 gene is ubiquitously expressed in the adult brain and seems to play a role in neurogenesis. A previous study has shown its implication in a translocation found in a mother and daughter, with schizophrenia and schizophrenia associated with mild learning disability, respectively. An anomaly of the *NPAS3* gene could lead to psychiatric disorders such as those reported.

P0382. A new case of cryptic unbalanced t(1:12)(q44;p13.3) translocation with polymicrogyria

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Neurological disorders and seizures have been reported for most of the patients with del(1q) syndrome, but polymicrogyria (PMG) has been described only in two patients affected by a familial unbalanced translocation with 1q44qter monosomy and 12p13.3pter trisomy. PMG is a malformation in which the brain surface is irregular and the normal pattern is replaced by multiple small gyri separated by shallow sulci, and it is possibly due to a defect in neuronal migration.
Here we describe a new case of an unbalanced t(1;12) translocation with PMG in a five-year-old child presenting with facial dysmorphisms, mental retardation, microcephaly, corpus callosum and cerebellar hypoplasia, abnormal feet, hypospadia and seizures.
No chromosomal anomalies were detected by standard cytogenetic analysis. Array CGH analysis, by Agilent 44K chip, unraveled a terminal 12Mb deletion of the q arm of chromosome 1 and a 3Mb duplication of the distal segment of chromosome 12p. Features of the proband were consistent with both the terminal del(1q) and the dup(12p) syndromes, except for PMG that has never been described in either rearrangement.
As PMG seems to occur in t(1;12) translocations, we speculate that this defect might be caused by concomitant haploinsufficiency of 1q44 genes and trisomy of 12p distal genes. Candidate genes mapping in these chromosomal regions and related pathways involved in neuronal migration will be discussed.

P0383. Radiprotective effects of medicinal plants' polyphenols on human lymphocytes *in vitro*

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The objective of the present study is to evaluate radioprotective properties of *Crataegus monogyna* fruits, *Cornus mas* leaves and *Gentianella austriaca* exctracts in cultured human peripheral blood lymphocytes *in vitro*, after irradiation with 2 Gy of ^{60}Co γ-rays. Plants were collected at the mountain Maljen in Serbia; air-dried, powdered and total phenolic content was analyzed. In *C. mas* leaves ellagic and gallic acid as dominant compounds were found, whereas *C. monogyna* fruit was rich in procyanidins and flavonoids. Main constituents of *G. austriaca* were ϒ-pyrones and secoiridoids. Significant radiorecovery potentials of *C. mas* and *C. monogyna* were observed, seen as reduced incidence of radiation induced micronuclei, reduced level of lipid peroxidation products and enhanced apoptosis. Enhancing the apoptosis of irradiated cells enables removal of damaged cells from irradiated tissue emphasizing physiological mechanism of cell death with no inflammation. Both extracts gently slow down the cell proliferation enabling more time for repair. Simultaneously, prolonged time for the DNA repair and elimination of heavily damaged cells via apoptosis will enable faster homeostasis among cells in irradiated tissue. *G. austiaca* polyphenols acts as strong antioxidant: significantly reduces lipid peroxidation, incidence of micronuclei, enhances apoptosis with no perturbation in cell cycle. *G. austriaca* posses remarkable protective properties, and should be examined further in detail. Investigation of its biological activity could be important for many other diseases where lipid peroxidation products have been reported. This study may contribute in the search for novel radioprotective agents.

P0384. Over 60 cases of PGD-FISH during years 1999-2007

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Over 60 preimplantation genetic diagnostic cases have been performed during years 1999-2007 using fluorescence *in situ* hybridization (FISH) method in collaboration between IVF-clinics in Helsinki area (both private and public) and Medix Laboratories. The indications for the IVF-PGD procedures were an advanced risk for an abnormal chromosome number/material. The advanced risks were due to either one the parents being a translocation carrier, advanced maternal age

or repeated failure in IVF treatments. The procedures included 9 AS-(aneuploidy screening), 13 sexing, 19 robertsonian translocation and 20 resiprocal translocation cases. The probes were chosen depending of the detectable abnormality and all of them were commercially available. The probes were tested either on the carrier's lymphocyte preparates in the cases of balanced translocation or on both spouse's lymphocyte preparates. Altogether over 361 embryos and 533 blastomeres were studied. In these the success rate was 68-82 % depending from the class of study (AS, sexing, reciprocal or robertsonian translocation). From the studied embryos the rate of normal embryos were 40 % in As-cases, 58 % (desired=girl) in sexing, 22 % in reciprocal and 41 % in robertsonian translocations. As a result of the IVF-PGD procedures 9 healthy babies have been born. Altogether clinical pregnancy rate/ET was for AS 37,5%, sexing 30%, robT 21% and balT 21% and take home baby rate/ET was 12,5%, 30%, 16% and 7%, respectively. To conclude PGD using FISH is a good option for couples having an advanced risk for abnormal chromosome content to have a chromosomally normal child.

P0385. FISH study of 45,X/46,XX mosaicism in patients with idiopathic premature ovarian failure

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Premature ovarian failure (POF) (OMIM 311360 and 300511) is a disorder characterized by amenorrhea and elevated serum gonadotrophin level before 40 years of age that accounts for about 10% of all female sterility.
Objective: to investigate the implication of low level 45,X/46,XX mosaicism in the development of idiopathic premature ovarian failure.
Patients: 81 cases of POF-affected women with normal karyotype: 37 with primary amenorrhoea and 44 with secondary amenorrhoea of median age 25 years (range14 - 41) were analysed.
29 women with normal reproductive histories, having at least one child were selected as controls. The subjects in this group were approximately the same age as the patients with POF.
Methods: Interphasic FISH was performed in peripheral blood lymphocytes using centromeric probe of X chromosome associated with centromeric probe of chromosome 18 as a control.
Only nuclei with two signals for chromosome 18 were analysed for the signal pattern of the X chromosome in order to exclude hybridization artifacts. For each woman, 500 cells were analysed.
Results: In the control group, X chromosome monosomy was detected in 0.95 to 3.5 % of cells (mean ± 2 SD, 2,33 ± 1,34).
Among patients, 15 (18,5 %) presented a significantly higher percentage of monosomic cells (>2,33 ± 1,34).
Conclusion: this study indicated that POF in some patients may be attributed to low-level 45,X/46,XX mosaicism. FISH is more sensitive than routine chromosome analysis in the detection of low-level X chromosome monosomy and could be recommended for all POF patients with normal karyotype.

P0386. An unusual supernumerary marker chromosome diagnosed following amniocentesis for advanced maternal age: cytogenetic characterization and phenotype.

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A 40-year-old woman was referred for amniocentesis at 16 WG. Amniotic fluid chromosome studies diagnosed mosaicism for a "de novo" small supernumerary marker chromosome (SMC) in a male foetus: 47,XY,+mar [13] / 46,XY [8].
The marker was characterized using several molecular cytogenetic techniques. M-FISH studies indicated that the SMC contained euchromatic chromosome 1 material and this result was confirmed by positive whole chromosome 1 painting. FISH with probes for centromeric chromosome 1 and subtelomeric 1p and 1q regions was negative. Microdissection followed by reverse chromosome 1 painting suggested that the marker contained pericentromeric 1p and 1q material. In the light of these results, the parents elected to terminate pregnancy.
Autopsy of the 25 WG foetus showed slight craniofacial dysmorphism, bilateral camptodactylia and rocker bottom feet, but no organ malformation. Variable degrees of mosaicism was confirmed by cytogenetic analysis on amniotic fluid at termination and on tissues at autopsy (skin, lung, heart, kidney).The cytogenetic characteristics and partial trisomy 1 phenotype are discussed.

P0387. Trabecular meshwork structure in Primary congenital glaucoma and Sturge-Weber syndrome

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Primary congenital glaucoma (PCG) is a genetic disease which manifests at birth or in infancy. PCG characterized by buphthalmos, high intraocular pressure, corneal edema and photophobia. We had done electrone-microscopy of seven trabeculectomy samples. In which one case was PCG with Sturge-Weber syndrome. Sturge-Weber syndrome is defined as facial port-wine stain in association with ipsilateral pial,vascular anomalies and inconstant ipsilateral choroidal vascular lesions with glaucoma. Materials: Seven cases of PCG were enroled in this study. One case was PCG with Sturge-Weber syndrome Method. To look for any cytogenetic abnormality, lymphocyte culture was set and chromosomes were analysed with GTG banding. After informed consent trabecular tissue were collected to look for any structural changes in trabecular meshwork. Surgical trabeculectomy tissues were sent for scanning electrone micropy. For EM study specimens were fixed in glutraldehyde fixative for 12 hrs and then post fixed in the osmium tetra oxide at 1% for 2 hrs. Tissue was dehydrated and mounted and gold plated. Scanning electrone microscopy was done. Results: Cytogenetically all patients were normal. EM pictures of trabecular meshwork in all patients showed trabeculardysgenesis and some unidentified spherical structures of varying size were seen in case with Sturge-Weber syndrome Conclusion: Due to trabeculardysgenesis IOP rises in the anterior chamber leading to glaucoma in PCG cases. In Sturge-Weber syndrome, spherical structures of varying sizes were seen, may obstructing the normal aqueous outflow. These structures are occupying the vital space and damaging structure of trabecular meshwork leading to blockages causing rise in IOP leading to glaucoma.

P0388. Array-CGH analysis of products of conception with high density oligonucleotide arrays

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Fifty percent of miscarriages are due to chromosomal abnormalities. Cytogenetic analysis of products of conception (POC) provides information about the cause of miscarriage and recurrence risk. This analysis depends on success in culturing of fetal tissue and preparation of metaphase cells. Tissue culture failures and suboptimal chromosomal preparations render the cytogenetic analysis unsuccessful in 10-40% cases. In addition, selective growth of maternal cells may lead to erroneous normal findings Comparative genomic hybridization (CGH) is a laboratory technique that enables assessment of relative DNA copy number changes between fluorescently labeled patient and control genomic DNA. Tissue culturing is not necessary for this assay and it has more resolution than karyotyping. There are some reports of CGH studies on POC specimens. These studies are either classical CGH studies or array-CGH studies on BAC arrays. So far no oligonucleotide array CGH study on POC specimens has been published. That is the reason we decided to perform a validation array-CGH study using custom oligonucleotide arrays from Agilent technologies. In this study, we analyzed 24 POC (fetal tissue and chorionic villi) specimens with known karyotype results and 6 tissue cultures failures. We were able to generate array CGH results from all samples. Cytogenetic results and CGH results were 100% concordant. In addition, previously undetected deletions were observed in two specimens (16p21 and 22q11.22) were after oligonucleotide array-CGH. These results indicate that oligonucleotide array-CGH can be used as a rapid diagnostic tool for analysis of POC.

P0389. An age based cytogenetic analysis in prostate cancer patients in Southern India

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Prostate cancer is a malignant tumor that arises in the prostate gland. It is located near the bladder and rectum, which creates and stores components of semen.Prostate cancer is the second most leading cause of death in men due to any cancer type in India. Human prostate cancer is characterized by multiple gross chromosome alterations. However the specific genes involved in the development of prostate tumors are still largely unknown. The chromosomal region largely affected in prostate cancer patients are 1, 6, 7, 8, 10, 13, 16, 17, 18, X and Y.

Since chromosomal abnormalities are involved in tumorigenesis, we selected karyotypic analysis in Prostate cancer patients in Southern India patients.. Prostate size in the patients is increased proportional to their age, so we performed an age based cytogenetic analysis. In the present study blood samples were collected from various hospitals in and around Tamilnadu.

Out of the 40 blood samples (Prostate cancer samples) 36% of the slides showed chromosomal aberrations such as deletions, translocations, mosaics and inversion. Among three groups Group II and Group III showed majority of chromosomal abnormalities when compared to Group I. In Group II ,46 XY,del (1q-),46, XY inv (16), 46,XY,t(6q;16q+), 46 XY,del(5p-), 46,XY,inv(9), 46XY,del(8q-) chromosomal aberrations were observed. In Group III ,46,XY,t(6q;16q+), 46,XY,/46,XY del (5p-), 46 XY,del (Yq-), 46 XY, del (1q-) are identified. The chromosomes mainly affected in the present study include 1, 6, 5, 8 and 16. Identification of chromosome alterations may be helpful on arising better therapeutic strategies in future.

P0390. Report of a patient with duplication of proximal 20q: clinical and cytogenetic characterization

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Partial trisomies of the long arm of chromosome 20 are rare. Most cases reported in the literature are the result of malsegregation of a parental translocation and are complicated by additional partial monosomy or trisomy of another chromosome region. Here we report the first child of healthy non consanguineous parents with pure 20q11.2 trisomy. He was first referred at age 2 months for facial dysmorphism (prominent metopic suture, epicanthic folds, peripalpebral oedema, small and thick ears, prominent cheeks) and standard karyotype appeared normal. He walked independently at age 25 months and speech delay was noted. At 3 years of age, measurements were within normal limits. Cytogenetic analysis of cultured peripheral blood lymphocytes with high resolution banding revealed a male karyotype with an apparent interstitial duplication in the long arm of chromosome 20 of band 20q11.2. FISH studies with a whole chromosome paint probe (WCP) specific for chromosome 20 confirmed that the additional material was originated from chromosome 20. FISH studies with M-BAND and BAC probes specific for the long arm of chromosome 20 confirmed that the duplicated band was restricted to band 20q11.2. The full karyotype was therefore 46,XY,dup(20)(q11.1q12).ish dup(20)(WCP20+,RP5-1184F4++,RP11-382A12++). Both parents had a normal karyotype, in favour of a de novo duplication. To the best of our knowledge, only four cases of pure 20q trisomy resulting from duplication have been reported. We compare clinical features and cytogenetic findings of our case with the previously reported observations.

P0391. Array CGH delineation of de novo pure partial trisomy 17q24.3-qter due to a terminal duplication in one-year-old girl

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Pure partial trisomy 17q24.3-qter is only described in a few cases, but it is an increasingly recognized clinical entity (Bridge et al., 1985; Orye and van Bever, 1985; Schwanitz et al., 1988; Ohado et al., 1989; Ghaffari et al., 1998). The characteristic features of this chromosome aberration include varying degrees of mental retardation, short stature, narrow forehead, macrostomia, a simple and long philtrum, low posterior hairline, and genital anomalies (Schinzel, 2001). Here we report a detailed clinical and molecular investigation of a one-year-old girl with a de novo dup(17)(q24.3-qter). She presented with severe developmental and growth delay, microcephaly, hypotonia and the following dysmorphic features: narrow forehead, upslanting palpebral fissures, hypertelorism, low-set posteriorly rotated ears with pronounced antihelix, large mouth, a simple and long philtrum, micrognatia, low posterior hairline, thenar hypoplasia, cutis marmorata, hypoplastic labia and prominent clitoris. No brain or inner organ anomalies were found. Conventional cytogenetics revealed an additional material on 17qter identified by 244k Agilent oligoarrays CGH analysis as a duplication of chromosome region 17q24.3-qter with a breakpoint between 66,580,674 and 66,678,854bp. Our case is the first pure duplication of chromosome region 17(q24.3-qter) confined by array CGH studies and it shows the usefulness of recently developed array CGH technology to accurately characterize chromosome abnormalities, especially when they are de novo.

P0392. A novel reciprocal translocation t(l ;17) detected in a woman with recurrent abortion

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In the present study, we present a novel reciprocal translocation t(l;17)(17pter.17p36.2::1p13 1pter)(1pter.1p13::17p36.2 17qter). A couple was referred to us for chromosomal analysis because of the history of two spontaneous abortions. Both husband and wife had a phynotypically normal child from their previous marriage. There had been no history of repeated spontaneous abortions in their families. Chromosomal study was performed on peripheral blood lymphocytes. A balanced reciprocal translocation between chromosomes 1 and 17 was observed in the wife. Therefore, the karytotype was assecced as 46,XX,t(l;17)(17pter.17p36.2::1p13 1pter)(1pter.1p13::17p36.2 17qter). The husband had normal karyotype. To the best of our knowledge these breakpoints have not been reported previously. Thus, we can assume that this is the first report of a translocation between chromosome 1 and 17, with such breakpoints

P0393. Importance of the correct designation of breakpoints for structural chromosomal aberrations in preimplantation genetic diagnosis strategies

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Introduction: Three couples were referred to our PGD centre where the male partner was a carrier of a balanced reciprocal translocation. PGD protocols were designed according to the provided karyotypes. During the workup it was found that the breakpoints reported in the karyotypes were incorrect.

Methods:

Case 1: 46,XY,t(14;16)(q13;q11.1) and oligospermia

Case 2: 46,XY,t(1;4)(q12;q33) and poor sperm parameters

Case 3: 46,XY,t(1;21)(q21.3;q22.1) and primary infertility

FISH protocols were designed using probes for the chromosomes involved in each translocation. Protocols were optimised on metaphase spreads from both partners.

Results: Using the individual FISH protocols determined from the karyotype, it was found that in all three cases the incorrect break points had been reporeted.

Case 1: Breakpoint on 16q11.1 was incorrect as the breakpoint was below this region. The derivative chromosome 14 would be missed in the balanced embryo.

Case 2: Breakpoint on 1q12 was incorrect as the breakpoint was at the centromere. This could have led to an extra signal for the centromere of chromosome 1 in a balanced embryo.

Case 3: Breakpoint on 1q21.3 was incorrect as the correct breakpoint was 1q12. This could have led to an extra signal for the centromere of chromosome 1 in a balanced embryo.

New protocols were designed and the patients underwent PGD.

Conclusions: This study highlights the need to test PGD FISH protocols on metaphase spreads from the patient to confirm the karyotype. Incorrect designation of breakpoints could lead to the use of an uninformative protocol which could result in a misdiagnosis

P0394. Evaluation of the recurrent miscarriage by chromosome's analysis in the Cytogenetic Laboratory of the Clinic Obstetric-Gynecology Hospital "Dr. Dumitru Popescu" Timisoara

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Due to the age of the future parents, the consensus on the moment of evaluation of a recurrent miscarriage has changed from after third or fourth to after second miscarriages. Chromosomal anomalies are known as one of the common causes of the spontaneous abortion. The problem identification and the diagnostic procedures include a large sample of practices: cytogenetic studies, studies regarding the anatomic integrity of the genital organs, studies concerning the diagnosis of the endocrine abnormalities, and the assessment of the blood coagulation protein/platelet defects.

In the Cytogenetic Laboratory of the Clinic Obstetric-Gynecology Hospital "Dr. Dumitru Popescu", founded in March, 2006, the chromosome imbalance can be diagnosed by cytogenetic investigations of virtually any tissue type. The cytogenetic studies of abortuses (7 cases), were carried on using entire abortus (2 cases), placental villi (2 cases), amnion (2 cases), and, for the first time in our country, fetal biopsy (3 cases). For advanced gestation, the fetus' blood in a sodium heparin was also utilized (2 cases). The culture success rate was increased by using more sample types for the same case (4 cases). The used culture techniques - fibroblast cultures, amnion cultures, placental villi cultures, blood cultures - were standardized and their processing was done according to the standard protocols. The chromosomes analysis was carried on (Ikaros-Metasystem) after the GTG banding. We also analyzed, during the same period, blood samples of 10 couples with 2 or more spontaneous abortions.

Results: aneuploidies (2 cases 45, X, and loss of various chromosomes), 2 cases of normal karyotype and 3 cases of culture failure. Among the couples we found 1 balanced translocation t(4;15), 1 case inv 9 (p12;q13) and normal karyotype for the rest.

P0395. Chromosome Abnormalities (ChrA) detected by conventional and high resolution (HR) cytogenetic assessment in tumor cells (TuC) and blood lymphocytes (BLy) from 18 patients with renal cell carcinoma (RCC)

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In 18 patients with diagnosis of RCC, 10 males and 8 females, mean age 59.9+/-11.2 years (range: 17 to 74), conventional and HR studies were carried out in Chr from TuC and BLy. A total of 322 metaphases in TuC and 534 in BLy of patients and 534 in BLy of 18 healthy controls matched by age and sex were studied. Results showed numerical and structural changes of Chr in patients with RCC ($p<0.001$).

Chr breakages were found in 60% of TuC and 10% of BLy of 15 and 7 patients.

In patients with RCC, CrA were found in TuC/BLy: acrocentric segregation in 6/0, polyploidies (4/0), poly> 60 (12/1), endoreduplications (6/2), double minute (6/2), dicentrics (3/0), triradios (3/0), quadriradios (3/0). Numerical clonal changes: +7 in 4/1, +7+7 (2/0), +7+8 (1/0), +8 (7/0), +10 (5/2), +11 (1/0), +13 (1/0), -3 (1/0) , -8 in 1/0, -10 (2/0), -X (2/0), -Y (4/3). A translocation t(3;8) (3p14.2-8q24) was found in TUC of one patient; deletion del 3p14 and a complex reorganization were also found in one patient in each case.

We conclude that ChrA are present in a high proportion of TuC and in a lower proportion in BLy of patients with RCC. These ChrA should be considered inespecific since could be seen in other conditions such as different types of cancer, hematooncological disorders and virus infections, and also induced by mutagenic agents.

Further studies are required to determined the clinical value of these changes from the prognosis and therapeutic viewpoint in RCC.

P0396. Subtelomere rearrangements in couples with repeated miscarriages

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Subtelomeric rearrangements, currently detected by FISH, STR, MLPA or Array-CGH approaches, are known to be associated with mental retardation and congenital malformations. Few reports describe subtelomere rearrangements in women with repeated miscarriages. By FISH analysis with a complete panel of subtelomere probes (Cytocell, UK; Vysis, USA; MP, France) we investigated 60 couples suffering for recurrent abortions (>2 aborted foetuses). All couples had normal karyotype and no clinical or genetic anomalies. In 2 couples (3%) we identified a subtelomeric rearrangement. In a couple referred for four consecutive 1st trimester spontaneous abortions, the spouse, nullipara, had a reciprocal translocation t(9;16)(q34;p13.3) de novo. In another couple with three 1st trimester spontaneus miscarriages and a healthy son, FISH using Multi-T probe device disclosed a 9q subtelomere duplication as an insertion onto chromosome 7p close to the apparently normal 7p subtelomeric region. The same rearrangement was also present in her son, in her brother and her mother who was reported as having experienced repeated abortions. FISH probes from other commercial sources were not able to confirm duplication of 9q subtelomeric region onto 7p. Fiber-FISH experiments revealed that the different commercially available 9q subtelomeric probes actually span different 9q regions being only the most distal one able to display the 9q duplication.

Further studies on large series of patients will allow to establish repeated miscarriages as an indication for subtelomeric analysis. The finding that various FISH probes reported as specific for the same subtelomeric region map at different chromosomal sites could be relevant for subtelomere analysis.

P0397. Case report: Patient with ring chromosome 4

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Ring chromosome formations are rare structural chromosome aberration. Most of them are sporadic cases. Patients with ring chromosome 4 are rarely observed. They associate concomitant loss of the telomeric 4p and 4q regions and lead to variable clinical manifestations depending on the size of the deleted chromosomal material. The authors report a 2 years old male patient with a near normal psychomotor development, some minor dysmorphism and with cytogenetic analysis from lymphocytes showing a mosaic karyotype 46,XY,r(4)[86]/46,XY[14]. Fluorescence in situ hybridization (FISH) study was also performed. We compare the phenotypic appearance of our patient with the previously reported cases of ring chromosome 4 in the medical literature.

P0398. Clinical features of a case with ring chromosome 18

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Rings of chromosome 18[r(18)] are frequent among ring chromosomes: depending on the size of the deleted regions in 18p and 18q, the clinical symptoms of r(18) correspond more or less to the typical signs of the 18p and 18q deletion syndromes. Ring chromosome 18 [r(18)] syndrome is characterized by mild to moderate learning disability, behavioural disorders, and various dysmorphic features.

Here we report an additional case of a 14 months girl with r (18). The girl was born at term after an uncomplicated pregnanacy and delivery. Birth weight was about 1.5 kg, length 48cm, and head circumference 36cm. The girls presented hypertelorism, hypotonia, epicanthal folds, abnormal fingers, low set ears, and abnormally growth teeth. Echocardiography indicated dilation of the aorta.

Karyotyping after lymphocyte culture at the age of 14 months revealed 46,XX,r(18)(q21.2qter). The parent had normal karyotype.

The clinical feature of our case is mostly compatible with the other reported cases of r(18) except the presence of abnormal teeth and heart problem. This report further contribute to to the clinical of the r(18).

P0399. Molecular Characterization of a case of ring chromosome 9

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Ring chromosomes are rare and are typically de novo;the syndrome associated to the 9 ring is not commonly observed and is characterized by constant signs, such as microcephaly, psychomotor retardation of varying entity and facial dysmorphism.
We report a boy, born of non-consanguineous parents in 2005, and referred to the genetics laboratory because of microcephaly. Cytogenetics study showed a karyotype interpreted as 46, XY, r(9). Parental studies showed the ring was de novo in origin.
C-banding showed the ring chromosome was monocentric. Using a painting chromosome 9 fluorescent in situ hybridization (FISH) probe we confirmed no other chromosome involved. To define the breakpoint on the 9p and 9q arms, FISH was performed with Telvysion 9p and Telvysion 9q probes covering both regions. We detected at least a 95Kb deletion in 9q but no deletion was detected with the telomeric 9p FISH probe.
To define the chromosomal breakpoints more closely, FISH-mapping with the RCPI-11 Human Male BAC Library using clones spanning chromosome 9 telomeres is undertaken.
Using FISH we have defined the loss of material more precisely and have shown that the subsequent monosomies are responsibles of the clinical evolution of the patient.

P0400. Inverted duplications associated with terminal deletions in six different ring chromosomes

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Inverted duplications associated with a distal deletion (inv dup del) have been reported for several chromosomes, but hardly ever in ring chromosomes. Characterizing, by array-CGH (Kit Agilent, 75 Kb and 6,5 Kb) and FISH, 25 different ring chromosomes in patients with phenotypic abnormalities, we identified in six of them inverted duplications associated with a terminal deletion at one end. At the opposite end no deletion was present in four cases, whereas a second deletion was present in two cases. Moreover, in one case with an inv dup del(13q), a further duplication of (13)(q12.21-3.1) was present in mosaic state.
Peripheral chromosomes analysis of the patients showed the following karyoptypes:
Case 1: 46,XX,r(13)(p11q34); case 2: 46,XX,r(15)(p11q26); case 3: 46,XX,r(18)(p11q23);
Case 4: 46,XX,r(13)(p11q34); case 5: 46,XX,r(22)(p11q26); case 6: 46,XX,r(18)(p11q23)
Mental retardation and dysmorphic features are common findings in the six patients.
Our results suggest that a more complex mechanism may be involved in the formation of some ring chromosomes and that ring formation may represent a new mechanism involved in the stabilization of broken chromosomes.
This new mechanism of ring formation has important phenotype/genotype implications since it implies that phenotypic correlations cannot be done assuming a simple deletion before having excluded this type of rearrangement.

P0401. Characterization of ring X chromosome by FISH: Role of X inactivation

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Some ring X [r(X)], have been described with MR. This is the result *of* functional disomy *of* the genes in the r(X) secondary *to* loss *of* the X-inactivation locus (XIST). We aimed *to* study the effect *of* r(X) on the clinical features, to correlate the presence of XIST and the pattern *of* X replications to the IQ level. The subject of this study was 54 cases with TS. Patients were subjected to clinical examination, estimation *of* I.Q. and cytogenetic analysis. Cases with atypical TS were further subjected to FISH using WCP for X , locus specific for XIST. , differential replication studies with BrDU for r(X).
13 cases had ring (X). By FISH, the origin *of* these rings was derived entirely from the X chromosome. XIST was found in all the rings. Ten cases had small rings, 3 had large rings. MR was found in 90% *of* cases with small r(X), the large r(X) had normal mentality. A significant relation was detected between the presence of r(X) and the lower I.Q.. Replication pattern showed the I.Q. level decreased with the presence of active r(X).
We conclude that abnormal stigmata different *from* the typical T.S. may suggest the presence of the r(X). The mechanism of the presence of active r(X) in our cases was not due to deletion of XIST gene and may be due to silencing of the gene due to interruption or mutation. Failure *of* inactivation does not necessarily lead to MR. We recommend more extensive studies on XIST.

P0402. Translocation Down syndrome; report of four de novo cases from Iran

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Down syndrome occurs in about one of 750 live births and is associated with a variety of karyotypes. Nearly 92.5% have simple trisomy-21, which is related to the maternal age. In about 4.8% the extra chromosome 21 material is present in the form of an unbalanced Robertsonian translocation or as an isochromosome of the long arm of chromosome 21, and does not vary with age. The remaining 2.7% are heterogeneous and include mosaicism, double trisomies and reciprocal translocations. In about three-fourths of translocation Down syndrome, neither parent is a carrier, and a mutation in the germ cells of one parent has caused the translocation. No one knows what causes these mutations.
We report four infants (three boys and one girl) from the four different families, all showed typical clinical features of the Down syndrome. The parental age of the four cases were between 20-30 years old. Chromosomal analysis were made, using the standard banding techniques, for the cases and their parents. The karyotypes of the three cases were 46,XY,der(21;21)(q10;q10),+21 and for the fourth one was 46,XX,der(14;21)(q10;q10),+21. The karyotypes of the parents of the four infants were normal.

P0403. Segregation and pathogenesis of balanaced/unbalanced homologous Robertsonian translocations, t(13;13), t(14;14); t(15;15), t(21;21) and t(22;22) - Case reports and review

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One in 900 humans is born with a Robertsonian translocation. The most frequent forms of Robertsonian translocations are between chromosomes 13 and 14, 13 and 21, and 21 and 22. Robertsonian translocations (balanced or unbalanced) involving acrocentric chromosomes, 13,14,15 and 21, 22 are well known chromosomal abnormalities leading to multiple congenital anomalies, infertility, repeated fetal loss, dysmorphism and mental retardation. However, homologous Robertsonaian translocations, t(13;13q),t(14;14),t(15;15) and t(22;22) are relatively rare. Carriers of balanced ROBs are at an increased risk of having chromosomally unbalanced, phenotypically abnormal offspring. These individuals are trisomic for one of the chromosomes involved in the translocation, with three copies instead of the normal complement of two. Carriers of ROBs are also at an increased risk of uniparental disomy (UPD), the inheritance of both chromosome copies from a single parent. Uniparental inheritance of some chromosomes has been shown to be deleterious due to the effects of imprinting (the differential expression of genes depending on the parent of origin).
Risk estimates vary depending on the type of rearrangement. Carriers of homologous acrocentric rearrangements are at very high risk of having multiple spontaneous abortions and chromosomally abnormal offspring. Parents of fetuses and children with unbalanced homologous acrocentric rearrangements are rarely found to be carriers or mosaic for the same rearrangement.

P0404. High frequency of robertsonian translocation in idiopathic infertile men of North Indian population.

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Robertsonian translocation (RT) is the most common structural rearragement of human chromosomes occurring at a rate of 1 /1000 live births. Of these, 60% inherit the rearrangement from one of their parents and 40% occur denovo. This proportion rises to 1% in infertile men & has been associated with infertility. This study was planned with the aim to determine the incidence of RT in men with spermatogenic arrest and to correlate if cases with such structural aberrations lead to recurrent ART failure. Our finding of chromosomal aberrations (3.7%) in the infertile males are in good agreement with literature of 3.3%. In this study on men with non-obstructive azoospermia and oligozoospermia, three men had 13q14q fusion. Thus the frequency of robertsonian translocation in our study was nearly 30 fold higher than in general population (0.1%). Although robertsonian translocation is likely to be found in chromosomes investigation of infertile men, their role in oligospermia is not clear. The testicular histology of the men carrying such a rearrangement shows a variable picture, ranging from severe impairment to near normality. Individuals carrying each of the ten possible nonhomologous robertsonian translocations of the five humans acrocentric chromosomes (13,14,15,21, & 22) have been reported, but two combinations, rob(13;14) & rob (14;21) are observed at a greater frequency than the rest (73% & 10)%,respectively. Since there is a high frequency of robertsonian translocations in infertile men drawing a genotype and phenotype correlation in these studies will help to asses the severity of spermatogenic arrest in these cases.

P0405. Incidence of satellite associations in lymphocytes of breast cancer patients

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Satellite association (SA) was studied in lymphocyte cultures from normal individuals and patients with primary breast carcinoma, totally 40 individuals and 1049 metaphases. The degree of satellite association was estimated by the frequency of cells exhibiting associations, by the number of associations per cell, and by the number of chromosomes in an association. The degree of SA was found to be significantly higher in patients than from those of control individuals (p=0.0001). Also there was a significant difference in the number of associations per cell between the two groups (p=0.0001). In the control group DD and DG associations were most frequent (41% for both) but in the patients DG was the most (58%). Scince satellite association is considered as a phenomenon which often causes nondisjunction, it may lead to chromosomal abnormalities in tumor cells.

P0406. Identification of Sex reversal syndrome using conventional cytogenetic technique

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Advance in experimental endocrinology, biochemistry, genetics, and molecular biology have all contributed to our understanding of the process of human sex differentiation in the decades.
Based on the recognition of the underlying anomaly in the process of sexual differentiation intersex disorders may be divided into abnormal gonadal determination and abnormal genital differentiation males with ambiguous genitalia but two differentiated testis are called MPH.
Females with ambiguous external genitalia but normal ovaries and normal internal genitalia are called FPH.
Abnormal gonadal determination is mainly dependent on sex chromosomal defects that can be detected by cytogentic analysis or by the DNA probes for genes located on the Y chromosome.
The XX males may be divided in to 3 subgroups:
46,XX males with the SRY gene(46,XX males without the SRYgene)and XX/XY mosaics.
DAX1 lies on the X chromosome. When it duplicates it causes an individual who is genetically male to develop physically as a female.
During the 8 years (from 1997 to 2006) we have reported 32 patients referred for sex reversal abnormality which was more common in the female group (n=16 , %0.7) compare to male population (n=10 , %0.4).
We conclude in here, that simple conventional cytogenetic methods are very helpful to identify male or female with sex reversal in addition molecular cytogenetic technology such as fish and molecular method to investigate the presence or absent of some critical genes such as SRY would be very useful to explain phenotype heterogeneity among sex reversal group male or female.

P0407. The MLPA method in diagnostic of *SHOX* gene and PAR1 deletions

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The short stature homeobox-containing gene (*SHOX*; GeneID:6473) is located in the major pseudoautosomal region (PAR1) of the human sex chromosomes. Extensive deletions encompassing the *SHOX* gene area or sequence changes that alter the gene product level or its functional properties were identified as the molecular basis of Léri-Weill dyschondrosteosis (LWD), Langer mesomelic dysplasia (LMD), and idiopathic short stature (ISS). In particular, approximately 60-80% of cases of LWD are due to such mutations. The rearrangement of sex chromosomes is the frequent cause of PAR1 deletions.
The presence and extent of *SHOX* gene and PAR1 deletions was detected using multiplex ligand-dependent probe amplification (MLPA) analysis in a group of patients with dyschondrosteosis phenotype. In two female probands previous cytogenetic studies had revealed the most frequent X;Y translocation, t(X;Y) (p22.3;q11). All other probands displayed normal karyotypes.
The MLPA analysis fully confirmed the cytogenetic results in both female probands with translocation. Moreover, the MLPA method was able to refine the extent of the Xp deletion and to specify the clinical prognosis in case of bearing a male foetus. We further observed a PAR1 deletion, including *SHOX* gene and its regulatory sequences, that does not exceed the pseudoautosomal region in a proband with a normal karyotype. A small deletion was detected covering the potential regulatory region of the *SHOX* gene in several subjects. However, its relevance to LWD phenotype has yet to be confirmed.
Our study proved the MLPA method as a rapid, sensitive, and suitable for detecting the *SHOX* gene and PAR1 deletions.

P0408. Analysis of immature germ cells in sperm from men with normal and impaired spermatogenesis

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The first step for looking at male infertility is usually confined to semen analysis. Such analysis gives valuable information about mature germ cells: their concentration, motility and morphology. However, semen is also useful souse of immature germ cell. More detailed information about spermatogenesis might be obtained by analysis of ejaculated immature germ cells using the method called Quantitative Karyological Analysis of Immature Sex Cells (QKAISC) (Kurilo, 1993). This method is used to determine the relative portion of germ cells at different stages of spermatogenesis and the stage of spermatogenesis block.
The samples of semen from 4 control subject (46,XY) and 24 patients with impaired spermatogenesis (46,XY) were analysed by means of QKAISC technique. In a control group the rate of immature germ cell in ejaculate was about 1,5%. Frequencies of spermatogenetic cells before and after MI division, and degenerated cell were 1,98%, 77,54% and 20,48% respectively. No difference between control subjects and patients with normal and slow abnormal semen parameters (8 subjects). In 6 patients with severe oligoastenoteratospermia and oligoastenosermia and 10 patients with azoospermia the increased number of degenerative spermatogenic cells and decrease number of spermatocytes II and spermatides were detected. The moderate correlation between concentration of spermatozoa in ejaculate and rates of spermatogenetic cells after MI division (+0,55; P<0,0058) and rate of degenerating spermatogenetic cells (-0,53; P<0076) was found. These results demonstrate the significance of QKAISC technique in perform-

ing of complex examination of ejaculate from patients with infertility. Supported by CRDF&RFBR.
Kurilo et al. Probl. Repr.(rus) 1995.v.3.p.33

P0409. Unusual small supernumerary marker chromosome (sSMC) in a woman with Triple X syndrome

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Small supernumerary marker chromosomes (sSMC) are small additional chromosomes. sSMC have been reported previously in four types of syndromes associated with chromosomal imbalances: in approximately 150 cases with Turner syndrome, 26 cases with Down syndrome and only one case each with Klinefelter syndrome and “Triple-X”-syndrome. Here we report the second case with an sSMC detected in addition to a Triple X karyotype.
The reported patient was referred to our clinic because of mild mental retardation, developmental delay, dysmorphic face. Three cell lines was detected, one with 47, XXX, one with 46, X, +mar, and one with 46, XX. The presence of a marker chromosome in this case generally implicates a sex chromosome origin. It may also originate from a non-sex chromosome.

P0410. Sex chromosome aberrations associated with reproductive failure problems

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Patients with sterility, primary amenorrhea, azoospermia or other reproductive failure have indication for cytogenetic analysis. The aim of this study was to evaluate the contribution of sex chromosomal abnormalities in such cases. We report here results of 22 patients, who visited Mother and Child Health Care Institute of Serbia “Dr Vukan Cupic” from 1996-2006.
Cytogenetic analysis was performed on 72-hour peripheral blood culture lymphocytes. Metaphases were karyotyped using G and C banding technique for chromosome identification. Molecular-genetic methods are used for solving cytogenetic dilemmas - FISH with X centromeric region specific probes (DXZ1) and PCR with primers for SRY gene and heterochromatin region of Y chromosome (HRY).
In 3 males with azoospermia karyotype: 47,XXY was found. At 6 cases of male sterility following sex chromosome aberrations were seen: 47,XXY; 46,i(X)(q10)Y; 46,XY[33]/47,XYY[4]; 46,XY[28]/47,XYY[8]; 46,X,der(Y)t(Y;Y)(p11.3;q11.1) and 46,X,r(Y)(p11.3;q12),inv(9)(p12q13)[22]/45,X,inv(9)(p12q13)[3]. In last two cases we provided presence of SRY gene and HRY using PCR method.
Following karyotypes: 46,X,i(X)(q10); 46,X,i(X)(q10)[30]/45,X[3]; 47,XXX[3]/48,XXXX[1]/46,XX[96] were associated with female sterility. Primary amenorrhea diagnosed in 11 female was combined with karyotypes:
45,X; 46,X,del(X)(q13); 45,X[34]/46,Xder(X?)[30] and 46,XY(8 cases).
To determine numerical aberration of X chromosome and define mosaic karyotype FISH with X - centromeric region probe was used. The presence of Y chromosome was confirmed with PCR.
Cytogenetic and molecular genetic approach is very important step to establish the genetic etiology in reproductive failure abnormalities. Our study shows that sex chromosomes aberrations are often associated with these disorders, which is crucial for genetic counseling and future possible preimplantation management.

P0411. Application of FISH to identification of subtelomeric rearrangement in cases of intellectual disability and in a couples with reproduction problem

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FISH technique with subtelomeric probes was applied for cytogenetic examination of 96 patients with normal karyotype obtained with routine banding methods. Patients were divided into two groups. Group I composed of 76 patients with intellectual disability, dysmorphic features; group II composed of 10 couples (20 individuals with history of several miscarriages and offspring with abnormal phenotype. In group I in 4 (5%) cases subtelomeric aberrations were found: 3 cases of subtelomeric deletion 1p36 (2 de novo and one due to inherited 1;12 translocation (with resulting monosomy of subtelomeric region1p and trisomy of subtelomeric region of 12q). In all 3 cases phenotypic features were characteristic of 1p deletion syndrome.
In the case No 4 balanced de novo translocation 19;22 was found. In the other 3 cases of group I subtelomeric polymorphic variants of chromosome 2q (inherited deletion) and 7q (inherited duplication) were found.
In group II three families with subtelomeric translocations were diagnosed: (7;14), (4;7) and (9;18): in two families chromosome 7q subtelomeric deletion was diagnosed (one of them prenatally); holoprosencephaly and associated abnormalities (cleft lip and palate, microcephaly) were observed in these cases (most probably sonic hedgehog gene being involved).

P0412. Molecular characterization of a mosaicism with a complex chromosome rearrangement: Evidence for coincident chromosome healing by telomere capture and *neo*-telomere formation

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Broken chromosomes, such as terminal deletions, must acquire new telomeric “caps” to be structurally stable. Chromosome rescue could be mediated either by telomerase through *neo*-telomere synthesis or by telomere capture. The first process is the main mechanism healing broken chromosomes in germline cells, the latter in somatic cells. Here, we describe a clinical and molecular study of a 14 year-old girl presenting with mental retardation, facial dysmorphism, urogenital malformations and limbs anomalies. High resolution G banding shows a *de novo* complex chromosome rearrangement in mosaic with two different cell lines, one cell line with a deletion 9pter and one cell line carrying an inverted duplication 9p and a non-reciprocal translocation 5pter fragment. Using array comparative genomic hybridization (a CGH), fluorescence *in-situ* hybridization (FISH) and polymorphic markers we deduced the most likely sequence of events that generated this complex mosaic. Surprisingly, we show evidence for simultaneous stabilization of rearranged chromosomes by telomere capture and neo-telomere synthesis. During embryogenesis, a double-strand break occurred on the paternal chromosome 9. Following mitotic separation of both broken sister chromatids, one acquired a telomere *via neo*-telomere formation, while the other generated a dicentric chromosome which underwent breakage during anaphase, giving rise to the del inv dup(9) that was subsequently healed by chromosome 5 telomere capture.

P0413. Breakpoints analysis of a balanced de novo translocation t(3;18)(q13.13;q12.1) in a patient with Opitz C trigonocephaly syndrome

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We present molecular characterization for a balanced translocation t(3;18)(q13.13;q12.1) in a male patient. He had clinical manifestations of the Opitz C trigonocephaly syndrome (OCTS) including trigonocephaly, a prominent metopic ridge, upslanting palpebral fissure, epicanthal folds, high-arched palate, thick and irregular alveolar ridge, long philtrum, redundant nuchal skin, a genesis of the corpus callosum, and hypotonia, who had been recently reported [Am. J. Med. Genet.

140A:1655-1657 (2006)]. Since responsible genes for the OCTS have not been isolated, though it is thought to be a heterogeneous condition, it would be important to investigate the precise structure for the breakpoints.
Fluorescence in situ hybridization (FISH) analysis identified a breakpoint spanning BAC clones on the chromosome 3q containing a gene encoding a member of the immunoglobulin superfamily (IgSF). Following FISH analysis by cosmids subcloned from the BAC clone plus Southern blot analysis demonstrated the breakpoint was in the gene. Inverse PCR and sequencing revealed the breakpoint was in exon 5 of the gene. Sequence analysis for the other breakpoint at 18q12.1 indicated that the translocation was occurred with 10-bp deletion in the exon 5. We could not find any genes around the other breakpoint according to the Genome Browser web site. Semi-quantitative RT-PCR analysis showed that the IgSF gene expression in B cells of the patient was reduced approximately 50% of the normal level.

P0414. Translocation (2;5) (p21-p15): Clinical findigs in a female case

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INTRODUCTION: The concurrence of this translocation involving a t(2;5) (p21-p15) has not been previously reported. We report a female case with this translocation.
CASE REPORT: The propositus a 5 years-old female, was the product of the second pregnancy from non-consanguineous parents. The mother presents an obstetric history of gravida: 2, abortion 1, caesarean 1. The pregnancy was interrupted by caesarea at 7 months of gestation by a premature rupture of membranes. At birth the weight was 2 kg, and multiple dysmorphic features were characterized by marked hypotonia, developmental delay and poor growth. She presented pyloric hypertrophic confirmed by barium studies. Surgical repair was performed at 4 months old without complications. The physical examination at this time showed height 15,500 gr (10-20 centile) weight 96.5 cm (3rd centile) and OFC 48 cm (10-20centile). Facial dismorfism characterized by strait biparietal diameter, prominent metopic suture, sparse eyebrows, hipertelorism, internal epicanthal folds, downslating palpebral fissures, high nasal bridge, broad nose, downturned corners of the mouth, thin superior lip and high palate; in hands: clinodactyly and camptodactyly of 5th fingers bilateraly was observed.
Hipoacusia was diagnosed by audimetry and craneal CT shown cochlear bilateral agenesia. Laboratory studies were normal. Standard chromosome studies on blood with 550-banded resolution revealed a karyotype of 46,XX, t(2;5)(p21-p15).
DISCUSSION: Few chromosomal translocation that involve chromosome 2 and 5 were reported, but all are related with other breakpoints (Kimberley 1999, Gilliam 2000). The molecular cytogenetic studies should be made in the patient to identify the genes involve in the translocation breakpoints.

P0415. Chromosomal Rearrangements in Patients with Reproductive Problem

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Pregnancy loss, particularly early miscarriage is a common problem that affects many women and their partners. Most spontaneous miscarriages are caused by an abnormal karyotype of the embryo. Balanced reciprocal translocations are present in 2-5% of couples who experience recurrent pregnancy loss. Structural chromosome rearrangements may result in unbalanced chromosome errors in pregnancies. We carried out cytogenetic investigations in 262 couples referred for recurrent spontaneous abortions (RSA). Chromosome analysis was performed on peripheral blood lymphocytes and GTG banding analyses at 550-600 band level. FISH technique was applied using WCP probes for 1 and 2 chromosomes.
We identified six cases of reciprocal translocations. Robertsonian translocations were detected in six cases (all carriers were female|) and pericentric inversion was found in one case. Sex chromosome abnormality was defined in one case with mosaic Turner syndrome. All cases of structural chromosome abnormalities caused the miscarriages, except one where a child with different clinical manifestation was born. As result of unbalanced gamete formation the translocation 46,XYt(4;9)(q26;p23) lead to partial trisomy of 4q26 region.
In our study the overall frequency of balanced chromosomal rearrangement as the main group of chromosomal aberrations was found to be 3,1%.
In conclusion we insist on the advantage of systematically performing a karyotype in case of RSA consisting on the detection of chromosomal abnormalities. The central concept in genetic counseling is the estimation of the probability of unbalanced progeny at birth and other unfavorable pregnancy outcomes as well the prognosis of success of assisted reproductive technologies.

P0416. Two cases of unbalanced offspring due to rare adjacent-2 segragation in non-relative translocation 4;15 carriers

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We report on two patients with partial trisomy of chromosome 4 and partial monosomy of chromosome 15 resulting from parental translocation 4;15. Translocation carriers were non-relative, other unbalanced offspring in these families have not been described although miscarriages and stillbirths have as well as liveborns been reported in family histories of both carriers. Further investigation with FISH has revealed different breakpoints on chromosome 15, proximal from Prader-Willi/Angelman specific region and distal, respectively. Obvious phenotypic difference was probably due to 15q11-13 loss of one of our patients.
Because of different segregation during meiosis I, various unbalanced karyotypes can arise in balanced translocation carriers. The frequency of unbalanced gametes varies from one translocation to another. Adjacent-2 segregation mode, in which each daughter cell gets chromosomes with homologous centromeres, is quite rare - on an average 5-10%. The type of unbalanced segregation depends on the nature of the chromosome involved and the breakpoints. Considering the pachytene diagram determined by breakpoints, the balanced translocation t(4;15)(q21;q11.2) and t(4;15)(q21;q13.1), respectively seems predisposed to chromosome imbalance through adjacent-2 segregation. Genetic counseling focused on prediction of the mode of imbalanced segregation will be discused.

P0417. Identification of a novel deletion in 3q23-25 in Treacher Collins Syndrome

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Treacher Collins syndrome (TCS) is a rare genetic disorder characterized by craniofacial deformities. TCS occurs in an approximate rate of 1:50,000 of live births. The disorder is inherited in an autosomal-dominant pattern. The typical clinical features include downward slanting eyes, sparse eyelash, micrognathia, cleft palate, microtia and notch in the lower eyelid called coloboma. More than 129 different mutations have been reported in patients with TCS. Patients with TCS were reported to be heterozygous for mutations in the TCOF1 gene, which is located in 5q32-q33.1. This gene codes for a protein of at least 1411 amino acids, treacle, which is a nucleolar phosphoprotein. The protein coded by this gene assists in protein sorting during particular stages in embryonic development, particularly that of the structures of the facial bones. There are reports with a small interstitial deletion of 3p,46,XY, del(3)(p23p24.12) and pericentric inversion inv(2) (p11.2q21) associated with TCS. We have done the cytogenetic analysis of a family with typical physical features of TCS. The high-resolution G-banding analysis has shown a small novel deletion in 3q23-25. This novel deletion in 3q23-25 has been found in two members of the family. Linkage of TCS to the 5q31.3q33.3 region has been established. We also confirm a de-

letion in 5q33 in all members of the family. This finding may represent a more severe manifestation of the mandibulofacial dysostosis. TCS is a heterogeneous entity, and evaluation and counseling of affected individuals should be undertaken with caution.

P0418. Partial trisomy 12p and deletion 5p: an overlap of dysmorphic features

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We describe a 2-year and 11 month-old girl who presented with dysmorphic facial features, short neck, supernumerary nipples, short wide hands, clinodactyly of the fifth finger, postnatal growth retardation, generalized hypotonia and developmental delay. Detailed clinical examination did not reveal associated congenital malformations. Cytogenetic evaluation on high resolution G banding showed aberrant chromosome 5 in all metaphases. The karyotype was designated as 4 6,XX,der(5),t(5;12)(5p15.3;12p12.2)mat. Molecular analysis confirmed deletion of subtelomere 5p and trisomy of subtelomere 12p.
A comparison of the clinical findings in our patient with previously described cases of pure 12p trisomies is presented. Pure trisomy 12p has a well delineated dysmorphic features and is often associated with different major malformations. Our patient did not display these typical features, phenotypic manifestations being more compatible with monosomy 5p. Rare structural rearrangements may lead to complex clinical presentations. Detailed clinical description of patients is needed in order to delineate the phenotype and improve genotype-phenotype correlation.

P0419. Newborn with partial trisomy 11q resulting from mother's t (11; 21) balanced translocation

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There is small number of reported cases with partial trusomy 11q, which do not result from the most frequently observed translocation in humans: t (11; 22).
We report a female newborn, the first child in family, with phenotype characteristic for partial 11q trisomy, present at birth: microcephaly, hypertelorismus, short, broad nose, long, prominent philtrum, high-arched plate, retraction of the lower lip, microretrognatia, low set, malformed ears, short neck, poor positioning of feet, wrinkled palm and feet skin and muscular hypotonia. Peripheral blood chromosomal analysis of index case and her family revealed partial trisomy 11q in newborn, resulting from rare mother's t (11; 21) balanced translocation. Further investigation of mother's family has shown that t (11; 21) was not present at her parents.
The cytogenetic and clinical finding of index case and her family will be presented.

P0420. DOWN SYNDROME IN A PATIENT WITH PARTIAL TRISOMY 21 RESULTING FROM A RARE MATERNAL SHIFT

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Down syndrome results generally from the presence of an extra copy of chromosome 21. Unbalanced translocations or abnormal recombinant chromosomes resulting from a pericentric inversion have also been observed. Here, we report on a child with a Down syndrome phenotype carrying a partial trisomy 21q22 resulting from "aneusomie de recombinaison" in a maternal shift. During pregnancy, the mother underwent amniocentesis because of pyelectasy detected by ultrasound examination. The fetal karyotype was considered as normal, 46,XY. At 5 years of age, due to a developmental delay, chromosomes studies were performed. The karyotype revealed an unusual short arm of one chromosome 21 suggesting the presence of extra genetic material. Because of the Down syndrome phenotype, FISH studies were performed using probes specific for the DSCR (Down Syndrome Critical Region). A third signal was observed on one chromosome 21p. Karyotypes of the parents as well as FISH studies were performed. The mother was found to be a carrier of a DSCR insertion onto the short arm of one chromosome 21. Shift or intrachromosomal insertion results from a three-break event leading to transposition of a chromosomal segment to another position in the same chromosome. Here the proband inherited an unbalanced recombinant chromosome following a crossing over in the maternal shift chromosome. Shifts occurring in the short arm of an acrocentric chromosome are rare. If the length of the segment is small, rearrangement mimics a short arm heteromorphism (pstk+). The present observation illustrates the importance of investigating an unusual short arm of acrocentric chromosomes.

P0421. Refinement of unbalanced aberrations of chromosome 7 in two severely affected children by multicolor banding (MCB)

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Although conventional cytogenetic analysis permits the diagnosis of the majority of chromosome aberrations, almost all of them require precision by molecular cytogenetic techniques. Here, we report on the application of MCB technique (FISH-based high-resolution multicolor chromosome banding) to refine two chromosome abnormalities involving chromosome 7 in two female children with severe mental retardation and congenital malformations. Initial standard karyotyping by GTG banding showed the first case to be a monosomy of chromosome 21 (45,XX,-21) and the second case to be a deletion of the chromosome 7 short arm, 46,XX,del(7)(p21p15). After the application of FISH with a set of site-specific probes for chromosome 21 following by use of whole-chromosome painting probes, the first case turned out to be an unbalanced translocation between chromosomes 7 and 21, but the breakpoints still were under question. To refine chromosomal aberrations in these cases, an MCB approach with microdissection-derived DNA probes for chromosomes 7 and 21 allowing painting of chromosomes at 550-band-resolution was applied. Thus, the first case was defined as partial monosomy 7q34-qter and 21pter-q22.13 due to an unbalanced translocation t(7;21) or 45,XX,der(7)t(7;21)(q34;q22.13),-21 and the second case was defined as 46,XX,del(7)(p21.2p15.2). Hence, we concluded that MCB technique was efficient enough for breakpoint precision in aforementioned cases. When seen in this perspective, one can further suggest MCB approach to represent an efficient tool for clinical molecular cytogenetics in addition to well-established FISH protocols with site-specific probes or newly introduced array CGH-based techniques. Supported in parts by INTAS.

P0422. Three unrelated cases with cryptic unbalanced subtelomere translocations

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Unbalanced subtelomere chromosome rearrangements are a significant cause of both isolated and familial mental retardation/multiple congenital anomalies syndromes with approximately 5 to 10% of over 3000 affected individuals tested worldwide. Here we report three unrelated cases with cryptic unbalanced translocations, t(1;11)(pter,qter), t(4:11)(pter;qter) and t(8;20)(pter;pter). Disballances were found by MLPA analyses. A combination of partial monosomy 1pter/trisomy 11qter was found in a one-month-old girl. She had a low birth weight, microcephaly, telecanthus, long eye lashes, blue sclera, broad nasal bridge, fleshy nose, smooth philtrum, down-turned corners of the mouth, thin lips, high palate, posteriorly rotated dysplastic ears, short neck, wide spaced nipples, abnormal dermatoglyphic patterns, long overlapping toes, generalized hirsutism, caudal appendage and patent ductus arteriosus. The second case, a two-month-old girl, presented with partial trisomy 4pter/monosomy 11qter. She had pyloric stenosis, thrombocytopenia, hepatosplenomegaly and following dysmorphic features: face/body asymmetry, pronounced hypertelorism, strabismus, wide nasal bridge, anteverted nostrils, high palate, midline cleft tongue, low set-up ears, clinodactyly. This clinical presentation overlaps previously described Paris-Trousseau type thrombocytopenia (#188025) due to partial monosomy 11q. The third case, a five-year-old girl, revealed partial monosomy 8pter/trisomy 20pter. She had mental retardation, obesity, hydrocephaly and following dysmorphic features: arched eyebrows, synophrys, tapering fingers, patchy skin depigmentation. Genealogical follow-up revealed in two of the cases additional

affected family members. The study included genotype-phenotype correlations and thus, contributed to clinical and molecular characterization of subtelomere aberrations. We showed the usefulness of recently developed technology for accurate diagnosis of submicroscopic chromosome abnormalities and their application in genetic counselling.

P0423. The 4P-syndrome. A Case description and literature review

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Wolf-Hirschhorn syndrome (WHS) is a rare developmental disorder associated with deletion of short arm of chromosome 4. Well-known genetic condition of WHS are typical facial anomalies, midline defects, skeletal anomalies, prenatal and postnatal growth retardation, hypotonia, mental retardation, and seizures.

Here we report a new case of WHS who referred to our clinic for cytogenetic investigation. The patient was a 9 month old baby boy with developmental delay, hypotonia, respiratory and heart problem, prominent eyes and forehead and delayed bone age. GTG banded karyotype reveald a deletion on segment of 4p (p15.31 pter). Due to a broad spectrum of possible morphologic abnormalities followed by mental retardation, prenatal diagnosis is very important. Postnatal recognition of the syndrome requires genetic counseling of parents and supportive multidisciplinary treatment.

P0424. Olfactory receptors gene clusters on 4p are not involved in the origin of Wolf-Hirschhorn syndrome-associated chromosome rearrangements.

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Wolf-Hirschhorn syndrome (WHS) is a multiple congenital anomalies/mental retardation (MCA/MR) syndrome caused by partial 4p monosomy. By analysing a total of 72 families, we found that 65 of 72 rearrangements (90 %) occurred *de novo*. Of these, 49 were further analysed by all telomeres (46) or by other locus-specific FISH (3). An isolated 4p deletion was detected in 36 (74 %), an unbalanced translocation in 9 (18 %), a dup/del 4p in 3 (6 %), and a der(4)(4qter→q32::4p15.3→qter) in one patient (2 %). With the purpose to investigate if hotspots for rearrangements exist, we performed FISH analysis with a total of 80 molecular probes, properly selected in individual patients, spanning the 4p15pter chromosome region. In particular, probes RP11-423D16 and RP11-751L19, delimiting the proximal OR on 4p, and probes 228a7 and RP11-324I10, delimiting the distal OR, were tested.

Looking at unbalanced translocations (n=16), both familial (n= 7) and *de novo* (n= 9), different pattern chromosomes were diagnosed, all autosomal. No hotspots were observed on 4p, with the exception of the t(4p;8p) translocations, that recurred within the proximal or the distal OR in 6 of 7 cases. Only one different *de novo* translocation, t(4p;7p), did occur within the distal OR. Of the remaining 56 rearrangements, consisting more often of isolated terminal deletions, only three were OR-mediated. A strong relationship was observed between parental origin and type of the *de novo* rearrangement. We conclude that ORs are not usually involved in the WHS-associated rearrangements, with the exception of t(4p;8p) translocations.

P0425. Molecular cytogenetic characterisation of an Xp duplication in a phenotypically abnormal girl with random X inactivation

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Partial duplications of the short arm of the X chromosome are relatively rare and have been described in males and females. We report the case of a 4 years and 10 months old girl presenting developmental delay, severe language retardation and dysmorphic features with macrocephaly (+1,8 SD), prominent forehead, wide palpebral fissures and anteverted nares. No pigmentary dysplasia of the skin was present. The external genitalia were normal. The karyotype completed by cytogenetic analysis with the Whole Chromosome Painting probe of chromosome X revealed a *de novo* partial duplication of the short arm of an X chromosome.

In order to further characterize the duplicated segment, we used a series of BAC probes extending from band Xp11.22 to Xp22.1. BACs from Xp11.23 to Xp11.4 were duplicated. The karyotype was finally 46,X,dup(X)(p11p11).ish dup(X)(p11.23p11.4)(WCPX+,RP11-416I6++,RP11-386N14++,RP11-466C12++). X inactivation status was studied using HpaII digestion of the AR and FMR1 genes. Unexpectedly, the two X chromosomes were found to be randomly inactivated, in the proband. Indeed, usually, in women with structurally abnormal X chromosome, the abnormal X chromosome is preferentially inactivated and those patients share an apparent normal phenotype. So, we speculate that in the present case, the phenotype of the patient could be explained by a functional disomy of the genes present in the duplicated region. We'll discuss the possible implication of these genes on the observed phenotype.

P0426. Familial pericentric Y chromosome inversion in Russian patient with trisomy 21

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We reported a patient with trisomy 21 and pericentric Y inversion. The proband, a Russian male newborn with a typical Down syndrome phenotype, was born to a 21-y.o. G1P1 mother and an unrelated 23-y.o. father. No genital abnormalities were mentioned in this patient.

We have examined the proband and his father, mother and grandfather. Chromosome analysis had been carried out on peripheral lymphocytes by standard techniques with GTG- and CBG- staining. Genomic DNA had been extracted from peripherical leukocytes using by standard protocol. Parents' origin of aneuploidy had been determined by analysis for four following polimorphic markers: D21S11, D21S1888, D21S1890, and D21S1895. Y microdeletion analysis had been performed using PCR amplifications for SRY, AMG/AMGL, ZFY/ZFX loci and 21 Y-specific STSs.

The proband's karyotype was 47,X,inv(Y)(p11.2q11.23),+21. Patient's father and patient's grandfather posses the same inverted Y chromosomes, mother had normal female karyotype. The extra chromosome was maternal origin is to determined by molecular analysis. PCR amplifications have shown an absence of sY1192 marker in proband and his father and grandfather. It is typically for b2/b3 deletions, partial deletions within AZFc region.

Remarkable, that deleted sY1192 locus falls in inverted repeat IR1 localization near or within Yq breakpoint of pericentric Y chromosome inversion. We suppose that revealed partial AZFc deletion may be due to pericentric inversion in ancestor Y chromosome. The transmission of this rearranged Y chromosome through three generations demonstrated, that at least some partial deletions (b2/b3 deletions) even in association with inv(Y) polymorphism not impair the male fertility.

P0427. Unbalanced translocation (Y;3) leading to 3p deletion in a dyslexic boy and his normal mother

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A 10-year-old boy was assessed for learning disability, language delay, dyslexia and hyperactivity. He was born at 32 weeks'gestation and required resuscitation. He walked at 19 months, suffered from frequent otitis media and was surgically treated for hypospadias. Standard karyotype displayed an abnormal chromosome 3 : add(3)(p25). The same abnormality was identified on the karyotype of his phenotypically normal mother.

FISH analyses showed that the additional material on chromosome 3p consisted of Y heterochromatin and Yq telomere. 3p subtelomeric probe (*Cytocell*, 230kb from telomere) was deleted whereas the *CRELD1* locus at 10 Mb from telomere was retained. So, both mother

and his son carried the same derivative der(3)t(Y;3)(q12;p25.3) leading to a 3pter deletion of less than 10 Mb.
Familial cases of Y translocation usually concern Y heterochromatin on acrocentric short arm. Involvement of a non-acrocentric partner has only been reported once to date.
3p deletion syndrome consists of growth retardation, microcephaly, facial dysmorphism (ptosis, broad nasal tip, micrognathia), mental retardation and occasional cardiac malformation, deafness and polydactyly. Size of deletion and phenotype are variable. Only two familial cases have been reported. In the present observation, 3p telomeric region is deleted in both mother and her child while only the child express clinical features that are not typical for 3p deletion syndrome. Therefore, it can be questioned whether this deletion really accounts for the patient's phenotype. Familial study and precise characterization of the deletion's size are in progress to shed light on this issue.

P0428. ZFX gene deletion in mosaic 45,X/46,XY female

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X/XY mosaicism is associated with a wide spectrum of clinical phenotypes varied from females with Ullrich-Turner syndrome (UTS), undervirilized males with mixed gonadal dysgenesis to normal virilized infertile males with azoospermia. At this the phenotype effects mainly depend on a presence of intact SRY gene and correlate with gonadal X/XY mosaicism.
We have examined patient with UTS. The proband was nine-year female referred to cytogenetic examination because of virilization. Beside short stature and clitoral hypertrophy, marked skin dryness was mentioned in this patient.
Chromosome analysis was performed using standard cytogenetic techniques with GTG- and CBG- staining. Molecular analysis was carried out on DNA extracted from peripheral leukocytes. PCR amplifications for SRY, AMG/AMGL, ZFY/ZFX loci and 21 Y-specific STSs have been performed.
Cytogenetic examination has found a presence of X/XY mosaicism. In 4 of 50 analyzed metaphases chromosome analysis has demonstrated a 45,X karyotype. PCR reactions have revealed a presence of SRY, AMG, AMGL, ZFY loci, and an absence of ZFX locus. Apparently patient had an Xp21 microdeletion included the ZFX gene. No Y chromosome microdeletions were found.
To our knowledge we have reported the first case of ZFX mutation. This gene encodes zinc finger protein lies within the critical region for ovarian failure and escapes X inactivation. Although we have found that in reported patient the XY cells were prevalent in blood lymphocytes, gonadal mosaicism may be essentially different. Furthermore, it is not excluded that ZFX gene loss may dismiss the SRY gene effect on the gonad differentiation.

P0428a. Novel matUPD15 identification by cytogenetic testing

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Prader-Willi Syndrome (PWS) is a multisystemic genetic disorder characterized by infantile hypotonia, feeding difficulties, distinct dysmorphic features, hypogonadism, psychomotor retardation and morbid obesity, with an estimated prevalence of 1 in 15,000 individuals. PWS is caused by the loss of function of paternal expressed genes within chromosome 15q11-q13, of which an interstitial deletion is observed in approximately 75% of the patients while a matUPD15 is noted in about 20%. The remaining 5% are due to a mutation or a translocation involving the 15q11-13 region.
Here we report on a newborn male with low-birth weight, severe hypotonia and poor sucking. Decreased fetal movements were noted during the 3rd trimester of pregnancy.
Chromosome analysis revealed normal male karyotype, with 2 identical marked chromosome 15P^{+}S^{++}. Parents' karyotypes were 46XX 15P^{+}S^{++} and 46XY, suggestive of a matUPD mechanism, with partial isodisomy. The newborn's FISH analysis (Q-Biogene probe), SNRPN region/ PML control, was normal. Molecular testing of polymorphic markers along chromosome 15 revealed a matUPD15, with heteroUPD15 from band q11.2 (D15S128) to band q21.1 (D15S659) and isoUPD at band q26.1 (D15S652). The cytogenetic results suggest isoUPD of the 15p arm, formed at the 2nd meiosis nondisjunction, and the molecular testing shows a double crossing over event in the 15q arm. To the best of our knowledge, this is the first report of detection of UPD15 using standard cytogenetic testing. We suspect that due to these unique findings, further clinical follow-up of this child may yield a better understanding of the genotype-phenotype correlation among PWS cases.

Po03. Prenatal diagnosis

P0429. Reporting Three Cases Of PND For Alpha thalassemia In Iran.

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Each normal person has 4α genes which are shown as αα /αα. Usually α_2 is more expressed than α_1 therefore deletion of α_2 or mutation on α_2 has more severe effect.

Being carrier alpha thalasemias are usually caused by deletion of one or two α-globins genes.

Alpha thal genotyped are usally shown like -α/αα (αthal 2); -α /-α or --/αα (α thal 1 or classical type). --/αα type is seen most in South East Asia. H-disease is caused by

--/-α form either by deletions or point mutations (e.g. --/$\alpha^T\alpha$).

Complete absence of 4α genes causes hydrops fetal is which requiring prenatal diagnose. We have performed three cases of PND for α-thal so far. In these cases the fetuses were diagnosed carriers (--/αα). In one case both parents were carrier of Med deletion (--^{med} /αα). The Hematological data of parents were:

Father: MCV=62/8 MCH=20/2 Hb=14/1 A_2= Normal

Mother:MCV=67/9 MCH=20/6 Hb=10/3 A_2=Normal

At the present time we think only need to perform PND for hydrops fealties.

Yet we need more strong evidence to do PND since most cases of Hdisease seen in adults by us were mild enough to exclude PND for similar cases.

Key words: Alpha thalassemia, Prenatal Diagnosis, H Disease

P0430. Whole-genome expression analysis of amniocytes

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Aims: The objective of our study was to investigate the ability of global gene expression array analysis from routinely collected amount of amniotic fluid. We also wanted to search for the differential gene expression profile of a polygenic disorder, the neural tube defect, which is the second most common birth defect in the world.

Material and methodology: We analyzed 6-17 mL of amniotic fluid. Samples were taken from seven pregnant women carrying fetuses with neural tube defect, diagnosed during ultrasound examination. Control samples were obtained from pregnant women who underwent routine genetic amniocentesis because of advanced maternal age (>35 years). Fetal mRNA from amniocytes was successfully isolated, amplified, labeled, and hybridized to whole-genome transcript arrays. Since the most significant epidemiological finding with respect to neural tube defects is the protective effect of maternal periconceptional folic acid supplementation, we also investigated specific folate-related genes.

Results: The detected differential gene expression profiles between cases and controls highlighted genes, like *SLA*, *LST1* and *BENE* genes, these might be important in the development of neural tube defects. None of the specific folate-related genes were in the top 100 associated transcripts.

Conclusions: This study demonstrates the ability of global gene expression array analysis from as small as 6 ml amniotic fluid, allowing the examination from routinely collected amniotic fluid samples so individual fetuses can be studied. The analysis of fetal gene expression differences might get us closer to decipher the complex genetic background of polygenic disorders.

P0431. Preimplantation genetic diagnosis for autosomal recessive polycystic kidney disease

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Autosomal Recessive Polycystic Kidney disease (ARPKD) is one of the most common hereditary renal cystic disease, and is caused by mutations in the PKHD1 gene. The diagnosis is often made late in pregnancy and sometimes at birth. Owing to the poor prognosis, there is a strong demand for prenatal diagnosis. Preimplantation genetic diagnosis (PGD) represents an alternative because it is done on cells taken from *in vitro* fertilized embryos at the third-day stage. Only healthy embryos are transferred, avoiding the physical and psychic traumatism of the termination of pregnancy in the case of an affected fetus detected later by prenatal diagnosis. We developed a single-cell diagnostic approach based on haplotype analysis in order to propose PGD to most couples, whatever the mutations may be.

Six linked markers within (D6S1714 and D6S243), or in close proximity to (D6S272,D6S436, KIAA0057, D6S1662), the *PKHD1* gene are tested in multiplex nested-PCR, using QIAGEN multiplex PCR kit, allowing identification of embryos carrying the high-risk haplotypes.

PCR analysis was first carried out on 50 single-lymphocytes. Amplification rate was excellent (100%), with an allele drop-out (ADO) rate ranging from 0 to 8%. Using this test, 4 PGD cycles were performed resulting in 18 biopsied embryos. Transferable embryos were obtained for three cycles, resulting in a pregnancy, and the birth of a healthy boy. The co-amplification of several loci increases the assay accuracy by allowing the detection of ADO, recombination and contamination. This simple diagnostic procedure can be applied to most patients at risk to transmit ARPKD.

P0432. Non Invasive Prenatal Diagnosis (NIPD): the suitability of locked nucleic acid (LNA)-modified allele-specific primers for detection of paternally inherited beta globin gene alleles

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Prenatal diagnosis (PND) with CVS sampling and amniocentesis involves a small risk of pregnancy complications. This may be avoided by NIPD, which for monogenic diseases is potentially based on detection of distinct paternally-inherited alleles of cell-free fetal DNA in the maternal circulation. However, methods for NIPD must address the problem that fetal alleles exist within an excess of maternal alleles (~5% versus ~95%) with low total-DNA concentration (~10-100pg/μl). This study investigates specificity and sensitivity of allele-specific PCR using LNA-modifed oligonucleotides for the most common Mediterranean beta-thalassaemia mutation (IVS-I-110G->A, HBB c.93+21G>A) when present in minority quantities relative to wild-type alleles (20%:80%). Design of PCR primers included an allele-specific primer (IVS1-110 G>A), LNA-modified at the 3' nucleotide (mutant base). Inclusion of a pBR322 RsaI-fragment and pBR322-specific primers monitored PCR conditions whilst overcoming the problem of co-amplifying genomic regions present in excess from the maternal genome. Reverse primers were labelled (Cy5.5), facilitating analysis on an automatic sequencer. PCR reactions analysed 200 diluted genomic-DNA mixtures with 80% normal and 20% mutant alleles (M/N), and 100 mutation-free (N/N) DNA samples (8-80pg DNA /reactions). 32 M/N and 32 N/N DNA samples were also analysed with non-LNA allele-specific primers under identical conditions. LNA-modified primers gave 2% false positives and no false negatives; non-modified primers gave false positive and negative rates >40%. The pBR322-specific band confirmed correct PCR conditions in all cases. These results indicate that LNA-modified allele-specific primers increase specificity and sensitivity.

P0433. Prenatal diagnosis of Carpenter Syndrome

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Background: Carpenter syndrome (CS) is a rare genetic disorder also called acrocephalopolysyndactily. The main features of Carpenter syndrome are craniosynostosis, congenital heart disease, obesity, extra fingers and/or toes (polydactyly), genital abnormalities, and short stature. Carpenter syndrome is an autosomal recessive disorder, for which the gene has not yet been identified. Objectives: To identify and characterize both craniofacial and limbs malformations associated with CS. Patients and Methods: A 28-year-old pregnant Caucasian female was referred at 21 weeks' gestation for a routine prenatal ultrasound. Fetal monitoring was made by ultrasound scans for fetal growth, congenital malformations, and amniotic fluid volume. We also collected informa-

tion about family medical history. Amniotic fluid samples were taken to perform prenatal cytogenetic diagnosis. Results: Ultrasound examination revealed a single fetus with an abnormal fetal craniofacial and limbs development: and oligohydramnios. Craniofacial abnormalities as a sonographic marker suggested the possibility of a chromosomal anomaly. More associated fetal anomalies were detected. Karyotype indicated a normal cytogenetic female: 46, xx. There was no family history of congenital anomalies. The couple chose to terminate the pregnancy. The family accepted autopsy. Autopsy findings confirmed the ultrasound diagnosis. Conclusions: The case was sporadic. The complex pattern of fetal anomalies identified and described suggested the possibility of CS. As the gene defect for Carpenter syndrome is unknown, the diagnosis of this condition remains a clinical one. The discovery of single or multiple fetal malformations requires not only complete echo graphic assessment, but also detailed post-abortum examination to allow optimal use of diagnostic aid programmes.

P0434. Our first steps in non-invasive detection of fetal Rh(D) using real-time PCR method

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Introduction: In the last ten years the detection of fetal origin cells and cell free fetal DNA in maternal circulation opened new horizonts in non ivasive prenatal diagnosis. The Rh(D) incompatibility is the most frequent blood group incompatibilities in the clinical practice, which can cause fetal anemia, hydrops and even fetal death. *Aims:* The aim of this study was to detect the fetal DNA in maternal circulation, to determine the Rh(D) status of the fetus. *Methods:* Blood samples and amnionic fluid samples were collected from 30 pregnant women, with Rh negative status, between 11-22 week of gestation presented for genetic amniocentesis at the 1st. Department of Obstetrics and Gynecology, Semmelweis University. After DNA isolation real-time PCR was performed in order to detect the exon 7 of the RhD gene located on the first chromosome (1p36.11.). *Results:* In 24 cases the PCR reaction gave same result in case of the DNA isolated from plasma and amniotic fluid, but in six cases the there was no PCR product of plasma samples and the product was detectable in amniotic fluid samples. The exon 7 was detectable in 25 cases, and there was no product in 5 cases. *Conclusions:* The real-time PCR method seems to be an easy and reliable method to determine the fetal Rh blood group. The sensitivity and specificity of the method in this study is in concordance with international data. The use of more than one probe could increase the sensitivity of the method.

P0435. Refined fluorescent STR quantification in cell free fetal DNA during pregnancy in physiological and Down syndrome fetuses.

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Real-time PCR is the most common approach to quantify cell free fetal (cff) DNA in maternal plasma. Analysis by the short tandem repeats (STR) has advantage to better recognize of different genotypes. But the quantity examination by Quantitative Fluorescent (QF) PCR of (STR) is limited to the only rough approximation.

This project, supported by IGA MZ CR NR7817-3, focused on more precise calculation of relative cff DNA amount tested by the STRs loci refined quantification directed to 21st chromosome.

The cff DNA was analysed on 475 samples from pregnant women with physiological fetuses in different stage of pregnancy (from 4gw- to 37gw) separately in three STRs systems (D21S1435, D21S1446 and pD) and also by gonosomal sequences (AMELX/Y). Thirteen samples with Down syndrome (DS) fetuses cff DNA were compared.

We optimized and assessed the Refined (R)QF PCR for STRs in particular locus. We modified calculations with respect to preferably amplificated short DNA molecules and to stutters. The cff DNA detection rate was 73.8% in minimally one of STRs if successful PCR amplification (88.4 %) is considered. The efficiency was decreasing from shorter to longer PCR fragments.

All three STR and gonosomal systems proved increasing of cff DNA during pregnancy. The stutter variability rate tended from shorter to longer STR system. Work manifested that DS samples had significantly higher amount of cff DNA. Our findings could contribute to the improvement of non-invasive prenatal diagnostics.

P0436. Cell-free fetal DNA quantification in maternal plasma at 16° and 17° week of gestation: the experience of I.R.C.C.S. Burlo Garofolo of Trieste

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During pregnancy, cell-free fetal DNA is present in maternal blood. Its quantity increases throughout gestation and with increasing maternal age. It represents an alternative source of fetal genetic material for non-invasive prenatal diagnosis.

It has been demonstrated that cell-free fetal DNA is significantly more present in women carrying fetuses with trisomy 21 and 13, bu not in trisomy 18.

Aim of this study was to quantify cell-free fetal DNA levels in plasma of pregnant women undergoing amniocentesis in the 2005-2006 period, in order to define its range and distribution at 16° and 17° week of gestation, and to correlate its concentration to fetal conditions.

Patients were recruited with informed consent and samples were collected before amniocentesis.

Fetal DNA quantification in maternal plasma was performed by real-time PCR on the SRY gene in male-bearing pregnancies. Fetal gender was ascertained by amniocentesis.

At all, we collected 260 samples, 125 of these carrying male fetuses. From cytogenetic analysis, all male fetuses were 46,XY, but one of these displayed a second level mosaicism; regarding fetal conditions, there were four cases with cardiac malformation, one case with renal malformation and diaphragmatic hernia, one case with corpus callosum agenesis, and one case with IUGR.

Preliminar data of fetal DNA analysis showed that the range of cell-free fetal DNA concentration in maternal plasma at 16 and 17 week of gestation was in line with literature. In cases of fetuses with malformations, range of fetal DNA concentration in maternal plasma was similar to that of normal fetuses.

P0437. Mechanisms of transport of the extracellular nucleic acids : study of choriocarcinoma cell line

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Extracellular DNA and RNA are present in normal and diseased human plasma.

They allow a novel approach to non-invasive diagnosis in the cancerology and antenatal fields but very little is known about their physiopathological mechanisms.

Our study was carried out on an in vitro human epithelial choriocarcinoma cell line : JEG-3. The cell line was first characterised, then a protocol was established for the separation of microparticles and exosomes in the supernatant fluid of a common culture. The protocol was validated using two techniques; electronic microscopy and flow cytometry.

The extracellular nucleic acids associated with the vesicles were then quantified. We were also interested in the localisation of the extracellular nucleic acids associated with the vesicles.

Study of culture supernatant showed the presence of extracellular DNA and RNA, quantifiable using real time quantitative PCR specific to the genes and transcripts analysed (*β-globin, SRY* for DNA and *GAPDH, HLA-G* for RNA).

We showed the presence of DNA and RNA associated with two types of vesicles . The analysis of specific and ubiquitous genes allowed us to demonstrate the different distribution of the genes and transcripts associated with the vesicles. Vesicular fractions were analysed following treatment with DNase and after permeabilisation. The percentage of the internal and external fraction for both genes associated with vesicules supported our hypothesis that the two genes have different modes of transport.

This work will be performed in order to evaluate the role of these extracellular nucleic acids in the intercellular communication through different vesicles. .

P0438. Is fetal DNA detectable in maternal urine?

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Objectives: The aim of this study was the detection, quantification, and correlation of cell-free fetal (cff) DNA in maternal urine and plasma in normal and complicated pregnancies during the third trimester.
Methods: 151 urine and plasma samples obtained from 96 women pregnant with male and 55 pregnant with female fetuses were collected and analyzed for cff-DNA using fluorescent PCR and quantitative real-time PCR. The concentrations of cff and total DNA in maternal plasma were correlated with maternal and obstetric parameters using appropriate correlation analyses.
Results: Y-chromosome specific sequences were detected in 31/96 (32.3%) urine samples collected from women pregnant with male fetuses using the DYS14-assay and in 6/96 (6.3%) urine samples using the SRY-assay for real-time PCR analysis. DNA was extracted from 1 ml maternal urine using the QIAamp®MinElute Virus Spin Kit (QIAGEN). No cff-DNA was detected in all 55 urine samples obtained from women pregnant with female fetuses. All 96 plasma samples obtained from women pregnant with male fetuses were tested positive for cff-DNA using real-time PCR. Cff-DNA exhibited a correlation with gestational age (R=0.244; P=0.018) and an inverse correlation with the latency between blood collection and birth (R=-0.218; P=0.036). Total DNA showed a correlation with placental weight (R=0.182; P=0.034) and pregnancies associated complications (R=0.280; P<0.001).
Conclusion: Our data confirm that cff-DNA is cleared by the kidneys in detectable amounts. Total DNA was found to be elevated in complicated pregnancies during the third trimester.

P0439. The association between choroid plexus cysts and cytogenetic abnormalities

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The choroid plexus is the area on the ventricles of the brain where cerebrospinal fluid is produced by modified ependymal cells. When a cystic form occurs inside the choroid plexus it is called a choroid plexus cyst (CPC). Cysts can be found in unilateral or bilateral form with different sizes. The choroid plexus cyst is a common variant and can be detected in utero incidentally by ultrasonographic examination during the second trimester. However it may be associated with an abnormal karyotype in approximately 3 % of fetuses. We aimed to evaluate the files of prenatally detected choroid plexus cysts between 2000-2006 years retrospectively. Among all referrals, CPC was found in 95 fetuses of which, 40 of them had bilateral and 55 had unilateral localization. All fetuses were in the second trimester and the mean gestation week was 18.83±1.77 (range 17-25 weeks). The mean maternal age was 27.90±3.82; 5 of them had an advanced maternal age (>35-year-old) and 4 of them had an increased maternal serum screening test result. Of 95 cases, 86 had isolated CPC. Nine of them had secondary ultrasonographic abnormality such as intracardiac ecogenic focus, cardiac hypertrophy, clinodactyly, renal pelvis dilatation, hydronephrosis, hyperecogenic kidney, ventriculomegaly and overlapping fingers. While cytogenetic investigation revealed karyotypic abnormality in 8 fetuses (8.42%), 88 were normal (41 fetuses were 46,XX and 47 were 46,XY). Among the abnormal karyotypes, 3 fetuses (3.16%) presented Trisomy 18. The other abnormal karyotypes were 46,XY[144]/47,XY+21[6], 46,XY,inv(9)(p11;q13) (2 fetuses) and 46,XY,15p+.

P0440. Fluorescence in situ Hybridization in prenatal diagnosis

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Samples of amniotic fluid from 49 patients were analyzed using Fluorescence in situ Hybridization (FISH) technique, applied on cultured and uncultured amniotic cells. Indications for FISH analysis on uncultured amniotic cells were: late weeks of gestation, abnormal ultrasound markers, abnormal serum-screening test, chromosome reciprocal translocation in parents and sex-chromosome related diseases. Analyses on 17 samples of uncultured amniocytes revealed two pathological karyotypes (11,8 %). FISH analysis on 32 cultured samples was used as adjunct technique after classical cytogenetical analysis. Indications for 24 samples were suspected chromosomal structural de novo and numerical aberrations, 5 were investigated for population variants of acrocentric chromosomes and 3 for parents carriers of reciprocal translocation.
From total number of 32 samples, 14 karyotypes were pathological (43,8 %) and 18 karyotypes were normal (56,2 %).
Following analyses on cultured amniocytes, 4 medically indicated abortions were recommended as well as 1 medically indicated abortion after uncultured amniocytes analysis.
FISH is a fast, powerful molecular cytogenetic technique used as an supplement to conventional chromosomal analysis.

P0441. Prenatal diagnosis of chromosomal abnormalities in Lithuania

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Objective. To ascertain the effectiveness of non-invasive prenatal testing of chromosomal abnormalities.
Methods. The risk for chromosomal defect was calculated by taking in account maternal age and gestation, first and/or second trimester serum biochemistry and first and/or second trimester ultrasound. The vast majority of women were tested in the second trimester. Cut-off for invasive testing was risk 1 in 250. For diagnosis karyotype analysis from cultured amniocytes and quantitative fluorescent polymerase chain reaction was performed.
Results. 827 amniocenteses were performed during the period of 4,5 years . Abnormal karyotypes were ascertained in 5.56% of cases. The structure of abnormalities was: trisomy 21-47.8%, trisomy 18-26%, mosaic karyotypes-8.7%, Robertsonian translocations-6.5%, reciprocal translocations-2.17%. In the group of women after non-invasive testing was born 5 babies with trisomy 21. The fetal loss after invasive procedures was 0.96%.
Conclusions. The effectiveness of non-invasive prenatal diagnostics of trisomy 21 was 81.5%. The vast majority of non-invasive and invasive tests should be performed in the first versus the second trimester of pregnancy.

P0442. Prenatal molecular diagnosis of Cockayne Syndrome

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Cockayne Syndrome (OMIM 216400) is a rare and multi-systemic recessive disease characterized by postnatal growth failure and progressive multiorgan dysfunction. Growth and developmental abnormalities become fully manifest in the second year of life. Progressive impairment of clinical features leads to severe disability, and death occurs within the first or second decade of life. CSA is caused by mutations in the 12 exons of ERCC8 gene, encoding a protein involved in the group of excision-repair cross-complementing process. Upnow 6 mutations has been characterised in the CSA gene. In our laboratory we examined a family with an affected son, died at the age of twelve, presenting typical clinical features. At the age of seven he weighed 9.360 gr and he was 48 cm high. The proband resulted compound heterozygote for two new mutations: a deletion *del1436-586* (segregating from the mother) detectable by RNA analysis and a missense mutation *C336G* located in exon 4 (from the father). Southern analysis was performed to characterised the extension of the deletion. Successively the mother undergoes prenatal diagnosis by transabdominal chorionic villus sampling (CVS) at week XII of pregnancy. Direct analysis on DNA and RNA was performed. A linkage analysis was also developed to study the segregation of the haplotypes, using markers located within an interval of 2.5cM comprising ERCC8 gene. This study allowed us to identify a foetus with a wild genotype confirmed at the birth by analysis on biological material isolated from placenta. This study documents the first molecular prenatal diagnosis of Cockayne syndrome.

P0443. The risk of cystic fibrosis with prenatally detected hyperechogenic bowel in Belarus population

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Hyperechogenic fetal bowel is prenatally detected by ultrasound during the second trimester of pregnancy in 0.1-1.8% of fetuses. It has been described to be associated with severe diseases, notably cystic fibrosis (CF). The incidence of CF in Belarus is 1:8000 newborns, much less than in West Europe. The aim of our study was to determine the risk of CF in a prospective study of 70 fetuses with hyperechogenic fetal bowel detected during the 2005-2006 years period in our Center. Fetal cells and parental DNA were screened for CFTR mutations. Two steps screening protocol was used. In the first step the most frequent mutations in Belarus CF patients - dF508 (61.6%); CFTRdele2,3(21kb) (6.8%) and 2184delA (4.1%), were analyzed in one multiplex PCR reaction. When one mutation had been detected, a direct sequencing strategy was applied. We found 6 affected fetuses, which gave us an 8.6% risk of CF when a digestive tract anomaly is observed at routine ultrasound examination. Mutations, associated with pancreatic insufficient CF, were the most frequent. In 6 CF cases 5 dF508 and 3 CFTRdele2,3(21kb) mutations were identified. The high incidence of CFTRdele2,3(21kb) can be explained by the more severe gastrointestinal expression of the disease in compound deletion carriers. Our results confirm that fetal bowel anomalies indicate a risk of severe cystic fibrosis and justify careful CFTR gene analysis.

P0444. Newborn screening for cystic fibrosis in the Czech Republic: observation of lower incidence of the disease

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An early diagnosis of cystic fibrois (CF) is considered as a favourable prognostic factor. Unless meconium ileus is present at birth, CF is could be often misdiagnosed. Increase of the age at diagnosis (ADG) in our country due to devolution of health care (prior to 1998 /median 0.58 years/; 1999-2005 /1.2 years/; p = 0.036) led us to initiate a pilot CF newborn screening project (NBS; two tier IRT/DNA; II/2005-XI/2006) covering ~62% of all newborns. Concentration of IRT was measured in 76,438 Guthrie cards and its level above the arbitrary cut off (75ng/ml) was found in 799 cases (1.05%). Positive cases were examined using a population specific *CFTR* mutation panel (~ 84% detection rate). In total, 12 CF patients were identified and the median ADG was 37 days (range 26-54). Interestingly, we also diagnosed previously unrecognised CF in 3 older sibs. 53 ,,IRT positive" newborns, that had only 1 *CFTR* allele, were subjected to follow-up sweat testing. Thus far, 45 cases were negative (ie. unaffected heterozygotes), while in one instance a borderline result indicated long-term monitoring. When using NBS data alone the incidence of CF was 1: 6,369, compared to the previously epidemiologically established value of 1:2,700. However, when respective prenatal diagnosis (PND) data from within study period were taken into account incidence increased to 1:3,900. Overall, our study proved that NBS is an efficacious tool for uniform diagnosis of CF and that its incidence could be lower due to systematic PND during the last decade. Supported by VZFNM00064203

P0445. Results of cytogenetical analysis of 1194 chordocenteses

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1194 chordocenteses were analyzed cytogenetically in a period of 22 years, using cytogenetical GTG-banding method. Indications for chordocenteses were late weeks of pregnancy (ultrasound markers, parents carrieres of reciprocal translocation, age, …) and amniocenteses confirming.

From total number of 1194 chordocenteses, 88 (7,37 %) were karyotypically determined as pathologic: 63 (71,5 %) numerical aberrations and 25 (28,4 %) structural aberrations. The most frequent numerical aberrations were trisomie of chromosome 21 (21/63; 33.3 %) and trisomie of chromosome 18 (20/63; 31.7 %).

Pathology in amniocenteses were confirmed with almost 100 % accuracy with chordocenteses. Considering other indications for chordocentesis, most pathologic karyotypes were confirmed at ultrasound markers indication (45/60; 75%).

P0446. Towards noninvasive prenatal diagnosis (NIPD) of trisomy 21 Down syndrome

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The development of non-invasive prenatal diagnosis (NIPD) of trisomy 21 Down Syndrome based on a maternal blood sample rather than the invasive procedures chorionic villus sampling or amniocentesis, is a long-term goal in reproductive care. Copy number counting of cell-free fetal DNA (cffDNA) sequences in maternal plasma presents a greater challenge than NIPD based upon detecting paternal sequences in cffDNA, already clinically feasible for X-linked disorders and RhD genotyping. One approach for identification of fetal DNA exploits differences in DNA methylation between maternal and cffDNA (the majority of which originates from placental syncytiotrophoblasts).

We describe the first identification and characterisation of a panel of chromosome 21-specific sequences (and reference sequences on other autosomes) that are differentially methylated between peripheral blood and placental tissue, DNA sequences which thus constitute candidate biomarkers for NIPD of trisomy 21 Down Syndrome.

To select DNA sequences to be screened for differential methylation between these tissues, we adopted three strategies (1) searching public databases for highly differentially expressed genes, (2) choosing 'random' promoter regions, and (3) choosing 'random' non-promoter regions. We screened these regions by methylation-specific restriction enzymatic and bisulfite-conversion assays, and hence identified a number of differentially methylated sequences located at 21q22.3 (AIRE, CLDN14, and ERG genes), at 1q32.1 (CD48 gene and FAIM3 gene), at 2p14 (ARHGAP25 gene) and at 12.24 (SELPLG gene). Clinical evaluation of the sensitivity and specificity for NIPD of trsiomy 21 Down syndrome is underway.

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P0447. Detection of median values of PAPP-A protein and free beta hCG in serum samples of pregnant women in north-west of Iran.

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Pregnancy-associated plasma protein A and the beta subunit of human chorionic gonadotrophin are well established markers used in combination with nuchal translucency to screen the pregnancies for Down syndrome and trisomy 18 in first trimester. However their median values are different according to the ethnic origin. In the present study the median values of free beta hCG and PAPP-A protein were obtained in the population of pregnant women of North-West of Iran, using serum samples prepared from 846 pregnancies at 11^{th}-13^{th} weeks of gestation. The results were also compared to those of similar findings on other ethnic origins. A significant difference was observed between the median values calculated in the present study and those reported for Asian and Caucasian ethinics. Our results indicate that employing the median values, present in available risk calculation soft wares for screening purposes, will result in underestimation of Down syndrome and trisomy 18.

P0448. Evaluation of indications of prenatally diagnosed Down syndrome cases between 2000-2006 years

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Down syndrome (DS) is the most commonly recognized genetic cause of mental retardation. The risk of trisomy 21 is mostly related to advanced maternal age. Our study aimed to determine the correlation

between prenatally diagnosed trisomy 21 incidences with the indications throughout 7 years.
The indications of the women who underwent cytogenetic prenatal analysis including amniocentesis (9737), chorionic villus sampling (95) and fetal blood sampling (229) were evaluated. The mean maternal age was 34.70±6.02 years and the mean gestation week was 17±3.85. Advanced maternal age was the most common indication (48.6%) in all prenatally diagnosed DS cases which is followed by abnormal ultrasound findings (18.6%) and increased maternal serum screening in triple test results (18.6%). Trisomy 21 was detected in a total of 70 cases. Chromosome analysis revealed 33 cases with 47,XY,+21; 32 cases with 47,XX,+21; 2 cases with 47,XX,+21,inv(9)(p11;q13), one with mosaic DS 47,XY,+21/48,XY,+3,+21, one with 46,XY,t(13;15)(q12 ;p11),+21 and one 46,XX,t(14;21).The distribution of the number of DS cases according to the advancing years were: 3/515 (0.003%) in 2000, 5/717 (0,697%) in 2001, 7/836 (0.837%) in 2002, 8/1285 (0.622%) in 2003, 21/1474 (1.424%) in 2004, 13/1316 (0.987%) in 2005, 13/1315 (0.988%) in 2006.
The mothers less than 35 years old who had a fetus with DS covered 47.8% of the cases, nevertheless, when the age lowered to 30 years, the ratio became 80.6%. The risk of having a child with DS increased in a gradual, linear fashion until 30 years old and showed a significant increase thereafter.

P0449. Down's syndrome screening in Saint-Petersburg. Ten years experience (1997-2006).

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Trisomy 21 or Down's syndrome is one of the most common genetic abnormalities with its risk progressively increase with maternal age. In many countries biochemical screening programs have been implemented, and the risk of Down's syndrome is calculated for each pregnancy. Total biochemical screening for Down's syndrome in Saint-Petersburg has been carried out in the second trimester of pregnancy since 1997. Assessment of risk relies maternal age and serum markers (AFP and HCG) concentrations. Also all pregnant women were subjected to ultrasound examinations on the 10-14 & 20-22 weeks of gestation and also for cytogenetic screening for chromosomal abnormalities. Basic results of screening programs for Down's syndrome since 1997 up to 2006 and some urgent problems in this area are outlined. Since 1997 36,9% DS fetuses (198/536) could be attributed to the women of 35 ages and more. Most of these cases - 61 % (102/166) were detected prenatally. Efficiency of DS detection in this group increased from 28.9% in 1997-98 up to 68% in 2004-06. About 18,7% of elder women rejected invasive PD for different reasons. Detection rate in 2-d trimester was 74,6% (cut off 1/360, FPR 6,8%). Since 2004 combined biochemical & US screening in the first trimester was initiated. Detection rate was 93.8% (30/32) (cut off 1/250, FPR 12.8%, average age 32.4+/-4.7). Pilot study of UE3 and inhibin A concentrations were performed for establishing of medians. Introducing of quadrotest will be useful for reducing of false positive results for the patients tested in the 2-d trimester.

P0450. Further results evaluation for the women with increased risk for congenital anomalies of fetus after biochemical prenatal diagnostic (PRISCA)

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PURPOSE: to evaluate real outcomes of pregnancy of women with increased risk of fetal chromosomal abnormalities after I or II trimester biochemical examination more than age risk.
METHODS AND RESULTS: Analyzing of medical documentation, contacts with women after birthing. The 371 women were examined by the PRISCA program during 2005.10.01-2006.10.01 year period. The risk factors were older age, not favorable anamnesis of pregnancy (congenital anomalies, miscarriages, sterilities and others), not favorable anamnesis of family or relatives. First trimester "double" test (PAPP-A + β-hCG) was done for 103 women, second trimester "triple" test was done for 268 and all two tests - for 24 women.
The increase risk for Downy and Edwards syndromes was got in 91 (24,5%) cases, according first trimester test - for 26, second - 63, two this - 12 women. The amniocentesis was offered in case when risk was higher 1:100 for women younger 40 years old and 1: 50 older 40 years old. This procedure was done for 15 women and 3 trisomies of chromosome 21(3,3% of all women with increased risk) were found using FISH method in combination with full cariotype of fetus. In other 74 cases of increased risk the further ultrasound examination was offered.
CONCLUSION: The greet importance is to evaluate the health status of newborns after birth and all outcomes of pregnancy in cases of increased risk according biochemical examination. These dates will show in full text of this work.

P0451. Prenatal diagnosis of trisomy 21 mosaicism with true chimerism

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The 32 year old female (gesta 1, para 0) had amniocentesis at 21 weeks gestation due to: increased nuchal translucency at ultrasound scan (6 mm at 13 weeks of pregnancy), abnormal alpha-feto-protein in maternal serum (0,44 MoM) and chromosomal abnormality in family history (an uncle with Down Syndrome). Chromosome analysis of amniotic fluid cultures showed a mosaic karyotype: 47,XX+21/46,XY. The couple was counseled for prenatal diagnosis of the mosaicism: we explained the possibility that the anomalous cell line could involve fetal tissues. To exclude the possibility of contamination with maternal cells (having suspected that a mosaicism with a very low line with no phenotypical modifications existed), we performed maternal karyotype, which turned out to be normal. After such investigations, the couple elected to terminate the pregnancy. Cord blood cultures showed two cell-lines: a normal cell-line of 46,XY and a 47,XX+21 cell line. We propose that this case represents an example of true chimerism. The most plausible mechanism underlyind this phenomenon is that two embryos (1 normal, male, and one trisomic, female) have performed a fusion in early pregnancy.

P0452. Introducing first trimester screening for chromosomal abnormalities in Estonia

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Prenatal diagnosis of genetic disorders in Estonia has been offered since 1990. Second trimester maternal serum screening was started in 1998. In 2001 first trimester ultrasound screening (NT) was introduced in some centers. Since February 2005 the combined first trimester screening (serum screening+NT) is offered in Tartu University Clinics. Our study included 1275 women at the first trimester of pregnancy. In 10^{+1} - 13^{+6} week of pregnancy PAPP-A and free ß-HCG were measured. Eighty-five percent of women underwent the combined 1st trimester screening (NT + PAPP-A and free ß-HCG). For individual risk calculation we used Prisca 4.0 software (distributed by Siemens Medical Solutions Diagnostics). All women had also 2nd trimester routine ultrasound screening in 19-20 week of pregnancy.
First 500 women underwent also routine 2nd trimester serum screening. From the beginning of 2006 stepwise sequential screening was developed for 1st and 2nd trimester screening. Screening positive (risk for trisomy 21 of ≥1: 270 at term) were 10,2% of tests based only on biochemical markers and 4,6% of combined screening tests. Eight women fulfilled criteria (risk for DS >1:50 and NT >2,5 mm or NT >3,0 mm) for early invasive diagnostics test (CVS). Chromosomal analysis (via CVS or AC) revealed 4 Down syndrome, 1 Edwards syndrome and 1 triploidy (69,XXX). During study period were found 2 "false negative" Down syndrome cases. The essential criteria for using 1st trimester screening wider in Estonia is the feasibility to measure NT during 12-13. week of pregnancy, which is now possible only in few centers.

P0453. Assessment of the Fragile X PCR assay in routine diagnostic practice

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Here we present our initial assessment of the Fragile X PCR kit (Abbott) in diagnostic practice. Sample genotype was determined using an

in house PCR protocol (BI 2720) and Southern blot analysis, while the assay was utilised in subsequent prenatal- and postnatal diagnoses. In 14 male-, 7 premutations and 7 full mutations and 18 female samples, 10 premutations and 8 full mutations, were detected. All expansions were independently confirmed and detection of PCR products within the premutation range was robust. However, 6 out of 15 full mutation samples were not visible on the agarose gel and two of them were even not conclusive on ABI 3100. In order to assess reliability of the TR/X ratio we have analyzed the homozygous or heterozygous status in a set of 23 females with known genotypes. Discrepancy was found in 6 out of 23 cases (26 %), which is in general agreement with previous reports. Subsequently, these results were taken into account in 3 different prenatal diagnostic cases. In two instances the results were negative, while in one case the expansion was exactly at the premutation / full mutation threshold (199 cgg+/-3cgg). There is a necessity to examine its methylation status in order to distinguish affected from the healthy carrier. In summary, this assay is promising product which allows rapid, albeit incomplete Fra X diagnostics. However, in house diagnostic validation is necessary for its optimalisation and manufacturer instructions should be strictly adhered to in order to assure consistent results. Supported by VZFNM 00064203.

P0454. Rapid diagnosis of hydatidiform moles by QF-PCR

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One possible cause of first trimester miscarriage is hydatidiform molar pregnancy, which is associated with a significantly increased risk of subsequent development of persistent gestational trophoblastic disease (GTD). Differentiating between partial (triploid, usually paternally derived) and complete (diploid, whether mono or dispermic, androgenetic in origin) mole is important because of their different prognosis and appropriate treatment therefore. However, the widespread use of ultrasound in the clinical managment of pregnancies has resulted in earlier detection and evacuation of moles, which makes the histopathologic clinical diagnosis that is based on subtle morphologic criteria more difficult to achieve. Nevertheless, other approaches seem to be reliable diagnostic methods. Hence, karyotyping or FISH may result useful in order to determine ploidy in hydatidiform moles as previously described. Moreover, genetic origin and classification of moles can be assesed by PCR-based methods.

Here, we present the molecular genetic diagnosis of complete and partial hydatidiform moles studied by multiplex QF-PCR with different specific chromosome polymorphic STR markers. From late 2005 on, all the first trimester curettage samples were provided by the gynaecological service of the hospital (whether suspicion of mole existed or not) in order to asess its chromosomal constitution and then were derived for anatomopathological studies. Correlation between genetic and histopathologic results was found in all of the cases except for one complete mole, whose anatomopathologic diagnosis failed.

Then, we propose the genetic molecular study as a rapid (24-48h), low-cost, less time-consuming, sensitive and reliable complementary diagnostic method of hydatidiform moles.

P0455. Intracardiac echogenic focus and cytogenetic abnormalities

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Intracardiac echogenic focus (ICEF) is defined as a discrete structure within the cavity of the heart that probably represents microcalcifications of the papillary muscles. ICEFs are usually observed during the routine fetal ultrasound examinations. It is commonly seen as a single focus in the left ventricule and the incidence of ICEF is given as 3-8 %. In most of the cases it is found as a normal variant however the association between echogenic foci and chromosomal abnormalities, particularly trisomy 21, has been reported in the literature. Our study aims to compare the association between echogenic foci and chromosomal abnormalities. Retrospective evaluation of clinical files of 27 cases whose referral reason was ICEF in their routine ultrasonographic screenings during the last four years was evaluated. The mean maternal age and the gestational age were 27.87±4.22 and 18.17±3.07, respectively. ICEF was the only ultrasonographic finding in 26 cases, while it was associated with other anomalies in one case. Chromosomal abnormality was found in only one case and had a karyotype of 46,XX,del(13)(q22),inv(9)(p11;q13). Fetal anomaly screening by ultrasonographic examination revealed nuchal translucency, nasal bone hypoplasia, hyperecogenic bowel, absence of the middle phalanx of 5th digit. Among the other karyotypes, 14 were 46,XX and 12 were 46,XY. There was no association between isolated ICEF and chromosomal abnormalities.

P0456. Characterization of the mitochondrial respiratory chain during human foetal development

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Mitochondrial disorders can have an early antenatal expression or undergo a symptom-free period. The aim of the present study is tracing the time point during pregnancy at which the foetal respiratory chain (RC) is being assembled and functions fully. The study was carried out on human foetuses aged from 9 to17 weeks of gestation, that were aborted because of genetic diseases that do not affect mitochondrial function. For each foetus, various tissues (brain, heart, liver, kidney and muscle) were examined. The research has focused on 3 main angles of the foetal respiratory chain.

Firstly, the assembly state of the foetal RC complexes was observed using Blue Native gels. Then, the protein profile of the RC complexes was examined using a cocktail of antibodies that allows tracing one subunit of each complex on SDS-PAGE. Finally, the enzymatic activity of the different RC complexes was measured.

The results show that as from 9 weeks of gestation all five complexes of the foetal RC are fully assembled (as compared with post-natal complexes) in all five tissues examined. Their protein composition for the subunits that were tested is similar; the ratio between them is constant and resembles that of the post-natal one. Finally, the enzymatic activity of these complexes is 2.5 times lower than that found for post-natal samples; however, the relative activity of each complex has the same pattern as observed in post-natal tissues.

Altogether, these results suggest that the respiratory chain is fully functional at early stages of human foetal development.

P0457. Rapid detection of chromosomal aneuploidies by MLPA in prenatal diagnosis

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Chromosomes aneuploidies of 13, 18, 21, X and Y account for the majority of abnormal fetal karyotypes in prenatal diagnosis. Conventional cytogenetic requires *in vitro* cell culture to obtain a karyotype with results available only after 10 to 15 days. Multiplex Ligation-dependent Probe Amplification (MLPA) is a novel method for detection of aneuploidies of chromosomes 13, 18, 21, X and Y by relative quantification of about 40 different DNA sequences in a single PCR reaction. On the basis of a retrospective clinical study, 204 uncultured amniotic fluid samples from women between 17 and 45 year's old and gestacional age between 12 to 31 weeks were analysed. The most frequent indications for fetal sampling were advanced maternal age, followed by positive biochemical screening for Down's syndrome and ultrasound abnormalities. The MLPA probe mix contain 8 probes for chromosomes 13, 18, 21 and X chromosomes and 4 probes for Y chromosome. Products were analysed by capillary electrophoresis and quantitative data were extracted from ABI Prism GeneScan Analysis Software.

No aneuploidies for chromosomes 13, 18, 21, X and Y were detected in 197/204 cases. Three cases showed trisomy 18 and 4 cases

showed trisomy 21. All the results were confirmed by conventional cytogenetic. No aneuploidies for chromosomes 13, 18, 21, X and Y were missed by MLPA. The efficiency of the method was not influenced by the gestational age.
MLPA is valid, simple, sensitive and cheap method of prenatal diagnosis of common aneuploidies within 24 hours, until the complete analysis of conventional karyotype.

P0458. A monosomy 8 cell line detected by FISH in a fetus with multiple abnormalities and mosaic trisomy 8 in chorionic villi

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Trisomy 8 mosaicism is associated with a variable phenotype, with neurodevelopmental delay, dysmorphic facial features, skeletal, renal and cardiovascular abnormalities being common features. Nevertheless, individuals with minor abnormalities and normal intelligence have been described.
Increased nuchal translucency thickness was detected at 13 weeks in the male fetus of a 27-year-old woman. At CVS, direct preparation showed a normal karyotype (46,XY), while in cultured cells mosaic trisomy 8 (47,XY,+8[11]/46,XY[53]) was found. At 15^{+6} weeks, ultrasound scan revealed bilateral cleft lip and palate with flat face and absence of the nose; no other abnormalities were evidenced. Pregnancy was terminated at 16^{+6} weeks.
Mosaic trisomy 8 was confirmed by standard karyotyping in placenta (47,XY,+8[13]/46,XY[87]) and amnion (47,XY,+8[16]/46,XY[68]), but not in umbilical cord (46,XY[100]). Pathological examination of the fetus confirmed cleft lip and palate with maxillary hypoplasia and showed gastrointestinal abnormalities, dysplastic kidneys and focal portal fibrosis in the liver. To confirm the presence of the trisomic cell line in fetal tissues, FISH was performed in liver and kidney samples using a chromosome-8 specific probe. In liver, two fluorescent signals were detected in 64% of nuclei, three signals in 13%, one signal in 23%; in kidney, 57% of nuclei showed two signals, 43% one signal. In control tissues, the presence of one signal was found in 3-10% of nuclei, suggesting that our finding reflected true mosaic monosomy 8.
In the case here described, multiple fetal anomalies were associated with a complex chromosomal mosaicism (trisomy/monosomy 8) with only trisomy 8 mosaicism detected at CVS.

P0459. Maternal MTHFR genotype, blood homocysteine levels and incidence of NTDs and Down syndrome

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Neural Tube defects (NTDs) and Down syndrome (DS) are common reason for malformations among newborns. Despite of the high frequencies, the etiology is still unclear. Some anomalies in the homocysteine metabolism and elevated blood homocysteine levels in mothers in combination with folate deficiency could be associated with NTD and DS appearance. The C677T and A1298C polymorphisms in Methylenetetrahydrofolate reductase (MTHFR) gene were described as an NTD risk factor and could cause elevated homocysteine concentrations. The frequency of C677T and A1298C substitutions were detected after restriction of the PCR products with HinfI and MboII, respectively. We have investigated 54 DNA samples from NTD mothers, 35 - from DS mothers and 60 - from control mothers. Plasma homocysteine levels were measured by chemiluminescence immuno assay. There was no statistical difference in allele frequencies according C677T. The observed frequencies of 1298C allele were 37.0%, 26.9% and 28.8% respectively. The frequency of AC genotype was statistically different between DS mothers and control and NTD mothers. Statistical differences were registered also between DS mothers and NTD mothers, regarding AA genotype. The measured mean total homocysteine level in the group of 49 pregnant control females was 3,14 umol/L, in comparison with the group of 11 women with NTD pregnancy (mean 10,2 umol/L). The data presented in this study failed to support the relation between MTHFR 677C>T and 1298A>C polymorphisms and risk of having a child with NTD and DS. Raised plasma homocysteine levels could be explained by folic acid deficiency, mutations in MTHFR genes or both.

P0460. Identification and characterization of DNA methylation sensitive markers for non invasive diagnosis of X linked aneuploidies.

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We are interested to the development of NIPD methodologies of X linked aneuploidies based on DNA methylation. We identified three new markers by "*in silico*" strategies. Among these markers, two are genes escaping X inactivation, and predicted to be expressed only in placenta; a third gene is X inactivated and expressed only in placenta as well. We next analyzed their expression by RT-PCR in placenta and blood: on this basis, the first, escaping gene was eliminated, given its expression in both tissues. The remaining analysis has been focused on the remaining two genes.
We confirmed their inactivation status, by somatic hybrids analysis: as predicted one gene is escaping, the other is subject to X inactivation. By bisulfite analsysis we demonstrated that the escaping gene is differentially methylated between blood and placenta, whereas the X inactivated gene, being not regulated by differential DNA methylation has been rejected.
Additional efforts will be necessary to complete the characterization of the marker we identified, and we will continue the search for additional markers. We plan to develop an MSP assay to check the differential methylation of these regulatory regions in blood and in placenta: later on, a Real Time PCR assay to determine the number of X and Y chromosomes of a certain sample. Our final goal is to adopt this strategy on plasma of donors for non invasive diagnosis of X linked aneuploidies.
This research is sponsored by CEE: SAFE: Special Non-Invasive Advances in Foetal and Neonatal Evaluation Network, contract LSHB-CT-2004-503243.

P0461. Foetal sex assessment in maternal plasma in the first trimester of gestation: large scale validation of the technique for clinical purposes.

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The knowledge of the foetal gender is an indispensable data for those couples at risk of an X-linked disorder. The possibility to determine the foetal sex from a maternal plasma sample collected in the early first trimester of gestation would avoid invasive prenatal procedures in some cases. For this reason, we have analyzed a large number of maternal plasma samples collected in the first trimester of gestation in order to know the earliest accurate gestational age to perform the study. The next step will be the application of this technique for clinical purposes.
Up to date, 185 voluntary pregnant women from the 5^{th} to 12^{th} week of gestation have participated in this study, having collected a total of 278 samples. Three replicas of each sample were analyzed by RealTime-PCR and the foetal gender assessment was established based on the presence/absence of the SRY gene. Three ways to validate results are being used: 1) By the analysis of a second blood sample within the first trimester. 2) By comparing with the prenatal tests results (CVS, amniocentesis or 20^{th} week ecography). 3)By comparing both a second sample + prenatal tests.
At the present moment, results from 138 out of 185 pregnant women have been confirmed obtaining a 100% of accuracy and specificity in samples collected from the 6^{th} to the 12^{th} week of gestation. Although the study has not finished yet, these results indicate the possible immediate application of the technique for clinical diagnosis in our hospital.

P0462. Increased Nuchal Translucency as a Prenatal Marker in Wolf-Hirshhorn Syndrome.

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Wolf-Hirschhorn syndrome is characterized by multiple congenital anomalies.
The syndrome is caused by partial aneupleoidy of the short arm of chromosome 4.
Patients are characterized by microcephaly, hypotonia, mental retardation, cardiac anomalies, club feet and characteristic dysmorphic facial features with cleft lip and palate, micrognathia, hypertelorism and midline defects.
Routine ultrasound imaging during second and third trimester pregnancy detects IUGR as well as most of the anomalies associated with Wolf-Hirschhorn syndrome. However, increased nuchal translucency (NT) has not been frequently described previously in this syndrome.
We present two young women who were referred to the genetic unit between 12 and 13 weeks gestation due to increased NT. Chromosomal analysis in both cases,
revealed deletion of the short arm of chromosome 4. In the first case hydrops developed and the women decided to terminate the pregnancy. In the second case early ultrasound screening at 15 weeks gestation, demonstrated multiple anomalies
and later there was fetal demise.
Increased nuchal translucency as a presenting symptom, has been previously described in relation with common chromosomal aneuploidies. Only rarely is it associated with other types of chromosome abnormalities. Our cases provide further evidence of a possible relationship between increased nuchal translucency and a chromosomal deletion syndrome.

P0463. Two prenatal cases of Pallister-Killian syndrome

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Pallister-Killian syndrome (PKS) is a rare sporadic syndrome. PKS is caused by a tissue-specific mosaicism distribution of supernumery isochromosome 12p.
Clinical findings in PKS include congenital defects (diafragmatic hernia, micromelia, hydramnios), mental retardation, seisures, streaks hypo or hyperpigmantation, facial dysmorphism.
We report two prenatal cases which illustrate the great variability of the tissue-specific mosaicism distribution. Both mothers were over 35 years old.
Case 1: Sonographic investigation showed nuchal translucency (NT) 2,6mm, ventricular dilatation, flat faces, micromelia. The section described diafragmatic hernia, low-set ears, hydrocephalus too. Tetrasomy 12p was found in 67% amniotic fluid cells and in 6% fetal blood cells.
Case 2: The socond trimestral screening test was positive, sonographic examination showed diafragmatic hernia. Tetrasomy 12p was found in 13% amniotic fluid cells, in 48% fetal blood cells and in 45% skin fibroblasts.
The cytogenetic investigation performed the diagnosis of PKS in both our cases by amniocentesis. M-FISH and mBAND analysis confirmed identification of isochromosome 12p, resp. i(12)(p10).
We discuss the difficulties of prenatal diagnosis due the variability of the tissue-specific distribution mosaicism and due the variability of the fetal phenotype.
The false negative results of PKS were reported on amniocentesis, on CVS but most often on fetal blood sample.

P0464. Verification of an improved strategy for combined first trimester screening and of commercial PAPP-A/proMBP complex examination for early detection of acute coronary disease

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Here we report verification of screening efficacy, complemented by aneuploidy risk evaluation, based on the degree of PAPP-A/proMBP and β-hCG deviations, including assessment of PAPP-A/proMBP complex as a biomarker of acute coronary disease (ACD). Analytes were examined in 2702 sera by Kryptor system/assays (Brahms), with trisomy 21/18 ascertainment by Lifecycle/Elipse software (Perkin-Elmer). Aneuploidy risk was evaluated according to analyte MoMs and NT deviations in 4 categories: I.: 0.6-1.9, II.: 0.5-0.59 and 1.91-2.0, III.: 1 analyte <0.5 or >2.0, IV.: both analytes <0.5 or >2.0. PAPP-A/proMBP Kryptor kit was used for sera examination in 51 controls, 110 stable coronary disease and 258 unstable angina pectoris (UAP) and STEMI and NSTEMI myocardial infarction cases. No autosomal or heterochromosomal aneuploidies were found in cat. I. Frequency of autosomal aneuploidies gradually increased from II. to IV. with the highest prevalence in cat. IV. Heterochromosomal aneuploidies were higher in cat. II. versus III./IV. These were detectable by analyte deviations only, whereas autosomal aneuploidies by increased NT, analyte deviation and software risk determination. Thus far, no case of fetal aneuploidy was missed by this strategy. Data from screening questionnaires enabled indication of counselling in 25% woman in cat. I. for improvement prenatal care because of other genetic, obstetric or exogenic risk factors. PAPP-A/proMBP examination proved, that serum levels were significantly increased only in UAP and myocardial infarctions ($p<0.004$ and $p<0.0005$, respectively). In ACD patients within <7 hours after the onset of chest pain increased PAPP-A/proMBP levels were an earlier marker than cTnI positivity. Supported by VZFNM00064203.

P0465. Experiences from FISH and PCR based preimplantation genetic diagnoses performed for 29 families

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We have employed both fluorescence in situ hybridisation (FISH) and PCR / fragment analysis techniques, to screen blastomeres for chromosomal aneuploidies or genetic mutations before transfer of embryos. Presently, we have performed 29 FISH- PGDs for 15 translocation families and three families with an X-linked disorder. In four cases, a second FISH round was done to screen for the most common trisomies. A total of 240 blastomeres from 215 embryos were analysed. Normal results were obtained for 71 embryos (33%). The success rate of the analysis was 71% (50/70) for reciprocal translocations, 90% (94/105) for Robertsonian translocations, and 88% (35/40) for sexing. Four pregnancies have been carried to term and five healthy babies have been born. One pregnancy is still pending.
We have performed PCR-based PGD analysis for seven Dystrophia myotonica (DM)) and three Fragile X syndrome (FRAXA) families. The CTG repeat region of the DMPK gene was successfully amplified in 70% (73/104) of the blastomeres. A total of 14 PGDs have been performed and two babies with normal DMPK alleles have been born. The CGG repeat region of the FRAXA locus has successfully been amplified in 17 out of 33 (52%) blastomeres. The method still relies on identifying the normal maternal allele in the blastomere. We have performed FRAXA PGD five times and one normal child has been born. The ongoing studies include the application of whole genome amplification, followed by routine mutation screening, for PGD. So far, we have used this method for one family with CNF.

P0466. Preimplantation Genetic Haplotyping (PGH) for monogenic disease.

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Amplification by Multiple Displacement Amplification (MDA) of DNA from a single cell provides sufficient material for genotyping at multiple loci. This has led to the development of Preimplantation Genetic Haplotyping (PGH), a generic approach to embryo testing that can be applied to any mapped inherited disease, regardless of the mutation;

misdiagnosis is reduced to the risk of a double recombination event.
Fourteen couples undertook 16 PGH cycles (10 cycles-cystic fibrosis (CF), 5-Duchenne and 1-Becker muscular dystrophy (DMD/BMD)), with a total of 79 embryos reaching biopsy, from which results were obtained in 71 embryos (89.9%). For CF, 5 couples were F508del heterozygotes, 1 couple were heterozygous for F508del and an unknown mutation (a previous child had CF), and two couples comprised an affected partner (F508del/R345H and homozygous F508del) and a F508del carrier, thus having a prior risk of 50% of an affected child. Results for diagnosed embryos were: 4 normal, 13 carrier, 6 normal/carrier, 12 affected, 3 affected/carrier and 4 ?aneuploid. Amplification of markers on one side of the gene only meant that for 6 embryos there was a residual risk (1-4%) of recombination between the gene and the marker amplified. For DMD/BMD, 7 embryos were normal female, 2 normal/carrier female, 4 carrier female, 5 normal male, 3 affected male, 2 ?aneuploid. Overall pregnancy rate was 53.8% per embryo transfer.
Further PGH marker panels for Fragile X syndrome, myotonic dystrophy, Huntington disease, spinal muscular atrophy, Alport syndrome and variants of globin (sickle disease and β-thalassaemia) have been developed for clinical use.

P0467. The single cell as a tool for genetic testing: credibility and precision

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Specimen for prenatal testing: chorionic villus sampling or amniocentesis are obtained during pregnancy thus the diagnosis of an affected embryo usually leads to pregnancy termination. Pre-implantation genetic diagnosis (PGD) is a procedure which involves the biopsy and testing of one cell following in-vitro fertilization and the implantation of unaffected embryos. The process minimizes pregnancy terminations in case of at risk couples.
The minute initial amount of DNA (single genome) generates technical difficulties such as amplification failure and allele dropout (ADO). Our objective was to measure and quantify those aspects of the single cells analysis. The study was carried out in single peripheral lymphocytes and embryonic culture cells. A set of nested PCR protocols was designed for five mutations of three genes: HEXA (Tay Sachs Disease): 1278+TATC, IVS12+1G>C and 805G>A. IKBKAP (Familial Dysautonomia): IVS20+6T and in the ASPA gene (Canavan Disease): 854A>C. In a total of 650 cells derived from heterozygotes to those mutations there was no significant difference in PCR efficiency between cell types although more failures were obtained in larger PCR products. ADO rate however was significantly lower in embryonic single cells compared to lymphocytes (average of 4.5 fold). We hypothesize that dividing cells demonstrate lower ADO rate due to their less compact nucleus structure. To test the hypothesis, lymphocytes were induced to divide by PHA and single cells were tested for ADO rate. The results showed a 1.8 fold lower ADO rate in the dividing compared to the non dividing cells.

P0468. Does the number of cells biopsied affect the implantation of embryos in preimplantation genetic diagnosis?

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Preimplantation Genetic diagnosis (PGD) offers couples with a known monogenic disorder a means of screening embryos for their inherited mutation with a view to transfer an unaffected embryo to establish a pregnancy. Analysis of single blastomeres biopsied from the embryo is fundamental to the technique. Contamination and allele dropout, the major problems of single cell PCR can be overcome and the efficiency and accuracy of the diagnosis can be improved by analysis of more than one cell from each embryo .
Between September 2003 and December 2006, 33 cycles of PGD were carried out for 6 disorders in 25 couples. Two cells were taken from every embryo that had 6 or more cells. If the biopsied cell had lysed before tubing or a nucleus was not seen, additional cells were taken. A total of 496 cells were biopsied from 243 embryos. A diagnosis was made in 163 (67%) embryos resulting in 44 embryos being transferred in 24 procedures. Out of the 44 transferred embryos, two (4.5%) had one, 33 (75%) had two, seven (16%) had three and one (2%) had four cells biopsied. From prenatal or postnatal analysis six out of 13 of the embryos that implanted could be identified. In four embryos 2 cells had been removed and in the remaining embryos it was three. This indicates that biopsing more than one cell improves diagnosis and can lead to implantation.

P0469. Preimplantation Genetic Diagnosis of myotonic dystrophy type 1

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Introduction: Myotonic dystrophy (DM1) is an autosomal dominant genetic disorder characterised by muscle weakness, myotonia, multisystemic lesions and hypogonadism, with an estimated prevalence of 1/8000 worldwide. The disease shows anticipation due to an expansion of a CTG repeat in the 3' untranslated region of the DMPK gene. Our centre is the only diagnostic centre routinely offering PGD for DM1 in the UK.
Methods: Embryos derived from IVF were biopsied on day 3 of development to allow collection and analysis of single blastomeres. Three different optimized fluorescent PCR protocols were applied in 22 clinical PGD cycles.
Results: Four out of the 22 cases were cancelled before oocyte retrieval due to either poor response to the IVF treatment or hyperstimulation. In another couple only two embryos were available for biopsy and PGD was cancelled. From the remaining 17 cycles, 217 oocytes were collected, 198 of them were inseminated of which 126 fertilized and 122 embryos were of sufficient quality for biopsy on day 3. A diagnosis was achieved for 82 embryos (26 normal, 56 affected). Twenty-two normal embryos were transferred in 12 cycles. Five pregnancies were established that have led to the birth of six healthy infants. The pregnancy rate per embryo transfer was 41.7 %.
Conclusion: PGD for DM1 is a practical and effective option for couples who wish to avoid termination of an affected pregnancy following prenatal diagnosis.

P0470. Activities of the ESHRE PGD Consortium (1997-2007)

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The European Society of Human Reproduction and Embryology Preimplantation Genetic Diagnosis (PGD) Consortium formed in 1997 to collect technical and outcome data, provide referral networks, survey and promote best practice. Data including fresh and frozen-thawed cycle details, pregnancies and babies were collected using a bespoke FileMaker Pro 5 database. Membership has increased steadily with a concomitant increase in number of centres reporting (16-50) and total number of cycles reported (392-2984) between reports 1 and 6. Cycle numbers for constitutional chromosome abnormalities and monogenic disorders have increased but a disproportionately large increase in preimplantation genetic screening (PGS) cycles reflects the increasing tendency for IVF laboratories to select the 'best' embryo for transfer by eliminating chromosomally abnormal embryos. Methodologies for technical aspects of the PGD process (including embryo biopsy and single cell diagnostics) are becoming more sophisticated, accurate and reliable ensuring extremely low misdiagnosis rates. PGD and IVF with sperm injection (ICSI)are comparable with respect to pregnancy complications and congenital malformation. The main complication, as with routine IVF, remains the risk of multiple pregnancy with concomitant higher morbidity and mortality. Aside from data collection, the Consortium has published best practice guidelines for PGD and PGS and recently, a joint report with the European Society for Human Genetics broadly examining the interface between genetics and assisted reproductive technology. To further improve preimplantation testing, studies are ongoing to investigate appropriate external quality assessment (multicentric evaluation of captured FISH images from single embryonic nuclei) and follow-up of children born following PGD.

P0471. Preimplantation Genetic Diagnosis for a Y-autosome translocation t(Y;8)(p11;q11).

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Reciprocal Y-autosome translocations are rarely observed, because implication of the Y chromosome in a translocation produces, most of the time, a meiosis blockage, which results in azoospermia.

However, when some spermatozoa are observed, assisted reproductive techniques (ART), particularly intra-cytoplasmic sperm injection (ICSI), can be proposed.

Because of the translocation, some of the spermatozoa may carry an abnormal chromosomal complement, involving a risk to obtain embryos with unbalanced karyotype. Preimplantation Genetic Diagnosis (PGD) can prevent this by selecting balanced embryos before transfer to the mother's uterus.

We present here a patient, carrying a t(Y;8)(p11;q11) translocation, who was referred to us for a PGD.

Because of the lack of data about segregation in Y-autosome translocations, analysis of patient's spermatozoa was performed by Fluorescent in situ Hybridisation (FISH) to estimate the risk of missegregation.

Simultaneously, to prepare for PGD, the FISH diagnosis method on single cell was improved by using a set of 4 probes: X centromere and Y heterochromatin labelled with Tetramethyl-Rhodamine, 8qtel labelled with dGreen, 8 centromere labelled with Spectrum Aqua.

Over 500 spermatozoa scored, 41.6% were found to be balanced. A PGD cycle was started, but diagnosis was not performed because of embryos development arrest at day 2.

Prior to any PGD attempt in such difficult cases, study of the chromosomal segregation establishes the proportion of balanced gametes, allowing to estimate chances of a successful ART. If too low, patients must be offered alternative options such as sperm donation.

P0472. A one year experience on PGD at Ege University in Izmir/ Turkey

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We would like to present our preliminary report of 1 year experience. Couples applied to the Family Planning and Infertility Research and Treatment Center for assisted reproductive technologies (ART) were referred to Medical Genetics Department for PGD and aneuploidy screening due to advanced maternal age, recurrent miscarriage, recurrent ART failure and balanced translocation carriers between the period of January 2006 and January 2007.

One or two blastomeres were aspirated on day 3 and analyzed using the fluorescent in situ hybridization (FISH) technique. Probes for chromosomes 13,16,18,21 and 22 were used for aneuploidy screening and individual specific probes were chosen for chromosomal translocations. Unaffected embryos were transferred on day 4 or 5.

There were 20 cycles for aneuploid screening (group 1) and 2 cycles for chromosomal translocation (group 2). In group 1, 118 embryos were biopsied successfully with a diagnosis rate of 84% and 39 unaffected embryos were transferred in 20 cycles, achieving 10 singleton pregnancies (implantation rate: 50%). In group 2, 8 embryos were biopsied with a diagnosis rate of 84% in 2 cycles and 1 balanced embryo transferred achieving pregnancy. Unfortunately we could not find any balanced embryo in the other cycle of our translocation group and cancelled the transfer. All antenatal amniocentesis confirmed the diagnosis. Post-natal physical examination showed no evidence of major abnormalities. PGD is an alternative method for having healthy children in selected couples with chromosomal abnormalities. In addition, PGD may increase the implantation rate in infertile couples and balanced translocation carreers seeking ART assistance.

P0473. Pre-implantation Genetic Diagnosis (PGD) for Genetic and Metabolic Disorders in Saudi Arabia

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Saudi Arabian culture is highly consanguineous, with cousin marriages accounting for 60-70%. Given the difficulties in management of genetic disorders, preventive measures for the suffering families by doing pre-implantation genetic diagnosis is undertaken. Over 30 cases are successfully prevented by PGD. The first of these disorders is Sanjad-Sakati Syndrome (SSS) OMIM# 24140, which is characterized by hypoparathyroidism, growth and mental retardation with a unique 12bp deletion. The second is Niemann Pick Disease type B (NPD-B) OMIM# 257200,) (acid sphingomylinase (ASM) deficiency) with more than 70 mutations reported in (SMPD1) gene, which presents with severe phenotype in Saudi Arabia. Four unique mutations are found in our Saudi families. A family with (W533R) mutation in the (SPMDI) gene suffering from a severe phenotype underwent PGD. The third disorder is Morquio's disease (MPSIV) OMIM # 253000, with severe classic phenotype with N-acetyl galactosamine-6-sulftase deficiency (MPSIV-A). More than 20 different mutations in (GALNS) gene reported in (MPSIV-A). A family with three affected siblings with severe classic (MPIV-A) with detected W195C mutation in the (GALNS) gene underwent PGD. In all these families PGD was undertaken using fluorescent PCR(F-PCR) and/or nested PCR with sequencing on a single cell, or Multiple Displacement Modification (MDA) to amplify the whole genome from a single cell. A singleton pregnancy ensure after transfer of one heterozygous and one/or normal embryo and prenatal diagnosis by CVS confirmed a normal pregnancy. This is the first report of successful PGD in different genetic disorders in Saudi Arabia, and the Muslim world.

P0474. QF-PCR: reliable and accurate for the rapid detection of the most common fetal chromosomal abnormalities in the first trimester

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Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) is a well established method for the rapid prenatal diagnosis of the most common chromosomal aneuploidies found in amniotic fluid samples. In recent years it has also been applied to chorionic villi samples (CVS) in the first trimester.

Since June 2005 to date 1272 CVS samples were tested with QF-PCR in our lab for the diagnosis of aneuploidies involving chromosomes 13, 18, 21 and X, Y. A total of 38/1272 (3%) fetal aneuploidies were detected and confirmed after chromosomal analysis of long-term cultures (LTC). Twenty four cases with trisomy 21 (1 mosaic case), 10 cases with trisomy 18, 1 case with trisomy 13, 1 case with Klinefelter, 1 case with triploidy and 1 case of a mosaic sex chromosome abnormality were detected. Maternal contamination was detected in 5/1272 (0,4%) samples and therefore no QF-PCR results were obtained. No false-positive or false-negative results were reported for the chromosomes tested. LTC analysis revealed an abnormal result for chromosomes not tested for by QF-PCR in 11/1272 (0,8%) cases. In another 5 (0,4%) cases there was a discrepancy between QF-PCR and LTC analysis due to in situ placenta abnormalities missed by sampling.

Based on our experience of two years, we consider QF-PCR to be a reliable, accurate, easy and relatively inexpensive method for rapid prenatal diagnosis in first trimester CVS.

P0475. Application of genetic methods in Prenatal Diagnosis: Experience in Iran

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Prenatal diagnosis has revolutionized prenatal care from the perspective of both the patient and the physician. For the patient, prenatal diagnosis provides genetic, anatomic, and physiologic information about the fetus or fetuses. The information can help individual to make deci-

sions regarding the pregnancy. For the physician, prenatal diagnosis provides vital information that can be utilized for better patient's management. Prenatal knowledge about genetic abnormalities enables the physician to tailor or manage the timing and mode of delivery for optimal maternal and fetal outcomes.
Great efforts have been put forth to devise more efficient genetic protocols. Because of its ability to provide results early in pregnancy, first-trimester screening is becoming increasingly more important. First-trimester screening provides the opportunity for early risk assessment and early diagnosis of fetal aneuploidy via chorionic villus sampling (CVS). Early diagnosis allows for pregnancy termination earlier in gestation, if the patient so desires,
While the advances in first-trimester genetic test are exciting, there will always be a role for second-trimester genetic test. Many patients may not present in time for first-trimester test. Likewise, many patients may not have access to providers skilled at CVS. In addition, a portion of patients may prefer amniocentesis to CVS.
In this talk, available genetic techniques for prenatal diagnosis in Iran will be discussed. In addition, from the prenatal diagnosis experiences in Iran, several other concerns noticed by genetecian such as fairness of access to genetic services, indications for prenatal diagnosis, confidentiality problems etc will be mentioned.

P0476. Needs assessment regarding decision about Down's syndrome prenatal testing: A systematic review of women, their relatives and health professionals' perceptions.

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Background: Population-based prenatal testing for Down's syndrome is being considered in the Province of Quebec. In order to elaborate effective decision support interventions, a systematic review was performed to identify the needs of women, their relatives and health professionals regarding decisions about prenatal testing for Down's syndrome.
Methods: PubMed, EMBASE, CINHAL and PsycINFO were searched for original studies in English or French. Studies were identified independently by two reviewers and discrepancies were resolved by a third one. Studies were included if they reported original data on difficulties and/or facilitators in making decisions about Down's syndrome prenatal testing, enrolled women, their relatives and/or health professionals and were conducted in real clinical situation. Content analysis is being currently performed by two reviewers using a taxonomy adapted from the Ottawa Decision Support Framework and quality of studies, assessed using Qualsyst validated tools.
Results: From 2390 potential titles, 64 publications covering 51 unique studies were included. The majority of studies were from UK (27%) followed by Canada and USA (12% each). Most targeted screening for Down's syndrome (41%). Most used qualitative methods exclusively (45%). Three studies were grounded in theory. Validated measurement tools were used in ten studies. Overall, the vast majority of studies targeted women (90%) followed by health professionals (12%) and partners (10%).
Conclusions: We observed an important gap in knowledge about the perceptions of health professionals and relatives. In order to elaborate effective decision support interventions for prenatal testing for Down's syndrome, future studies will need to address these gaps.

P0477. A supernumerary marker chromosome with a related pseudodicentric chromosome detected in a fetus during prenatal diagnosis

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Dicentric autosomes are considered as unstable constitutional chromosomes in humans. The presence of centromeres on the same chromosome leads to a high risk of attachment of the same chromatid to the mitotic spindle from opposite poles and to the formation of anaphase bridge during cell division. Therefore, breakage of the dicentric can occur.
We describe a fetus in which a minute supernumerary marker chromosome (SMC) was detected in addition to a larger pseudodicentri**c** chromosome. The case was a 12-week fetus with mosaicism for a normal and two abnormal cell lines: one had a dic (12;15)(q11.2;q11.2) chromosome, and the other had a minute SMC. Although the heart beat could be detected at the beginning of the pregnancy, the heart failed to fully develop and therefore therapeutic abortion was done at 16 weeks of gestation. Deletion of centromeric material was proposed as one mechanism of centromere inactivation in dicentric chromosomes. This SMC may be the result of a deletion event leading to inactivation of one centromere of a dicentric chromosome to generate a pseudodicentric chromosome. Therefore, this case suggests possible mechanisms for the origin of this SMC

P0478. Prenatal diagnosis of aneuploidies of chromosomes 13, 18, 21, X and Y by QF-PCR in the Republic of Macedonia

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The great majority of chromosomal abnormalities are due to aneuploidies of chromosomes 21, 18, 13, X and Y. The quantitative fluorescent (QF) PCR of selected small tandem repeat (STR) markers enables rapid and accurate prenatal diagnosis of these aneuploidies. Here, we present our results of the use of QF-PCR for prenatal detection of common chromosomal aneuploidies in 930 pregnancies at risk. The prenatal diagnosis was performed on genomic DNA isolated from fetal cells collected by amniocentesis and chorionic villus samples. All samples were analyzed by three multiplex PCR assays, amplifying a total of thirteen STR markers on chromosomes 21 (D21S1435, D21S1446, D21S1411 and D21S1414), 18 (D18S535, D18S1367, D18S978 and D18S386), 13 (D13S631, D13S258 and D13S1817) and X (DXS6803 and XHPRT). When these markers were uninformative, additional markers were used. Using this approach, we have detected 17 fetuses with trisomy 21, 11 with trisomy 18, one with partial trisomy 18, four with trisomy 13, three fetuses with Turner syndrome (45,XO) and one with Klinefelter syndrome (47,XXY). A polymorphic duplication was detected in two fetuses with STR marker D13S631; in both fetuses it was inherited from one of the parents. The parental origin of the aneuploidy was determined in 11 cases with trisomy 21, seven with trisomy 18, three with trisomy 13 and three with Turner syndrome. The origin was maternal in all complete trisomies and paternal in the partial trisomy 18 and the Turner syndrome cases. Triple X syndrome (47,XXX) was detected in a woman with a fetus with trisomy 18.

P0479. QF-PCR on Amniotic Fluid and Corionic Villi. Diagnostic troublesome results.

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Autosomal trisomies, which account for about 80% of significant abnormalities, can be detected within 24-48 hours by quantitative fluorescence (QF)-PCR. In the last four years we have used QF-PCR to assess relative allele dosage at polymorphic loci of chromosomes 13, 18, 21 and sex chromosome in more then 3.000 samples (80% amniotic fluid and 20% Chorionic Villus Samples-CVS). Samples of women with abnormal ultrasound referrals or from families with known chromosome rearrangements require full karyotype analysis, but if abnormalities are identified earlier this will aid the clinical management of pregnancy and will help to minimize the period of parental anxiety while awaiting for the diagnostic test result.
Mosaicism in CVS is well documented in the literature and it is detected in 1-2% of the CVS. Discrepancy between chromosome analysis of direct short-term cultures (cytotrophoblast cells) versus long-term cultures (mesenchymal cells) is very well known.
We describe here cases in which a discrepancy between QF-PCR and standard karyotype was observed in CVS analysis. In two cases QF-PCR showed the polymorphic markers of two X chromosomes (in

agreement with while direct short-term cultures karyotype), while the long-term cultures showed a 45,X karyopype. Moreover one case was partially informative because QF-PCR showed only one informative polymorphic marker for trisomy 18. The karyotype of the long-term cultures showed a trisomy 18. Our results confirm that QF-PCR technique is a rapid testing able to diagnose chromosome aneuploidy accurately in prenatal diagnosis on amniotic fluid but on chorionic villi more controversial results can be obtained.

P0480. Detection of fetal aneuploidies by quantitative fluorescent polymerase reaction

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Introduction: Multiplex quantitative fluorescent polymerase chain reaction (QF-PCR) analysis of amniotic fluid samples has been shown to be a useful tool in the detection of fetal aneuploidies, but has its limitations as it can not detect some fetal chromosome disorders of clinical importance.
Objective: To test the reliability of QF-PCR for the prenatal diagnosis of the common aneuploidies, to obtain data on the allele distribution of 7 different short tandem repeats in Hungarian population and to analyze the indications in which QF-PCR can be applied safely in prenatal diagnosis.
Materials and methods: At 4985 patients (25 twin pregnancies) undergoing amniocentesis we compared the results of QF-PCR with those of the conventional cytogenetic study. We have analyzed allele distribution of the applied STR markers. We compared the occurrence of chromosome disorders which can not be detected by amnio-PCR in cases with different indications of amniocentesis.
Results: 98.3% of amnio-PCR results were informative without false-negative and false-positive results. 9 samples (0.16%) were inconclusive because of borderline peak ratios of diallelic results. 126 chromosomal abnormalities of 152 were detectable by amnio-PCR. Amnio-PCR detected all chromosomal disorders in case of maternal age as indication for amniocentesis. In case of structural abnormalities detected by ultrasound, the chromosome disorder was not detectable by amnio-PCR in 23 cases.
Conclusion: All chromosome disorders of clinical significance were detected in case of advanced maternal age. The highest number of chromosome disorders that are not detectable by QF-PCR was in case of structural abnormalities detected by ultrasound.

P0481. Preparation of CVS for QF-PCR aneuploidy diagnosis and correlation with karyotype analysis of cultured cells

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Aneuploidy mosaicism has been reported to occur in up to 1% of chorionic villus samples (CVS), usually due to differences between the cytotrophoblast and mesenchyme cell lineages. Karyotype analysis of cultured metaphases from the mesenchyme gives an accurate prenatal diagnosis in the majority of cases. QF-PCR analysis of two separate villus tips taken from different regions of the CVS has proven to be an accurate predictor of fetal status regarding chromosomes 13, 18 and 21. Prior to June 2005 more than 3000 CVS were processed in our centre with no completely discrepant QF-PCR/karyotype results. However, in the following 6 months three such results were identified, all due to sample mosaicism and QF-PCR analysis of isolated cell populations. We therefore now test a small aliquot of dissociated cells prepared from 10-15 mg of CVS for cell culture. A case study has shown the mesenchyme to contribute between 40-50% of the DNA in these samples. Since this change in CVS preparation protocol, 1738 CVS have been tested by QF-PCR for autosomal trisomy, and 4 cases of mosaicism detected; 60% trisomy 13, 78% trisomy 18, 30% trisomy 21 and 35% trisomy 21. The first three cases showed non-mosaic abnormal karyotypes with only the final case showing mosaicism (46,XY,der(21;21)(q10;q10),+21[23]/46,XY[13]). In all cases the mesenchyme cell lines were detected by QF-PCR and there were no completely discrepant results. In summary, the QF-PCR analysis of dissociated cells from whole CVS has resulted in better representation of the CVS and greater concordance with the karyotype result.

P0482. DNA analysis with QF-PCR technique for confirmation of suspected fetal triploidy

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We report three cases of fetal triploidy diagnosed by QF-PCR analysis. The patients were referred to our Lab due to abnormal ultrasound scan of both the placenta and fetus at 14 wg, 22 wg and 20 wg respectively. QF-PCR technique was performed on DNA samples, extracted with commercial kit from amniocytes (case 3) and from fetal and placental tissue after termaniation of pregnancy (cases 1 and 2). Fourteen polymorphic STR markers (4 located on chromosome 21, 4 - on chromosome 18, 3 - on chromosome 13 and 3 on chromosomes X and Y) were amplified with Cy5-labeled primers. The observed electrophoretic profiles for all investigated STR markers showed diallelic or triallelic genotype concordant with trisomic pattern. No false negative results were observed. For case 3 DNA samples from parents were available and paternal origin of the additional chromosomal set was proved. The triploidy was confirmed by cytogenetic analysis performed after the termination of the pregnancy only in case 3. Our experience showed that QF-PCR analysis is a reliable technique for rapid prenatal diagnosis of triploidy when particular ultrasound anomalies are present and karyotyping is not available. A high-standard ultrasound examination was essential for the diagnosis of reported cases.

P0483. Evaluation of a non invasive prenatal RHD genotyping screening strategy

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It has been amply documented that fetal genotyping is feasible, allowing NIPD for the identification of RhD positive fetuses in RhD negative women. This approach now means that only those RhD negative women who are known to carry an RhD positive child will require treatment with anti-D IgG to prevent haemolytic disease of the newborn. We described our experience with plasma obtained from 250 RhD negative pregnant women between 10 and 28 weeks of gestation. 200 ul of maternal plasma is automated extracted by biorobot EZ1 (Qiagen, forencic card). The DNA eluate is tested in triplicate in RHD exon 7 and 10 real-time quantitative PCR. To reduce false negative due to low extracellular nucleic acids concentration in plasma in some extractions, we tested β-globin gene systematically. Plasmidic ranges for different genes were also used. A strategy based on β-globin quantification (cut-off at 1000 copies/ml) were established to perform this analysis in routine. All RHD NIPD were compared with RH genotyping cord blood. No false negative were obtained and two false positive cases were due to the presence of RHD variants. In these two cases, using plasmidic range permitted easily to confirm this diagnosis. In conclusion, automation and quality control of extraction step are essential to introduced this screening into restricted the antenatal anti-D immunoprophylaxys to women carrying RhD-positive fetuses.

P0484. Prenatal findings in a severe type of achondroplasia associated with mental retardation and acanthosis nigricans (SADDAN).

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Netherlands.
The detection of a skeletal dysplasia during pregnancy creates an intricate situation depending on the type of the skeletal dysplasia and the stage of the pregnancy. Establishing a diagnosis as exact as possible is important for support and management of the existing pregnancy and may have consequences for future pregnancies. Due to the time factor, a diagnosis is not always found during the on-going pregnancy. Additional information, such as follow-up after birth, skeletal X-rays, obduction and chromosomal and DNA-investigations is essential in such a situation. We present one postnatal and two prenatal cases of a rare skeletal dysplasia, where DNA-investigation confirmed the subtype of the skeletal dysplasia and generated important information about prognosis. In one prenatal case, MRI-examination of the pregnancy gave valuable additional information for the differential diagnosis.

P0485. β-Thalassemia Carrier Screening and Prenatal Diagnosis among Iranian Population

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Thalassemia is one of the most common monogenic disease worldwide and also in Iran. More than 200 different mutations have been reported to date, and each ethnic population has it's own cluster of common mutations. This is well illustrated by the experience of the National Thalassemia Screening Program in Iran. Molecular analysis of β-globin mutations has been performed by PCR-based protocols (ARMS, RFLP, DNA Sequencing) mostly for prenatal diagnostic in our center.DNA samples are usually tested for mutations like IVS-II-I, IVS-I-5, IVS-I-110,codon 5, codon 17,codon 41/42(-TTCT) and many common mutations and deletions.
In a population of **1954** prenatal diagnosis (PNDs) performed since mid 2000 and we have found 24% normal, 50.7% β-Thalassemia carriers and 22.9% affected (β-Thalassemia major).
Prenatal diagnosis has been an ongoing program in Iran since 1996 by a religious decree. Our center is part of the PND Network in Iran and the above PNDs have been performed as a part of the program to curtail β-thalassemia.
Keyword: Thalassemia , Prenatal Diagnosis , ARMS , DNA Sequencing

P0486. Quality control of prenatal sonography in detecting trisomy 18. The value of perinatal autopsy

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Introduction: Trisomy 18 (Edwards-syndrome) is the second most common autosomal trisomy. The second-trimester ultrsonographic examination followed by fetal karyotyping is the most relevant way of diagnosing this aneuploidy. However, sonographic findings have been demonstrated to have significant rates of fals-positive and fals-negative diagnosis. Detailed pathologic examination of aborted fetuses has important role in the quality control of ultrasonographic diagnosis. This study was designed to compare the prenatal ultrasound findings and postmortem pathologic findings of fetuses with trisomy 18.
Materials and Methods: 70 fetuses with trisomy 18 were diagnosed between 1990 and 2004. Sonographic and autopsy findings were compared by organ system and their correlation was assigned to 1 of 3 categories.
Results: There were 164 separate major structural abnormalities found on autopsy. Of them, sonography detected 72 (43.9%). Among major defects the agreement was more than 75% of all abnormalities of these systems: central nervous system (80%), abdominal abnormalities (87.5%) and cystic hygroma (100%). Whereas, the sensitivity of sonography was lower in these organ systems: cardiac system (66.6%), facial abnormalities (26.3%), urinary system (27.3%) and extremities (8.7%). The rate of additional findings at autopsy was 56.1% and involved mainly 2 organ systems: face (including ear) and extremities (including hands and feet). Some ultrasound findings (n=15) were not confirmed at autopsy in our series.
Conclusions: This study confirms that perinatal autopsy provides additional information in many fetuses with trisomy 18. In addition, examining the correlation between sonography and pathologic findings may indicate potential markers for sonographic screening of trisomy 18.

P0487. Characteristics of pregnancies with cytogenetic diagnosis of trisomy 21.

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Aim of the study: In this study we characterize the pregnancies with cytogenetic diagnosis of trisomy 21.
Materials and methods: We retrospectively looked at pregnancies in which trisomy 21 was found in chorionic villus sampling (CVS) or amniocentesis (AC) between 01.01.2002 and 31.12.2006. Data on maternal and paternal age ,reason for referral, fetal sex, fetal and parental karyotype were recorded.
Results: In this 5 year period, 73 prenatal cytogenetic diagnoses of trisomy 21 were made in our centre. It concerned 44 chorionic villus biopsies and 29 amniocenteses. In all but one case, there was free trisomy. Mosaic trisomy was present in 3 cases. Forty-one trisomic fetuses were male and 32 female. The main reason why cytogenetic diagnosis was performed, was maternal age (31/73), fetal anomalies on ultrasound (18/73) and second trimester serum screening (7/73). In 13 pregnancies there were 2 different reasons for referral.
The mean maternal age was 36 years, the mean paternal age 37.1 years.
For 53 parents, a karyotype was performed, being normal for 52 and showing a translocation for 1.
Discussion: Trisomy 21 is the most common chromosomal anomaly encountered in CVS or AC. Despite the small sample of this study, the experience in our centre confirms the common knowledge that it occurs more frequently in older parents and that in more than half of the cases there is an anomaly on ultrasound and/or biochemistry. For the majority of the pregnancies for which follow-up was available, termination of pregnancy was performed

P0488. Prenatal diagnosis of a marker chromosome derived from chromosome 3 in a fetus with growth retardation and microcephaly- a case report

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We present a rare prenatal case of an "incomplete" trisomy 3 rescue which resulted in an extra chromosome 3-derived marker chromosome in the foetus.
The patient was referred for prenatal cytogenetic diagnosis in chorionic villi because of advanced maternal age and a growth discrepancy between both foetuses in her twin pregnancy, with foetus II being too small for the gestational age.
Whereas foetus I had a normal male karyotype, STC-villi of foetus II showed 100% trisomy 3 and in LTC-villi an extra marker chromosome, derived from chromosome 3, was found in all analysed cells. In order to exclude confined placental mosaicism follow-up investigations in amniotic fluid cells were performed which showed the marker chromosome to be present in 100% of the cells. Parental karyotypes were normal.
FISH- and DNA-studies that were performed in order to characterize the marker chromosome and to elucidate the mechanism of formation, respectively, as well as the clinical data of the foetus will be presented.

P0489. The incidence of prenatally diagnosed Turner syndrome and their referral indications

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Turner syndrome occurring in 1/2000-5000 female live births is one of the most common chromosomal disorder. It is also a common reason in spontaneous miscarriages with an incidence of 10%. As 99% of embryos with 45,X karyotype result in spontaneous abortion, only 1% of Turner syndrome fetuses survive till birth. Increased nuchal translucency, cystic hygroma, and renal and cardiac defects are typical findings

of Turner Syndrome which can be determined by ultrasonographic examination. In this study 3595 amniocentesis, chorionic villus sampling and fetal blood sampling materials obtained during 1999-2006 were evaluated. Turner Syndrome was found in 16 cases (0.45%). The indications for prenatal diagnosis for those cases were cystic higroma in 8 cases (50.00%), missed abortion in 5 cases (31.25%) and advanced maternal age in 3 cases (18.75%). It has been reported that 40-60% of cystic hygroma cases are associated with Turner Syndrome. Among all prenatally detected cystic higroma cases (15) detected by ultrasonography during 8 years, 8 cases (53.33%) were found to have 45,X karyotype. The present study indicates the importance of cystic hygroma in the prenatal diagnosis of Turner Syndrome.

Po04. Cancer genetics

P0490. QMPSF : A novel method for detection of 1p19q deletions in gliomas

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Gliomas are the most common primary cerebral malignancies. Deletions of 1p and 19q chromosomes have shown to be predictors of chemotherapeutic response and better survival in oligodendrogliomas. Different techniques are available for the detection of these alterations including LOH, FISH and CGH. Despite good concordance exists in terms of sensitivity and specificity between these methods, all have specific limitations. The aim of our study was to describe a reliable novel technique for the detection of 1p/19q deletions in gliomas. For each chromosome arm (1p and 19q), we devised a multiplex PCR assay of fluorescent fragments corresponding to exons, which belong to genes located in the minimal deleted region. A control gene, located on chromosome 1q or 19p, is simultaneously amplified in each assay. PCR products are analysed on an automated sequencer and electropherograms generated from control and tumor samples are superimposed. We have searched for 1p/19q deletions by LOH and QMPSF (Quantitative Multiplex PCR of Short Fluorescent fragments) in a series of 50 patients with a glioma. We found that QMPSF, which does not require constitutional DNA, is a simple, rapid and reliable method to detect 1p/19q deletions. There was a good concordance with LOH data in (88% for 1p deletion and in 83% for 19q deletion). Furthermore, we show that QMPSF has a higher sensitivity than LOH and allows the detection of 1p/19q duplications.

In conclusion, QMPSF can be routinely used in diagnosis laboratories for the detection of 1p/19q rearrangements in glial tumors.

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P0491. Amplification of *ABL1* gene without *BCR/ABL1* fusion in two children with acute lymphoblastic leukemia

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The *ABL1* proto-oncogene, located on chromosome band 9q34 is the known translocation partner of the BCR gene on chromosome band 22q11, giving rise to t(9;22)(q34;q11). This is the most common chromosomal abnormality in Chronic Myeloid Leukemia (CML) and adult Acute Lymphoblastic Leukemia (ALL) patients. Otherwise, frequency of this translocation is only 3-5 % in childhood ALL patients. Amplification of *ABL1* gene is a rare event, that only 7 cases reported in the literature.

In this study, conventional cytogenetics (CC) and Flourescence In Situ Hybridization (FISH) studies were performed on two ALL patients. In the first case, which is a seven-year-old girl, amplification of *ABL1* gene, without *BCR/ABL1* fusion, was observed in 27% interphase nuclei in FISH analysis while metaphases were not found in CC. In the second case which is a ten year old boy, translocation of t(9;15) was detected in CC and interphase cytogenetic analysis by FISH showed the presence of the 10% *ABL1* gene amplification without t(9;22) translocation.

Our results indicate that, amplification of *ABL1* gene without *BCR/ABL1* fusion can be shown by FISH methods. This amplification might play role in leukomogenesis of pediatric ALL and may be used for disease monitoring

P0492. Oligo array CGH on 1, 5 and 10 year old FFPE breast cancer samples

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Microarray-based comparative genomic hybridization (array CGH) has become a powerful technique for studying DNA sequence copy number changes in breast tumors. The ultimate goal of these efforts is the identification of genomic markers that correlate with prognosis and prediction of clinical outcome. For many of these retrospective studies, formalin-fixed, paraffin-embedded (FFPE) tissues are the only samples available. DNA extracted from FFPE tissue can present challenges as it is often degraded and damaged. In this study, we extracted DNA from 24 FFPE breast tumor samples (mostly ductal invasive cancers): ten 1 year, eight 5 year, and six 10 year old. Traditional enzymatic labeling of degraded DNA samples can decrease the DNA fragment length still further and moreover can result in the introduction of enzymatic biases. Therefore, we chose a non-enzymatic labeling method - the Universal Linkage System (ULS™, Kreatech Biotechnology). Analysis of these samples on Agilent Human CGH arrays showed that the average probe-to-probe log ratio noise (DLRSD) was 0.33 for the 1 and 5 year old FFPE samples, indicating that high quality, reliable biological data were obtained. While the 10 year old FFPE samples showed higher average DLRSD values (0.53), the 60 mer probes still enabled detection of aberrations with a high level of confidence. Previously known aberrations associated with breast cancer, such as HER-2 (human epithelial receptor 2), PIK3CA (phosphatidylinositol 3-kinase) and CCND1 (cyclin D1) were detected in several samples. We conclude that the method described here is well suited for analysis of archival pathology specimens for biomarker discovery.

P0493. Cytogenetic findings in a group of patients with acute lymphoblastic leukemia

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Cytogenetic analysis in bone marrow samples of acute lymphoblastic leukemia (ALL) patients have both pathophysiologic and prognostic significance. In this retrospective study, we present the results of cytogenetic analysis of 348 ALL patients (243 children and 105 adults). Cytogenetic abnormalities were found to be more frequent in adults than in children, 23,8 % and 13,6 %, respectively. Structural abnormalities were more frequent than numerical abnormalities in childhood ALL (61.5 % vs 38.5 %) while numerical abnormalities were observed more frequently than structural abnormalities in adults (56 % vs 44 %). Overall, the most frequent cytogenetic abnormality in both childhood and adult ALL patients was t(9;22)(q34;q11) with a frequency of 12,3 % and 8,2 %, respectively. Monosomy 22 and trisomy 21 was the most frequent second and third cytogenetic abnormality in childhood ALL, followed by -7 and +8 at fourth place. On the other hand, - 21 was the most frequent second cytogenetic abnormality in adults, followed by -13, -15 and -20 at third place each with equal frequencies.

P0494. Cytogenetics in AML: results of 589 patients

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The role of cytogenetics in diagnosis and follow-up studies of acute myeloid leukemia (AML) is now widely recognized. However in contrast to chronic myelogenous leukemia (CML), the cytogenetics of AML is much more complex. More than 200 different structural and numerical changes have been found to be recurrent chromosome aberration in AML.

In this report, cytogenetic findings of 589 AML patients are presented. Novel translocations, deletions and numerical abnormalities were found besides of previously defined AML aberrations such as t(15;17), t(16;16), t(8;21), del11q, monosomy 7, and trisomy 8. Two complex, thirteen simple translocations, one inversion and three unbalance changes t(2;19;10)(q21;q13;p11), t(15;17;21)(q24;q23;q21) and t(1;11)(q32.1;q13.9), t(2;5)(q24.2;p13.3) t(2;21)(q11.2;q11.2) t(3;5)(q21;q33), t(3;18)(p13;q23) t(3;18)(q21;q23) t(5;22)(p13:p11) t(8;19) (q22;q13) t(9;17)(p11;p13), t(16;17)(p13;q21), t(7;17)(q11;p13) t(8;18)(p23;q21.1), t(12;18)(p11;q11) inv (13)(p12q32) del(2)(q34), del(2)(q37) dup(2)(q35;qter) respectively, were not reported before as AML related translocations. Some of these breakpoints were previ-

ously defined for different rearrangements in AML patients, and other ones have been reported to be loci of some genes which take place in hematopoiesis. Possible effects of these new cytogenetic findings on prognosis of AML will be discussed.

P0495. Case report of a rare AF9-MLL fusion gene in pediatric acute megakaryoblastic leukemia

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It is known that structural abnormalities involving the mixed -lineage leukemia (MLL) gene on 11q23 have been associated with hematologic malignancies.

In acute myelogenous leukemia (AML) the MLL gene fuses to more than 30 different partner genes. To the best of our knowledge only few cases of AML-M7 have been associated with MLL rearrangement. We herein report a rare case of pediatric AML-M7 with translocation t(9;11)(p22;q23) leading to the AF9/MLL fusion gene.

An 18-month old girl presented with anaemia and thrombocytopenia. In repeated bone marrow (BM) examinations, patient exhibited cytopenia and 15-25% infiltration with blast cells. Immunophenotyping revealed blast positivity for CD41, CD42b and CD61 and the leukemia was therefore classified as M7 according to FAB classification. Cytogenetic analysis of BM showed normal karyotype. FISH analysis was performed using the LSI MLL dual color break apart rearrangement probe (Vysis) at 11q23. 27,5% of analysed nuclei contained a MLL rearrangement. Patient was treated as per the BFM98 protocol for AML . Analysis at a later time and when patient achieved clinical and hematological remission, showed 4,2% positive nuclei while investigation of metaphases spreads revealed that the translocation involved the short arm of chromosome 9 (9p22) and the chromosome 11 at band q23. Chromosome 9 was hybridized, simultaneously with probe for ABL gene (9q34), as a marker.

The patient continued chemotherapy according to protocol and she is candidate for allogeneic bone marrow transplantation.

P0496. Mutation analysis of the *APC* and *MYH* genes in Spanish patients with Familial Adenomatous Polyposis

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Familial adenomatous polyposis (FAP) is an autosomal dominant inherited syndrome caused by germline mutations in the adenomatous polyposis coli (*APC*) gene. However, germline mutations in *APC* cannot be identified in about 20-30% of patients with classical FAP and up to 90% of those with attenuated phenotype (AFAP). Recently, germline mutations in the base-excision-repair gene, *MYH*, have been associated with some *APC* negative FAP and AFAP patients.

Thirty-four unrelated patients with a clinical diagnosis of multiple polyposis (15-100 colorectal adenomas) or classical FAP (>100 colorectal adenomas) were analysed for germline *APC* mutations. Patients without *APC* detected mutations were investigated for *MYH* mutations.

Eighteen families (54%) carried point mutations or large rearrangements in the *APC* gene. p.R805X and p.R216X mutations were found twice in unrelated patients. Phenotypic heterogeneity was observed for the R805X, associated with either AFAP or FAP, suggesting that modifier genes or environment could modulate FAP phenotype. Two new unclassified variants p.I1055M and p.R1171C were also identified.

Three biallelic (20%) and two monoallelic (14%) *MYH* germline mutation carriers were identified in 4 AFAP and 1 FAP families *APC* negative. The two most frequent germline mutations (p.Y165C and p.G382D) were observed as well as new genetic variants for which control populations studies were performed.

In this study 20% of the *APC* negative patients were found to be biallelic *MYH* germline mutation carriers. Therefore, *MYH* analysis is recommended for all *APC* negative families even if a recessive inheritance is not confirmed. However, mutations in *MYH* can not explain all cases of FAP.

P0497. Analysis of APC tumor suppressor gene promoter methylation status in adenocacinoma of stomach and in serum

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Adenocarcinoma of stomach has a high mortality rate . Its incidence rate in Ira is high. Often, the cancer is diagnosed when its is already advanced and has spread to other tissues, which makes treatment difficult. In order to early diagnose the disease attention is focused to the promoter methylation status of tumor suppressor genes. By applying Methylation Specific PCR(MSP) we studied the methylation status of the Adenomatous polyposis coli(APC) tumor suppressor gene in this type of cancer . MSP analysis of APC was done due to the fact that promoter methylation is an important way of APC inactivation. Our study indicates methylation of APC promoter in %66.6 of tissue samples. We further extended our study on to serum of such patients and our results indicate that MSP could be applicable to the serum which clinically is important for diagnosis and evaluation of treatment strategy.

P0498. The coumarins and furanocoumarins induced apoptosis and inhibit proliferation in human leukemic cells

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Coumarins and furanocoumarins are plant compounds exhibiting anticancer activities. A series of 15 coumarins and furocoumarins have been evaluated for anticancer activity in vitro on 6 human leukemic cell lines: HL-60, J45.01, Eol-1, 1301, U261, and ML1.

The cell cultures were stimulated with coumarin derivatives in various concentrations. As a negative control the cells were incubated without compounds. The cell growth was measured by using tetrazolium bromide (MTT) assay. Coumarins and furanocoumarins induced apoptosis in cells as indicated by dose- and time-dependent (1) cytochrome c release, (2) caspase activation, (3) DNA fragmentation, and (4) reduction of expression of anti-apoptotic genes: *BCL-2*, *MCL-1* and *BCL-xL*. Genes expression activity were detected by using real-time quantitative reverse transcription-polymerase chain reaction. After leukemic cells were treated with coumarins and furanocoumarins, the expression of *BCL-2*, *MCL-1* and *BCL-xL* mRNAs decreased.

Our studies show the influence of coumarins: xanthotoxin, o,o-dimetyl-fraxetin, heraclenine and phelopterine on apoptosis process in human lymphocytes in vitro. The compounds showed very good activity against different leukemic cell lines. Supported by grant of Polish State Committee of Scientific Research No 2 PO5F 04928.

P0499. Alterations in *ATP2A2* gene in correlation with colon and lung cancer

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Sarco/endoplasmic reticulum calcium transport ATPases (SERCA-type calcium pumps), proteins that accumulate calcium in the ER, play an important role in numerous signaling pathways controlling tumor growth, differentiation and cell death. Reports that SERCA2 haploinsufficient mice often developed cancer prompted us to study the involvement of the *ATP2A2* gene in human cancer development. We found 13 novel different alterations of the *ATP2A2* gene in 27 of 416 alleles of patients with two different types of cancer. Changes in *ATP2A2* were statistically significantly more common in patients with colon ($p < 0.0001$, OR = 25.3) as well as lung ($p = 0.046$; OR = 8.05) cancer. We found two missense mutations, two intronic deletions, an intronic insertion and 8 single nucleotide alterations, of which two were in the coding region, three in the intronic and three in the promoter region. We were able to detect lost or reduced expression of *ATP2A2* in all patients with alterations in the promoter region, as well as in patients with a combination of gene alterations. Our results suggest that germline alterations of *ATP2A2* may predispose to lung and colon cancer and that an impaired *ATP2A2* gene might be involved, directly or indirectly, as an early event in carcinogenesis.

P0500. Automated FISH analysis predicts good outcome in CML patients with Imatinib-induced remission and detects very low level leukaemic cell population with double *BCR-ABL* rearrangement.

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Seventy-six patients with CML, 69 in chronic phase, 1 in accelerate phase, and 6 in blastic crisis, were treated with imatinib mesylate (IM). The patients were studied for a 18-66 months follow up period using cytogenetics and FISH analysis with a dual-fusion BCR-ABL probe, and an automated FISH imaging system for rare cell events (BioView-Duet). Before IM treatment 50 patients showed Ph chromosome in all examined cells while in the remaining 26 patients a normal clone was also found. Complete or partial cytogenetic remission rate [CCR or PCR: t(9;22) absent or <35% of cells, respectively] overlapped complete or partial FISH remission rate (CFR or PFR: BCR-ABL absent or <35% of cells, respectively) in 74% of patients during the follow up period. However, CFR achievement within 12 months of treatment resulted in a disease-free second year of treatment in 97% of patients as previously reported using QF-PCR. Forty-three percent of CCR samples actually showed >0.5% leukaemic cells by FISH. Two cases showed 0.08% cells with double BCR-ABL (i.e. double Ph), undetectable by QF-PCR, which disappeared after increasing IM dosage. Moreover, FISH unravelled leukaemic cells in 21% of samples unsuitable for cytogenetic investigation from 9 patients who subsequently developed haematological relapse. In 11 patients with partial deletion on der(9)(q34) at treatment start, no CCR/CFR was achieved. Clinical and haematological relapse occurred in 5 cases, while in the remaining 6 a PCR/PFR was observed only after 30 months of treatment supporting a negative role of der(9) deletion on IM effect in CML patients.

P0501. Serial quantitation of bcr-abl transcripts predict resistence to imatinib in chronic myeloid leukemia

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Chronic myeloid leukaemia (CML) is attributed to a specific translocation between chromosomes 9 and 22. the resulting bcr-abl fusion gene codes for a fusion protein with tyrosine kinase activity leading to uncontrolled cell growth.
Imatinib, a bcr-abl inhibitor, induces apoptosis in CML cells by stabilizing the non ATP-binding form of bcr-abl ,and in turn phosphorylation of its sustrates .
Despite the excellent clinical results with imatinib in CML, some patients develop resistence .Several mechanisms of resistance have been described ,the most important in clinical samples appears to be the development of mutations in the kinase domain of bcr-abl. routine screening of patient's samples for bcr-abl kinase mutations is laborious and expensive, serial quantitation of bcr-abl transcripts is a useful tool to diagnose theses patients .
Here we report 15 patients diagnosed with chronic myeloid leukaemia and treated with imatinib 400mg daily . serial quantitation of bcr-abl transcripts in peripheral blood for a median followup of 36 months showed an emerging clone. indeed bcr-abl/abl ratios increased during the period of observation which is synonymous of resistence to treatment.
RQ-PCR studies can identify degrees of molecular response that predict long term stability in chronic myeloid leukaemia. and thus can be used as a screening strategy for mutation analysis.

P0502. Robotic Microscopy for Fully-Automated FISH Testing: Application in Bladder Cancer Identification and Recurrence Monitoring

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FISH analysis for the identification of bladder cancer has been well documented. Quantitation of chromosomes 3, 7 and 17 and deletion of 9p21, has been found to complement urine cytology raising the sensitivity of testing to >97% (specificity of >94%). However, FISH is labor intensive requiring a high skill level and somewhat subjective interpretation. An automated system for FISH analysis has the potential to reduce interpretation errors and decrease turn around times for patient results. We report here the use of a fully-automated, robotic fluorescence microscopy platform in the identification and analysis of cells, in urine sediment, for the diagnosis of bladder cancer or tumor recurrence.
100 patients suspected of having bladder cancer or bladder cancer recurrence were analyzed in a blinded study. A single slide was prepared from each patient's urine then analyzed manually on a fluorescence microscope. The slide was then loaded on the automated microscopy platform for FISH analysis. 50 of the slides were run on a second platform for intra-instrument comparisons.
97% of the samples showed complete concordance between the results obtained by manual and automated analysis. 19 samples were positive by both methods, 76 negative and 2 inconclusive. 2 samples were negative by manual analysis and inconclusive by automated and 1 sample was negative by automated analysis and inconclusive by manual. For the 50 slides run sequentially on two instruments, 49 gave the same result from both analyses. One slide was read as normal on the first instrument and inconclusive when run a second time, probably due to deterioration of the FISH signals.
These data suggest that an automated FISH analysis system is capable of providing accurate detection and enumeration of FISH signals in voided urine samples and has the potential to increase efficiency in the detection of bladder cancer and tumor recurrence.

P0503. Polymorphism of DNA repair genes XRCC1 and risk of brain tumors

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Background and Objective: DNA repair genes play a major role in maintaining genomic stability through different repair pathways. The current study was designed to evaluate the relation between polymorphism of DNA repair gene *XRCC1* and brain tumours. Their frequencies were also compared with patient's age, gender, smoking, alcohol status, family history, and tumor histopathology in a Turkish population.
Methods: We conducted a case-population base study including 135 cases of brain tumors and 87 population base age- and sex-matched healthy controls to examine the role of polymorphism of *XRCC1* Arg-399Gln gene, in the context of brain tumor risk for the Turkish population. Tumors were subdivided into 4 main groups that constitute 85% of brain tumors, according to tumor histopathological examination.. Group I; patients with glial tumors (n=71), Group II; patients with meningiomas (n=35), Group III; patients with pituitary adenomas (n=21) and Group IV; patients with metastases to the brain (n=8).
Results: There was no significant difference in the distributions of *XRCC1* Arg399Gln polymorphisms among the gliomas, meningiomas and the controls. However *XRCC1* Arg399Gln, polymorphism were significantly different in pituitary adenomas and metastases to brain. As metastases to brain were compared with primary brain tumors the difference was also significant.
Conclusion: These results suggest that the *XRCC1* Arg399Gln polymorphism may be a marker for the susceptibility to pituitary adenomas and metastases to brain.
Key Words: brain tumors, DNA repair gene, genetics, polymorphism, XRCC1

P0504. The role of XPD gene polymorphism in brain tumors

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Background and Objective: Deficits in DNA repair pathways may lead to tumorigenesis and pathogen defence. The current study was designed to evaluate the relation between polymorphism of DNA repair gene xeroderma pigmentosum group D (XPD) and brain tumors. We aimed to investigate it's role as susceptibility marker for brain tumors.

Their frequencies were also compared with patient's age, gender, smoking, alcohol status, family history, and tumor histopathology in a Turkish population.
Methods: We conducted a case-population base study including 136 cases of brain tumors and 86 population base age- and sex-matched healthy controls to examine the role of polymorphisms of XPD gene, in the context of brain tumor risk for the Turkish population. Tumors were subdivided into 4 main groups that constitute 85% of brain tumors, according to tumor histopathological examination. Group I; patients with glial tumors (n=72), Group II; patients with meningiomas (n=35), Group III; patients with pituitary adenomas (n=20) and Group IV; patients with metastases to the brain (n=9).
Results: There were no significant difference among groups as compared with each other (groups I-IV) and also compared with control healthy individuals in regards to XPD gene polymorphisms.
Conclusion: Lys751Gln polymorphisms of the *XP*D gene have not a functional consequence in brain cancer risk and do not have prognostic significance in patients with brain tumors either.
Key Words: brain tumors, DNA repair gene, genetics, polymorphism, xeroderma pigmentosum group D, XPD

P0505. BRCA1 and BRCA2 sequence variability in normal population in Croatia

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Hereditary breast cancer is characterized by an inherited susceptibility to breast cancer. The main candidates are BRCA1 and BRCA2 genes, which are acting as tumor suppressors, and are only genes known to be mutated in familial history of breast cancer. In familial cases an individual already carries a mutation in one of the alleles. Tumorigenesis is a result of inactivation of the second allele.
We are analyzing distribution of polymorphic variants of BRCA1 and BRCA2 genes in normal population to establish the reference values in healthy population, since no such screening has been undertaken in Croatia. We assume that by evaluating polymorphic forms of BRCA1 and BRCA2 predisposition to breast and ovarian cancer can be estimated.
Among a wide range of conventional mutation scanning methods based on conformational polymorphisms, we decided to use procedures based on high resolution melting approach and heteroduplex formation that give different melting profile and fluorescence, according to polymorphic or mutational variant.
This analysis is contributing to a program of an early detection and prevention of breast cancer in Croatia with intention of forming a database of a high penetrance susceptibility genes BRCA1 and BRCA2.

P0506. Haplotype Analysis of Recurrent BRCA1 and BRCA2 Mutations In Spain.

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Mutational analysis of BRCA1 and BRCA2 genes has been hampered by the large size of the two genes, and the frequent occurrence of unique mutations. Founder mutations for BRCA1 and BRCA2 have been identified in several populations, and haplotype analysis has provided evidence of a common ancestor. Several mutations (c.5272-1G>A from BRCA1, and c.3036delACAA, c.5344delAATA, c.5374delTATG and c.9538delAA from BRCA2) have been identified multiple times in our population, suggesting that could be founder in Spain.
Haplotype analysis using polymorphic markers spanning BRCA1 and BRCA2 loci was performed on index cases and on additional family members. Sixty-two BRCA1/2 positive families were genotyped. D17S855, D17S1185, D17S1323 and D17S1326 polymorphic microsatellites linked to the BRCA1 gene were analysed in seven c5272-1 G>A families. D13S171, D13S260, D13S1695 and D13S1698 STRs were typed in four different BRCA2 mutations c3036delACAA (36 families), c5374delTATG (11 families), c5344delAATA (4 families), and c9538delAA (4 families).
All carriers of c.5272-1 G>A and c.5374delTATG mutations shared the same haplotype at the four markers of BRCA1 and BRCA2, respectively, and thus supporting the possibility of a common ancestry. Results in c.3036delACAA families provide evidence of at least two distinct lines of transmission in Spanish population. This facilitates the setting up of a less expensive and less time-consuming strategy to test the BRCA genes in high-risk Spanish BC patients.

P0507. The complete deletion of *BRCA1* gene identified in the Slovak family by combination of sequencing, MLPA and array-CGH techniques.

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Pathogenic germline substitutions and short deletions/insertions in *BRCA1/2* account for the majority of hereditary breast/ovarian cancer cases, however, the large genomic rearrangements (LGR) also represent a substantial proportion of disease-causing changes. In this pilot study we demonstrate the specific case of Slovak family with breast/ovarian cancer, where sequencing analysis (SA) of *BRCA1* revealed the discrepancy of SNPs haplotypes and primarily indicated the presence of LGR.
Initially, the analysis of all exons of *BRCA1* was based on the combination of SSCP and SA. Subsequently, MLPA analysis (P002B; P087 kits) was performed. Finally, results were proved with array-comparative genomic hybridization (array-CGH).
SSCP and SA demonstrated presence of 11 SNPs in *BRCA1*, which indicated hemizygous status, and possible occurrence of LGR. The MLPA results showed reduction of peaks levels for each *BRCA1* exon. Array-CGH displayed a single deletion signal for *BRCA1* among set of 287 gene probes. Totally, 8 members of the family were analysed, in 3 of them LGR was confirmed.
We have discovered rare germline LGR affecting complete *BRCA1,* according to our knowledge this is the second time it was described. Although, the breakpoints of LGR were not identified yet, concerning family history it is evident, that clinical effect is comparable with small deletions/insertions and substitutions and leads to the loss-off gene function.
In conclusion, it is important to note that DNA analysis of *BRCA1/2* genes should be performed in all affected members of breast/ovarian cancer families concurrently, since the discrepancy in the SNPs haplotypes may indicate the presence of LGR.

P0508. Mutation screening of BRCA1 and BRCA2 in breast and breast/ovarian cancer families from the Slovakia.

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Germline mutations in the BRCA1 and BRCA2 genes have been shown to account for the majority of hereditary breast and ovarian cancers. We have screened high-risk breast and breast/ovarian cancer families from Slovakia using combination of the SSCP and direct sequencing techniques.
Mutational analysis of all exons and flanking intronic splice sites of BRCA1 gene was performed in 156 suspected families and subsequently in 47 BRCA1 negative families, the analysis of complete coding region of BRCA2 was performed.
Fourteen different types of BRCA1 pathologic mutations were identified in 31 breast/ovarian families. The most frequently found mutations were c.5266dupC (8 families), c.181T>G (5 families), c.68_69delAG (3 families) and c.843_846del4 (3 families), marked in the approved systematic nomenclature numbering. The novel BRCA1 mutation c.1166delG, which forms a stop codon in amino acid 393 giving rise to a truncation protein, was identified. This frame-shift mutation was identified in 2 patients suffered from breast cancer at the ages of 27 and 32 years, and in 2 subclinical probands.
Mutational analysis of BRCA2 gene showed presence of 3 distinct pathogenic mutations. The mutations c.3G>A, c.5946delT, c.9403delC were found in 3 families with strong family history for occurrence of breast cancer, all of them are already presented in BIC database.

In summary within 156 Slovak breast/ovarian families, we identified 34 families (21,8%) with pathologic germline BRCA1 and BRCA2 mutations. A novel BRCA1 mutation c.1166delG was not previously described in BIC database and published until year 2006 and we suspect it is of Slovak origin.

P0509. Germline BRCA2 large rearrangements are not a common cause of breast/ovarian cancer predisposition

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Screening for large deletions and duplications of the *BRCA1* gene is now recognized as an important part of routine molecular diagnosis in familial breast/ovarian cancer. The picture is less clear as to the value of screening *BRCA2* for such alterations. The available studies seemingly show that this is a worthwhile screening however they are based on patients selected for high probability of breast/ovarian cancer predisposition. No data are available for patients routinely ascertained in genetic units.

Therefore we embarked upon *BRCA2* large rearrangement screening on a large sample of 488 patients consecutively ascertained in our breast cancer family clinic for whom breast/ovarian cancer genetic-predisposition was 1%-96% (mean 53%) according to the Claus model. Obviously all these patients were tested negative for *BRCA1* point mutations and large deletions and for *BRCA2* point mutations. *BRCA2* large rearrangement screening was performed using a previously published QMPSF assay and included control samples bearing a *BRCA2* deletion and a trisomy 13 purchased from Corriel. All controls were successfully identified and no *BRCA2* rearrangement was found in these 488 patients.

These results strongly suggest that *BRCA2* large rearrangements are not a common cause of breast/ovarian cancer predisposition. Oncogenetic teams should now discuss if this screening should be proposed on a routine basis or only to highly selected patients. It could also be used as a second-line screening however this option could greatly delayed the results availability to the detriment of the genetic follow-up.

P0510. Breast cancer susceptibility association with a regulatory SNP in 3'utr region of E2F1 gene

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SNPs located in regulatory regions (rSNPs) have been proposed as potential causal SNPs in complex traits and diseases because of their role in modulating gene expression.

In the current work, we present the research on a rSNP located in 3'utr of E2F1, rs3213180, within a case-control association study, using up to 854 sporadic breast cancer patient and 553 control samples. A relative risk of 2.3 (95%C.I.=0.48-11.10), was estimated for rs3213180, not statistically significant, possibly due to the low Spanish MAF=0.05. It has been described that the expression of the E2F1 gene is modulated by c-Myc, which activates E2F1 transcription, but also blocks its translation through the activation of transcription of some miRNAs. These miRNAs recognize two binding sites at the 3'utr region of the E2F1 mRNA and delay the translation to protein, and rs3213180 is located at the 3'utr region, upstream of binding site 2.Luciferase expression assays using constructs with E2F1 3'utr have revealed a mild increment in expression in the presence of the minor allele (p-value<0.001) and a difference of expression with minor allele was again observed using constructs with binding site 2 mutated (p-value=0.02). Experiments with binding site 1 mutated are in progress and they will help to determine whether rs3213180 affects miRNAs binding as previous experiments are pointing out. Additionally, E2F1 protein expression by using western analysis on lymphocytes and immunochemistry staining on normal breast tissues from patients with different genotypes will be further discussed.

P0511. Search for a rapid pre-screening technique to identify heterozygous *BRCA* mutation carriers using gene expression profiling in peripheral blood lymphoytes

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Breast cancer is the most common cancer in women in the western world. Approximately 5-10 % of breast cancers are due to strong genetic susceptibility. Mutations in two major genes, *BRCA1* and *BRCA2*, account for at most 30 % of the families with hereditary breast cancer. Unfortunately, in most families there is little to indicate which gene should be targeted first for mutation screening, which is labor intensive, time consuming and often prohibitively expensive in poor and developing countries. We are investigating gene expression profiling to distinguish between *BRCA1* and *BRCA2* mutation carriers and non *BRCA* carriers. Heterozygous mutations in either *BRCA1* or *BRCA2* could dysregulate the DNA damage response and alert the immune system by inducing or inhibiting transcriptional activity of genes involved in the downstream signaling cascade. RNA from samples of blood peripheral lymphocytes from *BRCA1* mutation carriers and non-carriers from *BRCA1* families were hybridized on an Agilent pangenomic microarray of 44000 complementary cDNAs. Among 576 genes over- or under-expressed at least 1.5-fold between the two groups, Student's t-test with a Benjamini and Hochberg multiple testing correction ($p<0.05$) revealed 79 differentially expressed genes. Several of these genes are involved in transcription regulation or the BRCA1 signaling network and one is a stimulatory receptor of the innate immune system previously shown to be induced by DNA damage responses. We are now testing a larger sample set, as well as targeted experiments by RT-PCR to validate this preliminary data before studying gene expression profiles of *BRCA2* mutation carriers.

P0512. Germ line E-Cadherin mutation in familial lobular breast cancer

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Background: The cell surface glycoprotein, E-Cadherin (*CDH1*) is a key regulator of adhesive properties in epithelial cells. Germline mutations in *CDH1* are well-established as the defects underlying the cancer syndrome of Hereditary Diffuse Gastric Cancer (HDGC): an increased risk of lobular breast cancer (LBC) has been described in HDGC kindreds. We sought to investigate the frequency of germline *CDH1* mutations in LBC patients with early onset disease or family histories of breast cancer without diffuse gastric cancer. Methods: Germline DNA was analyzed in 23 women with invasive lobular or mixed ductal and lobular breast cancers who had at least one close relative with breast cancer or had themselves been diagnosed before age 45, had tested negative for a germline *BRCA1* or *BRCA2* mutation, and reported no personal or family history of diffuse gastric cancer. The full coding sequence of *CDH1* including splice junctions was PCR amplified and screened for mutations using DHPLC. Results: A novel germline CDH1 truncating mutation in the extracellular portion of the protein (517insA) was identified in one subject who had lobular breast cancer at age 42 and a first degree relative with invasive lobular breast cancer. Conclusions: Germline *CDH1* mutations can be associated with invasive lobular breast cancer in the absence of diffuse gastric cancer. The finding may have implications for management of individuals at risk for this breast cancer subtype, and compels clarification of the cancer risks in the syndrome.

P0513. Screening of *CDKN2A* and *CDK4* mutations in heterogeneous-phenotype breast cancer families presenting melanoma and/or pancreatic cancer.

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Breast cancer is hereditary in 10% of the cases, the majority related to mutations in *BRCA1* and *BRCA2* genes but only 20-25% of families screened are found positive, suggesting that other breast cancer susceptibility genes exist. Inherited mutations in the *CDKN2A* tumour suppressor gene, which encodes the $p16^{INK4a}/p14^{ARF}$ proteins, and in the cyclin-dependent kinase 4 (*CDK4*) gene, confer susceptibility to cutaneous malignant melanoma. The *INK4a/ARF* locus on chromosome 9q21, encodes for 2 unrelated and independently acting negative cell cycle regulators, $p16^{INK4a}$ and $p14^{ARF}$, arising from alternative first exons (1α or 1β respectively) and common exons 2 and 3. Besides melanoma, a high frequency of other tumours (pancreatic, nervous system, head and neck and breast) has been reported in $p16^{INK4a}/p14^{ARF}$ mutation positive melanoma families. The objective of this study is to analyse the presence of germline mutations in the *CDKN2A* and in *CDK4* genes, in 21 families with breast, melanoma and/or pancreatic cancer. Two families were negative for full *BRCA1/2* genetic screening. In the remaining 19 families, *BRCA1/2* and *CDKN2A*/*CDK4* screening are being done simultaneously, since *BRCA* mutation probability was under 25%. The entire *CDKN2A* coding region and exon 2 of the *CDK4* gene are being screened by direct sequencing. Results of our screening will be presented and will help determine if *CDKN2A* and C*DK4* screening is worth being done in heterogeneous-phenotype breast cancer families presenting melanoma and/or pancreatic cancer.

P0514. Screening of BRCA 1 gene in breast and ovarian cancer patients diagnosed at age under 40

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Since the identification of BRCA1 gene in 1994, there were numerous studies on prevalence and genetic background of breast and ovarian cancer. Introducing of new method for screening of large genomic rearrangements of BRCA 1 gene gave a new perspective to diagnosis, prognosis, therapeutical approaches and prevention of breast cancer. Tumor bioptic specimens from patients fulfilling a criteria of high to medium risk group for breast and ovarian cancer have been consecutively collected at Sarajevo Clinical Center during the period of two years.

DNA was isolated from tumor tissue samples using standard salting out procedure. After DNA isolation we introduced MLPA - Multiplex ligation-dependent probe amplification. 34 probe hybridization , ligation and PCR reaction was performed on Eppendorf Mastercycler gradient, and genotyping was performed on ABI PRISM 310 sequencer. Data analysis is performed using GeneMapper v. 3.2 and Coffalyzer software.

Results are displayed as RFU and peak area values and graphically as output of Coffalyzer.

P0515. *Prognostic value of chromosomes 1 and 8 aneusomy in invasive ductal breast carcinoma among Iranian women: An interphase FISH analysis*

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Breast cancer is amongst the leading causes of death in women worldwide and the most common cancer amongst Iranian women.

The aim of this retrospective study was to investigate the role of chromosomes 1 and 8 copy number assessed by Interphase Fluorescence In Situ Hybridization (FISH), as the genetic prognostic parameters in 70 Iranian women, aged 35 to 64 years(mean age of 48.1±*7.7)*, with primary sporadic invasive ductal breast carcinoma.

FISH, using centromeric probes for chromosomes 1 and 8 was applied to interphase cell suspensions all prepared from the carnoy fixed tumor cells and selected paraffin embedded tumor sections.

More than 10000 cells for chromosomes 1 and 8 were assessed. Aneusomies for chromosomes 1 and 8 were present in all the studied patients to different levels. The total abnormality rate for chromosome 1 was 47 percent , whereas for chromosome 8, this rate was 33 percent. This was similar to other reported cases .Statistical significant associations (<0.05) were demonstrated between hyperdiploidy 1 and trisomy 8 and patients' age below 50 years . Trisomy 1 and hyperdiploidy 1 showed significant correlation with poor survival. Larger tumor size and raised aneusomy 8 were significantly related. Patients with increased number of lymph node metastasis, involvement of lymph node metastasis and increased stage of the disease showed a poorer prognosis.

In conclusion, chromosomes 1 and 8 aneusomy alongside other clinical and pathological parameters may be considered as useful prognostic markers in invasive ductal carcinoma of breast.

P0516. Is telomere length in peripheral blood lymphocytes correlated with cancer susceptibility or radiosensitivity?

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Mean terminal restriction fragment lengths (TRF) in white blood cells have previously been found to be associated with breast cancer in case-control studies. In order to assess whether this marker could be used as a test for breast cancer susceptbibility, TRF length was measured in 72 individuals after treatment for breast cancer, 125 newly diagnosed breast cancer cases and 1804 unaffected controls between the age of 45 years and 77 years from the Twin Research Unit at St Thomas Hospital. Mean TRF was also tested for correlation with chromosome radiosensitivity and apoptotic response in the various groups.

After adjusting for age and sex, there was no significant difference in TRF lengths between the breast cancer treated and unaffected controls (6.859 kb versus 6.858, p=0.988) or between newly diagnosed breast cancer patients and unaffected controls (6.65 kb for cases versus 6.60 for controls P = 0.529). The only significant finding was a positive correlation between age-adjusted apoptotic response and mean TRF in newly diagnosed breast cancer patients (p=0.008).

This suggests that TRF lengths in white blood cells, is not a marker of breast cancer susceptibility and does not vary significantly between affected women who have or have not been treated for their cancer.

P0517. Genetic risk factors in non-BRCA breast/ovarian cancer families.

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Mutations in the BRCA genes account for less than 30 % of families at risk of hereditary breast and/or ovarian cancer. It is therefore imperative to identify the other genetic factors contributing to familial breast cancer, to better ascertain risk to individuals in these families and decide on appropriate preventive and/or early detection measures. Deleterious mutations or polymorphisms of many genes have been described as associated with breast cancer risk.

We analysed two genes for deleterious mutations, and snps or microsatellites in seven cancer-associated genes in 600 cases from non-BRCA1/2 breast/ovarian cancer families and in 400 women with no personal or familial breast/ovarian cancer history.

Deleterious mutations and missense variants of Chk2 were significantly more frequent in cases than controls. No mutations in XRCC2 were observed, although the variant allele His188 was significantly associated with cases. Polymorphisms affecting expression of IGF-1 and EGFR were significantly different between cases and controls. Polymorphisms in other genes were not associated with cancer. Multivariate analysis suggested that Chk2 mutation, the His188 variant of XRCC2, and the risk-associated alleles at EGFR and IGF-1 were independent risk factors. There was no apparent effect on age at diagnosis or type of cancer (breast vs ovarian or other) among carriers of risk alleles. Segregation analysis of Chk2 mutations and of the XRCC2 His188 variant are underway, as is the multiparametric analysis of additional genes. These results are an encouraging first step in uncovering the diverse genetic factors contributing to breast cancer risk in a polygenic model.

P0518. A map of nuclear matrix attachment regions within the breast cancer loss-of-heterozygosity region on human chromosome 16q22.1

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To explore the DNA domain organization of the breast cancer loss-of-heterozygosity region on human chromosome 16q22.1, we have identified a significant portion of the scaffold/matrix attachment regions (S/MARs) within this region. Forty independent putative S/MAR elements were assigned within the 16q22.1 locus. More than 90% of these S/MARs are AT rich, with GC contents as low as 27% in 2 cases. Thirty-nine (98%) of the S/MARs are located within genes and 36 (90%) in gene introns, of which 15 are in first introns of different genes. The clear tendency of S/MARs from this region to be located within the introns suggests their regulatory role. The genomic interval mapped has been identified as a possible region harboring tumor suppressor genes in both invasive ductal and invasive lobular breast carcinomas. The E-cadherin gene, which is located within this region, has been shown to be mutated in lobular breast carcinomas, resulting in loss of E-cadherin expression. However, in most cases of ductal carcinoma, E-cadherin is normally expressed, suggesting that other genes within chromosome 16q22.1 may be involved in the development of this tumor subtype. The construction of comprehensive S/MAR maps of the region using a panel of breast cancer cell lines may provide information on relevance for the etiology of breast carcinomas. Changes of positions of regulatory elements such as S/MAR within the region may allow an understanding of how the genes in the region are regulated and how the structural architecture is related to the functional organization of the DNA.

P0519. Expression of two testis-specific genes, TSGA10 and SYCP3, in skin cancer

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Cancer-testis genes are a group of genes expressed in testicular germinal cells and a range of human cancers. Testis specific gene A10 (TSGA10) is expressed in testis and actively dividing and fetal differentiating tissues. SYCP3 gene is supposed to be a testis specific gene and constitutes the core of the lateral elements of synaptonemal complex. It has role in regulating DNA binding to the chromatid axis, sister chromatid cohesion, synapsis, and recombination. *Methods:* In this study expression of TSGA10 and SYCP3 were investigated in 26 skin cancer using RT-PCR. Diagnosis of cancer was based on histopathological reports. *Results:* TSGA10 expression was observed in 66.4% of total of skin tumors including melanomas with 85.7%, Basal cell carcinomas (BCC) with 75% and Squamous cell carcinomas (SCC) with 40% positive expression of TSGA10, but, SYCP3 transcripts were not found in skin tumors. *Conclusion:* These results may get further insight into the potential role as cancer marker and as cancer testis gene implicated in tumorogenesis of skin tumors in the case of TSGA10

P0520. Evaluation of the relationship between *XRCC1* and *XPD* Polymorphisms and laryngeal squamous cell carcinoma (LSCC)

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Squamous cell carcinoma is the most common (90-95%) of all head and neck cancers (SCCHN), and laryngeal squamous cell carcinoma (LSCC) is one of the most common tumors of the upper aerodigestive tract. The incidence varies according to geographical area and most probably depends on specific environmental risk factors. Many studies reported that polymorphisms in DNA repair genes reduce their capacity to repair DNA damage and thereby lead to a greater susceptibility to cancer. DNA repair enzymes continuously monitor DNA to correct damaged nucleotide residues generated by exposure to environmental mutagenic and cytotoxic compounds or carcinogens. Our objective was to investigate the association, if any, between XRCC1 (X-ray repair cross-complementing 1) for Arg399Gln and XPD (ERCC2) for Lys751Gln polymorphic genotypes and LSCC, smoking and alcohol habits, tumor-node-metastasis (TNM) classification, and patient age. There was no significant difference in the genotype distribution of XPD between LSCC patients and their smoking and alcohol habits, tumor-node-metastasis (TNM) classification, and patient age and controls for each polymorphism ($p>0.05$). There was a significant difference between the genotype distributions of XRCC1 in patients and in control groups ($P<0.05$). Our result concluded that polymorphism in *XRCC1* codon 399 was associated with risk of LCSS in a sample of the patients while polymorphism in *XPD* codon 751 was not.

P0521. Isolation and characterization of the putative bladder cancer stem cell

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There is increasing evidence that the growth, spread and drug resistance of cancers is driven by a subpopulation of cancer stem like cells (CSCs) the only cells able to long-term self-renewal and generation of the phenotypically diverse tumour cell population. Current failure of cancer therapies may be due to their lesser effect on potentially quiescent CSCs which remain viable and retain their full capacity to restore the tumour. However, the development of new CSC-targeted strategies is currently hindered by the lack of reliable markers for the identification of CSCs and the poor understanding of their behaviour and fate determinants. We report the isolation and characterization of a putative bladder CSC population from human bladder tumors. Specimens were mechanically dissociated and digested by collagenase and plated at clonal density in serum-free medium containing mitogenic growth factors (basic fibroblast growth factor and epidermal growth factor). Karyotypic and interphasic FISH analysis were performed on fresh tumors and on tumor-derived cells after different times of culture. Preliminary results suggested a marked heterogeneity among different tumors and a cell selection after isolation and culture. After 2 days in vitro few proliferating clusters were observed that forms detached spheres. Experimental procedures are in progress to test the capacity of long term proliferation and differentiation. This is the first report at our knowledge on putative CSCs from human bladder tumors.

P0522. DNA copy number changes in gastric adenocarcinoma patients from Turkey

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Gastric cancer is one of the most common causes of cancer-related deaths in Turkey, but genetic changes underlying the development and progression of this cancer is poorly understood. In this study, DNA copy number changes were analyzed by comparative genomic hybridization (CGH) in 43 gastric adenocarcinoma patients from Turkey and correlated with tumor invasion, lymph node involvement and histological stage. Chromosomal imbalances were observed in 36 (84 %) tumor samples. Frequent gains were found on 1q, 7p, 7q, 8p, 8q, 11q, 13q, 16p, 20p, 20q and losses were found on 2q, 4q, 5q, 6q, 14q, 15q, 18q and Xq. High level gains were detected on 1q, 7q, 8p, 8q, 9p,13q and 16p. When all of the detected chromosomal imbalances were analyzed for the correlation with the histopathological factors, no statistically significant difference in the pattern of chromosomal imbalances were observed in tumor invasion. However, gain of 13q with a minimally overlapping region 13q21-q31 was found to be significantly higher in tumors with increased lymph node metastasis ($p<0.05$). In addition, gains of 6p, 9p and 10q were found to be correlated with stage I tumors ($p<0.05$). Minimally overlapping regions were defined as 6p21.1 and 9p21-p13 for gains of 6p and 9p respectively. 10q21-q23 and 10q25-qter, each of which was observed in two cases, were determined as minimally overlapping regions for gain of 10q. This data indicates that chromosomal imbalances play an important role in gas-

tric carcinogenesis and chromosomal regions identified in this study provide candidate regions for progression of gastric adenocarcinoma.

P0523. Mutations in CHEK2 gene in the Czech Breast Cancer Families

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Cell cycle regulator CHEK2 is an important breast cancer susceptibility gene. It seems that the contribution and spectrum of CHEK2 mutations varies from country to country.
Our aim was to detect CHEK2 1100delC mutation and genomic rearrangements in CHEK2 gene among patients with breast cancer from high-risk families with negative testing results of BRCA1 and BRCA2 genes.
Multiplex Ligation-Dependent Probe Amplification (MLPA) has been applied for examination of CHEK2 rearrangements and CHEK2 1100delC mutation. DNA from 180 breast cancer patients with no previously found BRCA1/2 mutation was investigated. In addition 273 BRCA1/2 negative breast cancer patients were investigate for the presence of CHEK2 exon 9-10 deletion using PCR reaction with specific primers. This deletion was originally reported in families of Czechoslovakian ancestry.
The 1100delC germline mutation was identified in one from 180 families tested (0,56 %) and the deletion of exon 9-10 was identified in 4 from 453 families tested (0,88 %). Both mutations lead to premature stop codon. The average age at the diagnosis of breast cancer in mutation carriers was 41,2 years and three of them reported a positive family history of breast cancer.
It is suggested that there is a two-fold increased risk of breast cancer in 1100C mutation carriers compared to noncarriers. However, the clinical impact of other CHEK2 gene mutations needs further investigations.
In the poster we present pedigrees of five breast cancer families that carry deleterious mutation in CHEK2 gene.
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P0524. Cytotoxic and cytogenetic analysis in a human cholangiocarcinoma cell line after resveratrol treatment

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Cholangiocarcinoma represents 3% of all gastrointestinal cancers, its incidence is increasing and therapies are dissatisfied. In vitro studies demonstrated that resveratrol (RES), a poly-phenol agent, inhibits proliferation of cancer cells in two-dimensional cell cultures, through an increase of apoptosis and cell destruction. New insights into tumor biology are produced by another in vitro model, the multicellular tumor spheroids (MCTS). We evaluated the activity of RES on a human cholangiocarcinoma cell line (SK-CHA-1) growing in two-dimensional and MCTS culture.
SK-CHA-1 cell line with pseudo-triploid karyotype (modal chromosomes number 71) and 12 different markers chromosomes have been exposed to different doses of RES (8, 16, 32, 64 microM) in both models. We investigated: 1) cell viability; 2) cell morphology; 3) plasmatic membrane damage through lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) medium release; 4) apoptosis through transglutaminase type 2 (TG2) activity 5) karyotype.
RES significantly reduced viability in both cell culture systems (from 89% to 16% vs 100% controls) and induced partial disaggregation of MCTS in a dose and time dependent fashion. In the culture media we found increased LDH levels (decupled vs controls) and ALP activity (quadruplicated vs controls), suggesting a plasma membrane injury; high levels of TG2 activity in treated cells suggested high pro-apoptotic stimuli (from 108% to 462% vs controls in both cell culture systems). No chromosomal changes have been observed.
In conclusion, the cytotoxic effect of RES on SK-CHA-1 cells supports further studies on the possible use of resveratrol as chemotherapic/chemopreventive drug in cholangiocarcinoma.

P0525. High-Resolution Genomic Profiling of Chronic Lymphocytic Leukaemia by Array CGH

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Chronic lymphocytic leukaemia (CLL) is the most common form of leukaemia in adults in the western world. Research into the genetic basis of CLL has successfully improved survival rate, but further advances are hampered by resolution limits of conventional genetic screening techniques such as cytogenetics, FISH, and metaphase CGH. We have applied high-resolution microarray CGH to analyse DNA copy number imbalances in a cohort of 50 CLL patient samples and identified genetic imbalances that have not previously been recognised, but correlate with disease progression and treatment outcome. Sample DNA was extracted from CLL cell populations with an average purity of 84%, and co-hybridised with normal reference DNA onto UCSF 1.4 Mb BAC arrays. Reference DNA was taken from the same individual for all array hybridisations, and we identified sites of normal copy number variations (CNVs) in this reference in a separate study by array hybridisation against 20 non-CLL samples. In addition, approximately 10% of CLL samples were also hybridised against DNA extracted from the same patients' non-leukaemic neutrophils. Known recurrent copy number changes, such as deletions of 13q14, 11q22-23, 17p13, and trisomy 12, were detected in 27 (54%), 9 (18%), 8 (16%), and 7 (14%) samples, respectively. Novel sites of recurrent copy number changes that are not recognised as sites of normal CNV and have not been previously correlated with CLL have also been identified. These sites have high potential to assist future improved clinical stratification and better adjusted treatment regimes.

P0526. Detection of ABL tyrosine kinase domain mutations in Turkish chronic myeloid leukemia (CML) patients treated with imatinib mesylate

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Chronic **m**yeloid **l**eukemia (CML) is a malignant hematopoietic stem cell disorder that is characterized by the Philadelphia (Ph) chromosome which results in fusion of BCR and ABL genes. BCR-ABL fusion gene encodes cytoplasmic BCR-ABL oncoprotein with a constitutive tyrosine kinase activity that enhances the proliferation of affected cell clone. Selective inhibitor of BCR-ABL tyrosine kinase namely imatinib mesylate (Gleevec, STI571) is used as an effective therapeutic agent. imatinib induces the BCR-ABL protein into the inactive conformation by binding to amino acids in the ABL kinase domain and blocks the binding of adenosine triphosphate (ATP). This prevents the transfer of phosphate from ATP and blocks the downstream signal pathways, leading to growth arrest or apoptosis. Targeted therapy with imatinib has led to revolution in the treatment of CML. However, some patients develope resistance to imatinib. Point mutations in the ABL kinase domain is the major mechanism of resistance in patients treated with imatinib and these missense mutations cause different degrees of resistance. In this study we've investigated the frequencies of T315I found in imatinib binding region, E255K found in P-loop region and M351T found in catalytic region mutations by ASO-PCR in 30 CML patients treated with an inadequate response to imatinib. In our study, T315I mutation is seen in %43 of patients, E255K mutation is seen in %10 of patients and M351T mutation is seen in %26.6 of CML patients. The relation between the mutations and the clinical outcome is important for predicting the treatment.

P0527. Karyotype monitoring during imatinib treatment in chronic myeloid leukemia

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The non-transplantational therapy in chronic myeloid leukemia (CML) has been dramatically changed by the introduction of imatinib mesyl-

ate. Glivec became the therapy of choice in chronic phase CML.
We present some preliminary results of cytogenetic evaluation within a study aiming the long term Glivec therapy monitoring.
Chromosomal studies were performed on GTG-banded slides obtained from bone marrow, after short term culture and standard preparation. FISH studies were done with BCR/ABL probes (Vysis), BAC FISH probe (8p23.1) and WCP probes (Vysis, Cytocell).
Cytogenetic evaluations were performed at 6, 12, 18 and 24 months. After more than 2 years of treatment most of the patients were assessed at 12 months intervals.
Sixty six out of 77 patients (86%) had advanced disease or a history of long term interferon-alpha and conventional chemotherapy in chronic phase CML. Eleven patients (14%) received imatinib as first therapeutic choice in early chronic phase. Among these patients, 2 had additional pre-treatment chromosomal changes [7q additional material of unknown origin and translocation t(8;14), respectively], and 2 had variant t(9;22): t(3;9;22) and t(1;9;22). None of the patients receiving front line Glivec therapy showed karyotype evolution so far. Instead, 5q deletion was seen in Ph negative cells of one patient, after 24 months of therapy.
The evaluation of cellular genome, prior to Glivec therapy, helps the prediction of response. Monitoring both Ph positive and Ph negative clones for chromosomal changes allows a better management of CML patients.
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P0528. Cytogenetic evolution patterns in CML post-SCT

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The cytogenetic evolution patterns in CMLs after allogeneic SCT are different from the ones observed in non-transplanted patients, a phenomenon suggested to be caused by the conditioning regime. We reviewed 131 CMLs displaying karyotypic evolution after SCT (122 allogeneic, 9 autologous), treated at Lund University Hospital or reported in the literature. Major route abnormalities (i.e., +8, +Ph, i(17q), +19, +21, +17 and -7) were seen in 14%, balanced aberrations in 61%, hyperdiploidy in 19%, pseudodiploidy in 79%, divergent clones in 14%, and Ph-negative clones in 21%. The breakpoints involved in secondary structural rearrangements clustered at 1q21, 1q32, 7q22, 9q34, 11q13, 11q23, 12q24, 13q14, 17q10, and 22q11. Cytogenetic abnormalities common in AML after genotoxic exposure, i.e., der(1;7)(q10;p10), del(3p), -5, del(5q), -7, -17, der(17p), -18, and -21, were only rarely seen post-SCT. Comparing the cytogenetic features in relation to type of SCT revealed that balanced aberrations were significantly more common after allogeneic than after autologous SCT (64% and 22%, respectively, $P = 0.03$). In addition, there was a trend as regards hyperdiploidy being more common after autologous ($P = 0.07$) and pseudodiploidy being more frequent after allogeneic SCT ($P = 0.09$).

P0529. Classification of chromosomal aberrations in a group of patients with chronic myeloproliferative disorders

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Chronic myeloproliferative disorders (CMPD) are thought to be clonal disorders arising in a multipotent hematopoietic progenitor cell. The diagnosis of an CMPD other than chronic myelogenous leukemia (CML) can be problematic due to a lack of clinically applicable clonal markers. It's suggested that in patients with classical CMPD phenotype, the hematopoietic stem cells appear to be polyclonal, suggesting that the chronic CMPD other than CML may actually be a genetically heterogeneous group of disorders. Here, we present the results of a retrospective data mining study for CMPD patients refered to our department for cytogenetic analysis to seek the clonality. We followed the the most established criteria for CMPD released by the WHO and Polycthemia Study Group for inclusions. The data was obtained from results of the bone marrow and peripheral blood samples of 274 patients diagnosed or suspected as CMPD between May 1998 and January 2007 analyzed with conventional /FISH cytogenetic methods. The cohorts were formed as CMPD?, ET, PV, CMML, IMF, and HES. The existence of Ph chromosome in conventional analysis and/or BCR-ABL fusions were determined as 4%, 2.8%, and 1.9% in CMPD?, ET, and PV subgroups and excluded. In a total of 337 analysis the frequency of structural-numerical abnormalities were determined (CMPD:30.9%-20.3 n=123; ET:13.8%-6.4% n=94; PV: 5.6%-9.3% n=54; CMML: 19.4%-45% n=40; IMF: 10.0%-15.0% n=20; HES: 16.7%-16.7% n=6). Structural and numerical gathered the leading chromosomal aberations were 17, 22, 11 for CMPD, 7, 17, 8 for ET, and 21, 8, 7 for PV.

P0530. Genetic aberrations appeared during the disease course may be associated with clinical progression in CLL patients

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Chronic lymphocytic leukemia (CLL) is the most common type of leukaemia in adults. Clonal aberrations are found in about 50% of cases by chromosome analysis and in about 80% of CLL cases by fluorescence in situ hybridization (FISH). Progression of disease is observed in some cases, but genetic factors involved are not completely known.
The aim of this study was to analyze the impact of genetic alterations that appear during disease evolution in clinical progression in CLL patients. Clinical parameters evaluated were: progression in stage, median survival time and need for treatment.
FISH using LSI D13S319/13q34/CEP 12 and LSI p53/ LSI ATM multicolor probe sets (Vysis) was performed on bone marrow or peripheral blood samples from 21 CLL patients at various time points during the disease course [median follow up: 5 years (2-14)].
Genetic aberrations occurred during disease development were observed in 5/21(24%) patients. Genetic alterations detected included: del (13q14) monoallelic (n=1), del (13q14) biallelic (n=1), del (13q14) monoallelic and biallelic (n=1), del (11q 22.3) (ATM gene) (n=2).
The median overall survival (OS) and need for treatment were not significantly different between patients with and without appearance of genetic aberrations during the disease course. The only difference between the 2 groups was a greater progression in stage in the former group (80% vs. 44%). It is remarkable that progression occurred despite the appearance of non high-risk genetic abnormalities (13q14 deletion).Although more cases are needed to confirm these results, our data suggest that genetic aberrations appeared during evolution of disease may be associated with clinical progression in CLL patients.

P0531. Genome copy number variations unique to CML

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Copy number variations (CNVs) are now a recognised feature of the human genome with unknown phenotypic consequences. Chronic myeloid leukaemia (CML) is a haematopoietic stem cell disorder defined by expression of the BCR-ABL fusion gene, a constitutively activated tyrosine kinase. We studied 49 CML samples at various stages of the disease by arrayCGH using a 1 Mbase BAC chip (SGI2600, Perkin Elmer). The aCGH profiles revealed a large number of single BAC imbalances where fluorescence ratios exceeded a ± 3 SD threshold. We limited our analysis to imbalances found in at least three samples. A total of 194 BACs met this criterion and were compared with the CNVs recently published (Redon[1] at al., Nature Genetics, 2006 and Database of Genomic Variations at http://projects.tcag.ca/variation). Many of the loci identified corresponded to the published data. However, among the 99 loci unique to our study, the two most frequent were BAC clones RP11-452O22 (35%) and RP11-89C6 (26%). Confirmed by Q-PCR, these imbalances are not reported elsewhere. CML occurs in about 1 per 100,000 of the general population, so it is unlikely that any constitutional CNVs offering a predisposition to CML would occur in the 270 individuals studied by Redon et al. Our results suggest that these newly identified unique CNVs, either constitutional or disease associated, are specific to CML and offer new insights into the biology of this malignancy.

P0532. Secondary chromosomal abnormalities within Philadelphia positive Chronic Myeloid Leukemia

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Chronic myeloid leukemia (CML) is a stem cell disorder characterized by Philadelphia chromosome (Ph), showing a progression into an agressive blast phase from a chronic phase. This progression is frequently preceeded by secondary chromosomal abnormalities which are considered as the cytogenetic signs of progression. In this respect a retrospective analysis of 162 cases of CML with Ph was performed in our department. In 43 of the cases, presence of extra chromosomal abnormalities was observed. The most frequent abnormalities were +8 (7 cases); -20, -21, -Y (4 cases each); -5, -7, -12, -13, del(17)(q25)(3 cases each); and -18, -19, i(17)(q10), del(17)(p13)(2 cases each). 28 different rearrangements such as t(2;8)(p21;q24), inv(4)(p14;q12), t(3;4)(q13;q31) not previously associated with Ph+ CML were observed. The cumulative data on cytogenetic studies of Ph+ CML will contribute not only to the prediction of the prognosis but also to the enhancement of monitoring responses to the treatment.

P0533. A unique complex translocations (9;22;6) in a patient with chronic myelogenous leukemia

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Complex chromosome translocations involve changes between three or more chromosomes and are found very rarely in the general population.
Chronic myelogenous leukemia (CML) is characterized in about 90-95% of cases by a karyotypic marker, the Philadelphia chromosome, originating from a reciprocal translocation of chromosomes 9 and 22, t(9;22)(q34;q11), and genetically resulting in the fusion of BCR/ABL gene. About 5-10% of Philadelphia positive patients with CML show various complex translocations involving the third chromosome in addition to chromosomes 9 and 22.
In this study we report a 20-year-old male patient with a diagnosis of CML with unusual and complex translocations involving chromosomes 9, 22 and 6.
Cytogenetic analysis was done on a 24-hour culture of bone marrow specimen. The cells were cultured by conventional methods and processed by standard techniques, using GTG-banding. The FISH were performed according to the manufacturers' directions, using whole chromosome painting probes.
The karyotype of our case was: t(9;22;6)(q34;q11;p21).
Apparently, the first translocation occurred between chromosomes 9q and 22q resulting to the formation of Philadelphia chromosome. Then the distal segment of the chromosome 6p has been translocated to the end of the derivative chromosome 9.
According to literature review and to the best of our knowledge, our case is a unique tranlocations which has not been reported.

P0534. Correlation between p53 protein accumulation and the development of colon cancer from Iran

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Colorectal cancer is the third cause of the cancer-related death in the world and the fourth most common cancer in Iran. The peak age is between 60-80 years old, while about 20% of cases occur under the age of 50. It is estimated that the annual incidence rate of colorectal cancer is about 150,000 and the death toll is 50,000 around the world. One of the basic mutation involved in these tumors include inactivation or alteration of p53 gene. Changes in this tumor suppressor gene are the basic event in many cancers. One of the methods to identify changes in this gene includes Immunohistochemistry (IHC). We studied the expression of p53 protein in cancerous and normal tissues from colon cancer patients for predicting the natural path of the disease and also developing cancerous cells in tissue.
In order to evaluate the highest risk patients, p53 nuclear accumulation evaluated by monoclonal antibody DO7 and Envision + Dual link+DAB (kit) in ordinary paraffin-embedded tissue sections. The method used to assess p53 status were immunohistochemistry (IHC), indicating accumulation of p53 in tumoral and normal tissue in colorectal cancer patients. P53 protein accumulation was seen in tumor tissues and normal cells adjacent to tumor cells. Our finding suggested that detection of p53 protein accumulation may play an important role in developing colon cancerous cells among cancer patients. It is suggested that detection of the p53 gene mutation can be investigate among colon cancerous cells and normal tissue adjacent to tumor cells in the future.

P0535. No association between the CHEK2 I157T allelic variant and colorectal cancer in Bulgaria

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In the development of colorectal cancer (CRC), genetic and environmental factors are involved, but the exact mechanism of carcinogenesis still remains unclear. The CHEK2 gene encodes a protein kinase that participates in the DNA damage response in many cell types. Three founder alleles have been described in this gene and all of them were associated with different cancer types.
The missense variant c.470 T>C, p. I157T has been associated with an increased risk of breast, colon, kidney, prostate, and thyroid cancer. This variant was also seen in healthy population controls.
Aim: To determine the frequency of the I157T alleles in Bulgarian patients with CRC and healthy controls.
Methods: The analysis was performed in a total of 299 patients and 273 healthy individuals. Samples were analyzed by PCR- RFLP. All mutations were further confirmed by direct sequencing.
Results: The I157T variant was found in 8 patients (2,7%) and 8 controls (2,9%). There was no association between the mutation and CRC. The patient group was then divided according to family history and each of them was compared to controls and in between, but again no significant differences were found in their frequencies. Different extra-colonic tumors were described in the pedigrees of three family cases. Conclusions: Our results from this case - control study do not confirm the role of the I157T mutation in the development of CRC in the Bulgarian population. Larger studies are required to investigate the tendency for higher frequency of different cancer types in mutation carriers.

P0536. Pattern of distribution of SNPs in DNA repair genes in colorectal cancer patients - a preliminary report.

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Epidemiological data shows that colorectal cancer (CRC) is the second most frequent cancer. One to two percent of all CRCs are caused by mutation in *APC* which leads to familial adenomatuos polyposis while about 5-10% of all CCR is caused by mutations in mismatch repair genes leading to hereditary non-polyposis colorectal cancer (HNPCC) syndrome. Although the participation of the mentioned above mutations in CRC development is obvious, in more than 85% of cases the genetic background remains unknown.
The involvement of "minor impact genes" such as XME and DNA-repair genes in aetiology of sporadic cancer is postulated by other authors therefore in our research we focused on analysis of polymorphisms in DNA-repair genes in CRC. On the basis of the recently published studies we hypothesise that it is not a single genetic alteration but a network of polymorphisms that plays a key role in the individual susceptibility to cancer. In order to verify this hypothesis we have chosen 11 genes involved in three different ways of DNA repair: base excision repair (BER- *OGG1, XRCC1*), nucleotide excision repair (NER- *XPA, XPC, XPD, XPG, XPF, ERCC1*) and homologous recombination repair (HR-*RAD51, XRCC3, NBS1*). The study group consists of 133 patients with CRC; 20 of them diagnosed with HNPCC or suspected of HNPCC and the rest with sporadic cancer.
We compare the frequency of polymorphisms between patients' and

control groups and we define an individual profile for every investigated person in order to establish the pattern of polymorphisms characteristic for CRC patients.

P0537. Molecular analysis of some genetic polymorphisms associated with colorectal cancer in Romanian patients

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Mutations in APC gene have not previously been characterized among Romanian patients with CRC. We analyzed blood and tumor samples collected from 32 patients (22 men and 10 women) and 32 relatives without CRC. We tested the presence of mutations in exons 6, 7, 12, 13, 14 and in regions B, L and W of exon 15 by PCR multiplex and 5 bp deletions at position 1061 by SSCP methods. We observed multiple deletions (e.g. in exon 6, 12, and in 15B, 15L and 15W regions) in a DNA extracted form the tumoral sample, but not in blood cell. We speculated that these mutations are an example of genomic instability described in malignancies. In one patient, we detected a deletion of exon 13 in DNA extracted from blood and tumoral tissues. For the patients analyzed until now we didn't find any mutation at codon 1061.
Association between the VDR gene polymorphisms and cancer development has been suggested by several studies. This study investigate genotype frequencies and association of the VDR Taq I and Apa polymorphisms with colorectal cancers in Romanian patients (n=32) in correlation with normal population (n=40). Prevalence of VDR Apa alleles and genotype frequencies in patients with colorectal cancer was similar to that in the normal population ; in the same time, for VDR Taq I prevalence was different to that in normal population (p 0,0083 , 95% CI , 1.2992<RR<5.2609). We need to increase the size of the lots in order to confirm our data.

P0538. Analysis of the microsatellite $(tttta)_n$ polymorphism of the *CYP11A1* gene in patients with prostate cancer

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The *CYP11A1* gene encodes a cytochrome P450 enzyme, which catalyses the first step in the synthesis of sex hormones. There is a microsatellite $(tttta)_n$ polymorphism in the promoter of *CYP11A*1 and the absence of the $(tttta)_4$ *CYP11A1* allele has been associated with clinically advanced prostate cancer. The aims of the present case-control study were *(i)* to examine the association of the number of tttta repeats with prostate cancer risk and aggressiveness and *(ii)* to test microsatellite instability. A total of 206 Slovenian males were enrolled in the study: 96 controls and 110 prostate cancer (CaP) patients with prostate gland confined (pT2) or extracapsular extended (pT3) cancer. Prostate tissue samples were collected in pairs of histologicaly normal and tumor tissue. Polymerase chain reaction followed by polyacrilamide gel electrophoresis revealed five *CYP11A1* $(tttta)_n$ alleles, corresponding to 4, 6, 8, 9 and 10 repeat units. The frequency distribution in the control group was similar to previously reported data for Caucasians. On the basis of the presence or absence of the $(tttta)_4$ allele, no statistically significant differences in genotype distributions were observed between the control and CaP group and no association was found with tumor aggressiveness. Furthermore, no microsatellite instability was observed in matched normal and tumor tissues, except for three samples that need further investigation. In conclusion, the $(tttta)_n$ *CYP11A1* polymorphism may not be a useful biomarker for prostate cancer risk aggressiveness prediction.

P0539. Polymorphism in cytochrome P450c17 (CYP17) is associated with increased risk of breast cancer

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The polymorphism in cytochrome P450c17 (CYP17), a key enzyme in the biosynthesis of estrogen, has been associated with breast cancer risk, but previous studies have been relatively small yet. CYP17 gene is reported to be associated with increased risk of breast cancer. This study evaluates the influence of genetic polymorphism of CYP17 on breast cancer susceptibility. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect the polymorphism, and the genotypes identified were assigned as homozygous wild type (A1A1), heterozygous variant (A1A2), and homozygous variant (A2A2).
Probably a statistically significant increased risk in carriers of homozygous A2 allele was found in woman ($P < 0.001$) in comparison with A1. However, no significant association between the genotype and breast cancer risk was observed among women with strong family history yet.
We genotyped 200 cases of primary breast cancer and population controls, all age of Iranian women, for the CYP17 polymorphism studied. Small overall association was found between CYP17 and breast cancer risk, for the A2/A2 carriers compared to the A1/A1 carriers until today.

P0540. The Methylation Analysis of *DAP Kinase (DAPK1)* Gene in Chronic Myeloid Leukemia Patients

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Cancer occurs as a result of various genetic and epigenetic mechanisms which cause loss/gain of functions of tumour suppressor genes, oncogenes, DNA repair and apoptosis genes. *DAP kinase* gene, which is an proapoptotic gene, induces the cell to apoptosis response to several internal and external apoptotic stimulants. Changes occurring in *DAP kinase* gene cause uncontrolled proliferation instead of going to apoptosis of the cell and cause development. Therefore *DAP Kinase* gene is described as a tumor suppressor gene. DNA methylation, one of epigenetic mechanisms that have as much importance as genetic mechanisms in cancer formation can prevent gene expression by silencing. Silencing of *DAP kinase* through this methylation mechanism can be observed in many solid tumour and hematopoietic malignancies. In this study methylation of DNAs of 35 patients with chronic myeloid leukaemia and DNAs of 25 healthy patients were analysed by Methylation Spesific PCR (MSP). As a result, it has been determined that 4 of 12 CML patients with drug resistance have hypermethylation and statistically the significant difference is established. However all of the CML patients with no drug resistance and all of the control sample groups DNAs don't have methylation. In this study although no correlation is found between hypermethylation of DAPK1 which is defined as a tumour suppressor gene and formation of CML, significant relation between hypermethylation of DAPK1and progress of CML and drug resistance is determined.

P0541. Mutation Analysis of the DBC2 Gene in Sporadic and Familial Breast Cancer

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The expression of the recently identified tumor suppressor gene, DBC2 (Deleted in Breast Cancer 2), is frequently extinguished in breast cancer cells or tissues. In the current study we have analyzed 100 sporadic breast cancer cases by PCR-SSCP, and DHPLC, followed by direct sequencing. An additional 17 breast cancer families, who were negative for the BRCA1/2 mutations, were analyzed by direct sequencing. Mutation analysis of the essential promoter region, all exons and exon/intron boundaries of the DBC2 gene was performed. Three novel mutations were observed in the promoter and 5'-untranslated region (UTR) of the gene; a G>A transition in the promoter at nt -238 from the transcription start site, and two tumor-specific mutations at nt -121C>T and nt +48G>A. No deleterious mutations were detected in the coding sequence of the gene in familial and sporadic breast cancer cases. The sequence variations found within the promoter and 5'-UTR region of the gene warrant expression analysis and screening more tumor samples at this region.

P0542. Differential methylation of *HLAIII* locus (6q21) in normal and cancer cervical tissues

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Epigenetic alterations, such as abnormal DNA-methylation patterns, are associated with many human tumor types. New techniques have been developed to perform genome-wide screening for alterations in DNA-methylation patterns to identify new tumor-suppressor genes and to find patterns that can be used in diagnosis and prognosis.

Non-methylated Genomic Sites Coincidence Cloning (NGSCC) allows to analyze tissue-specific distribution of the unmethylated CpG sites within the megabase(s)-long genomic DNA fragments (NGSCC; Azhikina et al., Mol Gen Genomics, 271: 22-32). We applied this technique to the *HLA III* locus (6q21), containing *D6S273*, the known as a marker of early genetic alterations in cervical neoplasias. Heterozygocity loss in this specific locus correlates with tumor progression and suggests that there are potential tumor-suppressor genes in this region of the chromosome 6 (Mazurenko et al., Mol Biol (Mosk), 40: 436-447).

With the use of NGSCC we constructed high density maps of unmethylated CpGs for both cancer and normal cervical tissues. We found the methylation patterns of CpG's in promoter region of *BAT2*, *BAT3* and *MICB* differ in normal and cancer tissues. These results were confirmed by bisulfite sequencing of the CpG islands of these genes.

Thus, the applied approach revealed characteristic tissue-specific features of large-scale distribution of unmethylated CpGs. The changes observed in this distribution might provide useful epigenetic markers of cancerous transformation.

P0543. Studying of adenovirus E1A gene silencing effects on HEK 293 cancerous cells using RNAi technique

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RNA interference (RNAi) is a natural gene regulatory mechanism widely used for studying gene function in a variety of species. Small interference RNA (siRNA) technology has been reported to produce post-transcriptional gene silencing (PTGS) in mammalian cells. Studies have been shown that siRNA expression mediated by vectors causes efficient and stable down regulation of gene expression, resulting in functional inactivation of the targeted genes. So in this study, we designed a human U6 promoter-driven mammalian expression vector to produce small hairpin RNA (shRNA) for *E1A* gene transcripts. To transfect HEK 293 cells by our designed plasmid, dendrosome, a new designed chemical compound was used. Using this technique, we got a system for stable expression of shRNA to reduce Ad5 E1A gene transcripts in these cells. In this report we will present the result of E1A silencing effects on cell cycle genes such as Rb1 by gene expression.

P0544. Mutational analysis of the beta-catenin gene in MSI(+) and MSI(-) endometrial tumors from bulgarian patients

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Activating somatic mutations in exon 3 of the ß-catenin gene (CTNNB1) have been identified in 11 - 25% of endometrial cancer patients. Attempts to systematize their distribution among microsatellite unstable MSI(+) and stable MSI(-) tumors have produced conflicting results. Tumor MSI has been reported at frequences from 17 to 45% for endometrial cancer.

In order to evaluate the frequency of exon 3 CTNNB1 mutations and their possible association with MSI status, we studied 35 patients with histologically confirmed stage I/II endometrial cancer.

DNA was extracted form fresh tumor tissue and from whole blood. For MSI status determination we used a panel of six polymorphic markers - BAT26, D2S123, D5S346, D18S35, FGA, and TP53. MSI(+) were defined cases where two or more markers showed instability. CTNNB1 exon 3 was direcly sequenced.

We detected MSI in 10 tumors (28.6%). Two of them (20%) harbored CTNNB1 mutations - a transversion at Ser 37 and a previously undescribed deletion of 48 bp corresponding to loss of codons 31 to 47. MSI(-) tumors had no mutations.

As a downstream transcriptional activator in the Wnt pathway, ß-catenin is regulated by phosphorylation on Ser/Thr sites encoded by exon 3 of the gene. Mutations at these residues often lead to nuclear accumulation of the protein and activation of target genes c-Myc and cyclin D1.

We identified mutations that alter or abolish Ser/Thr residues implicated in the down-regulation of ß-catenin. In our patient group their frequency is 5,7% and they are associated with MSI positive cases (p=0.021).

P0545. Identification of DNA methylation markers for detection and classification of colon cancer by epigenetic profiling

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Promoter methylation is thought to be an initial event in tumorigenesis. Especially right- sided colon cancer is associated with epigenetic modification of gene expression. The mismatch repair gene MLH1 is a prime target for epigenetic downregulation with a frequency of 40% in all right-sided colon carcinomas. Our aim in this study is to identify additional epigenetic markers to generate a panel that will allow the epigenetic detection and classification of colorectal cancer (CRC) and its precursor forms.

We carried out a genome-wide methylation study on clinically well-described colorectal adenocarcinomas, adenomas and paired normal epithelium. DNA from macrodissected fresh-frozen tissue was digested by the restriction enzyme MseI, linker-ligated and subsequently digested by two methylation-sensitive restriction enzymes. The remaining fragments were amplified, labeled and hybridized to our 9K clone library CpG island microarrays. Recently, we extended the screen to high-density CpG island tiling oligonucleotide microarrays.

We identified several loci aberrantly methylated with a high frequency in carcinomas using our 9K array. Thusfar three loci were verified using direct- and clonal bisulfite sequencing and are currently being studied in more detail. Interestingly, one locus was shown to be hypermethylated in carcinomas as well as adenomas. Possible clinical applications of these markers include early detection of cancer with applications such as in feces screening, and cancer prognosis.

P0546. FISH studies using bac clones of the EVI1 locus in 9 patients with hematological malignancies carrying 3q rearrangements.

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Chromosomal rearrangements involving 3q26 are a recurrent aberration in malignant myeloid disorders. Several of these rearrangements involve the *EVI1* oncogene or its surrounding sequences and are associated with a poor prognosis. In order to know whether the *EVI1* locus was rearranged in 9 patients with hematological malignancies carrying 3q abnormalities, fluorescent *in situ* hybridization (FISH) studies using BAC (bacterial artificial chromosome) clones were carried on. A dual color probe was constructed with 9 BACs; centromeric clones covering 1Mb and including *EVI1* gene were labeled with a red fluorescent dye and telomeric clones covering 1 Mb were labeled with a green fluorescent dye. From the 9 patients, two patients showed normal copies of the *EVI1* locus, four patients showed one *EVI1* locus rearranged and in all of them the breakpoint on 3q26 was telomeric to *EVI1* gene, one patient showed one copy of the *EVI1* locus translocated to another chromosome, one patient showed one copy of the *EVI1* locus rearranged and the other copy translocated and one patient showed one extra copy of the *EVI1* locus. FISH studies using the *EVI1* clones allowed

the detection in 4 cases of different 3q abnormalities not previously found by conventional cytogenetics. FISH analysis with BAC clones was found to be a useful tool to identify the chromosome breakpoints affecting *EVI1* locus in patients with 3q26 rearrangements.

P0547. Clinical and genetic characterization of Austrian FAP patients - A first report

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Background: Familial adenomatous polyposis (FAP) is an inherited colorectal cancer syndrome characterized by the early onset of numerous polyps and associated with germ-line mutations in the adenomatous polyposis coli (*APC*) gene.
Material and Methods: We analyzed a series of 128 unrelated Austrian families clinically diagnosed with FAP for mutations in the *APC* gene. Protein-truncation test and heteroduplex analysis were used as prescreening tests previous to DNA sequence analysis. Linkage analysis was performed with 4 polymorphic microsatellite markers.
Results and Conclusion: Medical examination revealed 69 (67.6%) patients with classical FAP symptoms and 33 (32.4%) patients with an attenuated or atypical phenotype. In 55.6% of the families a genetic defect was identified with at least one of the methods applied. The detection of a genetic defect in the *APC* gene was highly significantly associated with a classical phenotype, the detection of innumerable polyps, lesions of the retinal pigment epithelium (CHRPE) and desmoids. DNA sequence analysis identified 61 pathogenic *APC* mutations. Six mutations were detected in more than one family. Twenty-nine mutations are novel. Only a polymorphic fragment pattern could be associated with the inheritance of the disease in two families. This study is the first comprehensive report of *APC* gene mutations in Austrian FAP patients.

P0548. Association of biallelic *BRCA2* mutations in a child with rhabdomyosarcoma

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Growth retardation, microcephaly and solid tumour in childhood can be linked to Fanconi anaemia. Fanconi anaemia is an autosomal recessive heterogeneous condition, consisting in 11 complementation groups and involving at least twelve genes. Recently, biallelic mutations in *BRCA2* have been discovered in patients classified as Fanconi anaemia complementation group FA-D1.
We report on a further case presenting with rhabdomyosarcoma, intrauterine and postnatal growth retardation and microcephaly. This is the first child of non consanguineous parents. At the age of one year, cutaneous lesions were observed (naevi, haemangioma and cutis marmorata). The motor milestones were achieved normally. Karyotype was 46XX, but breakage studies were not performed.
At 1.5 year of age, an embryonic paravertebral rhabdomyosarcoma with vertebral extension and pulmonary metastasis was detected. The association of this solid tumour and the growth retardation was suggestive of Fanconi anaemia type FA-D1, despite no other typical features of this condition were observed.
Sequencing of *BRCA2* revealed two deletions: one in exon 8 (886delGT) and the other in exon 22 (9132delC). These two mutations are deleterious and have been previously reported in either Fanconi anaemia or breast cancer.
Heterozygous *BRCA2* mutation carriers are at risk of breast and other cancers. In the family pedigree, there were cases of breast cancer in the father's family and cases of prostate and pancreatic cancer in the mother's family. *BRCA2* molecular analysis is pending in the parents. We discuss the clinical phenotype of this rare form of Fanconi anaemia, and the complexity of the management in these cases.

P0549. *APC* and *MYH* mutations in Czech and Slovak FAP families

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Germline mutations in the adenomatous polyposis gene (*APC*) result in familial adenomatous polyposis (FAP) as well as an attenuated form of this syndrome. FAP is an autosomal dominantly inherited disorder predisposing to colorectal cancer.
We analyzed the *APC* gene for germline mutations in 59 Czech and 15 Slovak FAP patients. In addition, 50 *APC*-mutation-negative Czech probands and 3 probands of Slovak origin were screened for large deletions of the *APC* gene. Mutation screening was performed using denaturing gradient gel electrophoresis and/or protein truncation test. DNA fragments showing an aberrant electrophoretic banding pattern were sequenced. Screening for large deletions was performed by multiplex ligation dependent probe amplification (MLPA).
We identified 46 germline mutations among the 74 unrelated probands including large deletions. We reported 20 novel germline *APC* mutations and 3 large deletions encompassing the whole-gene deletions and/or exon 14 deletion.
Some of the *APC* negative FAP/AFAP cases have recently been found to be attributable to *MYH* associated polyposis (MAP), an autosomal recessive syndrome caused by mutation in the *MYH* gene.
We screened for germline *MYH* mutations in 120 *APC*-mutation-negative probands with classical and attenuated FAP. As a prescreening to detect DNA sequence changes, denaturing high performance liquid chromatography (dHPLC) was performed using the WAVE system (Transgenomic). Samples showing unique profiles were sequenced in both directions on ABI Prism 310 Genetic Analyzer (Applied Biosystems).
Altogether 10 previously reported changes and 8 novel genetic alterations in *MYH* gene, mostly in intronic sequences were identified.
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P0550. Florescent in situ hybridization (FISH) for the detection of Ph- positive clone in CML: Comparison with metaphases banding analysis

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Introduction: Chronic Meyloid Leukemia(CML) is characterized by the formation of BCR/ABL fusion gene, usually as a result of the Philadelphia(Ph) translocation between chromosomes 9 and 22.CML is characterized by the Philadelphia in more than 90% of cases. Material and method : In this study, we present our results of cytogenetic analysis with GTG banding and Fluorescent in situ hybridization(FISH) using dual color dual fusion probe CML patients registered at Taleghani hospital from March 2004 to March 2006 ,IRAN.
Results: GTG banding metaphases was Carried out in 30 Patients Blood /Bone marrow. Ph translocation showed in 26(87%). About 23(76.6%) of Ph- positive patients displayed the typical D-FISH signal pattern, 17 included metaphase FISH and the rest has only interphase FISH results.
Conclusion: Fluorescence in situ hybridization has become a widely used method for studying Ph translocation .

P0551. The role of CDH1 gene variants in inherited predisposition to gastric cancer

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The CDH1 gene is a main suppressor of inherited gastric cancer. Nevertheless only a small faction of familial gastric cancer cases is due to CDH1 mutations. The genetic reason for the other inherited gastric cancer cases is unknown at present time. One possibility is that CDH1 variants confer gastric cancer risk. In particulary it is known that CDH1 -160C/A promoter polymorphism is connected with functional activity of CDH1.
We investigated significance of CDH1 -160C/A and 2076C/T SNPs for

gastric cancer risk among probands with familial gastric cancer without CDH1 mutations by case-control study. An association of 2076T variant with gastric cancer was found (P=0,007). The risk value for -160C/A was not significant. However a significant excess of genotypes including both -160A and 2076T among probands in comparison with control sample was revealed (OR=3,33; P=0,009). The risk was the highest if the variants -160A and 2076T composed a haplotype (OR=13,6; P=0,003).
The data suggest a role of CDH1 -160C/A and 2076C/T SNPs in a predisposition to gastric cancer, especially when both the variants -160A and 2076T occur on the same chromosome.

P0552. Differences in gene expression between transitional cell carcinoma of the human bladder with short and prolonged recurrence-free period using oligonucleotide microarrays

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The prediction of tumour recurrence in patients with superficial bladder tumours is inaccurate. For the improvement of recurrence prognosis in patients with superficial bladder cancer we investigated gene expression and identified differences between superficial bladder tumours without recurrence during period of two years (5 patients) and with early recurrence (7 patients), which might explain differences in the biology and clinical outcomes of these groups of TCC. High-density oligonucleotide microarrays (29,019 genes) were used to analyze the transcript profiles of 12 superficial bladder tumours: 11 pTa and 1 pT1, grading 2 G1 and 10 G2. Statistical analyses were applied to investigate the ability of the genes to identify patients without recurrence during period of two years and with early recurrence. Initial screening using the GeneSpring and Bioconductor software tools revealed a putative set of about 120 genes associating with the recurrence class. Significant differences were observed by HOXA10, GPNMB, TCN1, H19, FABP3 and PLOD2 genes. Besides, we integrated the microarray dataset with additional background knowledge, in order to algorithmically mine for differential-expression patterns in terms of the Gene Ontology functions and processes as well as known regulatory pathway memberships. Our results indicate that it may be possible to identify patients with a high risk of disease recurrence at an early stage using a molecular profile present already in the superficial tumours.
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P0553. Genetic disorders related neoplasia in childhood

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Background. Despite the fact that neoplasia is not a deterministic genetic disease, the majority of childhood malignancies result from errors that take place during early stages of cell differentiation, tissue maturation and organ development.
Objectives. We aimed at evaluating the frequency of association between neoplasia and some genetic disorders, recognized for their increased susceptibility role and predisposition for neoplasia in order to provide a basis for risk assessment and counseling of families prone to develop neoplasia.
Patients and methods. The study has been undertaken on all patients with neoplasia admitted in the IIIrd Pediatric Clinic, Timisoara between 1981-january 2007.
Results. We analyzed 750 patients aged 1 month - 20 years. 730 patients (97,33%) had cancer and 20 patients (2,67%) had benign tumors. We diagnosed 27 patients (3,6%) with neoplasia and concomitent genetic disorder: Down (5), Peutz-Jegers (1), von Hippel-Lindau (1), Beckwith-Wiedemann (2), Rubinstein-Taybi (1), Nijmegen breakage syndrome (2), Kostmann agranulocytosis (1), Fanconi anemia (1), Ataxia-telangiectasia (1), Bourneville disease (3), Neurofibromatosis type I (2), Proteus syndrome (1), Poland syndrome (1), minor anomalies (5 cases). Types of neoplasia developed by the patients with genetic disorders were dominated by solid tumors in 15 cases (55,5%) and non-lymphoblastic leukemia in 12 cases (44,5%).
Conclusions. The proportion of genetic disorders associated neoplasia is significantly higher than in normal population. Therefore the identification of risk factors in a family should be the first step for individualized counseling and monitoring, able to assure the detection in early stage of the disease.

P0554. The Italian External Quality Control Programme for Adenomatous Polyposis of the Colon (APC gene): five years experience

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Familial adenomatous polyposis (FAP) is a rare autosomal dominant inherited disease (incidence 1/8000)[1]. More than 90% of families affected by FAP have a mutation in the tumor suppressor gene *APC gene* (5q21) (OMIM 175100). Mutations in this gene are characterized by 100% penetrance, although there is a variation in phenotypic expression of the disease[1].
According to a 2004 survey of the Italian Human Genetic Society, about 264 *APC* gene molecular genetic tests were performed by Italian laboratories[2].
The Italian External Quality Control Programme (IEQC), financially supported by the Ministry of Health and coordinated by the Italian National Institute of Health - Istituto Superiore di Sanità, started in 2000 in order to improve the quality of molecular genetic tests in Italy[3]. In the frame of the IEQC, about 50% of public laboratories performing *APC gene* tests have been monitored[2].
The number of responding public laboratories versus enrolled laboratories during the five years was 6/8, 7/8, 7/7, 7/7, 5/7 from 2001 to 2006 respectively; on average of 93,3% of 192 samples were correctly genotyped.
Currently methods used by laboratories to detect mutation were direct sequencing, SSCP, PTT and DHPLC.
Written reports were not homogeneus among laboratories, although a new form of written report was proposed to laboratories in 2004.
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P0555. Cytogenetic and Moleculer cytogenetic Analyses (I-FISH, M-FISH) of Glial Tumors

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Glial tumors are the most common tumors of the central nervous system, affecting individuals of all ages but the underlying genetic changes that give rise to these tumors are still poorly understood. Combination of conventional cytogenetic and molecular cytogenetic (FISH, M-FISH) analyses identifies consistent chromosomal rearrangements and copy number changes of related genes in this group of tumors METHODS: Chromosome preperation was carried out on primary cultures of the 23 primary glial tumor samples using standard cytogenetic techniques. Karyotyping, interphase fluoresence in situ hybridization (I-FISH) by using LSI p53(17p13.1), LSI p16(9p21)/CEP9, LSI 1p36/1q25, LSI19q13/19p13, LSI EGFR/CEP 7, LSIPTEN (10q23)/CEP 10, and LSITOP2A /HER2 /CEP17 gene specific probes and Multiplex-FISH (M-FISH) were applied for chromosome rearrangements and specific gene deletions/ amplifications. RESULTS: Karyotype analysis could be performed in 16/23 cases (70.0%), and complex chromosomal anomalies were seen in all tumors. The most frequently seen aberrations were monosomies of chromosomes 1p, 6, 9, 10, 11, 13, 14, 16, 17, 19 and Y, trisomies of chromosomes 5p,7,12 and 15. Double minutes were significant. The most frequently seen amplifications were TOP2A, HER2 and EGFR genes (43% each), followed by 19q13 region amplification (30%). Chromosomes 9p21 (35%),17p31 (26%), 1p36 (17%) and PTEN gene deletions were diagnosed. Chromosomal rearrengements such as t(9;Y), .t(7;10), t(21;17), t(15;9) were revealed by M-FISH analysis. CONCLUSION: Not only cytogenetic analysis, but also improved molecular cytogenetic techniques can increase our

abilities to progress toward effective strategies of molecular diagnosis and classification of glial tumors.

P0556. Study of genetic profile and intratumoral patterns of clonal evolution in gliomas

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Diffuse infiltrating gliomas are the most common tumors of the central nervous system, being their clinical diagnosis based almost exclusively on tissue histology, which relies mainly upon the World Health Organization (WHO) classification scheme. However, a variable clinical behavior is observed even in the same histological category and diagnostic variability can occur due to the heterogeneous nature of these tumors. So, the association between histologic and molecular classification will be helpful to give prognostic and predictive information.

The main objective of our study is to identify molecular genetic alterations that may account for the heterogeneity of gliomas, which will permit to establish the genetic profile and patterns of clonal evolution in individual patients. To correlate the chromosomal instability with the gene expression profiles of these tumors, the tumor samples are firstly studied by interphase fluorescence *in situ* hybridization (iFISH) analysis and then submitted to c-DNA microarrays.

A total of 40 glioma patients were already analyzed. In all cases, iFISH studies were performed on fresh tumor samples for the detection of numerical/structural abnormalities for 10 loci in 8 different chromosomes (1p36, 7q11, 7p11, 9q34, 9p21, 10q23, 13q14, 17p13, 19q13, 22q11). Among these 40 tumor samples, the mRNA from 20 samples was already extracted and submitted to c-DNA microarray studies, which are being performed.

Identification of homogeneous prognostic subgroups and definition of clonal evolution patterns may be valuable for diagnosing and for predicting prognosis and response to treatment in patients with glioma.

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P0557. Surveillance of women at high risk of gynecological cancers: Ten years results from one surveillance center

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Backround: Women at high risk of ovarian or endometrial cancers are recommended to be under programmed surveillance once a year. The efficacy of the screening has not been proven. The role of screening instead of prophylactic surgery has been questionable.

Population and Screening: A cohort of 730 000 inhabitants in Northern Finland was included in the study. 453 families contacted the Department of Clinical Genetics. 137 women were classified as high risk for gynaecological, breast or colorectal cancer and were taken for surveillance program. The women with BRCA1 or BRCA2 mutation were screened semiannually and other risk-groups annually with TVUS and CA125 measurements. Mammography and Ultrasound or MRI was performed once a year.

Results: Main indications for surveillance were: Family history of breast and ovarian cancer: 39%; Breast cancer at young age: 19%; HNPCC mutation: 20%; and BRCA1 or BRCA2 mutation: 14%.

Prophylactic surgery was performed on 16 women (12%): Eight LH+BSO, six BSO and two mastectomies. 19 cancers and two premalignant lesions were found: Seven breast cancer, two endometrial cancers, one ovarian cancer, one endocervical cancer, five colorectal cancers and three miscellaneous cancers (sarcoma, leukaemia, skin cancer). 70% of the cancers were diagnosed during the first two years surveillance period. 60% of the breast cancers and 80% of the colorectal cancers were diagnosed in their advanced stage. Gynecological cancers were diagnosed in their early stage.

Conclusion: In the screening and counselling program for women at high risk in gynaecological cancers the role of prophylactic surgery should be considered.

P0558. Real time quantification of human telomerase reverse transcriptase mRNA in liver tissues from patients with hepatocellular cancer and chronic viral hepatitis

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Telomerase activity (TA) and human telomerase reverse transcriptase (hTERT) mRNA expression was determined, in liver tissues from patients with hepatocellular carcinoma (HCC; n=13), chronic viral hepatitis (CVH) B (n=19),C (n=13) and in 17 patients without liver disease in whom liver biopsy was performed during cholecystectomy (control group). TA was evaluated using TRAP assay and hTERT mRNA expression was assessed using the LightCycler technology. TA was detected in all HCC tissues compared to 15.6% of CVH ($p<0.001$) and none of controls ($p<0.001$). TA levels and hTERT mRNA were higher in HCC compared to CVH ($p<0.001$) and normal livers ($p<0.001$). hTERT mRNA expression was correlated with TA ($p<0.05$). CVH patients that tested negative for TA and hTERT mRNA had significantly lower disease duration (58±85 months) compared with those tested positive (144±50 months; $p<0.05$). Detection of TA and quantification of hTERT mRNA expression in liver tissues could be useful and additional markers for HCC diagnosis and may serve as prognostic markers for HCC development in CVH patients. However, we were not able to draw general conclusions at this moment, as the number of CVH patients positive for hTERT mRNA was relatively small. Real-time quantification of hTERT mRNA expression as a diagnostic/prognostic marker in patients with chronic hepatitis B and C and its relationship with hepatocarcinogenesis needs further evaluation.

P0559. E-box methylation in hTERT promoter interferes with c-myc binding and predicts survival in early stage cirrhotic HCC patients.

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Early detection of hepatocellular carcinoma (HCC) is essential for the successful treatment of patients, however the molecular pathogenesis of the disease has not been elucidated. Human telomerase reverse transcriptase (hTERT) has been found up-regulated in HCCs, while it is not expressed in normal liver tissues. So far no mechanism describing the regulation of hTERT in liver tissues has been reported. In the present study we determined hTERT promoter methylation status in liver tissues and cell lines, correlated it with hTERT and c-myc expression levels and estimated its clinical significance in early stage cirhhosis HCC patients. Fifty-four patients were enrolled in this study: 27 with HCC, 13 with chronic viral hepatitis and 14 without liver disorders. hTERT methylation status was detected by MethyLight analysis and sequencing, while hTERT and c-myc expression levels were assesed by real-time PCR. hTERT expression levels were strongly correlated with DNA methylation levels in all patients ($r=0.912$, $p<0.001$) and c-myc expression levels in HCC patients ($r=0.851$, $p<0.001$). Specifically, hTERT promoter was found methylated in all CpG sites where c-myc is binding in the first E-box. Moreover, hTERT methylation status showed an independent prognostic significance for overall survival in HCC patients ($p<0.0001$). We propose that hTERT methylation status correlates with its expression in liver tissues and HCC cell lines and that E-box methylation in hTERT promoter inhibits c-myc binding and subsequently hTERT upregulation. Additionally, hTERT methylation levels correlate with overall survival of HCC patients suggesting that methylation status could be used as a prognostic marker of HCC progression.

P0560. Adaptation of hereditary breast/ovarian cancer genetic counselling and testing service in Lithuania

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ania, [3]The Institute of Oncology Vilnius University, Vilnius, Lithuania.
The germline mutations in the BRCA1 and BRCA2 genes account for the majority of hereditary breast and ovarian cancers. We present our approach for the adaptation of hereditary breast/ovarian cancer genetic counselling and testing service in Lithuania. In the year 2006 we developed strict referral criteria for high/moderate-risk patients.
During appointment detailed three-generation genealogy is collected; pathological records are verified; genetic and risk information is communicated; availability of genetic testing and its limitations are discussed; informed consent is obtained; management and supportive options are provided.
BRCA1 testing is initiated with direct sequencing for three common mutations (c.4153delA, c.5382insC, c.300T/G), recently reported in Baltic and Slavic populations. 11 female patients diagnosed with breast cancer (<=35 year) and one with family history of ovarian cancer have had referred so far. 5382insC mutation was prevalent in three and c.4153delA mutation in one cases (33% overall).
We propose absolute indications for common mutations testing: a) breast cancer diagnosed before the age of 35 and ER negative or first degree relative/second through male; b) medullary breast carcinoma and c) invasive, nonmucinous epithelial ovarian tumor regardless the age.
After the exclusion of common mutations, DGGE prescreening for the genes prioritized according Manchester scoring system with variants confirmation by direct sequencing, as well as MLPA gene dosage analysis, are performed. We found several polymorphic variants in BRCA1 gene: c.3232A/G, 3667A/G, 4427T/C, 4956A/G. No BRCA1 or BRCA2 genes rearrangements were detected.

P0561. MSH2 deletions and anticipation effects in HNPCC

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Hereditary non-polyposis colorectal cancer (HNPCC) is caused by mutations in mismatch repair genes. Using multiplex ligation-dependent probe amplification, we identified three MSH2 deletions in Italian patients with HNPCC (proband A exons 1-3, proband M exon 8, and proband C exons 1-6). Deletion breakpoints sequencing allowed us to develop rapid PCR-based mutation screening, which confirmed the presence of the deletions in several affected and asymptomatic individuals. While the exon 8 and exon 1-3 deletions are novel, the MSH2 1-6 is identical to the one recently documented in two branches of another unrelated Italian family (family V+Va). Haplotype analysis showed that the kindreds C and V+Va (both from northeastern Italy, both displaying clinical features of the Muir-Torre syndrome) shared a common haplotype, indicating that the MSH2 1-6 deletion is a novel founder mutation. Families A,C, M, and V+Va all showed progressively earlier cancer-onset ages in successive generations. Analysis of 23 affected parent-child pairs in the four kindreds showed median anticipation of 12 years in offsprings' onset of cancer ($p < 0.0001$). No birth cohort effect was found. We have started to analyze by means of a cloning-sequencing approach the instability levels in PBLs and normal tissues from several both symptomatic and asymptomatic mutation carriers belonging to one of these families. Preliminary results suggest that the age at cancer diagnosis correlates with the accumulation of instability in HNPCC target organs. These data represent the first significant evidence of anticipation effects in HNPCC families carrying MSH2 deletions.

P0562. Genetic testing in paragangliomas, the PGL.NET study

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Purpose: Germline mutations on *SDHD, SDHB* or *SDHC* genes cause Hereditary Paraganglioma. In 2003, the PGL.NET network has launched a multicentric prospective study to test the practice of the *SDH* genetic testing in every patient affected by head and neck or thoracic-abdominal or pelvic paragangliomas (PGL) in France.
Patients and Methods: 321 affected patients were genotyped in 3 years. 86 had a positive family history. *SDH* genetic testing was performed by *SDHD*, *SDHB* and *SDHC* direct sequencing and by the search for large genomic rearrangements by quantitative multiplex PCR of short fluorescent fragments (QMPSF).
Results: We identified a *SDH* germline mutation in 188 patients (58.6%). 104 patients had a mutation on *SDHD,* 74 on *SDHB*, and 10 on *SDHC*. 79 different mutations were found among whom 4 large deletions comprising one to several exons (~5%). Age at the diagnosis of the first PGL was 36.2 years-old for the mutation-carriers compared to 48.9 for the non-mutation carriers. A head and neck PGL was diagnosed in 98.1% of the *SDHD* subjects and in all the *SDHC* subjects, but only in 40.5% of the *SDHB* subjects. A thoracic-abdominal location was present in 64.9% of the *SDHB* and in 17.3% of the *SDHD* subjects. A malignant PGL was diagnosed in 43.2% of the *SDHB* subjects and only in 3.8% of the *SDHD* subjects.
Conclusion: The PGL.NET study data suggest a clinical-focused strategy for the paraganglioma genetic testing. The benefits of such genetic testing in pre-symptomatic subjects is currently under evaluation.

P0563. SNP screening for *HMGA1* mutations in 55 Dachshunds

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HMGA non-histone proteins participate in a wide variety of cellular processes including regulation of inducible gene transcription, integration of retroviruses into chromosomes, and the induction of neoplastic transformation and promotion of metastatic progression of cancer cells.
For various malignant tumours over-expression of *HMGA* was shown to be characteristic suggesting a relation between high titer of the protein and the neoplastic phenotype. Due to the similarities of human and canine cancer the dog has joined the common rodent animal model for therapeutic and preclinical studies. For use as a model system, in previous studies we characterised the canine *HMGA1* gene completely and screened the protein coding sequences of twelve canine breeds for SNPs. In a Dachshund sample the screening revealed a transition from A to G in exon 7 leading to an amino acid exchange from threonin to alanin causing a mutated HMGA1 protein.
Herein we report the screening of 55 Dachshunds for the specific mutation affecting the cancer related *HMGA1* gene to elucidate if the observed exchange is frequently existent in the Dachshund population. Genomic DNA was isolated from 55 collected Dachshunds samples using the QiaAmp kit. A specific genomic PCR reaction was established allowing the amplification of the complete exon 7 and flanking regions of intron 6 and 7, respectively. Direct sequencing of the purified PCR products revealed that the observed mutation is absent in the studied cases. Combining both studies indicates that the observed HMGA1 mutation is rare in Dachshunds.

P0564. Missense mutations in the MMR genes *MLH1* and *MSH2* and their clinical significance.

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Mutations in the mismatch-repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2* are responsible for Lynch syndrome (HNPCC), the most common form of autosomal dominant genetic predisposition to colorectal cancer. Germline mutations in *MLH1* and *MSH2* account for approximately 90% of detected mutations in families with HNPCC. We analyzed a cohort of patients selected on the basis of Bethesda criteria for microsatellite instability (MSI) and/or immunohistochemistry (IHC) for detection of the proteins encoded by *MLH1*, *MSH2*, and *MSH6*. We identified 9 mutations in *MLH1* and 16 mutations in *MSH2* through sequencing analysis of the whole coding sequence and multiplex ligation-dependent probe amplification (MLPA) method. Seven were missense mutations (4 in *MSH2* and 3 in *MLH1*), that represent a chal-

lenge for the clinician and for the genetic counselor, who often can not use them for the management of Lynch syndrome families. Several parameters can be evaluated to gain insight into the significance of such unclassified variants (UVs): familial segregation analysis, absence of the variants in control samples, presence of other pathogenic mutations, amino acid conservation, and functional and mRNA analyses. None of these variables can be used alone to predict the significance of UVs in a single case, but combined evaluation can be clinically useful. We report our experience on the interpretation of these UVs and the clinical management of patients and their families.

P0565. Functional screening of *MLH1* and *MSH2* unclassified variants in a large cohort of French HNPCC families reveals an important fraction of splicing mutations

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Numerous unclassified variants (UVs) are detected in the mismatch repair genes *MLH1* and *MSH2* involved in hereditary nonpolyposis colorectal cancer (HNPCC) and cannot be used for genetic counselling. We have undertaken their systematic screening by developing a functional splicing assay using patient DNA. The genomic region of interest, either mutant or wild-type, was PCR-amplified and inserted into the intron of an expression vector. After transfection into HeLa cells and total RNA extraction, the effects of mutations on splicing were evaluated by RT-PCR and systematic sequencing. We have examined 85 different UVs detected in 84 French HNPCC families (54 missense, 10 silent, 3 deletions of a single codon and 18 intronic variants) and found that 26% affect splicing. Five variants are at positions distinct from splice sites, suggesting the presence of regulatory elements such as exonic or intronic splicing enhancers (ESE, ISE). We cloned the short regions (~30 bp) containing the putative exonic regulatory elements into the strictly ESE-dependent central exon of a modified β-globin expression vector and demonstrated by cell transfection assays and RT-PCR that these sequences indeed contain splicing enhancers. This led us to examine in this ESE-dependent splicing assay other *MLH1* and *MSH2* exonic mutations detected in HNPCC patients and previously described as affecting splicing and found that the regions affected by these mutations do contain ESE elements. In absence of reliable bioinformatics predictions, the sequential use of these two functional assays represents a valuable tool for the interpretation of UVs found in these and other genes.

P0566. The significance of *BCL-2* and *IAP f*amilies genes expression in diagnostics of human acute leukemias

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The aims of work: (1) The expression levels designation of the genes regulating the apoptosis process *IAP and BCL-2* families genes in the normal bone marrow cells and the human acute leukaemias cells. (2) The evaluation of the reciprocal correlations of *IAP* and *BCL-2* families genes expression, typical for individual types of leukaemias. (3) The analysis of the potential dependence between the genetic investigations results and the clinical and laboratory parameters.Conclusions: (1) The gene expression levels of the *IAP and BCL-2* families in normal bone marrow cells and the human acute leukaemias cells differed in the individual types of acute leukaemias and had prognostic significance. (2) In ALL the *MCL-1* gene showed the highest expression. (3) In M0/1 AML the *BCL-xL* gene showed the highest expression. No dependence between gene expression levels and the survival, however the correlations between the *BAX* or *BCL-w* genes expression with CD34 antigen expression were affirmed; (4) In M2 AML the *MCL-1* gene showed the highest expression. The dependence of the *SURVIVIN* gene expression higher level on the bad prognosis for a patient was affirmed. (5) In M3 AML the *MCL-1* gene showed the highest expression. The dependence of the *SURWIWIN, NAIP* or *MCL-1* genes expression higher levels on the poor prognosis for a patient were affirmed. (6) In M4 AML the *NAIP* gene showed the highest expression. (7) In M5 AML the expression top-level for the *MCL-1* gene was affirmed. Supported by grant of Polish State Committee of Scientific Research No 2 PO5B 064 30.

P0567. I171V mutation in the NBS1 gene is associated with predisposition to cancer.

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Homozygous mutation 657del5 within the NBS1 gene is responsible for the majority of Nijmegen breakage syndrome (NBS) cases. NBS patients are characterized by increased susceptibility to malignancies mainly of lymphoid origin. Recently it has been postulated that heterozygous carries of 657del5 NBS1 mutation are at higher risk for cancer development. The aim of the study was to analyse the frequency of I171V mutation in NBS1 gene in 135 children with acute lymhoblastic leukemia, 258 women with breast cancer, 176 patients with larynx cancer, 103 with second primary tumours and 385 healthy individuals. I171V mutation was present in 22 cancer patients compared with only one in healthy individuals. This constitutes 3.27% in studied patients with malignancies and 0.26% in the control group (P=0.0006; relative risk, 0.657; odds ratio, 0.077; 95% confidence interval, 0.01-0.57). Since DNA was isolated from non malignant cells, all mutations found in cancer patients appeared to be of germinal origin. It can be concluded that NBS1 allele I171V may be a general cancer susceptibility gene.

P0568. Intracranial germ cell tumors: association with Klinefelter Syndrome and analysis of X/Y chromosome aneuploidies in the tumors

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Extragonadal germ cell tumors (EGCT) arise in specific midline regions mainly in the anterior mediastinum, intracranial germ cell tumors (IGCT) occur mainly in male children and adolescents. X chromosome polyploidy and X hypometilation have been suggested as a mechanism for malignant transformation independently of the histological type. On the other hand, several reports associated EGCT with Klinefelter syndrome (KS). Recent reports indicate that KS patients have a relative risk (66.7) for development of malignant mediastinal germ-cell tumors and around 8% of male patients with primary mediastinal tumors have KS, corresponding to 50 times of the expected frequency. Trying to record the frequency of KS and to confirm the presence of X chromosome polyploidy in XY IGCT, 13 paraffin embedded tumoral and normal tissue specimens in XY cases with IGCT were studied using FISH. We confirm KS in two cases (8%) demonstrating that this constitutive aneuploidy could be related to carcinogenesis. A low percentage (1%) of X and Y chromosome polyploidy was observed in all cases. In the XY patients the aneuploidy could be involved in the tumorogenesis, however, malignant transformation arise through the accumulation of multiple and different genetic abnormalities.

P0569. Inactivation of the laminin gamma 3 chain (*LAMC3*) gene in various cancers

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We have identified *LAMC3* gene promoter CpG island among those abnormally methylated in breast cancer by methylation sensitive arbitrarily primed PCR. Gene expression analysis by real-time RT-PCR has revealed extremely frequent (94%) decrease/loss of *LAMC3* expression in breast cancer samples. Loss of expression was also detected in the MCF7 breast cancer cell line. We have performed the fine mapping of the CpG island and designed a PCR assay for *LAMC3* methylation detection. Its promoter region appeared to be unmethylated in control samples and methylated in several breast cancer samples, but the frequency of this abnormal methylation was surprisingly

low: 8% (8/98 samples). Loss of heterozygosity (LOH) study with the intragenic microsatellite marker *D9S313* revealed *LAMC3* locus deletions in 8/48 (17%) breast cancer samples. Methylation and LOH studies were also performed for bladder and clear cell renal cancers, demonstrating higher frequencies of *LAMC3* molecular alterations. *The study was supported in part by Applied Biosystems, USA.*

Molecular pathology of the LAMC3 gene in breast, renal and bladder cancers

	LOH			Methylation	Expression
	Total number of samples tested	Number (%)of hetero-zygotes	Number (%) of samples with LOH		
Breast cancer	58	48 (83%)	8 (17%)	8/98 (8%)	29/31 (94%)
Clear cell renal cancer	75	49 (65%)	10 (20%)	8/34 (24%)	n/a
Bladder cancer	77	45 (58%)	12 (27%)	13/45 (29%)	n/a

P0570. Angiotensin Converting Enzyme (ACE) Insertion/Deletion (I/D) Gene Polymorphisms in Leukemic Hematopoiesis

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Renin angiotensin system (RAS) represents an autocrin/paracrin system affecting normal and neoplastic hematopoiesis. In this study, we investigated angiotensin converting enzyme (ACE) insertion/deletion (I/D) gene polymorphisms, which may affect the behavior of the local RAS in hematological neoplastic disorders.

Our results indicated alterations in the polymorphisms of major RAS component, ACE in leukemic hematopoiesis. ACE ID/II genotype frequency is increased in leukemic patients compared to the controls. Disease risk in patients with insertion (ID or II) is increased (chi-squared, p=0.008, OR: 3.2[1.3-7.9]). ID/II genotype was found in 80.4 % of the patients while it was 55.9 % in the control group. Hence, our study firstly provided evidence that the ACE ID/II gene polymorphism may be linked to the development of leukemia as a clue of activated local RAS in leukemogenesis.

Leukemias * ACE Gene Polymorphism Crosstabulation

			ACE-Polymorphisms		
			DD	ID/II	TOTAL
Leukemia	ALL	Count	1	7	8
		% within ALL	12,5%	87,5%	100,0%
		% within ACE-pol	25,0%	22,6%	22,9%
	AML	Count	1	9	10
		% within AML	10,0%	90,0%	100,0%
		% within ACE-pol	25,0%	29,0%	28,6%
	CML	Count	2	15	17
		% within CML	11,8%	88,2%	100,0%
		% within ACE-pol	50,0%	48,4%	48,6%
Total		Count	4	31	35
		% within leukemias	11,4%	88,6%	100,0%

P0571. Molecular-genetic analyses of children with hematological malignances in Bashkortostan Republic, Russia

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Hematological malignances are the most common cancer in childhood. Chromosome rearrangements determine the prognosis of diseases and play an important role in the choice of treatment protocols, allowing the control of minimal residual disease during and after treatment. During the 2005-2006 years, 34 bone marrow samples of patients with leukemia tested to the presence of chromosomal aberrations by the method of reverse transcription-polymerase chain reaction.

We detected three patents with t(1;19) E2A/PBX among 26 ALL samples. This translocation defines an unfavorable prognosis. Patients with t(1;19) received high-risk treatment protocol, that permit to reach molecular remission and decrease risk of relapses.

Among ALL patients - three were with t(12;21) TEL/AML1. This translocation defines a favorable prognosis. Patients with t(12;21) received standard-risk treatment protocol, that permit to reach molecular remission and decreases the risk of complications.

Among ALL patients - one had a t(9;22) BCR/ABL p210. This translocation defines a very unfavorable prognosis. This child has received high-risk treatment protocol in combination with STI571 (Glivec) 400 mg daily.

Among 6 AML patents, one was with AML-M3, translocation t(15;17), PML/RARA confirmed the diagnose. This patient has received AML-APL protocol in combination with ATRA (Tretionin).

We detected t(9;22) BCR/ABL p210 translocation in two samples in patients with chronic phase of CML. These patients have received treatment with Glivec 400 mg daily. After 6 and 12 months of treatment molecular remission was achieved, t(9;22) was not detected.

Thus, molecular-genetic analyses plays an important role in diagnostics, choice of treatment and prediction of relapse in hematological malignancies.

P0572. Cytogenetics, immunophenotype and biomolecular parameters-particularities in acute lymphoblastic leukemia

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Background. Acute lymphoblastic leukemia (ALL) refers to a group of heterogeneous diseases with different clinical expression and prognosis. Therefore, patients require precise and complete initial evaluation of risk factors including cytogenetics, immunophenotype and biomolecular assessment in order to plan an individualised, optimal treatment regimen.

Objectives. We aimed at evaluating the biological profile of leukemic cells and its particularities in ALL.

Patients and methods. We analyzed cytogenetics, cell surface markers and biomolecular parameters in connection with clinical parameters (sex, age, risk factors, ethnic group, central nervous system leukemia, mediastinal mass) in 156 ALL patients aged 0-18 years, treated in our Clinic between 1990-2002.

Results. Compared to data from literature, in our study group we noticed no differences between sex distribution (56% vs. 57% being males), a lower proportion of patients belonging to 2-6 years age group (53.8% vs. 77%) and a double proportion of patients older than 6 years (41.6% vs. 20%). Frequency of medium and high risk forms was higher in study group (75.6% and 10.2% vs. 59.46% and 10.65%) and also the incidence of L3-ALL (3.3%). Biomolecular assessment revealed a comparable proportion of patients with t(9,22)-BCR-ABL mutation (1.28%) and a higher proportion of t(14,11) MLL-AF4 (2.56%). Leukemic cells expressed lymphoid CD10 marker in 35.07% vs. 63.01%. There were no differences between central nervous system involvement in newly diagnosed ALL (3.8% vs. 4%). These data can explain the worse prognosis of our patients.

Conclusions. Our results provide practical information that should be taken into account for planning individualized treatment.

P0573. Molecular basis of the Li-Fraumeni syndrome (LFS): an update from the French LFS cohort

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The Li-Fraumeni syndrome represents one of the most devastating genetic predispositions to cancers and is characterized by a wide spectrum of early-onset malignancies (sarcoma, brain tumour, adrenocortical tumour, breast cancer, leukemia, lymphoma, gastric carcinoma, gonadal germ cell tumour, colorectal and lung cancer). We

have performed the extensive analysis of *TP53*, based on complete sequencing of the 11 exons and on QMPSF, in 370 families suggestive of LFS, fulfilling the French LFS network criteria (J. Med. Genet. 2001). We detected in 73 families (20%) a germline alteration of *TP53* corresponding, in most of the cases (96%), to point mutations or small deletions or insertions, widely distributed between exons 3-11 and in 4% of the cases to complete or partial genomic deletions (4%). These results constitute a definitive argument demonstrating that LFS results from a haploinsufficiency at the *TP53* locus. If most of the families presented the classical LFS wide tumour spectrum, the presentation of some kindreds was remarkable, mimicking *BRCA* families. The earlier development of tumours in *TP53 wt/mt* mice compared to *wt/-* mice (Cell 2004) led us to compare the age of tumour onset between patients harbouring missense mutations (57 patients) and those carrying other alterations (35 patients). As predicted by the murine models, we indeed observed a significant difference between both groups (19.4 y *vs.* 29.8 y). These results confirm that missense mutations not only inactivate the transcriptional activity of the wild-type protein but have also an additional oncogenic effect.

P0574. Telomere length in peripheral blood cells of germline TP53 mutation carriers is shorter than that of normal individuals of corresponding age.

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A decrease in the age at cancer onset and increase in cancer incidence in successive generations in Li-Fraumeni syndrome (LFS) families with germline TP53 mutation have been previously described. In this study we analysed a possible relationship between telomere length and cancer onset in TP53 mutation carriers. Telomere length was measured using real-time quantitative PCR in 20 carriers of germline TP53 mutations and in 83 unrelated healthy individuals and the relative telomere/single copy gene (T/S value) was calculated. According to the age at blood sampling, patients and controls were divided into two age groups: children and adults. The telomere length was correlated to TP53 mutation status, and the telomere shortening in patients to the age at cancer onset. *t*-test and linear regression were used to analyse the data. Compared to healthy controls, telomere length was significantly shorter both in the child (P=0.001) and adult (P=0.034) germline T53 mutation carriers. Although statistically significant correlation between telomere shortening and the age at cancer onset could not be observed, there was a trend of shorter telomeres in mutation carriers affected in childhood compared to those affected later in life. Cancer therapy as well as sex differences were unlikely to affect the results. Statistically significant telomere shortening was observed in mutation carriers, providing a possible link between TP53 mutation, telomere length, predisposition to early-onset cancer and anticipation in Li-Fraumeni syndrome. Supported by grants MSM0021620813 and MZO00064203.

P0575. Truncation of MBD4 predisposes to chromosomal translocations and alters the response to therapeutic agents

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We previously identified a novel genomic instability phenotype of multiple reciprocal chromosomal translocations in a MLH1-defective, microsatellite unstable (MSI) colon cancer cell line (HCA7) and, further, showed that it was unlikely to be directly caused by the mismatch repair (MMR) defect in this cell line. To gain insight into the molecular basis to this novel translocation phenotype, we examined coding and splice-site nucleotide repeat tracts in DNA repair genes for mutations by direct sequencing together with RT-PCR expression analysis of the associated transcript. The material was a selected panel of 8 MSI cell lines including HCA7. A strong candidate identified through this approach was MBD4 as it showed a homozygous truncating mutation associated with substantial loss of the transcript in HCA7 not seen in the other lines. In previous published studies, heterozygous MBD4 mutations were observed in up to 89% of sporadic MSI microdissected colon tumor foci. Using MFISH, we here show that over-expression of the truncated MBD4 (*MBD4^{tru}*) in DLD1, a MSH6 defective, MSI human colon carcinoma cell line predisposed these cells to acquire structural chromosomal rearrangements including multiple reciprocal translocations after irradiation, reminiscent of those seen in HCA7. We also found that over-expression of *MBD4^{tru}* in DLD1 alters the colony survival after exposure to cisplatin, etoposide and gamma rays. These data suggest a wide role for MBD4 in DNA damage response and maintaining chromosomal stability.

P0576. Identification and characterization of microRNAs on common genomic instability regions in breast cancer cells

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Various loss or amplification regions are known in breast tumors. An intensive search for potent tumor suppressors or oncogenes located in these regions continues. MicroRNAs (miRNAs) are ~18-24 nt that regulate protein expression by binding to complementary sequences in the 3' UTR regions of mRNAs. We hypothesized that miRNAs located on genomic instability regions in breast cancer cells may contribute to breast tumorigenesis. First, by using bioinformatics, we mapped miRNAs and candidate miRNAs to reported genomic instability regions. We found more than 38 known miRNAs and 35 candidate miRNAs in these regions. To further confirm loss or amplification of miRNA genes in these regions, we performed semi-quantitative PCR in 20 breast cancer cell lines, 2 immortalized mammary lines and 2 normal controls. Our results showed 85% of selected miRNAs (29 of 34 known miRNAs) were either lost or amplified in at least 2 different lines and 61% (21 of 34 known miRNAs) in at least 3 lines. Interestingly, most of these alterations were found to be amplifications even in regions reported to harbor losses in breast tumors.
Further studies are underway to verifiy deregulation of these miRNAs. Functional analysis will also clarify potential targets of these miRNAs. Our results will help us better understand the biological roles of miRNAs during breast tumorigenesis and will potentially lead to new diagnostic, prognostic and therapeutical findings in breast cancer.

P0577. Missense mutations in genes predisposing to colorectal cancer : Muddying the diagnostic and prognostic waters

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Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC, Lynch syndrome) are classical colorectal cancer predisposition syndromes. FAP is generally due to mutations in APC, whereas HNPCC is caused by mutations in mismatch repair (MMR) genes, primarily MLH1, MSH2, MSH6 and PMS2. Although most mutations are nonsense or small deletions/insertions, thus predicted to result in protein truncation, missense mutations have also been well-documented. Depending on patient selection criteria, the proportion of missense mutations is approximately 30-32% for MLH1, 15-18% for MSH2, 5-12% for (classical) APC.
We present two families which demonstrate the diagnostic and prognostic dilemmas associated with missense mutations in genes for FAP and HNPCC, which have led us to formulate somewhat different suggestions for laboratory evaluation and clinical management. Patient 1 developed colon adenocarcinoma at 24 and multicentric thyroid carcinoma at 30. Sequencing showed a missense alteration in MLH1: G22A (65G->C), also present in her healthy 54-year-old-mother. Rather surprisingly, both tumors were Microsatellite Stable (MSS) and conserved expression of the MLH1 protein. This constellation has been described previously in Lynch syndrome families, pleading the question of whether missense mutations are truly pathogenic and whether the mechanism of malignant transformation is other than impaired DNA repair.
Patient 2, who presented at age 67 with rectal bleeding, had approximately two dozen polyps on colonoscopy. Histologically, several were tubulo-villous adenomas, suggesting attenuated FAP. Sequencing identified a missense alteration in APC: L1129S(3386->C). Family history was positive for polyps. Discussion has centered on management, which generally includes colectomy in FAP.

P0578. Somatic mitochondrial DNA mutations in gastric intraepithelial neoplasia: a histopathologic and biomolecular study.

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We analysed molecular changes in the displacement loop (D-loop) region of mitochondrial DNA in 24 gastric intraepithelial neoplasias (GINs) from a high gastric cancer risk area in Northern Italy. Helicobacter pylori (H. Pylori) infection was assessed by histological examination. The mtDNA D-loop was amplified and sequenced from matched paraffin-embedded gastric mucosa and GIN samples. The GINs were divided into two groups. Group A, with H. pylori-positive gastritis, contained 7 patients, and Group B without H pylori gastritis, contained 17 patients. Group A had a larger proportion of high-grade lesions than B (p= 0.004). Group B had a larger proportion of cases with mtDNA D-loop mutations than A (p=0.004). We found that 15 (62%) of the 24 patients with GINs harbored tumor-associated somatic mutations as following: a G>A transition at nucleotide position (np) 16004; an A>C transversion at np 16013; a T>G transversion at np 16045**;** a C>T transition at np 16380**;** a C>T transition at np16495 (2 cases); an A>G transition at np 16510**;** an A>G transition at np 16515; a T>G transversion at np 10; a 1-base-pair insertion at np 12 (12.1 insA); an A>G transition at np 13; a C>G transversion at np 552; a C>G transversion at np 572; a T>G transversion at np 579; a previously described C>T transition at np 559. These results provide further evidence for the morphologic and mtDNA biomolecular heterogeneity of GINs, and suggest the existence of two distinct pathways to gastric cancer - corpus-dominant H pylori gastritis and the atrophy-metaplasia pathway.

P0579. The role of Multidrug Resistance-associated Protein 1 (MRP1) expression and its polymorphisms in Iranian pediatric acute leukemia patients : impact on treatment outcome

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Occurrence of cross-resistance to structurally and functionally unrelated drugs, called Multidrug Resistance (MDR), is a main cause of failure in the chemotherapeutic treatment of malignant disorders. Several mechanisms of MDR have been identified; one of these is the overexpression of adenosine triphosphate (ATP)-dependent membrane proteins that function as drug-efflux pumps. The multidrug resistance protein MRP1 is a member of the superfamily of ATP-binding cassette (ABC) transporters. MRP1, a 190-Kd protein, is encoded by the MRP1 gene located on chromosome 16p13 and has been shown to transport a broad range of organic substrates, such as glutathione (GSH) conjugates and other anionic conjugates.

Our aim was to investigate the possible association between MRP1 gene expression level and its polymorphisms and clinical outcomes in Iranian pediatric patients. In a retrospective study, we analyzed samples obtained from 42 ALL similarly treated pediatric patients to assess whether the overexpression and/or function of this protein and also correlation between SNPs and Overexpression correlate to treatment failure and therefore affects clinical outcome. In this regard total RNA and DNA was isolated from peripheral blood of ALL acute leukemia patients and ten healthy individuals. MRP1 gene overexpression was detected in 12 patients using Real-Time RT-PCR and compared to the type of response to chemotherapy.

We also have investigated the associationn between MRP1 mRNA level and other clinical characteristics including cytogenetic subgroups and FAB subtypes. We also studied the effects of 4 SNPs (in exon 16 and 20) of MRP1 gene on its overexpression using PCR-SSCP methods followed by sequencing.

P0580. Neurofibromatosis, lymphoma and cavernoma in a child with homozygous genomic deletion in the *MSH2* gene

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Heterozygous mutations in one of the DNA mismatch repair genes (*MLH1, MSH2, MSH6, PMS2*) cause hereditary non polyposis colorectal cancer (HNPCC). Homozygous or compound heterozygous mutations in those genes have been reported in about 20 families and cause patchy pigmented skin anomalies reminiscent of neurofibromatosis I (NF1) and childhood malignancies (both haematological and HNPCC-related).

We report a 3 years old boy, born to first cousin parents, referred for NF1 who presented with café-au-lait spots and Lish nodules of the irides. No mutation was found in the NF1 gene. He subsequently developed an intracerebral cavernoma treated by surgery, and a lymphoblastic lymphoma. A metastatic colon cancer was diagnosed in his father at age 29 but no other familial history was consistent with hereditary non polyposis colorectal cancer. We identified a homozygous deletion of exon 8 of the *MSH2* gene in this child. Both parents were heterozygous for this deletion. This is the first report of a homozygous genomic rearrangement in the *MSH2* gene. All clinical features of this child, but the cavernoma and Lish nodules, are consistent with those previously described. No vascular malformations have been described so far in this syndrome so it is unclear if this association is coincidental or not.

P0581. Mutation screening of the *MUTYH* gene by high resolution melting analysis

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High-resolution melting analysis (HRMA) is a mutation detection method based on the principle that the melting curves of DNA fragments vary depending on base composition. Heterozygous samples can be detected with high sensitivity, whereas homozygosity for a nucleotide change may not lead to significant curve shape or melting temperature changes compared to homozygous wild-type samples. We have evaluated whether HRMA could be used for the identification of homozygous mutations in the *MUTYH* gene, implicated in the autosomal recessive form of intestinal polyposis (*MUTYH*-associated polyposis; MAP). HRMA was first tested on a set of 30 samples of known genotype at exons 7 and 13, where the mutations 495a>g (Y165C) and 1145g>a (G382D) are located. These account for about 70% of *MUTYH* mutations among Caucasians. In order to generate a condition of artificial heterozygosity, test samples were mixed with a homozygous wild-type reference control sample and amplified by PCR. Samples heterozygous or homozygous for the two common mutations, as well as for other exon 7 and 13 sequence variants, could be identified by HRMA. The experiments were then repeated without mixing the test samples with the reference control DNA. We found that homozygous mutant samples could be consistently distinguished from homozygous wild type samples also under these conditions. We applied HRMA as mutation screening test in a series of samples of unknown genotype. The results obtained were confirmed by sequencing, indicating that HRMA is a sensitive and reliable method for the identification of mutations in the hotspot exons of the *MUTYH* gene.

P0582. Cytogenetic evaluation of 506 myelodysplastic syndrome patients

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Myelodysplastic syndromes (MDS) are clonal hematologic malignancies with associated genetic abnormalities. The heterogeneous genetic changes have a role in MDS pathogenesis. Hence, cytogenetic testing is important in the diagnosis and prognosis of these disorders. Cha-

racteristic chromosomal abnormalities in MDS are del(5q), -5, del(7q), -7, +8, del(11q), del(12p), del(13q), del(17p), del(20q), +21. The aberations in chromosome 5 and 7 are the most frequent cytogenetic findings. In the present study, we defined cytogenetic changes with respect to their predictive value. We retrospectively analyzed cytogenetic characterization of 506 consecutive patients with suspected or confined MDS referred to our department from different centers. There were 254 male and 252 female patients with an age-range of 0 to 87 (median 54). For each case, bone marrow or peripheral blood cells were cultured in vitro for 24h at 37^{0}C. Chromosome spreads were obtained following standard techniques. In 140/506 (28 %) patients the quality of the slides were insufficient for evaluation. The number of abnormal and normal karyotypes were 256 (62 %) and 140, respectively. Of 226 patients, 161 (71 %) showed a single anomaly (-7, +8, etc.) The most common abnormality was pertaining to chromosome 7. Complex karyotypes (≥ 5) were found in 13 patients (6 %). Hypodiploidy detected in 86 (38 %) of the patients.
Our results corroborate that cytogenetic findings are in a heterogeneous state and conventional cytogenetic analysis are important in the diagnosis and prognosis of MDS cases.

P0583. Analysis of JAK2(V617F) Mutation in Turkish Patients with Myeloproliferative Disorders

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Polycythemia rubra vera (PCRV), essential thrombocythemia (ET) and agnogenic myeloid metaplasia (AMM) are clonal myeloproliferative disorders arising from a multipotent progenitor. The recent identification of a V617F mutation in the JAK2 tyrosine kinase gene in a high proportion of patients suffering from MPDs may provide confirmation of a diagnosis. Janus kinase 2 (JAK2) is a cytoplasmic tyrosine kinase that transduces signals, especially those triggered by hematopoietic growth factors such as erythropoietin, in normal and neoplastic cells. Here we analyzed JAK2(V617F) mutation in 41 Turkish patients with myeloproliferative disorders. METHODS: DNAs from bone marrow samples of the patients were extracted and then PCR reactions by using F:5'-TGC TGA AAG TAG GAG AAA GTG CAT- 3` and R:5'- TCC TAC AGT GTT TTC AGT TTC AA-3' primers and sequencing analysis were performed. RESULTS: A single point mutation (Val617Phe) was identified in JAK2 in 19 (73.0 %) of 26 patients with PCRV, six (50%) of 12 with ET, and two (66.0 %) of three with AMM. Total V617F mutation prevelance was 66.0% (27/41). CONCLUSION: JAK2V617F mutations are present in almost all patients with PCRV, and in approximately half of those with ET and AMM. This molecular abnormality takes an increased importance in the knowledge of the physiopathology of MPDs, particularly in PCRV and also in prognosis of the patients. However, further studies are necessary to answer many questions regarding the role of a single allele in three clinically distinct disorders, the mechanism of activation of JAK2V617F, and the pathogenesis of JAK2-negative myeloproliferative disorders.

P0584. *NANOG* expression in Ewing's sarcoma and desmoplastic small round cell tumor

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The initiation and development of cancer include several molecular events like accumulation of genetics and epigenetic alterations. Since the 19th century, several researchers have noted the homologies between cancer and embryonic cells. The hypothesis that cancer arise from adult stem cells can be supported by the studies showing gene expression profile similar to normal stem cells. The transcription factor *NANOG* maps on the chromosome 12 in humans, it is uniquely expressed in embryonic stem (ES) cells and in germ cell tumors, and it is important for self-renewal. Knockout of this gene provokes the differentiation of epiblast cells into parietal endoderm cells, in mouse. The expression of *Nanog* in the NIH3T3 transformed these cells, suggesting an oncogenic potential of this transcription factor. Some reports have demonstrated the expression of *NANOG* in seminoma and breast carcinoma. We identified the expression of *NANOG* transcripts in samples of Ewing's sarcoma and desmoplastic small round cell tumor by RT-PCR assay of RNA extracted from fresh tumor samples. This data suggests that this factor is involved in other kinds of cancer. Take together, the expression of *NANOG* in these tumors, and the effects of their disruption in animal models suggest that this gene could be a new therapeutic target for cancer.

P0585. Molecular variants of the NBS1 gene in glial and embryonal brain tumors in children

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Mutations in the *NBS1* gene, which is involved in double-strand DNA repair, are associated with an autosomal recessive disorder, Nijmegen breakage syndrome. Several studies strongly suggest that *NBS1* heterozygosity may be associated with elevated risk of some cancers.
The aim of the study was to identify molecular variants of the *NBS1* gene in a total of 109 children with glial (juvenile pilocytic astrocytomas, astrocytomas anaplasticum, glioblastomas) and embryonal brain tumors (medulloblastomas, atypical teratoid/rhabdoid tumors, supratentorial primitive neuroectodermal tumors). Blood and tumor DNA samples from all patients were screened for the presence of the two most common mutations, c.657del5 (p.K219fsX234) and c.643C>T (p.R215W), in exon 6 of the *NBS1* gene. In addition, in glial tumor tissue exon 5, and in embryonal tumor tissue exons 3, 5, 7, 8, 10, 13, 14, were investigated. Molecular studies consisted of PCR-SSCP and sequencing analyses.
Of 53 patients with glial tumors, two heterozygous cases with mutations in exon 6 of the *NBS1* gene, c.657del5 (p.K219fsX234) and c.643C>T (p.R215W), were identified. In the group of 56 patients with embryonal brain tumors, two heterozygotes for mutation c.511A>G (p.I171V) in exon 5, and three heterozygotes for a common mutation, c.657del5 (p.K219fsX234), in exon 6 of the *NBS1* gene were found. Moreover, five frequent polymorphisms: c.553A>G (p.E185Q), c.2016A>G (p.P672P), c.1197C>T (p.D339D), IVS12-7A>G, IVS10+45delA, were revealed. The frequency of sequence variants of the *NBS1* gene in children with glial and embryonal brain tumors differs from that observed in the general population.
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P0586. Evidence for amplification of the mutant NBS1 allele in tumour samples from NBS-heterozygotes

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Mutations of the NBS1 gene cause an increased tumour risk in NBS-homozygotes as well as in NBS-heterozygotes. However, in the latter case the underlying mechanisms are completely unknown. To approach this problem, ten tumour samples of 9 different NBS-heterozygotes from Poland were analyzed by FISH and RT-PCR. The tumour samples were derived from carriers of the 657del5 mutation or the R215W variant. Interphase FISH was performed on isolated nuclei from paraffin embedded tumour samples by means of a Cy3-labeled BAC-probe containing the whole NBS1 gene region. A BAC-probe flanking the NBS1-region and/or a chromosome 8 centromeric probe were hybridised as controls. Four tumour samples showed a deletion of the NBS1 gene due to a monosomy 8. In contrast, three tumours demonstrated an amplification of the NBS1 gene due to trisomy or even pentasomy of chromosome 8. Additionally, two samples showed both, deletions as well as amplifications of the NBS1 gene in different cells of one tumour. RT-PCR analyses on 4 tumour samples demonstrated a loss of the NBS1 wild type allele and a gain of the NBS1 mutant allele. In this context it is important to note that the 657del5 mutation is hypomorphic because a 70kDa amino-terminal truncated fragment is produced by alternative initiation of translation at a start codon upstream of the deletion through which it is brought into frame. We speculate that overexpression of this fragment might exert a dominant negative effect. This and/or loss of the NBS1 wild-type allele are possible mechanisms for tumorigenesis in NBS-heterozygotes.

P0587. Differential expressed genes in favourable versus unfavourable neuroblastoma tumors

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Neuroblastoma (NB), a childhood tumor originating from neural crest cells in the sympathetic nervous system, has a complex biological heterogeneity depending on clinical stage and age at diagnosis. In order to screen for genes involved in tumour development, a global micro array expression analysis was performed on six NB tumours (three favourable and three unfavourable). Data indicated that the expression levels of several important players in the noradrenalin biosynthesis pathway were significantly lower in unfavourable NB tumours compared to favourable. The 95 most significant genes with a fold change above 2.0 between groups were picked out for verification with real-time PCR (with TaqMan Low Density Array cards, Applied Biosystems) on tumours included in the micro array study. Thirteen additional tumours were also analyzed by real-time PCR in order to explore if the expression pattern is applicable to a larger group. The preliminary results show that transcripts encoded by *solute carrier family 6* (*SLC6A2*), *transcription factor AP-2 beta* (*TFAP2B*), and *chromosome 5 open reading frame 13* (*C5ORF13*) all show a distinct down-regulated pattern in unfavourable tumours versus favourable. The protein encoded by *SLC6A2* is an important mediator in the noradrenalin biosynthesis pathway. Both TFAP2B and C5ORF13 (also known as P311) are known to induce the expression levels of cell-cycle regulator P21. Also, TFAP2B has been shown to regulate expression of genes required for development of tissues of ectodermal origin, such as neural crest. These findings insist us to further explore these genes and their involvement in neuroblastoma development and progression.

P0588. Screening 80 unrelated neurofibromatosis type 1 patients for deletions of the *NF1* gene.

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Neurofibromatosis type I (NF1) is a common autosomal dominant disorder affecting 1/3,500 individuals. The major diagnostic features include café-au-lait spots, neurofibromas, axillary/inguinal freckling, and Lisch nodules. Various constitutional mutations of the *NF1* gene have been found in NF1 patients. However, no clear genotype-phenotype correlation has been established so far, except for patients with deletions of the entire *NF1* gene who have a more severe phenotype, including facial abnormalities, mental retardation, developmental delay, early development of numerous cutaneous neurofibromas, and plexiform neurofibromas. We have developed the panel of intragenic microsatellite markers (D17S1307, D17S1849, TAGA/TAGGint27a, ACint27b and GTint38) for detection of NF1 region gross deletions and indirect NF1 DNA-diagnostics. Moreover, we used 3 polymorphisms (702A>G, 1528-29delT and 5546-19T>A) for the definition of NF1 locus heterozygosity. A total of 80 unrelated patients who met the diagnostic criteria for NF1 were included in our study. In five patients all markers were homozygous and one patient had a deletion (TAGA/TAGGint27a, GTint38). Parents DNA was accessible only for three of five potential carriers of deletions. Among the latter, two of three had no severe phenotype characteristic for loss of the entire *NF1* gene. Based on the heterozygosity of each marker, the probability of random homozygosity of eight markers in one individual could be calculated as 0.00027. It is possible to explain such contradiction by the presence of a small number of allelic variants of marker D17S1307 and prevalence of one allelic variant (52 %) of marker D17S1849.

P0589. Expression analysis and investigation of single nucleotide polymorphisms in putative NF1 modifying genes

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominantly inherited tumor diseases. Several symptoms of NF1 as the dermal neurofibromas show a high interfamilial variability in classical NF1. In the familial spinal neurofibromatosis, a very rare variant of NF1, the number and onset of neurofibromas is obviously decreased. On the other hand, patients with microdeletions spanning the NF1 and several contiguous genes have an earlier onset and a higher number of dermal neurofibromas as classical NF1 patients. To explain this variability the existence of NF1 modifying genes in this region has been proposed. Our expression studies in dermal neurofibromas revealed four putative NF1 modifying genes: CENTA2, UTP6, C17orf79 and RAB11FIP4. We investigated the expression level and SNP variability of these genes in more detail. First results will be presented.

P0590. Disruptive mitochondrial DNA mutations in complex I subunits are markers of oncocytic phenotype in thyroid tumors

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Oncocytic tumors are lesions composed of cells characterized by mitochondrial hyperplasia particularly common in the thyroid gland. Because of their distinctive features they represent a good model to study the role of mitochondria in tumorigenesis. We recently demonstrated the association between two pathogenic mitochondrial mutations and a defective biochemical phenotype in a cell line model of oncocytoma. To understand whether specific mitochondrial DNA (mtDNA) mutations are associated with the oncocytic phenotype we sequenced the entire mtDNA in 45 oncocytic thyroid tumors (HCTs), 5 oncocytic breast tumors and 52 control cases (21 non-oncocytic thyroid tumors, 15 breast carcinomas and 16 gliomas) utilizing a recently developed technology (Applera). Thirteen oncocytic lesions (26%) presented disruptive mutations (nonsense or frameshift), whereas only 2 samples presented such mutations in the non-oncocytic control group (3.8%). In one case with multiple thyroid nodules analyzed separately a disruptive mutation was found in the only nodule with oncocytic features. In one of the 5 oncocytic breast tumors a disruptive mutation was identified. All disruptive mutations were found in complex I subunit genes and the association between these mutations and the oncocytic phenotype was statistically significant (p=0.001). To study the pathogenicity of these mitochondrial mutations, primary cultures from oncocytic tumors and corresponding normal tissues were established. Molecular and biochemical analysis and electron microscopy showed that primary cultures derived from tumors bearing disruptive mutations failed to maintain the mutations and the oncocytic phenotype. We conclude that disruptive mutations in complex I subunits are markers of thyroid oncocytic tumors.

P0591. P53, H-ras, c-myc, c-erbB2 and bcl2 analysis in oral squamous cell carcinomas

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A comprehensive study of molecular changes during oral carcinogenesis has been performed on 60 specimens of squamous cell carcinoma (OSCCs). P53 and H-ras have been subjected to mutational analysis by SSCP followed by sequencing. C-myc and c-erbB2 have been tested for amplification using differential PCR and bcl2 gene expression has been determined by immunohistochemistry.

Mutations in one or more exons of the p53 gene have been found in 60 % of cases. H-ras codons 12/13 mutations have been detected in 22% of cases, while c-myc and c-erbB2 amplification has been recorded in 35% and 32% of specimens, respectively. Bcl2 expression has been registered in 68% of OSCCs.

We confirmed a positive association between the presence of p53 mutations and bcl2 expression, as well as a significant correlation between c-erbB2 amplification and bcl2 expression. No statistically significant correlation was found between molecular and histopathological changes (p53 mutations showed a borderline correlation with histological grades).

P0592. Different profiles of BRCA1 and BRCA2 SNPs association with breast/ovarian cancer risk among sporadic cases and BRCA1 mutation carriers

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The BRCA1 and BRCA2 genes are the main suppressors of inherited breast and ovarian cancer. The molecular basis for cancer localization in either the breast or in the ovary is unclear. We speculate that cancer localization may be modified by different variants of BRCA1/2 genes.

We studied 4 samples of patients for SNPs in the BRCA1 and BRCA2 genes. Patients with sporadic breast and ovarian cancer, patients with the same types of cancer with BRCA1 mutations and a control sample. The BRCA1 E1038G and BRCA2 N372H, H1256P coding variants, and a SNP located in the 5'-untranslated region were investigated. In a sample of sporadic breast cancer patients, an association of the 1038EG variant with cancer risk was found (OR=2,11; P=0,016). There was no difference in four SNPs frequencies between BRCA1 mutation carriers with breast cancer and control sample. This may means that no modifications are required for breast cancer localization under BRCA1 mutations. In sporadic ovarian cancer 203AA homozygous variant was associated with cancer risk (OR=6,59; P=0,0016). An association of 1038GG (OR=0,12; P=0,019) and 203GA (OR=0,29; P=0,0033) were revealed for cases of ovarian cancer with BRCA1 mutations.

In conclusion, the data suggest a possibility distinguish four groups of patients with breast or ovarian cancer by profiles of BRCA1/2 SNPs association with cancer risk. The risk of sporadic breast and ovarian cancer is associated with different SNPs. The BRCA1 mutations caused breast cancer independent of the SNPs studied. We conclude that BRCA1/2 polymorphic variants may act as modifiers of the magnitude of ovarian cancer risk of BRCA1 mutations.

P0593. Coexistence of copy number changes of different genes (erbB-1, erbB-2, CMYC, CCND1 and ZNF217) in ovarian tumors

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The aim of this study was to establish the frequency of combinatorial and separate copy number changes of erbB-1, erbB-2, c-myc, CCND1 and ZNF217 in ovarian tumors from different phenotype; icroarray of 507 ovarian tumors was analyzed by fluorescence in situ hybridization. A total of 47 tumors were successfully analyzed for all 5 loci, among them 38 were malignant, 7 were tumors with low malignant potency (LMP) and 2 were benign. Normal gene copy numbers of all loci were established in 12 malignant tumors (31.6%), in 2 of LMP tumors (28.6%) and in both adenomas. At least one aberration was found in 26 malignant tumors (68.4%) and in 5 LMP tumors (71.4%). Single abnormalities were detected in 14 malignant tumors (36.8%) and in all aberrant LMP tumors, while double or higher abnormalities were found only in malignant tumors (31.6%). The most frequent genetic change both in malignant and LMP tumors was copy number increases of c-myc. Coexistence of two or more abnormalities was more frequent in serous (64.3%) than in non-serous (40%) tumors (p<0.19). Copy number changes of c-myc and CCND1 were predominantly found separately, while copy number increases of erbB-1, erbB-2 and ZNF217 were usually combined with another aberration. We concluded that, in particular, c-myc gain is early event in ovarian carcinogenesis, whereas gains of erbB-1, erbB-2 and ZNF217 are late events. Serous cancers are genetically more instable than non-serous cancers. About 70% of ovarian cancers have unbalanced alterations in some of the analyzed loci.

P0594. The right cutoff point: is it possible to improve the predictive value of p53 immunohistochemistry?

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p53 immunohistochemistry (IHC) has been performed in thousands of malignant tumors but its clinical impact is still unclear.

We have already demonstrated that the p53 genotype assessed by sequencing is predictive for response to preoperative cancer therapy. Aim of the present study was to evaluate whether p53 IHC is of any use for predicting response to p53 dependant treatments.

Using one of the most frequently applied antibodies for IHC, pAb1801, we calculated different cutoff points for scoring positivity, ranging from >0% to >80%, for their correlation with the response to preoperative cancer therapy.

In 106 patients (22 lung cancer, 49 rectal cancer and 35 breast cancer patients) a mutant p53 genotype has been shown to be significantly associated with treatment failure. A positive IHC calculated with a 10% cutoff did not show any correlation with treatment response in lung and in rectal cancer patients, respectively. Only in breast cancer patients, treatment failure was related to the presence of a positive IHC staining, but specificity - the probability of recognizing responders - was lower than calculated by sequencing.

Raising the cutoff improved specificity, but we could reach 100% in breast cancer only. Furthermore, correlation of p53 IHC and treatment response was found in breast cancer patients only.

We conclude that the specificity of p53 IHC to detect responders to cancer therapy may be improved by changing the cutoff. However the later decreases the sensitivity of recognizing nonresponders. Therefore, in contrast to p53 sequencing, p53 IHC does not qualify for clinical application.

P0595. Correlation between genetic polymorphisms of GST-P1, p53, COX-2 and overexpression of p53 in esophageal squamous cell carcinoma from Iran

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The incidence of esophageal cancer (EC) is depended on the geographic differences. It is due to specific environmental factors and chemicals. Northern Iran is the highest risk area for this type of cancer, but the specific environmental factors are very important as determinants of esophageal cancer risk in Iran is not clearly defined. It is demonstrated that single nucleotide polymorphisms (SNP) in various genes revealed a correlation between the presence of specific allelic variants and cancer susceptibility in diverse malignancies. Furthermore, studies of polymorphisms in cancer susceptibility genes included GST-P1, p53 and COX-2 in various types of cancer were shown. Therefore, in this investigation, we assesed the role of genetic polymorphisms of *GST-P1*, *p53*, *COX-2* in risk of developing esophageal squamous cell carcinoma from Iran.

The *p53* Pro72Arg, *GST-P1* Ile105Val and COX-2 -765G>C genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) and direct DNA sequencing analysis in healthy controls and patients. Also, Immunohistochemistry was performed to determine p53 expression. Among the EC patients subjects, the frequency of G allele for *p53* gene, A allele for GST-P1 gene and C allele for COX-2 gene were high. Accumulation of p53 protein in tumor cells was not correlated with investigated genetic polymorphisms.

This preliminary study support a hypothesis that *GST-P1*, p53 and COX-2 variants are involved in development of esophageal cancer from Iran. However nothing is known so far about the status of *GST-P1*, *p53* and COX-2 genetic polymorphisms and its association with overexpression of *p53* in EC from Iran.

P0596. Is 16bp intron 3 polymorphism for p53 gene associated with esophageal cancer in Iran?

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The p53 protein is considered a key combination in countering stress messages such as DNA damage. This protein is a transcription factor and it can induce cell growth arrest or apoptosis to protect genome and cell against genotoxic damage. Also, the p53 gene is involved in the etiology of malignant disease. It is recorded that most cancers carry *p53* gene mutations. The frequency of esophageal squamous cell car-

cinoma (ESCC), which it is predominant cancer in esophagus, is very high in northern Iran. The incidence pattern of esophageal cancer is different among Iranian population and up to 171/100,000. So, identification of *p53* gene mutations and polymorphisms is very important to find the major causes of this type of cancer in Iran. Several studies were described the polymorphism in intron 3 of the p53 gene (16bp intron 3 insertions) and susceptibility to several types of cancer and diseases. This type of polymorphism can change the expression of p53 gene.
In this investigation, we assessed allele frequency of *p53* polymorphism in intron 3 among Iranian ESCC patients and healthy controls. The *p53* genotypes were determined by direct DNA sequencing analysis in healthy controls and patients. Among the ESCC patients subjects, the 16bp intron 3 polymorphism for *p53* gene were significantly higher than healthy controls. Our finding suggested that *p53* genotype may play an important role in developing esophageal tumor among ESCC patients. It is suggested that protein expression of the p53 gene can be investigate among Iranian ESCC patients in the future.

P0597. Epigenetic regulation of shc1 protein expression, and genetic variation within the SHC1 gene in breast carcinomas.

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The SHC1 gene encodes three isoforms: p66shc, p52shc and p46shc. Both p52/46shc activate the mitogen activated protein kinase pathway (MAPK), in contrary to p66shc which downregulates MAPK. Moreover p66shc plays an important role in apoptosis and response to oxidative stress by transferring signal from p53. Despite that p66shc and p52/46shc are involved in different cell functions, they all play an important role in tumorigenesis.
Western blot analysis of cytosol fractions from breast tumours revealed that p66shc protein expression is decreased for most of the samples. Sequencing of bisulphite treated DNA from the same patients showed that the level of methylation inside the p66shc promoter region correlates with protein expression. Additionally, samples which did not fit properly to this thesis underwent mutation analysis. Exon 1 and the promoter region of SHC1 were sequenced in 20 breast tumours, but no common mutation was found. There was discovered mutation within the Sp1 binding site for one of the samples, but a single nucleotide change did not affect expression of p66shc. Moreover, SNP analysis was performed, comprising four known and two new polymorphisms within the common part for all 3 SHC1 isoforms. 200 DNA breast cancer samples and 252 DNA samples from healthy individuals serving as controls were analyzed. Two independent haplotypes were identified based on the SNP genotypes. Despite the presence of two rare polymorphisms, one of the discovered haplotypes may have a protective effect against breast cancer. The genotype was twice as frequent in the control population as in the tumour samples.

P0598. p73 regulates PTEN genic expression

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p73 belongs to a family of p53-related nuclear transcription factors that includes p53, p73 and p63. Differentially from p53, p73 is expressed as two NH2-terminally distinct isoforms, due to a second downstream promoter: transcriptionally active (TAp73) and transcriptionally inactive (ΔNp73) forms. The ΔNp73 isoforms can act as dominant negative inhibitors of the TA isoforms and p53, so TA and ΔN show pro- and anti-apoptotic properties, respectively. Moreover additional complessity is generated at the COOH terminus because p73 undergoes multiple COOH-terminal splicing (α being full-length isoforms). PTEN is a dual-specificity phosphatase and has a tumor suppressor function. This study investigates the regulation of PTEN expression by p73 in thyroid cells because in thyroid carcinomas both decrease of PTEN expression and increase of p73 expression have been previously shown. Stable thyroid cell line was generated expressing inducible p73 isoforms, these cells showed incremented PTEN protein upon over expression of TAp73α and β; instead cell line expressing ΔNp73α showed decremented PTEN protein. Cell transfection studies indicated that TAp73α and TAp73β can activate PTEN promoter, instead ΔNp73α inhibits it, in a p53 independent way. Moreover the ΔNp73α inhibitory action is prevalent on TAp73α and TAp73β activatory actions. This evidence supposes that in thyroid cancers, in which there are a high ΔNp73α expression respect to the normal thyroid tissue, ΔNp73α could reduce PTEN expression even in presence of the others activating isoforms.

P0599. Searching for polymorphisms in paclitaxel elimination pathway influencing drug efficacy and toxicity

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Paclitaxel is an anti-cancer drug widely used in the treatment of a variety of solid tumors, including breast, ovarian and lung. There is a large interindividual variation in the efficacy and adverse effects of this drug, mainly hematologic toxicity and neurotoxicity. It has been proposed that polymorphisms in genes encoding drug-metabolizing enzymes and drug transporters might contribute to the differences in paclitaxel outcome. Thus, the aim of this work is to identify SNPs involved in paclitaxel elimination that could influence its efficacy and toxicity. Paclitaxel elimination is mediated by hepatic metabolism and biliar excretion: the uptake of paclitaxel into the hepatocytes is mediated by the organic anion transporting polypeptide (OATP)1B3, in the liver cytochrome P450 2C8 (CYP2C8) and CYP3A4/5 hydroxylate paclitaxel and the P-glycoprotein mediates biliar excretion. Thus, we selected variants in these genes and genotyped them in more than 80 oncology patients treated with paclitaxel: four *CYP2C8* SNPs from which two are in the coding region; one *CYP3A5* SNP causing alternative splicing; one *CYP3A4* promoter SNP influencing transcription; two coding *OATP1B3* SNPs and three *MDR1* SNPs previously associated with altered activity. Neurotoxicity was evaluated in the patients and the influence of clinical variables, such as total paclitaxel dose, time to neurotoxicity and time to recovery were taken into account in the study. Then, the genotypes were compared to the clinical data of the patients, in order to assess their effect. In conclusion, the identification of genetic variants involved in paclitaxel elimination could be useful to predict drug effects.

P0600. Molecular genetic analysis of pheochromocytomas and paraganglioma in Czech patients

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The pheochromocytoma is tumor arising in adrenal or extra-adrenal sites and occurs as a sporadic form or, less frequently, in familial setting as a part of inherited syndromes. Paraganglioma of the head and neck occurs mostly sporadically and also in syndromic or nonsyndromic familial settings. To date four susceptibility genes for pheochromocytoma have been reported that included RET proto-oncogene, VHL tumor suppressor gene and recently identified genes SDHB and SDHD for succinate dehydrogenase subunit B and D respectively. Mutations in these genes can predispose one to pheochromocytoma and paraganglioma.
All established genes were analyzed to investigate possible genetic cause of pheochromocytoma and paraganglioma in the population of Czech patients. Among 100 patients we have found several previously described mutations or polymorphisms. Besides that one novel mutation was found in the SDHB gene in a child patient. We conclude that a large part of diseases could be be due to mutations in an unidentified gene(s).
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P0601. Methylation-associated *PHOX2B* gene silencing in human neuroblastoma

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Neuroblastoma (NB), an embryonic tumour originating from neural crest cells, is one of the most common solid tumours in childhood.

Although NB is characterized by numerous recurrent, large-scale chromosome rearrangements, the genes targeted by these imbalances have remained elusive. We recently identified the paired-like homeobox 2B (*PHOX2B*, MIM 603851) gene as disease-causing in dysautonomic disorders including Congenital Central Hypoventilation Syndrome (CCHS), Hirschsprung disease (HSCR) and NB in various combinations. Most patients with NB due to a germline heterozygous *PHOX2B* gene mutation are familial and/or syndromic. *PHOX2B*, at chromosome 4p12, does not lie in a commonly rearranged locus in NB. To evaluate the role of *PHOX2B* in sporadic, isolated NB, we analysed 13 NB cell lines and 46 tumors for mutations of coding and promoter sequences, loss of heterozygosity (LOH), or aberrant hypermethylation of *PHOX2B* (13 cell lines and 18 tumors). We identified no mutation but LOH in about 10% of the cases and aberrant CpG island methylation of the 500 bp *PHOX2B* promoter region in 4/31 (12.9%). Altogether, both germinal and somatic anomalies at the *PHOX2B* locus are found in NB.

P0602. Breast cancer and the promyelocytic leukemia gene

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Introduction: The *PML (*promyelocytic leukemia) protein is a tumor suppressor gene that encodes a multifunctional protein with critical tumor suppressive functions such as induction of apoptosis, growth arrest and cellular senescence. Its expression is downregulated in about one third of tumors, including breast cancer. With respect to these facts we tested the hypothesis that germline mutations of the *PML* gene might predispose to breast cancer.

Patients and methods: Thirty five patients fulfilling the criteria for *BRCA1/BRCA2* gene mutation analysis in whom no germline *BRCA1/BRCA2* gene mutation was found were included into the study. Genomic DNA was isolated from peripheral blood. Nine exons of the *PML* gene were screened for mutations using heteroduplex analysis. Samples with fragments of abnormal mobility were sequenced using ABI prism 3100 genetic analyser.

Results: No germline mutation was found in the *PML* gene in any of the patients.

Conclusion: Our results suggest that predisposition to breast cancer is probably not associated with germline mutations of the *PML* gene. Acknowledgements: The project was supported by IGA MZ CR NR/9092-3/2006.

P0603. Array CGH of dic(7;9)(p11-13;p11-13) in B-cell precursor acute lymphoblastic leukemia reveals clustered breakpoints at 7p11.2-12.1 and 9p13.2

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The dic(7;9)(p11-13;p11-13) is a recurrent chromosomal abnormality in acute lymphoblastic leukemia (ALL), mainly of B-lineage. Although more than 20 dic(7;9)-positive ALLs have been reported to date, the molecular genetic consequences of this aberration are unknown. We performed high-resolution (32K) genome-wide array-based comparative genomic hybridization (array CGH) and locus-specific fluorescence in situ hybridization analyses on three cases with dic(7;9) in order to characterize the breakpoints on 7p and 9p. The analyses showed a clustering of breakpoints at 9p13.1-13.2 in all three cases and at 7p11.2 in two; the array CGH revealed two different breakpoints - 7p12.1 and 7p14.1 - in the remaining case. Based on the present results this abnormality should hence be designated dic(7;9)(p11.2-12.1;p13.1-13.2). Unfortunately, lack of material precluded further molecular genetic studies, and it thus remains to be elucidated whether the pathogenetically important outcome of the dic(7;9) is formation of a chimeric gene or loss of 7p and 9p material.

P0604. POET (Prevention of Endometrial Tumours), a randomised control trial of the effect of the Mirena Intrauterine System (IUS) with surveillance, versus surveillance alone, on the development of atypical endometrial hyperplasia (AEH) and carcinoma (EC) in women with Lynch Syndrome aged 35-65y.

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Women with Lynch syndrome have an increased risk of developing endometrial cancer (up to 65% lifetime risk, 20% before 50y). The Mirena progestogen-releasing intra-uterine system greatly reduces the thickness of the endometrium. This study proposes to evaluate whether treatment with the Mirena for 4y reduces the rate of detection of AEH and EC in women with Lynch Syndrome on surveillance by annual transvaginal ultrasound (TVS) and EB. Secondary study outcomes include determination of the sensitivity and specificity of surveillance, the age-related incidence of AEH and EC in women with Lynch syndrome, the premalignant pathway to carcinoma, the psychological effects of this management protocol and any adverse effects of surveillance and use of the Mirena IUS. We piloted the Mirena IUS for 6 months in 15 women, average age 42y (24-56y). 9 experienced mild, 4 moderate and 2 severe pain on insertion. One IUS was removed for excess uterine bleeding; 7 women had spotting and 7 no endometrial bleeding. EB was successful before and after the trial period; all post-Mirena biopsies showed decidualisation of the endometrium. 5 women chose to continue for longer with the Mirena.

This study is funded by CR-UK for 5y. in the UK, where we have initiated it as a multi-centre National study. We welcome expressions of interest to initiate wider international collaboration.

P0605. Detection of PML-RARA rearrangement by RT-PCR in an acute promyelocytic leukemia without evidence by FISH.

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Acute promielocytic leukemia (APL) is characterized by a reciprocal translocation, t(15;17), resulting in fusion of the genes promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARA). This translocation is detected in about 70-90% of patients by conventional cytogenetic methods. There are also masked or cryptic t(15;17) that may be generated by submicroscopic insertions of PML or RARA or more complex rearrangements. Those masked PML-RARA fusions can be identified by molecular analyses such reverse transcriptase-polimerase chain reaction (PCR) and fluorescence in situ hybridization (FISH).

We have studied the case of a 55-years-old woman that shows clinical, morphological and inmunophenotypic features of APL. The PML-RARA was not evident on FISH test, while RT-PCR revealed the presence of PML-RARA transcript (bcr-3). The probe used was PML-RARA dual color dual fusion (Vysis). It was not possible to perform the chromosomal analyses because of the coagulation of the sample of bone marrow. The patient was treated with a standard protocol for APL that includes All Trans Retinoic Acid (ATRA) and chemotherapy.

The FISH probes used in this case are a good molecular testing to detect cryptic, variants or complex PML-RARA rearrangements, but depending on the size of the insertion of PML-RARA, the target could be so small that it would not hybridize with the probe or even hybridization itself might not generate a fluorescent signal large enough to be visualized by FISH. Thus, a combination of molecular testing FISH and PCR could avoid false negative results in presence of masked PML-RARA fusions.

P0606. Overexpression of *CD24* and *c-myc* in prostate cancer tissue samples obtained by needle biopsy.

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Objective: Altered *CD24* and *c-myc* expression was reported in different cancers. We decided to establish a quantitative real-time PCR

method to measure the expression of these genes in prostate cancer tissues and compare it to a non-cancerous samples.
Subjects and methods: Prostate tissue samples were collected using needle biopsy from 21 prostate cancer (PCA) and 10 benign prostate hyperplasic (BPH) patients. RNA was isolated; cDNA synthetized; *CD24* and *c-myc* expressions were determined by quantitative real-time PCR method. The expression of *phospholipase 2A* gene was measured for normalization of the gene expression results. Serum prostate specific antigen (SPA) levels were determined by microparticle enzyme immunoassay (MEIA) method.
Results: PSA levels were significantly different between the PCA and BPH groups, 252.37±308.33 ng/ml vs. 3.5±2.14 ng/ml (p=0.001). *CD24* expression was 33.65±47.39 ng/µl in prostate tumorous and 4.00±4.25 ng/µl in the BPH group (p=0,014), while the *c-myc* expression was 88.85±114.95 ng/µl in the prostate tumorous and 17.08±21.75 ng/µl in the BPH group (p=0.028).
Conclusion: Overexpression of *CD24* and *c-myc* was observed in prostate cancer samples using quantitative real-time PCR method. *CD24* expression could be an independent marker on tumour invasion and metastasis, with *c-myc* which plays also role in cell cycle regulation and metastasis development.

P0607. Significance of VDR, MTHFR and Insulin Gene Polymorphisms in Prostate Cancer in Turkish Population

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Prostate cancer is an increasingly common disease for which there are few well-established risk factors. Family history data suggest a genetic component; however, the majority of prostate cancer cases cannot be explained by a single-gene model. AIM: We examined genetic polymorphisms in Vitamin D receptor (VDR), methylenetetrahydrofolate reductase (MTHFR) and insulin genes in a case-control study of prostate cancer in Turkish population. METHODS: VDR gene BsmI and FokI, insulin gene PstI and MTHFR gene A1298C and C677T polymorphisms were analyzed using DNAs from peripheral blood samples of 100 prostate cancer patients and 200 controls with benign prostate hyperplasia. RESULTS: No significant difference was seen in all genes, but the significance for A1298C polymorphism is in the edge of range. CONCLUSION: None demonstrated any significant variation in distribution within these genes and therefore we concluded that VDR, MTHFR and insulin genes polymorphisms might not be major risk factor for prostate cancer in Turkish population.

P0608. Molecular genetic alterations in tumor epithelium and tumor-associated stromal cells of prostate cancer.

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We investigated epigenetic changes and allelic imbalance (LOH/AI) in prostate cancer epithelia, tumor-associated stroma and prostate intraepitelial neoplasia (PIN) in prostatectomy specimens. Adenocarcinoma epithelia, foci of PIN and benign prostate hyperplasia (BPH) and stroma adjacent to tumor tissues were isolated from 34 whole-mount prostatectomy specimens of patients with pT1-T4 stage prostate cancer by using laser capture microdissection.
The methylation status of *p16, HIC1, N33* and *GSTP1* genes were evaluated using methylation-sensitive PCR. We found high levels of gene methylation in the tumor epithelium (78, 89, 33, 70%) and high-grade PIN (57, 71, 33, 71%) and some methylation in BPH (25, 25, 13, 0%) located adjacent to tumors. All investigated genes were methylated with very similar frequencies in adjacent stroma. There is no methylation in BPH and stroma from patients with hyperplasia.
Microsatellite allelotyping was performed using 5 polymorphic markers for regions 8p22, 16q23 and 13q14. The frequencies of LOH/AI were higher in the corresponding stroma (Table). LOH/AI in tumour epitelium at 16q23 and 13q14 were significantly associated with stage, Gleason score, metastasis, but no positive correlation between LOH/AI and clinicopathologic parameters in adjacent stroma was observed. The epithelium LOH/AI frequencies at all loci were increased from stage I to IV, in contrast, stromal LOH/AI frequencies were diminished respectively.

Marker	LOH/AI frequencies			
	Tumour	stroma	PIN	stroma
D8S1731	15/31 (48%)	16/28 (57%)	1\13 (7.7%)	1\9
D16S534	15/30 (50%)	16/29 (55%)	3\9 (33%)	1\6
D16S422	14/25 (56%)	9/18 (50%)	1\8 (13%)	1\6
RB1.20	11/30 (37%)	15/26 (58%)	2\9 (22%)	2\7

The finding of frequent genetic and epigenetic alterations in tumor-associated stroma suggests a more important role for stromal fibroblasts in prostate cancerogenesis than was previously appreciated.

P0609. Aberrant expression of *TGIFLX/Y* gene in prostate cancer

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Prostate cancer is the most common malignancy and the second leading cause of cancer in men. Genetic studies have revealed this disorder to be heterogenous in nature. The molecular biology of prostate cancer and its progression is characterized by aberrant activity of several regulatory pathways at the cellular level, and the surrounding tissue of the prostate.. Alterations at the DNA, RNA and/or protein expression levels of molecules involved in these pathways are all potential candidate markers of prognosis and therapeutic response. It is known that homeobox genes encode transcription factors which regulate multiple developmental processes. These homeodomain proteins play an important role in determining cell fate. *TGIFLX/Y* is a homeobox gene, contains two genes; *TGIFLX* (X-linked) and *TGIFLY* (Y-linked). The biological function of these genes is unknown. In order to determine the potential function and involvement in normal and abnormal development, the expression pattern of *TGIFLX/Y* in prostate cancer tumors was investigated. In this study a RT-PCR assay for detection of *TGIFLX/Y* mRNA was carried out. Two different types of clinical samples including 100 prostate tumors and 95 benign prostate hyperplasia (BPH) were studied. RT-PCR analysis reveals different patterns of *TGIFLX/Y* gene expressions among prostate cancers. In some tumor samples the expression of both genes was detected. However, in some cases either no expression or just one gene was detectable. In contrast, no expression of *TGIFLX/Y* mRNA in BPH samples was observed. Our results suggest possible *TGIFLX/Y* involvement in prostate cancer development .

P0610. Cowden syndrome in Norway

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We report the results of diagnostic and predictive testing in all families possibly having Cowden syndrome or families with breast and thyroid cancer registered at the Norwegian cancer family clinics.
Cowden syndrome (multiple hamartoma syndrome, MIM 158350) is characterized by multiple hamartomas in the skin, mucous membranes, breast, thyroid and endometrium. Patients with Cowden syndrome have increased risk of breast cancer, thyroid cancer and endometrial cancer. In 1997 germline mutations in PTEN were demonstrated to cause Cowden syndrome. By analyzing the computerized medical files in our department we identified families with a diagnosis of Cowden syndrome, all families suspected to have Cowden syndrome, and all families with a combination of breast and thyroid cancers. The other Norwegian genetic centres contributed their families with Cowden stigmata.
PTEN mutations were found in all six families meeting the clinical criteria for Cowden syndrome, in none of the two families assumed to have Cowden syndrome but not fulfilling the criteria, and in none of the eight families with a combination of breast and thyroid cancers. Mutations in PTEN were identified in all affected members of the families fulfilling the Cowden syndrome criteria. 56 persons were tested, 19 were identified as mutation carriers.

Penetrance of PTEN mutations was high and expressions were Cowden syndrome stigmata in early infancy or childhood and cancer in adolescence or early adulthood. All families but one were small, and de novo mutations were found. Outside clinically identifiable Cowden families, PTEN mutations may not contribute to inherited breast cancer.

P0611. Analysis of TP53 Arg72Pro and MDM2 SNP309 T>G polymorphisms as modifier factors in hereditary retinoblastoma.

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Retinoblastoma (RB) is the most common primary intraocular malignancy in children, arising by two-hit inactivation of the *RB1* gene. Hyper-expression of negative p53 regulators was recently found as a frequent somatic event. Most *RB1* mutation carriers develop multifocal RB. However, they show a broad range of phenotypic variability (age of onset, involvement of one/two eyes and needed therapy). To test the hypothesis that this may in part results from variable function of genes involved in cell cycle and apoptosis, we investigated the effect of two functional polymorphisms, one in *TP53* (Arg72Pro) and the other in the promoter of *MDM2* (SNP309 T>G), on the age of tumour diagnosis. The *TP53* Pro allele has been shown to have a weaker proapoptotic activity and to influence age of onset in Lynch syndrome; the *MDM2* 309G allele to accelerate tumour formation in Li-Fraumeni syndrome and sporadic cancers. We tailored specific Pyrosequencing (C) assays for the two SNPs and genotyped 43 RB patients with a characterized or suspected *RB1* germline mutation (i.e. familial and bilateral cases). At univariate analysis, neither the *MDM2* nor the *TP53* SNP did reveal significant differences on RB onset. We then evaluated their combined effect through modelling estimates. Patients with the GG-ProPro genotype showed an earlier tumour onset compared to those with GG-ArgArg or GG-ArgPro. An increased number of patients is needed to firmly establish this association. This approach could help in better characterizing the role of the p53 pathway in RB carcinogenesis, and defining more accurate prognosis for patients.

P0612. RNASEL gene polymorphisms and the risk of prostate cancer: a meta-analysis

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Studies revealing conflicting results of the role of RNASEL polymorphisms Glu265X, Arg462Gln and Asp541Glu on prostate cancer risk led us to perform a meta-analysis on published data investigating the association of these polymorphisms and the prostate cancer risk.

We performed a meta-analysis of 5 studies with Glu265X genotyping, 7 studies with Arg462Gln genotyping and 6 studies with Asp541Glu genotyping. The overall results of the meta-analysis suggested no major influence of these variants on the prostate cancer risk. However, analysis of the Asp541Glu polymorphism by ethnicity showed that the genotypes with Glu variant including Asp/Glu (Familial cases vs. Control: OR=1.38, 95% CI=1.04 - 1.82; Sporadic cases vs. Control: OR=1.26, 95% CI=1.07 - 1.48; Prostate cancer vs. Control: OR=1.29, 95% CI=1.12 - 1.48) and Asp/Glu+Glu/Glu (Familial cases vs. Control: OR=1.37, 95% CI=1.10 - 1.70; Sporadic cases vs. Control: OR=1.24, 95% CI=1.07 - 1.44; Prostate cancer vs. Control: OR=1.27, 95% CI=1.13 - 1.44) increased the prostate cancer risk in Caucasians, thus suggesting a dominant model for the Glu variant. Analyses by subpopulation also showed no evidence of effects of either Arg462Gln or Glu265X on the prostate cancer risk in Caucasians.

Our meta-analysis of available studies indicates that as compared with genotype Asp/Asp, the Glu variant at the Asp541Glu polymorphism increases the risk of prostate cancer by no more than 2-fold in Caucasian subjects, regardless of family history of the disease. This suggests that genuine genetic effects of this polymorphism probably account for only a small part of prostate cancer in the Caucasian population.

P0613. Chracterization of chromosomal alterations in paraffin-embedded salivary gland tumors by comparative genomic hybridization.

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Salivary gland tumours (SGTs) are rare tumors of the neck and head with an overall incidence in the Western world of approximately 2.5-3/100.000/year. SGTs involve the major glands (parotid, submandibular and sublingual) and the minor glands (oral mucosa, palate, uvula, floor of mouth, posterior tongue, retromolar area and peritonsilla area, larynx and paranasal sinuses).

SGTs are remarkable for their histopathologic and biologic diversity; they include benign and malignant tumors of epithelial, mesenchymal and lymphoid origin.

Although exposure to ionizing radiation has been implicated as a cause of SGTs, the etiology of most of these tumors cannot be determined; moreover it's difficult to determine the prognosis and select the optimal therapeutic modality. The study of molecular pathogenesis of SGTs is a challenging task because of the rarity and histopathological diversity of these malignancies.

Comparative Genomic Hybridization (CGH) is a powerful tool for detecting chromosomal aberrations in archival paraffin embedded tumor samples.

CGH analysis, performed on four adenoid cystic tumor samples (with different hystotypes) and one pleomorphic adenocarcinoma , revealed fourteen aberrations (on average 2.8 per case). Gains involved chromosomal regions 3q28-29, 4q35, 6q27, 10q15, 18p11, 20p13,22q12-13; losses involved 2q36 -q37, 6p25, 11p15, 22q13. The correlation of CGH results with clinical-pathological data and a comparison with literature data will be discussed.

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P0614. Gene copy number profiling mucoepidermoid carcinomas of salivary glands by using array comparative genomic hybridization

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The recent development of DNA microarray- based comparative genomic hybridization has greatly facilitated the identification of chromosomal regions of imbalance and the target genes in them. The purpose of this paper is to identify whole genome aberrations in mucoepidermoid carcinoma (MEC) of salivary gland and to corelate genetic differences and histologic in mucoepidermoid carcinomas. We performed an oligonucleotide based array-comparative genomic hybridization (aCGH) survey in 9 cases of low-and 5 cases of high-grade mucoepidermoide carcinomas. All cases of low-grade MECs had no genomic aberrations while in high-grade MECs were seen a complex for copy number aberrations including 3q amplification.

In conclusion, by using aCGH, we defined differences of copy number aberrations between the histologic grades of the MECs that might be a directly influence to the behavior of these tumors.

P0615. *SEMA6B* is a candidate tumor suppressor gene on 19p13.3

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It was previously suggested, by the results of high-resolution 19p13.2-13.3 allelotyping in breast carcinomas, that this region might contain at least four tumor suppressor genes corresponding to the peak frequencies of loss of heterozygosity (LOH). We have recently found one of the 19p13.3 genes, *SEMA6B*, to be abnormally methylated in breast cancer. The *SEMA6B* promoter CpG island was methylated in 37/98 (38%) tumors and in 3/98 (3%) adjacent apparently normal tissue samples (p<0,0001). Gene expression analysis performed by real-time RT-PCR revealed *SEMA6B* downregulation in 11/25 (44%) breast cancer samples, upregulation in 1/25 (4%) and unchanged ex-

pression (compared to the adjacent tissue) in 13/25 (52%) samples (p=0,0006). Expression was normal in the MCF7 cell line and absent in the T47D cells. *SEMA6B* LOH studies are hampered by the absence of the informative microsatellite markers within the gene. Thus we have elaborated a single nucleotide primer extension-based method allowing semiquantitative analysis with capillary electrophoresis of fluorescently labeled products (SNaPshot, Applied Biosystems). Two intragenic SNPs with the heterozygosity close to 50% were selected for the LOH analysis, one located within exon 1 of the gene (rs2304213) and the other intronic (rs4807602). By this approach *SEMA6B* LOH was detected in 31/98 (32%) samples, indicating high frequency of deletions in this locus. Taken together, our data suggest that *SEMA6B* is a strong candidate tumor suppressor gene on 19p13.3. Recently identified tumor suppressor function of a number of other members of the semaphorin family supports this suggestion. *The study was supported in part by Applied Biosystems, USA.*

P0616. Expression study of receptor tyrosine kinase targets of Imatinib mesylate in skull base chordomas

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Chordoma is a rare embryonal neoplasm arising from notochordal remnants. The current therapy is a combination of surgery and radiotherapy; chemotherapy is not applied due to its reported low efficacy. Evidence on the efficacy of Imatinib mesylate (STI571), an inhibitor of the tyrosine kinases c-kit and PDGFRs, has been reported in five chordoma patients expressing PDGFRβ. We determined the expression of *PDGFRA* and *PDGFRB*, encoding PDGFRα and PDGFRβ receptor subunits respectively, by means of RT-PCR in 15 skull base chordomas including a primary tumor and its recurrency. Fourteen tumors expressed both genes, while the recurrency expressed only *PDGFRB*. We then performed immunohistochemistry of PDGFRα and PDGFRβ in 11 chordomas, which stained stromal cells and focally some tumor cells. In order to identify a possible autocrine or paracrine loop, we determined by RT-PCR the expression of their ligands *PDGFA* and *PDGFB*, which were found to be both expressed in 8 tumors, while *PDGFB* was observed in one sample. Because homodimeric or heterodimeric PDGF ligands activate both homodimeric or heterodimeric PDGFRs, it can be hypothesized that in 9 chordomas PDGFRα and/or PDGFRβ are activated. *KIT* mRNA was detected in three chordomas, while its ligand *SCF* only in one tumor, not expressing *KIT*. The expression study will be extended to additional chordomas to verify whether PDGFR expression in stroma is a characteristic of this kind of tumor. Protein phosphorylation status will be determined in suitable samples. This work provides new insight on STI571 targets in chordoma and might contribute to address pharmacological and clinical research.

P0617. Mutation Analysis of RAP1A in Sporadic Breast Cancer

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Breast cancer is one of the most common neoplasms and is the main cause of death due to cancer among females in civilized and in many developing countries.

Among the genetic abnormalities, Loss of Heterozygosity (LOH) is frequently observed in 1p13.3, the region that is involved in the sporadic type of breast cancer. The tumor suppressor gene, RAP1A, which has a critical role in contact inhibition, is located at this locus. In this study, 50 sporadic breast cancer tumor specimens were examined using PCR-SSCP analysis and sequencing. No deleterious mutations were observed in the promoter (500 base pairs upstream of the transcription initiation site), and the protein-coding exons including exons 3, 4, 5, 6 and 7. We observed variations including an A>T transversion in intron 3 at nt + 29 and nt + 44 (4%). These observations indicate that mutations are not a common cause of RAP1A abnormality in sporadic breast cancer. Other means of RAP1A inactivation such as promoter hypermethylation need to be investigated in sporadic breast cancer.

P0618. Detection of hypermethylated APC DNA in squamous cell carcinoma of esophagus and possible application as a molecular marker

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Squamous cell carcinoma of esophagus (SCCE) is a very lethal type of cancer with the highest incidence rate in Iran. For effective treatment of SCCE, early detection and availability of molecular markers could be very helpful. Methylation of cytidine nucleotide in CpG islands of genes is a mechanism of transcriptional regulation. The importance of this event becomes prominent especially in case of tumor suppressor genes which leads to inactivation of these genes. Evaluation of methylation status of the Adenomatous Polyposis Coli (APC) tumor suppressor gene has formerly been shown to be well applicable as a molecular marker for adenocarcinoma of esophagus. Regarding to this, an optimized method of Methylation Specific PCR was applied to analyze the methylation status of promoter region of APC in patients of SCCE with different stages of tumorogenesis. Extracted DNA from tumor and normal tissues of SCCE were digested with appropriate restriction enzyme, treated with sodium bisulfite, and amplified in a two-step PCR applying methylation specific primers. According to our results, all normal tissues were observed to be unmethylated, while 45% of tumor tissues were methylated such that 35% were homozygous and the 65% were heterozygous. Indeed hypermethylation of APC was seen even in early-stage tissues. These results indicate that hypermethylation of APC gene provides an important mechanism for APC inactivation and its involvment in esophagous cancer, so APC promoter hypermethylation changes could provide a molecular marker system for the detection of esophagus cancer and could be applied in combination with other molecular markers.

P0619. Novel splice isoforms of STRADα differentially affect LKB1 activity, complex assembly and subcellular localization

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STRADα is a pseudokinase which forms a heterotrimeric complex with the scaffolding protein MO25 and the tumor suppressor serine threonine protein kinase LKB1. Mutations in LKB1 are responsible for the Peutz-Jeghers Syndrome (PJS) which predisposes to hamartomatous polyposis and are also observed in some sporadic tumors. The LKB1/STRAD/MO25 complex is involved in the regulation of numerous signaling pathways including metabolism, proliferation, and cellular polarity of human intestinal epithelial cells.

Cell polarization, together with tissue-restricted transcription, represents the main feature of enterocytes differentiation. Since a full-length STRADα transcript has not been reported so far in these cells, we evaluated the expression of endogenous STRADα in 5 colorectal cancer cell lines characterized by their diverse ability to differentiate *in vitro*.

We report herein the absence of full-length STRADα in these cells, and the discovery of several novel splice isoforms of STRADα that differentially affect the kinase activity, complex assembly and subcellular localization of LKB1.

P0620. Construction of a new chimeric receptor for T_cell therapy by using single domain antibody

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The specific activation of the immune system to control cancer growth has been a long-lasting goal in cancer immunotherapy. Employment of T cells for tumor therapy is an attractive approach. In order to redirect and enhance the ability of the patient's own immune cells to fight cancer, the chimeric receptor (CR) approach that endows lymphocytes with antibody specificity was developed. The CR consists of scFv that is linked through an extracellular linker to transmembrane

and cytoplasmic domains of lymphocyte triggering moieties.The most important problem of this type of immunotherapy is scFv, because of its poor solubility and stability, the big size and unstable linker. Part of the humoral immune response of camels and lamas is based on heavy-chain antibodies where the light chain is totally absent. These unique antibody isotypes interact with the antigen by virtue of only one single variable domain, referred to as VHH. VHH have these unique features:

1) Solubility and stability

2) Specificity and binding to their antigen with nanomolar affinity

3) Recognizing unique conformational epitopes that are currently out of reach for conventional antibodies

4) Homology to human VH sequences that causes VHH is not immunogene for human

We are developing a new construct which expresses a CR containing VHH instead of scFv. This construct might provide opportunities to develop a new generation of T cell therapy that hope to solve some of the problems of T_cell therapy.

P0621. A novel approach for the determination of T-cell clonality using microarray

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T-cell lymphomas account for 15-20% of all lymphoid malignancies in Western countries. Determination of T-cell clonality has important for the differential diagnosis between malignant and non malignant T-cell proliferation.

We developed a new method for the post-PCR analysis of TCR-γ rearrangements using hybridization on oligonucleotide microchip. The TCR-γ has become a favorite target for T-cell clonality assays because the γ-chain undergoes rearrangement before the α- and β-chains and, consequently, is rearranged in all lymphocytes.

The microchip contains oligonucleotide probes for all variable (V) and joining (J) gene segments involved in rearrangements of the TCR-γ locus. To estimate the accuracy of the method, we examined 49 samples of patients with lymphoproliferative disorders and 47 samples of normal donors. The results of hybridization on the microchip displayed 100% coincidence with the results obtained by other methods. The sensitivity of the method is sufficient to determine 10% of clonal cells in the sample.

Furthermore this biochip has been used for determination of the of Vγ and Jγ genes frequencies. Among the Vγ gene segments, those of family 1 were most commonly used (total frequency 62%), while the lowest frequency (3.5 %) was observed for the family 4 gene segment Vγ11. As for the Jγ gene segments, the Jγ1 and Jγ2 gene segments are most commonly used in clonal rearrangements: their frequency was 81.6% in the total rearrangements with the Jγ gene segments.

The results demonstrate the principal possibility of detecting T-cell clonality in patients with T-cell lymphoid malignancies by hybridization on the microchip.

P0622. "Familial and sporadic Thyroid Tumors: new variants in candidate genes and their relevance for the oncocytic phenotype."

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Familial Non-Medullary Thyroid Cancer (fNMTC) is associated with some of the highest familial recurrence among all cancers. Inheritance patterns indicate that fNMTC is transmitted as an autosomal dominant trait with reduced penetrance, but a multigenic inheritance is not excluded. TCO (Thyroid tumor with Cell Oxyphilia), a predisposing locus for recurrence of oxyphilic/oncocytic tumors, characterized by the presence of oncocytic cell, rich in mitochondria, was previously mapped to the 19p13.2 region.

In order to identify the TCO gene, genes on chromosome 19p13.2 were analyzed in patients of the families contributing to linkage. We screened 9 genes with a role in tumor development and/or mitochondrial functions: MUC16, DNMT1, ICAM1, EIFS4, MBD3L1, SMARCA4, ILF3, TYK2, ZNF358. We identified new variants in the coding region of MUC16, a member of the mucin family and highly expressed in thyroid tumors. Haplotype analysis and functional study of these variants is currently ongoing and results will be presented.

In parallel, we previously identified disruptive mutations in a panel of sporadic oncocytic thyroid tumors in genes of complex I subunits encoded by mitochondrial DNA. These changes correlated with the biochemical defects identified in oncocytic neoplasia. However, the mutations do not account for all the cases of thyroid oncocytic tumors, suggesting that other nuclear changes might be involved. We are currently screening nuclear genes encoding for the complex I subunits and for PolG, the mitochondrial polymerase. Results of the screening will be presented.

P0623. Tumor necrosis factor gene -308 (G→A) polymorphism and risk of Non-Hodgkin lymphoma

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Common genetic variants in inflammatory response genes can affect the risk of developing Non-Hodgkin lymphoma (NHL). The tumor necrosis factor (TNF) is central in proinflammatory response and therefore represents a strong candidate for mediating the risk of developing immunologicaly related malignant disease. Recent data have suggested that TNF -308 (G →A) polymorphism might be associated with increased risk of NHL. Genotype AA is associated with higher serum levels of soluble TNF.

This study investigated the potential influence of TNF -308 polymorphism on the etiology on NHL in Serbian population. A total of 48 NHL patients and two control groups consisting of 102 diabetes patients and 120 healthy blood donors were included in the analysis. All subject were genotyped for TNF -308(G→A) by PCR-RFLP analysis. One of 48 patients (2%) was homozygous (AA), and 13 of 48 patients (27 %) were heterozygous (GA). In control group consisted of diabetes patients, 3 of 102 (3%) were homozygous and 28 of 102 (27%) were heterozygous and in healthy blood donors control group, two of 120 (2%) were homozygous and 30 of 120 (25%) were heterozygous. Our study found no association between TNF-308 polymorphism and risk of NHL:the differences in allelic and genotype distribution was not statistically significant among tested groups. However, hypothesis on involvement of TNF -308 polymorphism in NHL deserves further investigation on a larger cohort of patients. In future studies additional polymorphisms within the TNF gene and inflammatory response genes should be taken into consideration

P0624. TP53 mutation frequency analysis in breast cancer against other carcinoma types

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The aim of this study was to compare occurrence of somatic mutations at codons 248 and 273 of TP53 gene in patients with breast cancer to patients diagnosed with other types of cancer.

TP53 is a tumor suppressor gene involved in control of cell division and mutations in the gene are considered to represent the most common genetic alteration in human cancers. These mutations may damage the normal function of TP53 as a transcription factor and the induction of repair or apoptosis may be diminished. Consequently, genetic alterations may accumulate in the cell.

Codons 248 and 273 are positioned at DNA binding domain of TP53 gene and mutation that are to be tested are single base substitution A>G in exons 7 and 8. As a result amino acid sequence is changed from Arg-Gln and Arg-His respectively.

This study included 100 subjects, half of which are breast cancer affected individuals and rest are patients with other cancers. Genetic material was extracted from bioptic tissue specimen. Mutations occurrence in TP53 (codons 248 and 273) was determined by PCR analysis using single strand conformation polymorphism and restriction enzyme digestion. Pathohistological findings obtained from clinic were correlated with molecular alterations. Genetic data were evaluated in respect

to available biological markers values (ER, PR, Her2 status, lymph nodes status, age, etc).

P0625. Identification of tumor-suppressor loci that may contribute to the pathogenesis of uveal melanoma

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Uveal melanoma (UM) is the most common primary intraocular neoplasm. More than 50% of uveal melanomas are linked to large deletions or monosomy of chromosome 3, but the specific genetic mechanisms responsible for the malignant behavior of UM are still unknown. OBJECTIVE: To identify tumor-suppressor loci that may contribute to the pathogenesis of UM. METHODS: Loss of heterozygosity on chromosome 3 was investigated by PCR-based microsatellite analysis in 45 tumors and related to clinical data. Microsatellite analysis was performed using markers at 3p25.3, 3p21.3, 3p14.2, 3q26.3, 13q14, 9p21.2-p21.3, close to or within the *VHL, RASSF1, FHIT, TRAIL, RB1, CDKN2A* loci. The methylation status of the *VHL, RASSF1, FHIT, RB1, CDKN2A* promoter regions was analyzed using methyl-sensitive PCR. RESULTS: In the majority of cases, LOH on chromosome 3 was detected at all informative markers, indicating of monosomy 3 (20 of 45 tumors). *RASSF1* promoter methylation was detected in 10 of the 45 (22%) patients with primary UM regardless of LOH status. No hypermethylation of the *VHL* and *FHIT* promoter regions was found. Neither LOH at *RB1* locus, nor hypermethylation of the *RB1* promoter region was found. Methylation of the *CDKN2A* promoter and LOH in its locus occured in some tumors. CONCLUSIONS: These data show that monosomy 3 could be the most common event in UM development, potentially of clinical relevance. LOH and promoter hypermethylation of Rb1-pathway genes are rare events in UM. Further studies are necessary to understand if the *RASSF1* promoter methylation could have a pathogenic effect in UM.

P0626. Inactivation of the *VHL* gene in sporadic clear cell renal cancer

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Renal cell carcinoma is the most common variant of the kidney cancer, which accounts approximately 75% patients with this disease. The majority of those tumors are characterized by inactivation of the *VHL* gene suppressor as a result of mutations, allelic deletions and/or methylation. We have conducted the complex molecular-genetic analysis of 64 samples obtained from patients with the clear cell renal cancer. *VHL* mutations were detected by SSCP and subsequent sequencing, loss of heterozygosity was analyzed using STR-markers D3S1317 and D3S1038, methylation was tested by methylsensitive polymerase chain reaction. All revealed variations were statistically analyzed in respect to the parameters of primary tumors in various groups of patients. Seventeen *VHL* somatic mutations were detected, 12 from which were described for the first time. Allelic deletions of *VHL* were found in 31.6%, and methylation - in 7.8% samples of the renal cancer. As a whole, *VHL* inactivating events were presented in 46.9% cases of disease, in 51.7% - among renal cancer patients with stage I. We have not observed any association of mutations, loss of heterozygosity and methylation with clinical-pathological parameters of disease. Results of this investigation can be used in creation of a molecular markers system of the renal cancer, for example, a methylation panel of suppressor genes, they can be applied in investigation of properties of various *VHL* mutations.

P0627. Molecular genetic testing in Von Hippel-Lindau syndrome: limitations and implication for genetic counselling.

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Von Hippel-Lindau (VHL) is a dominantly inherited cancer syndrome caused by mutations in the *VHL* tumour suppressor gene. Tumours associated to VHL are haemangioblastomas of brain, spinal cord, and retina, renal cell carcinoma, pheochromocytoma and endolymphatic sac tumours. Manifestations and severity are highly variable within and between families. Molecular genetic testing is indicated for at-risk subjects in order to identify those deserving surveillance. We present two kindreds with clinical diagnosis of VHL, molecular genetic testing and counselling. Proband of FamS had bilateral renal carcinoma, kidney and pancreatic cysts, a cerebellar syndrome since infancy and neck-face dystonia, but no brain or spinal haemangioblastomas. Family history: daughter (renal and pancreatic cysts), son (testicular cysts), maternal aunt (metastasic tumour), and cousin (pheochromocytoma age 30). A mutation not previously described in *VHL* was found in the proband and both affected children: c.261_263delATGinsGCT (W88L). A third, 9-year old child, tested negative for W88L. The mutation was not present in her cousin. In FamV two individuals fulfilled VHL criteria (brain and spinal cord haemangioblastomas, pancreatic and renal cysts). No mutations were identified in *VHL* after sequencing and dosage analysis by MLPA. Genetic counselling was requested for a 28 year-old, asymptomatic sibling. None of the affected individuals showed retinal angiomas, one of the cardinal features of VHL. These families exemplify practical situations in which genetic counselling may be hampered: 1) Novel mutations without proven pathogenicity, 2) Tumours of the VHL spectrum in family members not co-segregating the mutation (phenocopies) and 3) Failure to identify the causative mutation.

P0628. A frequent XPC mutation in xeroderma pigmentosum patients from North Africa

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Introduction. Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder that is associated with a germline nucleotide excision repair defect. Patients exhibit extreme sensitivity to sunlight and a 4000-fold increased frequency of skin cancers. Seven XP complementation groups and a variant group have been identified, three of which are believed to be more frequent (XPC, XPA, XPD-ERCC2).
Methods. We investigated the role of XPC, XPD and XPA genes in eight unrelated consanguineous XP families from Maghreb (six from Morocco, two from Tunisia). All index cases presented typical XP symptoms including pronounced photosensitivity, multiple basal cell carcinomas (80%), and malignant melanomas (20%). DNAs was extracted after informed consent and initially subject to microsatellite analysis by studying 2 microsatellites located in each three XP gene. Homozygous patients were further analysed by sequencing the entire gene coding sequence on a ABIPRISM 3130.
Results: A previously reported and recurrent homozygous nonsense mutation in the XPA gene, R228X, was found in a patient from Tunisia. Surprisingly, in the remaining seven unrelated families (87%), we identified the same homozygous frameshift mutation, c.1643_1644delTG, p.V548AfsX572. This mutation was previously reported in two other unrelated families from Maghreb. XPC haplotype analysis highly suggested a common fondator effect.
Conclusion. Our study suggests a predominant involvement of XPC as a XP susceptibility gene in countries from North Africa. The high frequency of the frameshift XPC mutation could, if confirmed, simplify the molecular diagnosis of XP patients from Maghreb, and facilitate prenatal diagnosis if requested by the families.

Po05. Molecular and biochemical basis of disease

P0629. The relationship between the -765G>C COX-2 polymorphism and the development of cutaneous inflammatory process

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Cyclooxygenase-2 (COX-2) is up-regulated in epidermis inflammatory processes and plays an important role in control of keratinocytes proliferation and differentiation. We analysed the association between COX-2 polymorphism (-765G>C) and molecular markers that quantify the inflammatory reaction.

The study was conducted on 67 subjects, divided into 3 groups: 26 normal subjects in group 1, 28 patients with cutaneous malign tumors in group 2 and 13 patients with psoriasis in group 3.

The intensity of inflammatory process was assessed by determining the seric levels of C-reactive protein (CRP), fibrinogen and alpha1-acid glycoprotein (AAG). The -765 G>C variant of the COX-2 gene was genotyped by restriction endonuclease digestion of polymerase chain reaction products.

Patients carrying the -765C allele had markedly lower seric levels of CRP, AAG and fibrinogen vs patients homozygous for -765G in the groups 2, 3 respectively.

The authors suggest that there is an association between COX-2 polymorphism (-765G>C) and the amplitude of cutaneous inflammation, probably as a result of the alteration of binding sites from COX-2 gene promoter for various transcription factors that participate in the initiation and progression/resolution of inflammatory process.

P0630. Progressive Familial Intrahepatic Cholestasis type 3 (PFIC3): evidence of allelic heterogeneity and of a possible evolutionary marker for mammalian ABCB4 genes

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PFIC3 is an autosomal-recessive disorder due to mutations in the ATP-binding cassette, sub-family B, member 4 gene (*ABCB4*). ABCB4 is the liver-specific membrane transporter of phosphatidylcholine, a major and exclusive component of mammalian bile. The disease is characterized by early onset of cholestasis with high serum-glutamyltranspeptidase activity, that progresses to cirrhosis and liver failure before adulthood. We analysed 68 children with a PFIC3 phenotype by sequencing of the entire open reading frame of *ABCB4* gene.In 13 patients mutations were found on both alleles, whereas in 5 cases only one mutated allele was identified; in total, 31 different mutated *ABCB4* alleles were observed with 25 new mutations. Exon 17 showed a higher frequency of mutations (20.7%). The elucidation of the three-dimensional structure of bacterial homologues allows to locate the position of the mutated amino acids in the predicted ABCB4 tertiary structure. The mutations are located in the NBDs (8 mutations, accounting for the 27.6% of the total), in the TMDs (10 cases, 34.5%) and in the ICDs (9 cases, 31%); only, one mutation was found in the EC4 and one in the linker region. Our results indicate a broad allelic heterogeneity of the disease with exon 17 that, as recently demonstrated for the closely related paralogous *ABCB1* gene, could contain an evolutionary marker for mammalian *ABCB4* genes in the seventh transmembrane segment (TM7). The low detection rate of *ABCB4* mutation in our patients (about 26%) suggest genetic heterogeneity of PFIC type 3 disease.

P0631. ABCB4 molecular characterization in patients with Intrahepatic Cholestasis of Pregnancy (ICP): is high serum gamma-glutamyltranspeptidase (gammaGT) level a marker for unambiguous genetic deficiency?

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Intrahepatic cholestasis of pregnancy (ICP) is a disorder that occurs mainly in the third trimester of pregnancy and is characterized by pruritus, elevated fasting serum bile acids (>10µmol/L), and spontaneous relief of signs and symptoms within 2 to 3 weeks after delivery. It is regarded as a benign disease with no meaningful consequences to the mother but associated to an increased perinatal risk with increased rates of fetal morbidity and mortality. To date, γGT is considered a marker to differentiate between ICP due to ABCB4 deficiency (high γGT) from ICP due to ATP8B1 or BSEP deficiency (normal γGT). ABCB4 mediates the translocation of phospholipids across the canalicular membrane of the hepatocyte, a process that is of crucial importance in protecting cholangiocyte membranes from high concentrations of detergent bile acids. We enrolled 26 women with ICP phenotype and all the 27 coding exons of *ABCB4* were sequenced. In three women we identified heterozygous missense mutations: two of them are already described (T424A, R590Q) in others ABCB4 deficient phenotype and one is a new mutation (L627V); all three mutations are located in the very well conserved Nucleotide Binding Domain. Our patients had serum γGT values within the normal range. Our finding indicate that *ABCB4* mutations are present not only in ICP patients with high γGT but also in women with normal γGT; these results are in agreement with the recent finding by linkage analysis of a splice site mutation in a large ICP Mennonite pedigree (Schneider G. et al.; Hepatology 2007; 45(1):150-8).

P0632. A missense CNGA3 mutation which is rare in Western populations is frequent among both Muslim and Oriental Jewish patients with achromatopsia

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Achromatopsia is a heterogeneous autosomal recessive condition characterized by the absence of cone function causing reduced visual acuity, nystagmus, photophobia and lack of color vision. Three causative genes have been identified so far. Aiming to study the genetic basis of achromatopsia in the Israeli population, we recruited 9 Arab-Muslim and 7 Jewish families with the disease. Clinical evaluation included a full ophthalmologic examination, color vision testing and full-field electroretinography. We excluded the most frequently reported achromatopsia-causing mutation, c.del1148C in the CNGB3 gene, in all studied patients. In contrast, a rare missense CNGA3 mutation (c.1585g>a, V529M) that was reported in only three patients worldwide, was found homozygously in patients from 5 families and heterozygously in patients from 2 additional families. Interestingly, the V529M mutation was found in both Arab-Muslim families (6/18 chromosomes, 33%) and Oriental Jewish families from Iraq, Iran, Buchara, and Afghanistan (6/8 chromosomes, 75%). On the other hand, it was not found in any of the 6 analyzed chromosomes from Ashkenazi-Jewish origin. The mutation was also absent in 13 patients with the clinical diagnosis of cone dystrophy. Haplotype analysis using markers located within the CNGA3 gene suggested two different CNGA3 haplotypes in both groups. In summary, we report here the identification of a specific CNGA3 mutation which is the cause of disease in 44% of Israeli achromatopsia cases. Our results demonstrate genetic differences in the etiology of achromatopsia between the Israeli population and the American / European populations that were thus far reported in the literature.

P0633. Acute intermittent porphyria: impact of newly found mutations on the biochemical and enzymatic protein properties

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Acute intermittent porphyria (AIP) is a low-penetrant autosomal dominant inborn error. It results from the half-normal activity of porphobilinogen deaminase (PBGD, EC 4.3.1.8), the third enzyme in the heme biosynthetic pathway. AIP is manifested by life-threatening neurovisceral attacks. Acute attack can be provoked by various factors such as drugs, hormones, and alcohol, and is accompanied by massive accumulation of porphyrin precursors in the urine. To date, over 300 different mutations have been found in the PBGD gene.

During systematic genetic analysis of Czech and Slovak AIP patients, we found four novel mutations (610 C>A, 675 delA, 750 A>T, 966 insA) and five previously reported mutations (76 C>T, 77 G>A, 518 G>A, 771+1 G>T, 973 insG) in eight unrelated families. Mutational screening was performed by PCR, denaturing gradient gel electrophoresis (DGGE), and DNA sequencing.

To establish the effects of these mutations on the protein structure of PBGD, we expressed mutant constructs with the described mutations in *E. coli,* and we analyzed their biochemical and enzymatic properties. All purified enzymes carrying causative mutations had relative activities lower then 1.0 % of the average level expressed by the normal allele.

The identification and characterization of these novel mutations within the PBGD gene of the newly diagnosed AIP patients provide insight into the molecular heterogeneity of AIP. Investigation of the effects of the present mutations on the protein structure and function provide further understanding of the molecular basis and interaction of the molecules in the system.

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P0634. Neurodegeneration in X-Adrenoleukodystrophy: a mitochondrial disease?

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X-linked adrenoleukodystrophy (X-ALD), is the most frequent inherited monogenic demyelinating disease (minimal incidence 1:17,000). X-ALD leads to death in boys or to motor disability in adults (adrenomyeloneuropathy or AMN). The gene mutated in the disease (ABCD1) is a peroxisomal ATP-binding transporter of very-long-chain-fatty acids (VLCFAs), whose accumulation in plasma and tissues is the hallmark of the disease. We have generated and characterized mouse models for X-ALD, by classical knockout of the ABCD1 gene. These mice exhibit a late-onset phenotype closely related to AMN patients, with neurodegenerative features (excitotoxicity, microgliosis, axonal degeneration, slower nerve conduction velocity and psychomotor impairment), that begins at 15 months of age (1,2,3). The pathogenesis of X-ALD is largely unknown, so are the mechanisms of toxicity through VLCFAs. Using microarrays, Q-PCR and Western Blots of mouse spinal cords, we have identified and confirmed dysregulation of oxidative stress routes and mitochondria depletion as early events in the pathogenesis. Indeed, markers of oxidative lesions of lipids (MDAL), glucides (CML) and proteins (AASA) are upregulated in mutant spinal cords. Treatment with VLCFAs of glial and neuronal cultures induces ROS generation and decrease of mitochondria membrane potential. Ex-vivo organotypic spinal cord slice cultures recapitulate closely the pathogenic events seen in spinal cords, and will constitute a powerful screening tool for therapeutic agents, and for deciphering molecular cues underlying neurodegeneration in X-ALD.

(1) Pujol et al, Hum Mol Genet. 2002 Mar 1;11(5):499-505; (2) Pujol et al, Hum Mol Genet. 2004 Dec1;13(23):2997-3006; (3) Ferrer et al, Hum Mol Genet 14(23):3565-77 (2005)

P0635. Functional analysis of secretion-impaired fibrinogen mutants rescued at low temperature: implications for treatment of protein-misfolding disorders.

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Congenital afibrinogenemia is a rare bleeding disorder characterised by the complete absence of fibrinogen in blood. To date, 72 causative mutations accounting for complete fibrinogen deficiency have been identified in the three fibrinogen genes, *FGA*, *FGB* and *FGG*. Among these, 9 missense or late-truncating nonsense mutations have been shown to specifically impair secretion of fully assembled fibrinogen molecules, implying the existence of a quality control for fibrinogen secretion. We previously revealed opposite roles for the homologous betaC and gammaC domains in secretion and demonstrated that secretion-impaired fibrinogen mutants are retained in a pre-Golgi compartment. The aim of this study was to restore the secretion of these mutants and study the properties of the rescued fibrinogen hexamers. We found that the secretion defect of two missense mutants but not that of late-truncating nonsense mutants can be partially corrected by incubating cells at low temperature (27°C) in a co-transfected COS-7 cell model. By contrast, exposure of cells to the chemical chaperones 4-phenylbutyrate (4-PBA), dimethyl sulfoxide (DMSO) and triethylamine N-oxide (TMAO) failed to rescue the secretion of any mutant. The mutants rescued at low temperature could be incorporated into fibrin clots and formed factor XIII-mediated gamma-gamma dimers in contrast to a dysfibrinogenemia mutant, used as a negative control for these assays. However, plasmin digestion analyses revealed abnormal patterns for the mutants compared to normal fibrinogen, suggesting that the rescued mutants have a non-native conformation. Our data underline the importance of careful functional investigations before validating chemical or pharmacological chaperone-based therapies for misfolded proteins.

P0636. *CACNA1F* gene mutation is behind Åland Island eye disease

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Åland Island eye disease (AIED), also known as Forsius-Eriksson syndrome, is an X-chromosomal recessive retinal disease characterized by a combination of fundal hypopigmentation, decreased visual acuity due to foveal hypoplasia, nystagmus, astigmatism, protan color vision defect, progressive myopia and defective dark adaptation. We have previously localized AIED to the pericentromeric region of the X-chromosome, but the causative gene is still unknown. In this study, we screened the *CACNA1F* gene from genomic DNA and lymphoblast mRNA to identify the mutation underlying the disease phenotype in the original AIED family. A novel deletion in the *CACNA1F* gene was identified, covering exon 30 and portions of the flanking introns. Expression studies indicated that the particular exon was excluded from the mRNA. This *CACNA1F* mutation co-segregated completely with the disease phenotype in the AIED family and was not observed in 121 control chromosomes. Mutations in *CACNA1F* are known to cause the incomplete form of X-linked congenital stationary night blindness (CSNB2). Since the clinical picture of AIED is quite similar to CSNB2, it has long been discussed, whether these disorders might be allelic or form a single entity. *CACNA1F* mutations have been identified in patients with an AIED-like phenotype, but previous studies have failed to reveal any *CACNA1F* mutation in patients of the original AIED family. Our results now show that AIED is caused by a novel deletion mutation within *CACNA1F*.

P0637. Gene conversion leading to a severe Adenylate Kinase deficiency.

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We describe a family in which a 15-year-old boy displays a non-spherocytic chronic haemolytic anaemia associated with a mental retardation, and a Willebrand disease. This phenotype led us to measure the

activities of erythrocyte enzymes including the Adenylate Kinase 1. The proband had an AK1 deficiency (OMIM:103000) with an activity equal to 0. This rare recessive disorder is due to mutations in AK1 (GenBank J04809; 9q34.12). Molecular screening of the AK1 gene showed the proband to be homozygous for a missense mutation within exon 4 (c.63G>T; p.Lys21Asn) whereas the mother was heterozygous for this mutation and the father wild type. To explain this genetic discrepancy, we searched a familial inconsistency and/or a deletion in the AK1 paternal allele in the proband. Microsatellites analysis on chromosomes 1 to 5 showed an exclusion of paternity; and using QMPSF method we found that the proband was not hemizygous for the AK1 exon 4. We also showed that the proband had two different chromosomes 9, by studying haplotypes for several microsatellites tightly surrounding the AK1 locus, ruling out the hypothesis of a consanguinity. Considering the rarity of the AK1 deficiency (only 9 families described including 3 molecular characterizations), the most likely event leading to this genotype should be a conversion of the paternal allele.

Enzymes activities and haplotypes surrounding AK1 locus (9q34.1)

	Proband	Mother	Father
AK1 (N: 164-228 UI/gHb)	0 UI/gHb	108 UI/gHb	292 UI/gHb
Hexokinase (N: 0.74-1.14 UI/gHb)	1.00 UI/gHb	0.80 UI/gHb	0.85 UI/gHb
D9S290 D9S159 D9S1831 2.5 Mb D9S179 D9S1847 D9S164	**262**/262 **301**/299 **258**/258 AK1 **247**/233 **190**/182 **91**/96	**262**/260 **301**/293 **258**/243 AK1 **247**/241 **190**/182 **91**/97	

P0638. Elevated alpha synuclein mRNA levels are associated with alcohol dependence

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Dopaminergic neurotransmission has been suggested to be a main system mediating alcohol dependence. However, genetic factors that contribute to risk for alcohol addiction are unknown. We hypothesize that genetic variations of gene expression of key proteins of dopaminergic system determine the basic phenotypes associated with alcohol dependence. Several studies have linked alcohol dependence phenotypes to chromosome 4. One candidate gene is a gene coding for alpha synuclein (SNCA). Therefore, the main goal of our study was to investigate whether the expression of alpha synuclein gene is associated with alcoholism. The SNCA mRNA expression level was measured by quantitative polymerase chain reaction in the peripheral lymphocytes of 26 male alcoholics and 20 nondrinking healthy control subjects from North West region of Russia. The expression differences between control and alcohol were evaluated using one-way ANOVA the SPSS 12.0 software package. The SNCA expression in patients with alcoholism (1,2 ΔCT; SD 0,53; p=0,006) was significantly higher when compared with healthy control subjects (0,8 ΔCT; SD 0,4). In contrast, no differences were found for mRNA expression for transcription factor Nurr1(NR4A2) in lymphocytes between these groups (p=0,09). We suggest that increased level of alpha synuclein might contribute to the development of alcohol dependence in addicted patients.

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P0639. Sixteen novel mutations identified in *COL4A3*, *COL4A4* and *COL4A5* genes in Slovenian families with Alport syndrome and benign familial hematuria

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Alport syndrome and benign familial hematuria are type IV collagen inherited disorders. Mutations in *COL4A5* are generally believed to cause X-linked Alport syndrome, while mutations in *COL4A3* and *COL4A4* genes can be associated with the autosomal recessive and dominant type of Alport syndrome or benign familial hematuria. In view of the wide spectrum of phenotypes, an exact diagnosis is sometimes difficult to achieve. This study involved screening each exon with boundary intronic sequences of *COL4A3*, *COL4A4* and *COL4A5* genes by optimised PCR-SSCP analysis in 17 families with Alport syndrome and in 40 families diagnosed as having benign familial hematuria. Twelve different mutations were found in the *COL4A5* gene in Alport syndrome patients, comprising nine missense mutations, a splice site mutation, a mutation causing frameshift and a nonsense mutation. One of the missense mutations (p.G624D) was present not only in one family with Alport syndrome, but also in five families with suspected benign familial hematuria. Three heterozygous mutations in the *COL4A3* gene (two missense and one frameshift) and four heterozygous mutations in *COL4A4* (two splice site, one in-frame deletion and one missense) were identified in patients with benign familial hematuria.

P0640. Cytoskeleton proteins are modulators of mutant tau-induced neurodegeneration in drosophila

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Tauopathies, including Alzheimer's disease (AD) and fronto-temporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), are a group of neurodegenerative disorders characterized by the presence of intraneuronal filamentous inclusions of aberrantly phosphorylated tau. Tau is a neuronal microtubule-associated protein involved in microtubule assembly and stabilization. Currently, the molecular mechanisms underlying tau-mediated cellular toxicity remain elusive. To address the determinants of tau neurotoxicity, we first characterized in *Drosophila* the cellular alterations resulting from the overexpression of a mutant form of human tau associated with FTDP-17 (tau V337M). We found that the overexpression of tau V337M, in *Drosophila* larval motor neurons, induced disruption of the microtubular network at presynaptic nerve terminals and changes in neuromuscular junctions morphological features. Second, we performed an extensive misexpression screen to identify genetic modifiers of the tau V337M-induced neurodegeneration. The screening of 1250 mutant *Drosophila* lines allowed us to recover 30 modifier genes, including several components of the cytoskeleton, and particularly from the actin network. Furthermore, we found that numerous tau modulators identified in our screen were involved in maintenance of synaptic function. Taken together, these findings strongly suggest that disruption of the microtubule network in presynaptic nerve terminals could constitute early events in the pathological process leading to synaptic dysfunction in tauopathies.

P0641. Possible mechanism of mirror symmetry motifs forming in amyloidogenic proteins

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In our previous investigation we showed that mirror symmetry motifs play role in abnormal fibrillogenesis of several proteins. Now we suggest the mechanism of such motifs forming. This mechanism is similar to mechanism of the expansion diseases developement. So we show that expansion of repeats in DNA sequence may lead to mirror symmetry motifs forming. A computational method for mining shared short repeats in protein sequence was developed. Testing of our in silico method on sequence of prion protein and alpha-synuclein allowed us to find known repeats in amyloidogenic determinants of these proteins. This method can be useful for mining of potential amyloidogenic determinants in proteins. Proposed mechanism allow us to suggest a role of short DNA repeats in forming of protein-protein interaction sites during evolution.

P0642. A novel frameshift mutation in SLC12A6 in patients with Andermann syndrome.

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Andermann syndrome (OMIM 218000) or agenesis of the corpus callosum with peripheral neuropathy (ACCPN) is an autosomal recessive trait characterised by severe progressive motor and sensory neuropathy, mental retardation and a variable degree of agenesis of the corpus callosum. The disease is rare, but has a high prevalence in the province of Quebec, Canada due to a founder effect. Up to date five different protein-truncating mutations and one missense mutation were found in the gene that encodes the solute carrier family 12 (potassium/chloride transporters), member 6 (SLC12A6)(1,2).
Here we report a Turkish family with a novel homozygous truncating mutation in SLC12A6 in 2 sibs. The patients have agenesis of the corpus callosum and motor and sensory neuropathy consistent with the diagnosis of Andermann syndrome. Mutation screening was performed by sequencing analysis of all coding exons and intron/exon boundaries of SLC12A6 (NM_133647.1). In exon 21, a 1-bp deletion was detected at nucleotide position 2902 (c.2902delC). This mutation is predicted to cause a frameshift and a premature stop (Arg968fsX6), deleting the K-Cl co-transporter domain of the SLC12A6 protein. The mutation was homozygous in both patients; the asymptomatic parents were heterozygous for the sequence variation. This study extends the spectrum of SLC12A6 mutations observed in patients with Andermann syndrome.
1. Howard HC et al. Nat Genet 2002; 32(3):384-392.
2. Uyanik G et al. Neurology 2006; 66(7):1044-1048.

P0643. Exclusion of SARA2 gene in 2 families with genetic lipid malabsorption syndrome: Anderson's disease (or Chylomicron Retention Disease).

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The study of naturally occurring mutations in hypocholesterolemic patients presenting with lipid malabsorption syndrome has been useful in identifying new target for lipid-lowering therapy. Anderson's disease (or Chylomicron Retention Disease), is a very rare lipid malabsorption syndrome, usually diagnosed in early infancy, characterized by an inability to export dietary lipids as chylomicrons due to a recessive genetic defect in lipoprotein secretion. Recently, the molecular basis of this defect was shown to be due to mutations in the SARA2 gene encoding the Sar1b protein. This protein is involved in the vesicular transport between the endoplasmic reticulum and the Golgi apparatus. We report here 4 patients from 2 families, clearly diagnosed as Anderson's disease patients. They had low levels of cholesterol and of lipid soluble vitamins. No chylomicrons were secreted after a fat load, and alpha and betalipoproteins were 50% of normal. Endoscopy showed a typical white stippling-like hoar frosting covering the intestinal mucosal surface. Ultrastructural examination of intestinal biopsies showed an accumulation of free lipid and lipoprotein-like particles, reflecting the secretory defect. Direct sequencing of the 7 exons of the SARA 2 gene revealed no mutation. This result thus excludes, in these patients, SARA2 as the molecular basis of the lipoprotein secretory defect and suggests the existence of at least another gene. Proteins involved in the intracellular processing of chylomicron secretion would be good candidates.
Informed written consent was obtained from all participants or from their legal guardians (RBM 93018, CCPPRB 94002).

P0644. Two novel mutations in the AR gene in Greek patients with complete androgen insensitivity syndrome

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Androgen Insensitivity Syndrome (AIS) is an X-linked disorder, characterized by incomplete or absent virilization in 46XY individuals, caused by mutations along the Androgen Receptor (AR) gene. We report on two novel mutations, one frameshift (Gln711ArgfsX787) and one missense (Leu881Pro) detected in two unrelated patients referred with complete AIS. Patients 1 and 2, aged 9 and 14 years old respectively, had a 46, XY karyotype but exhibited a complete female phenotype. Both presented with inguinal hernia, while patient 2 also reported primary amenorrhea. PCR reaction was performed for exons 2-8 of the AR gene followed by direct sequencing. The Gln711ArgfsX787 mutation, revealed in patient 1, concerns a single nucleotide deletion (2494delA) at codon 711. This frameshift mutation results in glutamine to arginine substitution at that position as well as in the formation of a premature stop codon at position 787. Further molecular analysis revealed the same mutation in patient's mother as well as in two affected with CAIS maternal aunts. The Leu881Pro mutation, detected in patient 2, is a single base alteration (3004 T>C) that results in leucine to proline substitution at position 881. Molecular analysis of the patient's mother revealed normal alleles in her peripheral blood thus implying that the mutation is either de novo or the result of a possible gametic mosaicism. Both mutations are reported for the first time and provide further evidence on the phenotypic outcome of various AR mutations.
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P0645. G743E mutation in the androgen receptor gene detected in a familial primary amenorrhea

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Androgen insensitivity syndrome (AIS) is an X-linked genetic disorder of male sexual differentiation caused by mutations in the androgen receptor (AR) gene. The defects of receptor function that have been characterized fall into two categories. The first are those that disrupt the primary sequence of the AR. The second is that which is caused by single amino acid substitutions within the AR protein. Mutations in the AR gene result in a wide range of AIS phenotypes.
We report here a new case of amino acid replacement G◊E at codon 743 in exon 5 within the hormone binding domain of the AR in a 17-year-old pubertal female who consulted for primary amenorrhea. She had a family history of amenorrhea (2 aunts) but no history of ambiguous genitalia. She had fully developed breasts, pubic hairs, and no axillary hair (Tanner stage M4, P3, A0). External genitalia were a normally sized clitoris, normal labia majora and minora. The following serum levels of hormones were determined: Testosterone, 9,6 ng/ml; estradiol, 50 pg/ml and LH, 5,6 mUI/ml. A sonogram of the pelvis demonstrates the absence of uterus and the presence of testes in the inguinal canal. The karyotype was 46,XY. This female phenotype with pubic hair is defined as partial AIS.
At our knowledge, this mutation was identified in only one case led to complete AIS. With the advent of molecular analysis of the AR gene, it was hoped that a correlation between a molecular defect and a particular phenotype could be established.

P0646. Analysis of molecular defects in Polish Angelman Syndrome patients

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Angelman Syndrome (AS) is a rare neurogenetic disorder that is caused by impaired *UBE3A* gene expression resulting from changes in the parental genomic imprinting or specific mutations. Four known types of molecular defects might be responsible for the AS: deletion of 15q11-q13 region, paternal uniparental disomy, imprinting defects or *UBE3A* mutations. The imprinting defects may result from microdeletion of the imprinting center or somatic mosaicism. The aim of the study was the identification of molecular defect in the patients with clinical manifestation of AS.
Two hundred twelve patients with clinical symptoms of AS were included in the study. First, the analysis of imprinting defect was done with PCR-based DNA methylation analysis of *PW71* and *SNRPN loci* (MS-PCR). When the mosaic methylation pattern was detected, the quantitative methylation assay (QAMA) has been performed (28 cas-

es). The patients with the normal methylation pattern were qualified to the analysis of the *UBE3A* coding sequence.
The QAMA analysis did not reveal somatic mosaicism in any of the patients selected for this procedure. In patients without imprinting defect several single nucleotide polymorphisms have been found (T/G in intron 7, T/C in intron 9, A/G in exon 13, insT in intron 6). In one patient, the G>A substitution in exon 6 was found that changes alanine into threonine within hydrophobic domain of the protein.
The molecular analysis performed for AS patients revealed several new genetic variantsin *UBE3A*. Moreover, the QAMA method was developed for the mosaicism detection and can be used to diagnostic procedure of AS.

P0647. RSPO4 mutations cause autosomal recessive anonychia and cluster in the furin-like cysteine-rich domains of the Wnt signaling ligand R-spondin 4

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Congenital anonychia is a rare autosomal recessive disorder characterized by absence of finger- and toenails. In a large German non-consanguineous family with four affected siblings with isolated total congenital anonychia we performed genome-wide mapping and showed linkage to 20p13. Analysis of the RSPO4 gene within this interval revealed homozygous and compound heterozygous mutations in this and other families with autosomal recessive anonychia of different ethnic origins. All mutations were not present among controls and shown to segregate with the disease phenotype. RSPO4 is a member of the recently described R-spondin family of secreted proteins that have been shown to activate the Wnt/ß-catenin signaling pathway known to be evolutionary conserved and pivotal for embryonic development by regulation of cell morphology, proliferation, and motility. Given that all but one RSPO4 mutations detected so far, affect the highly conserved exons 2 and 3 and adjacent introns, it can be postulated that RSPO4 mutations cluster in the furin-like cysteine-rich domains. This is in line with experimental data proposing that for ß-catenin stabilization a shortened R-spondin 4 protein comprising just these two furin-like regions is sufficient. Our findings add to the increasing body of evidence indicating that mesenchymal-epithelial interactions are crucial in nail development and put anonychia on the growing list of congenital malformation syndromes caused by Wnt signaling pathway defects. To the best of our knowledge, this is the first gene known to be responsible for an isolated, non-syndromic nail disorder.

P0648. Somatic APC mosaicism in FAP quantified by SNaPshot analysis

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Somatic mutational mosaicism presents a challenge for both molecular and clinical diagnostics and may contribute to deviations from predicted genotype-phenotype correlations. During APC mutation screening in 1248 unrelated patients with familial adenomatous polyposis (FAP), we identified 75 cases with an assumed or confirmed de novo mutation. Prescreening methods (PTT, DHPLC) indicated the presence of somatic mosaicism in eight cases (11%).
Sequencing of the corresponding fragments revealed very weak mutation signals, pointing to the presence of either nonsense or frameshift mutations at low level. All mutations were confirmed and quantified by SNaPshot analysis: in leukocyte DNA taken from the eight patients, the percentage of mosaicism varied between 5.5% and 77%, while the proportion of the mutation in adenomas was consistently higher.
The eight mutations identified as mosaic are localised within codons 216-1464 of the APC gene. According to the known genotype-phenotype correlation, patients with mutations in this region exhibit typical or severe FAP. However, six of the eight patients presented with an attenuated or atypical polyposis phenotype.
Our data demonstrate that in a fraction of FAP patients the causative APC mutation may not be detected due to weak signals or somatic mosaicism that is restricted to tissues other than blood. SNaPshot analysis was proven to be an easy, rapid, and reliable method of confirming low-level mutations and evaluating the degree of mosaicism. Part of the deviations from the expected phenotype in FAP can be explained by the presence of somatic mosaicism.
The study was supported by the Deutsche Krebshilfe.

P0649. Apolipoprotein A5 T-1131C alleles in pediatric patients with obesity and metabolic syndrome

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The prevalence of the metabolic syndrome is elevated among obese children and adolescents. The aim of present study was to examine the distribution of -T1131 C allele of apolipoprotein A5 gene in pediatric patients with metabolic syndrome and obesity. A total of 76 children with metabolic syndrome and 64 with obesity were studied. In both groups the serum triglycerides were significantly higher as compared with 116 pediatric, or with 189 adult controls ($p<0.05$). Triglyceride levels in children carrying -1131C allele were significantly increased, compared to the subjects carrying -1131T allele ($p<0.05$). In obese children the prevalence of the ApoA5 -1131C allele frequency was approximately two fold elevated compared to the healthy pediatric and adult controls (10.9% vs. 6.40% and vs. 5.60% respectively; $p<0.05$); while in metabolic syndrome patients the prevalence of the ApoA5 -1131C allele frequency did not differ from the healthy pediatric or adult controls (6.60% vs. 6.40% and vs. 5.60%). Multiple regression analysis model adjusted for total serum cholesterol levels, BMI, triglycerides revealed that the ApoA5 -1131C variant confers risk for the development of obesity in children (OR at 95% CI was 5.95, range 1.48-23.97, $p<0.012$). Since the -T1131C variant was found previously to confer risk for adult metabolic syndrome, increased prevalence of this variant in pediatric obese subjects suggest, that perhaps these children are the future selected candidates to develop metabolic syndrome in adulthood.

P0650. Apolipoprotein A5 T-1131C variant confers risk for metabolic syndrome

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Objectives: The promoter region T-1131C mutation of the recently identified apolipoprotein A5 (ApoA5) gene has been shown to associate with increased triglyceride levels in several studies. This variant was found to confer risk for development of ischaemic heart disease and stroke. The gene is in linkage disequilibrium with factors known to correlate with impaired glucose homeostasis; one of the criterias of metabolic syndrome. Therefore, the distribution of the ApoA5 -1131C allele was examined in patients with metabolic syndrome. *Methods and results*: PCR-RFLP assays were performed to detect the -1131C variant in a total of 122 patients affected with metabolic syndrome and 210 controls. The triglyceride levels of patients carrying -1131C allele were significantly elevated in carriers than in non-carriers in both groups (3.40 ± 0.59 mmol/l vs. 2.40 ± 0.14 mmol/l in the metabolic syndrome patients, $p<0.05$; 2.10 ± 0.19 mmol/l vs. 1.22 ± 0.05 mmol/l in the controls, $p<0.05$). The serum cholesterol levels did not differ between subjects with C allele and non-carriers in either group. In metabolic syndrome patients the prevalence of the ApoA5 -1131C variant was increased compared to the healthy controls (12.7% vs. 6.20%, $p<0.05$). Logistic regression analysis model adjusted for differences in age, gender, total serum cholesterol levels, acute myocardial infarcts and stroke events revealed that the ApoA5 -1131C variant confers risk for the development of metabolic syndrome (OR: 2.458 at CI: 95%: 1.294-4.671; p=0.006). *Conclusions*: The data presented here show that bearing ApoA5 T-1131C variant represent independent risk factor for metabolic syndrome.

P0651. Androgen insensitivity syndrome: mutations in exon 3 of *AR* gene in patients with partial and mild forms

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Androgen insensitivity syndrome (AIS) is one of the entities of absent or deficient masculinization in individuals 46, XY. It can present a clinical spectrum [complete (normal female phenotype), partial (P) or frankly ambiguos, and mild (M) or normal or near-normal male], depending on alterations of the androgen receptor gene (*AR*), located on chromosome Xq11-12. The objective of the present study was to evaluate patients referred to the University Hospital, School of Medicine of Ribeirão Preto (USP), with a clinical diagnosis of AIS. The medical records of 31 patients were reviewed, and 14 patients with a probable diagnosis of AISP and eight with AISM were submitted to cytogenetic analysis and studied by PCR-SSCP, for each of the eight exons of the *AR* gene. One novel point mutation not previously reported in the literature involving exon 3 was detected in an AISP patient (Met623Ile). Three other point mutations previously described in the same exon were identified: one patient with AISP (Arg607Gln), one patient with AISM (Gly589Gln), and one patient with AISM (Tyr602Pro). Based on these results, it was verified that it is possible to systematically employ SSCP-PCR as a very useful screening tool of patients referred to medical services, for the confirmation of the etiopathogeny of the disease, the clinical management of patients and genetic counseling.

P0652. Genotyping of patients with arrhythmias

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Electrical heart disorders are caused by problems with the electrical system that regulates the steady, rhythmic beat of the heart. Some arrhythmias are dangerous and cause sudden cardiac death. Other type of the electrical heart disorder is the Long QT Syndrome (LQTS). LQTS is associated with two cardiac muscle ion channels: voltage-gated K^+ channels and voltage-gated Na^+ channels. To the voltage-gated K^+ channels belong I_{Kr} and I_{Ks} channels. I_{Ks} channels consist of KvLQT1 (*KCNQ1*) as an α subunit and minK (*KCNE1)* as a β subunit, while I_{Kr} channels have HERG (*KCNH2*) subunit and MiRP1 subunit.
Common genetic variations might modify arrhythmia susceptibility in the general population. Polymorphisms located within the gene coding region can directly influence the structure of its protein product, while others located within the gene regulatory sequence can influence the regulation of its protein expression levels. For this reason it is important to study polymorphisms of genes associated with LQTS (play important role in standard heart function) in sudden cardiac death survivors.
The mutation analysis of *KCNQ1* and *KCNH2* was performed using methods multiplex-PCR, multiplex SSCP (single strand conformation polymorphism) as the screen method, and automated sequencing. In the group of of 20 patients (sudden cardiac death survivors), we have identified some characteristic nucleotide polymorphisms (Y662Y, I489I, F513F, IVS13+22G>A, IVS8+40GG>CA).
Prevalence of cardiac ion channel genes polymorphisms seems to be much more common than hypothesized thus far.
This work was supported by grants IGA MZ CR NR/9340-3, MSM 0021622415 and by the Czech Society of Cardiology.

P0653. Identification and functional characterization of three novel mutations in desmocollin-2 gene associated with arrhythmogenic right ventricular cardiomyopathy

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Mutations in genes encoding desmosomal proteins have been reported to cause arrhythmogenic right ventricular cardiomyopathy (ARVC), an autosomal dominant disease characterised by progressive myocardial atrophy with fibro-fatty replacement. We screened 54 ARVC probands for mutations in desmocollin-2 (*DSC2*), the only desmocollin isoform expressed in cardiac tissue. We identified three heterozygous mutations (c.304G>A (p.E102K), c.631-10C>T and c.1034C>T (p.I345T)) in three probands and in five family members. The two missense mutations p.E102K and p.I345T map to the N-terminal region, relevant to adhesive interactions. In individual carrying the c.631-10C>T mutation, cDNA analysis failed to detect aberrant transcripts, and real-time quantitative RT-PCR showed an approximately 50% reduction of *DSC2* expression, implicating haploinsufficiency as the operant mechanism in this *DSC2* mutation.
To evaluate the pathogenic potentials of the *DSC2* missense mutations detected in patients affected with ARVC, full-length wild-type and mutated cDNAs were cloned in eukaryotic expression vectors to obtain a fusion protein with green fluorescence protein (GFP); constructs were transfected in neonatal rat cardiomyocytes and in HL-1 cells. Unlike wild-type DSC2, the N-terminal mutants are predominantly localised in the cytoplasm, thus suggesting the potential pathogenic effect of the reported mutations.
Mutations in *DSC2* gene in patients with ARVC provide further evidence that many forms of ARVC are due to alterations in cell-cell adhesion.

P0654. Assay of determination of the frequency of different desmosomal genes in Arrhythmogenic right ventricular cardiomyopathy

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Background. Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a rare disease characterized by fibrofatty replacement in the right ventricular. It is a familial disease in 30-50%, with autosomal dominant inheritance. Genes encoding desmosomal proteins were identified as responsible for ARVC. Preliminary studies suggest that plakophilin-2 (PKP2) is involved in about 11-47%, desmoglein (DSG2) in 10% and desmoplakin (DSP) in 5-16% of ARVC. Mutation in Plakoglobin gene (JUP) is reported in autosomal recessive Naxos disease. Objectives. Our aim was to identify mutations in PKP2, DSG2, DSP and JUP genes and estimate their prevalence in ARVC patients. Methods. For 23 independent patients with classical ARVC, after DNA extraction from blood samples, mutation screening analysis was performed by direct sequencing of PKP2, DSG2, JUP and DSP genes. Results. PKP2 gene: Eight causal mutations (3 frame shift, 2 small deletions, 1 insertion, 2 missense) and 3 additional missense variants were identified in 10/22 index patients. DSG2 gene: three mutations were found in 6 / 16 patients (1 frame shift and 2 missense mutations). Among 19 analysed patients, two missense mutations were identified in DSP and none in JUP. Conclusions. We reported high prevalence of PKP2 and DSG2 gene mutations among ARVC patients with respective frequency of 45% and 37%. Rarely, we found mutations in DSP gene (1%) and none in JUP gene in our population. These results highlight the frequency of the PKP2 and DSG2 gene involved in ARVC and suggest the interest of molecular analysis of this gene for clinical purpose.

P0655. ARX syndrome is due to a variable duplication size at the critical region

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We describe a novel polyalanine tract expansion mutation of the ARX gene, a 27-bp duplication of exon 2, which segregates in a newly identified family.
The index case was ascertained due to psychomotor retardation, hypotonia and myoclonic seizures with normal brain MRI. Additional affected boys were reported in this family, which is otherwise compatible with X linked inheritance. Using PCR and sequence analysis on peripheral blood cell DNA, a 27-bp duplication of the critical region of

exon 2 of the ARX gene, starting from position c.430, was detected. This mutation affects the same region as the recurrent 24-bp duplication. Mutation segregation and haplotype analysis shows that this mutation arose *de novo* in the grand mother on the great grand paternal chromosome. Our data also suggest that the grand mother might be a somatic mosaic for the 27-bp duplication. The 27-bp mutation was transmitted steadily, i.e. without signs of somatic instability, through 2 generations leading to the affected proband.
Conclusions: 1. ARX gene mutation analysis is strongly recommended in families with yet undefined X linked mental retardation. 2. The critical region of exon 2 may involve at least two variable duplication sizes, 24bp and also 27 bp. 3. Post zygotic duplication leading to mosaicism in the carrier female, may be a possible mechanism in the evolution of the ARX condition.

P0656. Hypometylation at the KvDMR in clinically normal children conceived by assisted reproductive technologies

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Recent evidences have suggested an association between assisted reproductive technologies (ART) and alterations in the mechanism of genomic imprinting. Beckwith-Wiedemann syndrome (BWS) is a congenital overgrowth disorder characterized by abnormalities in the 11p15.5, mainly hipomethylation at the KvDMR imprinting control region. A 4-9 fold increased incidence of BWS has been reported in children conceived by ART when compared to naturally conceived children. In the present study, we investigated the methylation pattern at the KvDMR, by the methylation-specific PCR approach (MS-PCR) in samples obtained from 18 clinically normal children conceived by ART. Abnormal methylation pattern (hypomethylation) at KvDMR was found in three children. Paternal uniparental disomy (UPD) was considered unlikely by the identification of normal methylation pattern (mono-allelic) at H19DMR in all patients. Although the limited sample size and the qualitative aspect of methylation obtained by the MS-PCR approach, we speculate that hypomethylation at the KvDMR is not sufficient to cause the clinically aspects of BWS and might be present in clinically normal children conceived by ART. Additionally, follow-up studies are indicated to determine the consequences of this unusual hypomethylated pattern at the KvDMR in these cases.

P0657. Sperm Mitochondrial DNA is better than blood in pathogenesis of asthenozoospermia

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Mitochondria play an important role in cellular energy metabolism, free radical generation and apoptosis. The number, shape and size of mitochondria vary from tissue. In addition number of mitochondrial DNA also vary. Mitochondria thus contain their own genome and their own transcription, translation and protein assembly machinery. Mutation in mitochondrial DNA has been known to cause several neuromuscular diseases, cardiomyopathies but their role in pathogenesis of spermatogenetic arrest and impaired sperm motility is not known. In the present study we analysed mitochondrial mutation in blood and sperm mitochondrial DNA. The mitochondrial DNA mutation in sperm DNA was higher (32 substitutions) as compared to blood (20 substitutions). A comparison of the sequences of the above genes with a reference sequence revealed a total of 32 nucleotide substitutions in the sperm mtDNA but not in the DNA from the blood cells. Of the 32 substitutions in sperm DNA, 7 were in COI, 12 were in COII, 4 were in ATPase8, and 9 were in ATPase6. This may be due to ROS mediated damage. As sperm has just 10-100 copies of mitochondrial genome,mutations in these result in early phenotype manifestation. In such cases sperms of men with impaired motility are due to disruption of OXPHOS pathway. The effects of these mutations to correlate the mitochondrial DNA mutation with phenotype are required. This will aid in providing comprehensive counseling and most adapted therapeutics to the couple.

P0658. Association Analysis MMP9 Polymorphisms with Asthma in Mexican population

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Asthma is an inflammatory disease of the small airways of the lung. Asthma has now reached epidemic proportions, with more than 10% of children being affected in many westernized societies. This disease has been characterized by chronic and allergic airway inflammation, which induces cytological and histological changes in the in the arirway structure overtime. Those changes have been called airway remodeling, involving changes in the extracellular matrix, collagen and elastin; airway smooth muscle globet cell hyperplasia; subepitelial fibrosis; and hyperplasia and hypertrophy of airway smooth muscle cells. Recent studies have demonstrated the molecular and cellular mechanisms of remodeling. Matrix metalloproteinases (MMPs) and the metalloproteinase inhibitor (TIMP)-1 play an important role in tissue remodeling. Patients with severe or uncontrolled asma show increased activity of these proteins in the bronchial biopsy specimens, blood, induced sputum, and brochoalveolar fluid. Our aim was to investigate whether MMP9 and TIMP1 polymorphisms are associated with asthma in Mexican Pediatric patients. We performed a case-control study in 110 Mexican patients with clinical diagnosis of asthma and 250 control individuals without allergy or asthma history. We genotyped 3 SNPs in the MMP9 and TIMP1 genes using the 5´ exonuclease assay (TaqMan) for allelic discrimination. The TIMP1 polymorphism did not show association with asthma. However, both MMP9 SNPs analyzed (rs2274755G/T and rs2274756G/A: R668Q) which are in linkage disequilibrium were significantly associated with the risk of childhood asthma (5.2% vs 2%, P=0.003; OR= 2.30; 95% CI = 1.30-4.06). Our results shown that the MMP-9 gene might be involved in the development of asthma in our population.

P0659. Role of mitochondria in Ataxia-Telangiectasia: Investigation of mitochondrial deletions and Haplogroups

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Ataxia-Telangiectasia (AT) is a rare human neurodegenerative autosomal recessive multisystem disease that is characterized by a wide range of features including, progressive cerebellar ataxia with onset during infancy, occulocutaneous telangiectasia, susceptibility to neoplasia, occulomotor disturbances, chromosomal instability and growth and developmental abnormalities. Mitochondrial DNA (mtDNA) has the only non-coding regions at the displacement loop (D-loop) region that contains two hypervariable segments (HVS-I and HVS-II) with high polymorphism. We investigated mt-DNA deletions and haplogroups in AT patients. In this study, 24 Iranian patients suffering from AT and 100 normal controls were examined. mt-DNA was extracted from whole blood and examined by 6 primers for existence of mitochondrial deletions. We also amplified and sequenced the mtDNA HVS-I by standard sequencing techniques. mtDNA deletions were observed in 54.1% (13/24) of patients (8.9 kb deletion in all samples, 5.0 kb in one and 7.5 kb in two patients), representing mtDNA damage which may be due to oxidative stress in mitochondria. Our results showed that there is no association between mtDNA haplogroups and AT. This data may indicate involvement of mitochondrial damage in the pathogenesis of AT.

P0660. ABCA1 transporter in atherosclerosis development.

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Abnormalities in reverse cholesterol transport (RCT) are involved in arterial atherosclerosis (AA). ABCA1 transporter is a key protein of RCT and facilitates cholesterol efflux from peripheral cells to high density lipoproteins particles. However, the genetic factors that contribute in ABCA1-mediated RCT are not well understood.
It is reasonable to propose that polymorphic alleles of ABCA1 as well as genetic variation in ABCA1 gene expression may contribute to AA. Our aim was to study correlation between polymorphic markers C69T, C(-17)G, 319insG, R219K of ABCA1 gene, the level of ABCA1 gene

expression and AA development. We have investigated association of C69T, C(-17)G, 319insG, R219K genetic variants with atherosclerotic lesions among patients with angiographically verified atherosclerosis (N=108). Carriers of 319insG allele of ABCA1 gene have less atherosclerotic lesions than individuals that did not have this allele (p=0.01).
For measuring ABCA1 gene expression, we employed real-time quantitative polymerase chain reaction using cDNA isolated from peripheral leucocytes of patients with angiographically verified AA (N=9) and controls (N=5).
We have discovered 2-fold reduction in mRNA levels (mean) of ABCA1 gene in patients comparing with controls (0.928 versus 0.470, p=0.007).
We suggest that ABCA1 gene expression level is a significant risk factor in AA development.
The study was supported by Russian Foundation for Basic Research (grant 06-04-49609).

P0661. The p22 phox gene is transcriptionaly regulated by NF-KB and AP-1 in human aortic smooth muscle cells

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Objective: Accumulating evidence demonstrates the involvement of the oxidative stress in the pathophysiology of several major cardiovascular diseases including atherosclerosis, hypertension, stroke or heart failure. However, the molecular mechanisms accountable for the increased production of reactive oxygen species remain uncertain. Among others, NADPH oxidase is one of the most important sources of superoxide in vascular cells. NF-kB and AP-1 transcription factors are down stream targets of NADPH oxidase-derived radicals, which control the expression of inflammatory and immune genes, matrix remodeling, apoptosis, and cell proliferation. Here we investigate the role of NF-kB an AP-1 itself in the regulation of $p22^{phox}$, an essential subunit of NADPH oxidase, in human aortic smooth muscle cells.
Methods and results: Computer analysis revealed the presence of potential NF-kB and AP-1 *cis*-acting elements in the human $p22^{phox}$ gene promoter. Deletion analyses together with transcription factor pull-down assays demonstrated that NF-kB and AP-1 are particularly important in the regulation of $p22^{phox}$ transcriptional activity. Real time PCR and Western blotting analysis showed that $p22^{phox}$ mRNA and protein expression are significantly down-regulated by NF-kB and AP-1 inhibitors or decoy oligodeoxynucleotides.
Conclusions: Taken together, the present study provides evidence that NF-kB and AP-1 transcription factors are important regulators of the NADPH oxidase essential subunit, $p22^{phox}$, in human aortic smooth muscle cells. Given the importance of reactive oxygen species in vascular physiology and pathology, members of this transcription factor family may be important therapeutic targets in the treatment of cardiovascular diseases.
This work was supported by the Romanian Academy (grant no. 56/2006).

P0662. Serotonin transporter gene polymorphisms and intraplatelet serotonin concentration in autism

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Serotonin is released in pre-synaptic membrane and can reach specific receptors in post-synaptic membrane or be partially reuptaked by its specific transporter at the synaptic cleft. Serotonin circulating levels and its effects depend on specific serotonin transporter activity whose efficacy changes with its gene polymorphisms. In that way,
serotonin transporter gene may be a likely candidate in autistic disorder. The aim of this study is to determine a possible association between serotonin tranporter gene polymorphisms 5-HTTVNTR and 5-HTTLPR and autism and to evaluate intraplatelet serotonin concentration.
Patient group was made of 118 individuals (3,6 and 41 years) subdivided in 82 with idiopathic autism, 15 with pathology associated autism, 15 with PPD-Nos and 6 with Asperger syndrome. Control group was made of 68 individuals (45,57±11,23 years).
We studied 5-HTTLPR and 5-HTTVNTR by PCR and intraplatelet serotonin concentration by ELISA.
We found no significant differences in genotype and allelic frequencies between patient subgroups and between patient and control groups in both polymorphisms.
we found a significant higher serotonina concentration in the patient group when compared with control individuals. We did not find significant differences between the different genotypes of both polymorphisms and serotonin concentration.
In this study we did no find any association between serotonin transporter gene polymorphisms and autism.
Autistic patients have higher intraplatelet serotonin concentration, which may contribute for their characteristic behaviour disturbances.
Intraplatelet serotonin concentration is independent of serotonin transporter gene polymorphisms.

P0663. Prevalence of complete and partial AZF deletions in Russian infertile men

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The aim of presented study was to evaluate a prevalence of complete and partial AZF deletions in Russian infertile men. We have examined a cohort of 446 men from infertile couples. The semen analysis had been performed according to the WHO recommendations (WHO, 1999). The patients with chromosome abnormalities were excluded from this study. Complete AZF deletions have been detected according to Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions (EAA/EMQN, 1999), with some modifications. Molecular analysis for partial deletions within AZFc region has been performed using multiplex PCR amplifications with following STSs: sY142, sY1192, sY1197, sY1291, sY1206, and sY1125.
Complete AZF microdeletions and partial AZFc deletions have been found in 6.3% and 12.1% examined patients, respectively. 'Classic' AZFc (b2/b4) deletions comprised 86% complete AZF deletions. In other cases complete AZFa (n=2) and AZFb+c (n=2) deletions have been identified. In contrast to partial AZFc deletions the complete AZF deletions were identified only in azoospermic and severe oligozoospermic patients (sperm count ≤5mln/ml). Between examined men with partial AZFc deletions the sperm parameters varied from asthenozoospermia, oligozoospermia to azoospermia. The commonest type of Y chromosome microdeletions was b2/b3 deletion. This partial AZFc deletion has been revealed in about 9% examined men. In 2.5% patients the gr/gr deletions have been found. Three unclassified partial AZFc deletions were characterized of an absence of sY1197; sY1192 and sY1206; sY142 and sY1197 loci, respectively.
We have demonstrated high prevalence of both complete AZF deletions and partial AZFc deletions in Russian infertile men.

P0664. Microarray-based mutation analysis of Bardet-Biedl genes in Spanish patients

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The Bardet-Biedl syndrome (BBS) is a complex and genetically heterogeneous disorder with diverse clinical manifestations (early retinal dystrophy, polydactily, mental delay, obesity and hipogonadism). Until now, 12 BBS genes have been described (*BBS1-BBS12*), with evidence for at least one more gene implicated. Its inheritance pattern is not clear, since there are families that show a complex inheritance of the syndrome. Therefore, genetic analyses of BBS are complicated To overcome this challenge we screened our population with a genotyping microarray comprehending 9 BBS genes (*BBS1-BBS10*) (Asper Ophtalmics, Estonia) that contains 110 disease-associated changes and other SNPs currently known, enabling simultaneous detection of the BBS genes variants.
Until now, we have analyzed 44 BBS samples, with at least one mutation identified in 41% of them. The distribution of the mutated alleles identified was: 1 patient with three mutated alleles, 9 patients with two mutated alleles, and 8 patients with one mutated allele. The most prevalent mutation was the M390R, accounting for 36% of all the mutations detected.
The BBS genotyping microarray is a robust, cost-effective, and comprehensive screening tool for genetic variation analysis, suitable for

the study of one heterogenetic? syndrome as BBS. Nevertheless, it is necessary to search for new mutations to update the chip and improve its clinical application.
Project: FIS PI060049

P0665. A digenic defect on both the chloride channels ClC-Ka and ClC-Kb mimics a Bartter like type IV Syndrome.

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Usually mutations in the BSND gene product, a protein called barttin, are responsible for a Bartter syndrome associated with sensorineural deafness (Bartter type IV or BSND), an inherited tubulopathy kidney disease. The BSND syndrome is characterized by a marked polyhydramnios, severe renal salt wasting and deafness.
There is only one exception, previously reported, where a patient was affected by sensorineural deafness and renal Bartter phenotype without BSND mutations; simultaneous mutations in both CLCNKB and CLCNKA genes (coding for ClC-Kb and ClC-Ka chloride channels respectively) were demonstrated to be the disease-causing mutations.
We describe here a new patient who has a digenic disorder due to a contemporary combined impairment of two closely related genes, CLCNKA and CLCNKB, leading to a phenotype that combines a severe renal salt wasting and sensorineural deafness. The molecular analysis performed on the BSND, CLCNKB and CLCNKA genes revealed the absence of mutations in the former gene and a homozygous deletion of exons 1-6 for the CLCNKB gene and of exons 7-19 for the CLCNKA.The simultaneous disruption of both ClC-Ka and ClC-Kb chloride channels leads to a syndrome clinically not distinguishable from Bartter type IV. This event should be due to the tight topology of the highly homologous CLCNKA gene which might predispose to an unequal crossing over leading to partial or complete deletions of the CLCNKB gene. We hypothesize that this chimaeric resulting gene interferes with the correct function of both the channels, and leads to a Bartter IV-like phenotype.

P0666. Evaluation of the stress effect on expression of BEST-5 gene in cultured hepatocytes and cloning of this gene

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Liver has important roles in body metabolic regulation and for this reason hepatocytes are used worldwide. Investigations showed that isolation of hepatocytes causes activation of stress related genes. The aim of this study was to study the stress related expression of BEST-5 following hepatocytes isolation and culture.The BEST5 gene is cloned and analysed for the first time from isolated and cultured rat hepatocytes. Very little is known about this gene and almost nothing is known about its function. RNA was isolated from hepatocytes after 3h culture and used for generation of PCR products corresponding to the BEST5. cDNA generated was cloned into pCR®2.1 plasmid vector. Following transformation into TOPO10 oneshot® cells, the cells were grown in LB agar plates containing X-Gal and ampicillin, overnight at 37°C. To confirm that the plasmids contained inserts of the correct size, the vectors obtained from mini-preparations were digested with the desired restriction enzymes. Sequencing was performed for the gene. RT-PCR and Northern blotting analysis showed that BEST5 mRNA is expressed, 3h after isolation and culture of primary hepatocytes (3h) BEST5 mRNA was observed until 5h of culture and then there is no detectable band of BEST5 at further time points. Comparison of expression of the level of mRNA of BEST5, when data statistically were analysed, there was a significant difference between the expression of BEST5 mRNA expression at 3h with 0h, 24h, 35h and 48h of culture ($P<0.001$). **Key words:** BEST-5, Stress, Hepatocytes, RT-PCR, Northern Blotting

P0667. A new IVSI-7 A→T beta globin mutation discovered in three beta carrier subjects and in a beta thalassemia intermedia patient.

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Beta thalassemia is a very important health problem in Italy not only associated to the Italian population but also to other different ethnic groups due to the increased Asian and African immigration. In fact the considerable level of immigration in our country over the last decade has obviously led to significant social and economic changes as far as public health is concerned. So far more than 250 different mutations have been found in the beta globin gene. We report a new beta thalassemia mutation detected in four members of two different families of Italian origin. In the first family two subjects were beta thalassemia trait (mother and her daughter) while on the second one the A->T substitution at IVS1 nucleotide 7 was identified in an adult woman in the heterozygous state and in her child related to the codon 39 mutation. The genetic compound codon 39/IVS1-7 was found in a fifteen years old boy with the thalassemia intermedia phenotype.
The new mutation here described seem to be completely in superposition with the haematological phenotype associated to the IVS1-6 mutation in carrier state. It is assumable that this mild phenotype can be related to the progressive distance between the mutation and the consensus sequence of the first intron donor site of the β gene, relieving the negative consequence of the efficiency of the splicing mechanism.

P0668. A Novel Cryptic Splice Site in IVSI-110 β-thalassemia

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The IVSI-110 β-thalassemia mutation was first described in 1981 and is one of the most common β-globin splicing mutations found in patients of Greek or Cypriot origins. This mutation is the result of a G to A substitution at position 110 in the first intron. This mutation has previously been reported to generate an aberrant 3' acceptor splice site, which is preferentially recognised by the spliceosome over the normal 3' acceptor splice site. The IVSI-110 mutation leads to a 90% reduction in normal β-globin chain synthesis causing transfusion-dependent disease in homozygous patients. Our group recently reported the creation and characterisation of 'humanised' transgenic mice containing the IVSI-110 human β-globin locus. Using human β-globin-specific RT-PCR, we noted two human β-globin-specific aberrant spliced products. We observed quantitative differences in the aberrant β-globin specific RT-PCR spliced products in peripheral blood and bone marrow-derived cells from 'humanised' IVSI-110 transgenic mice and IVSI-110 human patient-derived peripheral blood cells. We attribute the relative differences in the level of aberrant splice products to mRNA instability. In this study, we confirm by cloning of RT-PCR products and DNA sequencing the identity of the novel activated cryptic 3' acceptor site in mice and also in humans containing the IVSI-110 mutation. We conclude, that the identification of novel cryptic splice site indicates that IVSI-110 splicing is more complex than previously reported, and is worthy of further investigation.

P0669. Combination of Hb Knossos [Cod 27 (G-T)] and IVSII-745 (C-G) in a Turkish Patient with Beta-Thalassemia Major

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Beta-thalassemia is the most common disease among hemoglobinopathies in Antalya, Turkey, as well as worldwide. Mutations found in Turkish beta-thalassemia patients constitute a heterogeneous group, consisting mostly of point mutations. Only in very rare cases, deletions or insertions cause affected or carrier phenotypes. Hb Knossos (beta 27 (B9) Ala-Ser) is a rare variant with a normal HbA2 level. In this study, we aimed to investigate the effect of compound heterozygosity for Hb Knossos [Cod 27 (G-T)] and IVSII-745 (C-G). To our knowledge, this is the first report of such a combination related with beta-thalassemia major phenotype in a Turkish family, where Reverse Dot Blot Hybridisation (RDBH) and DNA sequencing analysis were used. Heterozygous inheritance of the mutation results in mild beta-thalassemia

phenotype, whereas homozygous inheritance leads to intermediate β-thalassemia. As a result, the compound heterozygosity of Hb Knossos with IVSII-745 appears as the cause of the beta-thalassemia major phenotype in our case. In conclusion, we suggest that combination of these mutations in beta-globin gene is important in expression of beta-thalassemia major phenotype in a marriage between a beta-thalassemia carrier and a person who has Hb Knossos and its like some other silent beta-thalassemia mutations are not associated with an elevated HbA2 level.

P0670. Genetic diagnosis in the Birt-Hogg-Dubé syndrome

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Birt-Hogg-Dubé (BHD) syndrome is an inherited autosomal dominant genodermatosis characterized by predisposition to cutaneous fibrofolliculomas, lung cysts leading to pneumothorax and renal cell carcinomas. The age-dependent penetrance and the great variability of the clinical manifestations even within a given family suggest that BHD remains underdiagnosed. The disease is caused by heterozygous germline mutations in the BHD gene, encoding fibrofolliculin. Mutations are identified in 85% of BHD patients. All mutations previously reported putatively lead to protein truncation. More than 50% of mutations are frameshift located in a C-poly-tract in the exon 11.

We have performed genetic diagnosis with DNA sequencing of BHD and Multiplex PCR/Liquid Chromatography for detection of rearrangement of this gene. We have analyzed 100 unrelated index cases with suspected or confirmed clinical diagnosis of BHD syndrome and identified 14 germline BHD different mutations in 24 probands: 8 frameshifts with two recurrent mutations (one duplication in exon 11 was found in 9 patients, one deletion in the exon 9 in three patients), 3 nonsense mutations, two splice site mutations, one missense mutation and one rearrangement. Ten were novel mutations including the missense mutation and the large rearrangement that have not yet been described in BHD. Presymptomatic diagnosis was then performed in 47 relatives revealing 34 gene carriers at whom clinical surveillance including kidney imaging has been proposed.

We discuss our results according to patients' clinical manifestations and clinical indications of research of BHD mutations.

P0671. The Bloom's syndrome helicase interacts with the MutS homolog, MSH4, during meiosis

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Bloom's syndrome (BS) is a recessive human genetic disorder characterized by marked genetic instability associated with a greatly increased predisposition to cancers of most types. The gene mutated in BS encodes a 3'-5' DNA helicase (BLM) identified as a member of the RecQ family. BLM is involved in the cellular response to DNA damage and stalled replication forks. Moreover, several studies support a role of BLM during meiosis and it is noteworthy that human males homozygous for BLM mutation are infertile. However, the specific function of BLM during meiosis remains unclear. Here, we provide biochemical evidence indicating that BLM interacts with MSH4 during meiotic prophase I. These data are consistent with cytological analyses showing that both BLM and MSH4 foci are located on chromosome cores at the same stages of the meiotic prophase I. MSH4 is a meiosis-specific MutS homolog known to participate to several steps of meiotic recombination process. MSH4 acts first at the step of homologous chromosomes synapsis and, in ensuing steps, to promote meiotic crossovers. Based on the biochemical properties of BLM and MSH4, we consider the possibility that interaction between BLM and MSH4 may have implications in the regulation of meiotic recombination events which is crucial since impaired meiotic recombination process may result in nondisjunction of homologous chromosomes at the first meiotic division and formation of aneuploid germ cells.

P0672. Semax exerts vasotropic and neuroprotective effects in experimental brain ischemia

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We have analyzed the effects of Semax (synthetic peptide Met- Glu-His-Phe-Pro-Gly-Pro; its N-terminus represents a fragment of adrenocorticotropic hormone - ACTH 4-7) and its C- terminal fragment Pro-Gly-Pro (PGP) on functional morphology and proliferative activity of cells from different cerebral regions of the rats subjected to global brain ischemia. The study was carried out on brain of 2-month-old male Wistar rats (n=45). After 15 minutes of irreversible bilateral common carotid artery occlusion the animals were exposed to intraperitoneal injection of Semax, PGP or 0,9% NaCl. The repeated introductions were made at 1h, 4h and 8h after operation. Animals were decapitated at 30 min and 24 h after operation. Intact and sham-operated animals were used as a control group. The sections were stained with hematoxylin and eosin for histological analysis. Murine monoclonal antibodies to PCNA (proliferating cell nuclear antigen) were used for immunostaining of proliferating cells. In intact animals, comparative morphofunctional analysis of effect of Semax or PGP has indicated that both peptides have exerted vasotropic effects based on activation of capillary network. The increase of intensity of immunopositive reaction of proliferating cells demonstrates trofic action of Semax on ependyma of ventricles, cells of blood-brain barrier and neuroglia. In this study, among these investigated peptides only Semax has manifested neuroprotective action on animals with acute stroke. Semax decreases ischemic injury of neurons and prevents progress of local foci of destruction of neuronal tissue. Furthermore, signs of vascular stasis decrease in the ischemized rat brains under Semax trearment.

P0673. Estrogen receptor (ER) beta2 negatively regulates the transactivation of ERα in human breast cancer cells

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Estrogens, by binding to and activating two estrogen receptors (ERs), ERalpha and ERbeta, are critically involved in the development of the mammary gland as well as breast cancer. An isoform of ERbeta, ERbeta2 (also called ERbetacx), with an altered C-terminal region, is co-expressed with ERalpha in many human breast cancers. In this study, we generated a stable cell line from MCF7 breast cancer cells expressing an inducible version of ERbeta2, along with endogenous ERalpha, and examined the effects of ERbeta2 on the ERalpha protein levels and function. We showed that ERbeta2 inhibited ERalpha-mediated transactivation via ERE and AP-1 sites of reporter constructs as well as the endogenous genes pS2 and MMP-1. Chromatin immunoprecipitation (ChIP) assays revealed that ERbeta2 expression caused a significant reduction in the recruitment of ERalpha to both the pS2 and MMP-1 promoters. Furthermore, ERbeta2 expression induced proteasome-dependent degradation of ERalpha. The inhibitory effects of ERbeta2 on ERalpha activity were further confirmed in HEK293 cells that lack functional endogenous ERs. We also demonstrated that ERbeta2 can interact with ERalpha both *in vitro* and in mammalian cells which is compatible with a model where ERbeta2/ERalpha heterodimers are targeted to the proteasome. Finally, in human breast cancer samples, we observed that expression of ERbeta2 significantly correlated with ERalpha-negative phenotype. Our data suggest that ERbeta2 could influence ERalpha-mediated effects relevant for breast cancer development including hormone responsiveness.

P0674. STAT6 and ADAM33 single nucleotide polymorphism in association with Bronchial asthma

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Bronchial asthma is one of the most frequent childhood chronic ailments, defined on the basis of epidemiological, physiological, patho-

logical and immunological criteria.
Immunopathological process induces both local and general changes and can negatively influence child development.
Main aim of this project, supported by IGA MZ CR: NR8383-3/2005 is to test if the bronchial hyperreactivity level is associated with certain alleles or haplotypes of genes (ADAM 33 and STAT 6) involved in immune system activity in course of allergic illness development and bronchial epithelium remodelling .
Examination of responsible polymorphisms of ADAM 33 and STAT 6 genes was accomplished by sequence analysis in 20 children with asthma bronchiale, their sibs and parents and 20 control healthy children.
Statistical evaluation of association of genes polymorphisms with asthma bronchiale occurrence and bronchial hyperreactivity level in children will be presented by our poster.
Examination of polymorphisms positively associated with allergic reaction could become a routine part of diagnostics. The description of influence of particular sequence changes could contribute to additional insight to the bronchial hyperreactivity genetic background.

P0675. Functional consequences of eIF2B mutations in cells from affected patients

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The Childhood Ataxia with diffuse Central nervous system Hypomyelination (CACH)/Vanishing White Matter (VWM) syndrome is an autosomal recessive leukodystrophy characterized by a progressive spastic ataxia and a CSF-like aspect of the white matter on MRI. Severity is correlated with age at disease onset and with episodes of rapid deterioration following febrile infection or head trauma. Mutations in the five subunits of the eIF2B translation initiation factor (alpha to epsilon) are the most frequent cause of the disease. eIF2B is involved in the first step of protein synthesis by activating the translation initiation factor eIF2 thanks to its guanine nucleotide exchange activity (GEF activity). By regeneration of active eIF2, eIF2B is a key factor in the cellular stress response. We demonstrated that GEF activity of eIF2B is decreased 63 CACH/VWM patients lymphoblastoid cell lines (lIb) in correlation with disease severity whereas no significant decrease was observed in 10 primary fibroblasts. Determination of lIb GEF activity is relevant to prescreen patients eligible for eIF2B sequencing. In contrast, no difference in reticulum endoplasmic stress responsse was observed between eIF2B-mutated and control lIb whereas mutated fibroblasts expressed an enhanced response to stress in comparison to control ones. Therefore we initiated a differential transcriptomic study (on pangenomic chips arrays) on 10 couples (mutated patients *versus* controls) with or without cellular ER-stress. The first results suggest the involvement of multiple pathways.

P0676. Primary calpainopathy, the new mutation detected in the CAPN3 gene (the case of Moldavian LGMD2A patient).

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Introduction. Limb-girdle muscular dystrophy type 2A (LGMD2A) is an autosomal recessive muscular disorder caused by mutations in the gene coding for calpain 3, a calcium-dependent protease. While a large number of CAPN3 gene mutations have already been described in calpainopathy patients, the diagnosis has recently shifted from molecular genetics towards biochemical assay of defective protein.
Methods. Mutation search was conducted using SSCP and direct sequencing methods.
Rezults. We perform direct sequencing analysis in 8 exons (exons 4, 5,10,11, 12,20, 21 22) in Laboratory of DNA-diagnosis (Moscow) of patient with clinical features of LGMD,2A. The onset of the disease is 16-17 age. Involvement was first evident in either the pelvic, with asymmetry of wasting when the upper limbs were first involved. Spread from the lower to the upper limbs occurred within 26-27 years. Pseudohypertrophy of the calves was not established. Biochemical analysis: elevated levels of serum creatine kinase [hyperCKemia]. By Molecular -genetics analysis we detected two mutation in heterozygous occurrence of a missense and deletion mutations: c.550del A (exon 4) and c.1302C>T (exon 10). These mutations are no conjunction with a frame-shift mutation. The c.1302C>T mutation did not descript in the mutation data base. We suggested that this mutation have the important role in clinical phenotype (compound effect).
Conclusion: Our results illustrate the importance of DNA analysis for reliable establishment of mutation status, and provide a new insight into the process of genotype-phenotype correlations of LGMD2A patients.

P0677. Molecular and clinical study of Bulgarian patients with Limb-girdle muscular dystrophy type 2A (LGMD2A, Calpainopathy)

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A total 24 LGMD2A Bulgarian patients (22 unrelated families) were diagnosed on DNA. Two of them were reported previous [Richard et al., 1999]. In about 40% of the cases the first clinical diagnosis was different from LGMD2, namely DMD/BMD and SMA. The affected females are two times more than the male patients. Calf hypertrophy, early contractures, proximal muscle weakness in the four extremities simultaneously, pelvic/shoulder muscles weakness were more frequently found in male patients. Females present with later onset, slowly progression, milder course of the disease, become wheelchair bound later than male patients. Heart involvement was seen in 2 cases. Interfamilial and intrafamilial variability were also observed.
We detected 12 *CAPN3* gene mutations. Five of them (42%) fall in exons 4 and 7 and cover 70% (28/44) of the affected unrelated chromosomes.
The most frequent mutations are c.550delA (50%), p.Glu323X (7%) and the big deletion del2-8 (5%). They were found in 68% (15/22) of the families and cover 61% (27/44) of the affected chromosomes. The rest mutations were found in single cases and five of them (p.Arg118Gly, p.Arg169Gly, p.Gly333Asp, c.1811_1812delTC, c.1981_1984delATAG) were detected only in Bulgarian patients. In six families the second mutation was not found.
Calpainopathy occurred the most frequent LGMD2 form in Bulgaria which accounts for at least 50% of all LGMD2 cases. We developed a systematic approach for DNA diagnostics of LGMD2A, starting with mutation screening in exons 4 and 7. The introduced method permits to perform an adequate prophylaxis of the disease in our country.

P0678. Distinct spectrum of *CFTR* mutations and IVS8 poly (T) variants in Persian males with congenital bilateral absence of the vas deferens

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Congenital bilateral absence of the vas deferens (CBAVD) is a frequent cause of obstructive azoospermia and nearly 75% of patients have at least one detectable *CFTR* mutation. To study the *CFTR* gene mutations in Persian CBAVD patients with presumed low cystic fibrosis (CF) frequency, we analyzed 112 Persian CBAVD and 7 CUAVD males from Iran with 52 fertile males as control. All 27 exons and their flanking sequences was analyzed using a combination of the SSCP and direct sequencing. Forty-six of the 112 patients (41.07 percent) had two mutations in the *CFTR* gene, 41 of them had the *5T* allele. Forty-three patients (38.39 percent) had a mutation in one copy of *CFTR* gene. In 23 patients (20.53 percent) no *CFTR* mutations were found. IVS8-*5T* was observed with TG12 or TG13 haplotypes, on 61 chromosomes thus confirming the association of this variant with CBAVD in Persian patients. Screening for the IVS8-*5T* and F508del together led to the identification of more than one-third of alleles. Exon 9 skipping was strongly joined with *5T*/*5T* genotype, the rate of normal *CFTR* mRNA increased by having IVS8-*9T* $(TG)_{9-10}$ and IVS8-*7T* $(TG)_{10-11-12}$. We could detect one novel nonsense mutation (K536X) in the NBD1 region and two novel missense mutations (Y122H & T338A) in the M2 and M6 regions of *CFTR* gene. The combination of the *5T* allele in one

copy of the *CFTR* gene with a cystic fibrosis mutation in the other copy is the most common cause of CBAVD in Persian population.

P0679. Molecular genetic diagnostics of celiac sprue from frozen biopsy material and significance of multi-disciplinal cooperation

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Celiac disease (CD) is a multi-factorial disease characterised by a lifelong abnormal immune response showing the features of an autoimmune reaction to gluten, causing morphological changes in the intestinal mucosa to occur in sensitive individuals. CD manifests itself through various atypical symptoms and can be masked by a symptomatology leading to a diagnosis of another disease. Prevalence range from 1/200-1/300 individuals. The disease is closely associated with HLA class II alleles. About 95 % of the persons with CD possess HLA class II alleles DQA1*0501 and DQB1*0201/202 (coding heterodimer DQ2) and DRB1*04 (in close relation with heterodimer DQ8).

The diagnostics of CD is currently based on assessment of clinical symptoms, serologic tests, histological and enzymohistochemical examinations of the duodena mucosa. The spectrum of diagnostic approaches at our laboratory is supplemented with a molecular detection of selected risk HLA alleles, which may substantially contribute particularly to the differential diagnostics of infiltrative type CD (type 1 according to the Marsh´s modified classification), which occurs both in CD-diagnose patients being on a gluten-free diet, CD-manifest relatives (,,potential CD“) and Duhring-dermatitis-herpetiformis-diagnose patients. The presence of risk HLA alleles was determined using the PCR method.

The cooperation between pathologists and molecular geneticists substantially contributes to vindicating the clinical diagnosis of CD, particularly in cases unclear in histological terms as well as in potential and latent forms of the disease. It makes possible to find other risk individuals among the relatives of CD patients. The absence of these examinations may result in a diagnostic unclearness.

P0680. Expression of Centa2 during early heart development: a new candidate gene for the onset of cardiovascular malformations

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We previously showed that cardiovascular malformations (CVMs), including pulmonic stenosis, septal and valve defects, have a higher incidence in patients (pts) with NF1 microdeletion syndrome, compared to classical NF1 pts, likely owing to 17q11.2 region haploinsufficiency. RT-PCR and Northern blot analysis of 9 genes within the NF1 deleted region showed that Centa2, Suz12 and C17orf40 are expressed in human fetal and mouse embryonic heart. In situ hybridization on mouse whole embryos and sections allowed us to asses their expression pattern during development. Centa2 was expressed in encephalon, gut, otic vesicles, developing limbs and heart; in particular, a strong hybridization signal was detected in heart at 9-9.5 dpc, when the heart tube begins to loop and endocardial cushions, primordia of the valve leaflets and membranous septa are forming; Centa2 expression in heart continued until the last stage analyzed (15 dpc). Suz12 was found in encephalon, pharyngeal arches and developing limbs, with a weak signal in heart at 10 dpc. C17orf40 was expressed in otic vesicles, pharyngeal arches and limbs, but not in heart. Preliminary results following RT-PCR and in situ hybridization experiments on zebrafish showed expression of Centa2 in adult heart.

These findings suggest that Centa2 might be involved in heart development and in CVMs onset. Expression studies of Centa2 will be extended to zebrafish embryos and immunohystochemistry with anti-Centa2 antibodies on mouse sections will be performed to provide further evidence on the involvement of Centa2 in heart development and CVMs.

P0681. Clinical phenotype in a Portuguese patient with a deletion of the entire coding region of the connexin 32 gene

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X-linked Charcot-Marie-Tooth disease (CMTX) is a peripheral nerve disorder that has been linked to mutations in the connexin 32 (Cx32) gene (*GJB1*). Cx32 works as a gap junction protein found in myelinated peripheral nerve and mutations in the protein are predicted to interfere with the formation of functional channel in a dominant negative manner. The majority of *GJB1* mutations are missense mutations, while a minority is nonsense mutations or small deletions. CMTX is characterized by a moderate to severe motor and sensory neuropathy in affected males, and usually mild to no symptoms in carrier females. Here we report an 18 year old Portuguese male patient, with walking difficulties, prominent muscular atrophy of muscles below the knee, lower limbs with ‘invert champagne bottle’ appearance and with steppage gait, associated with a deletion that, at least, eliminates the Cx32 gene entire coding sequence. A few families with deletions of the *GJB1* gene have recently been reported [1,2]. This rare mutation is described for the first time in a CMTX Portuguese patient. As in the previous reported cases, the CMTX clinical phenotype of this patient is similar to the ones associated with missense or nonsense mutations in this gene. However, he has a complete absence of the Cx32 gene and, therefore, a model of dominant negative inactivation of the function of other gap junction does not apply, at least in this case.

[1] Nakagawa M. *et al,* J Neurol Sci. 2001, 185: 31-7

[2] Takashima H. et al, Acta Neurol Scand. 2003, 107:31-7

P0682. Mitochondrial Coupling Defect in Fibroblasts from Patients with Mfn2-Related Charcot-Marie-Tooth Type 2a

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The mitofusin 2 gene (mfn2) encodes a dynamin GTPase, located in the outer mitochondrial membrane, which is involved both in the maintenance of the mitochondrial network and in the modulation of cellular energy balance. Mutations of mfn2 may account for at least a third of the cases of Charcot-Marie-Tooth disease type 2A (CMT2A). In this study, we investigate mitochondrial cellular bioenergetics in *MFN2*-related CMT2A. Methods: mitochondrial network morphology and metabolism were studied in cultures of skin fibroblasts obtained from four CMT2A patients harboring novel missense mutations of the *MFN2* gene. We studied intracellular reactive oxygen species, mitochondrial membrane potential (ΔΨm), respiratory parameters, rate of mitochondrial ATP synthesis and ATP/O ratio. Results and interpretation: No alteration of the morphology of the mitochondrial network was observed. In contrast, the mitochondrial energetic metabolism was greatly altered in the fibroblasts from patients with *MFN2* mutations. We found a significant coupling defect leading to reduced OXPHOS efficacy (reduction of ATP/O). However, this uncoupling did not lead to a deficiency in ATP production since it was compensated by an increase in the basal respiration. In other words, the ATP production was maintained, although at a higher energetic cost. A 30% decrease of the ΔΨm was also observed in fibroblasts patients as a direct consequence of impaired mitochondrial coupling. These results suggest that the sharply reduced efficacy of oxidative phosphorylation in *MFN2*-related CMT2A may contribute to the pathophysiology of the axonal neuropathy.

P0683. Duplication of the Xq28 region including *GDI* and *FLNA*, but not *MECP2*, in a family with moderate MR and ataxia

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The advent of new techniques such as array-CGH and MLPA has led to the characterization of several new cryptic microdeletions and duplications as the cause of mental retardation syndromes (MR). By high resolution chromosome X specific array-CGH, we identified small duplications of the *MECP2* locus as the underlying cause of a distinct

severe MR phenotype in males (Van Esch et al. AJHG, 77, 2005). Most of these duplications comprise, next to the *MECP2* gene, several other MR-related genes, such as *L1CAM*, *GDI* and *FLNA*. However, delineation of the minimal critical region and detection of a twofold increased expression of *MECP2* mRNA in the patient-derived cell lines compared to controls, pointed to an increased dosage of *MECP2* as the major cause of the MR phenotype in these families.
Here we present a large X-linked MR family with a small duplication at Xq28 harboring the *GDI* and *FLNA* genes, but not the *MECP2* gene, segregating in the family. The MR phenotype consists of mild to moderate MR and ataxia as cardinal features.
Further screening of patients for this duplication, not including *MECP2*, revealed one additional family. This finding illustrates that a duplication of the *GDI* and/or *FLNA* genes can also result in an MR phenotype, and might play an additive effect in the larger duplications also including *MECP2*. The clinical data of this second family, delineation of the extent of both duplications as well as proposing the most likely candidate gene will be presented.

P0684. Functional analysis of pancreatitis-associated missense mutations in the pancreatic secretory trypsin inhibitor (SPINK1) gene

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Variations in the SPINK1 gene [encoding pancreatic secretory trypsin inhibitor (PSTI)] are associated with chronic pancreatitis. We have recently determined the functional consequences of three missense mutations which occurred within the signal peptide sequence of PSTI by Western blotting analysis of wild-type and mutant PSTI expressed in Chinese hamster ovary cells. Here, this approach was extended to analyse seven missense mutations [p.N34S, p.G48E, p.D50E, p.Y54H, p.P55S, p.R65Q, and p.R67C) occurring within the mature peptide of PSTI. This analysis enabled us to classify these missense mutations into three categories. The first category comprises the p.N34S and p.P55S polymorphisms, both of which occurred in evolutionarily non-conserved residues, involved amino acid substitutions with similar physico-chemical properties, and did not cause any significant reduction in terms of PSTI mature peptide expression. The second category contains only the p.R65Q missense mutation, which occurred in a well-conserved residue, involved the substitution of a positively charged amino acid by a non-charged one, and caused about 60% reduction of protein expression. The third category consists of p.G48E, p.D50E, p.Y54H, and p.R67C, all of which occurred in strictly conserved residues, involved charged amino acids, and caused complete or nearly complete loss of PSTI expression. Having excluded the possibility that the reduced protein expression may have resulted from reduced transcription or unstable mRNA, we surmise that these missense mutations may have disturbed the intracellular transportation of their respective mutant proteins. This is suggestive of a possible unifying pathological mechanism underlying both the signal peptide and mature peptide mutations.

P0685. Triplication/duplication of the trypsinogen locus in patients with hereditary and idiopathic chronic pancreatitis

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Hereditary pancreatitis (HP) is a rare autosomal dominant disorder. Apart from an early age of onset and a positive family history, HP is clinically indistinguishable from other forms of chronic pancreatitis. HP as well as idiopathic chronic pancreatitis (ICP) have previously been reported to be caused by 'gain of function' missense mutations in the cationic trypsinogen (PRSS1) gene. These findings as well as other complementary observations potentiated the importance of a tightly regulated trypsin activation/inhibition balance for pathophysiology and suggested that trypsinogen may be sensitive to a gene dosage effect. We therefore surmised that an increased copy number of the PRSS1 gene located at chromosome 7q34 might account for some of the unidentified families with HP. Using semi-quantitative fluorescent multiplex PCR and FISH, we identified a triplication of a ~605 kb segment containing the PRSS1 gene on chromosome 7 in five out of 34 French HP families, in which no known point mutations in the PRSS1, PRSS2, SPINK1 and CFTR genes had been found. We also investigated trypsinogen copy number variations in 1246 French Caucasians with ICP. The ~605 kb triplication and a novel duplication of the trypsinogen locus were detected in 10 and four subjects with ICP, respectively. Of the 14 events, 12 (6%) were found in the 202 ICP patients whose age of disease onset was <20 years. Our results thus have revealed a novel molecular mechanism (i.e. increased copy number or gene dosage) causing HP and ICP; and demonstrated that chronic pancreatitis is also a genomic disorder.

P0686. Mutations screening in patients with different types of hereditary motor and sensory neuropathies

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Hereditary motor and sensory neuropathy (HMSN), including Charcot-Marie-Tooth (CMT) disease, is a common disability impairing the peripheral nerve function in 1:2500 persons. We have provided the molecular genetic analysis of duplication/deletion of chromosome 17p11.2 including PMP22 gene encoded Pmp22 protein which plays a crucial role in the development and maintenance of compact myelin in autosome dominant HMSN patients. We identified 17p11.2 duplication/deletion using STR markers from 17p11.2 region (D17S122, D17S921, D17S1358, and D17S2226). For fragment STRs analysis Cy5-labeled primers and the "ALF express II" were used. Duplications were found in 19 CMT type 1A families form Ukraine. We detected the deletion in 2 patients of one family with hereditary neuropathy with liability to pressure palsies (HNPP type). The CMTX1 is X-linked type of HMSN associated with mutations in GJB1(Cx32) gene coding for the gap junction protein connexin 32. CMTX1 may arise due to incorrect trafficking of Cx32 protein or reduction in the gap junction mediated communication pathway in PNS. So the next aim of our investigation was the screening of Cx32 gene mutation variants. We have developed and applied denaturing gradient gel electrophoresis (DGGE) analysis of Cx32 exons 1B and 2 for suspected X-linked CMT patients. DGGE analysis revealed 4 aberrant Cx32-probes. We analysed and identified these probes by direct sequencing.It has been sown the PMP22 duplication/deletion and Cx32 gene mutations association with CMT1A, HNPP and CMTX1 clinical phenotypes. The methods of 17p11.2 duplication/deletion detection and Cx32 mutation variants analysis in Ukraine have been developed.

P0687. Early onset Charcot-Marie-Tooth disease caused by Leu239Phe mutation in the GDAP1 gene in three Russian families: evidence for a founder effect

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Mutations in the ganglioside - induced differentiation-associated protein 1 (*GDAP1*) gene are common a cause of the Charcot-Marie-Tooth (CMT4A) disease with autosomal recessive mode of inheritance. To date more than twenty mutations in the *GDAP1* gene have been reported in patients suffering from the demyelinating, axonal or mixed form of Charcot-Marie-Tooth disease.
24 patients from 20 unrelated families with severe early onset polyneuropathy and possible autosomal recessive inheritance were screened for mutations in *GDAP1* gene by SSCP analysis.
In one affected family we revealed c.458C>T (Pro153Leu) mutation. A c.715C>T transversion at codon 239 (Leu239Phe) was detected in seven affected subjects from five apparently unrelated families. All patients had early onset and axonal type of CMT disease.
Haplotype analysis of the *GDAP1* locus for markers D8S279; D8S1776; D8S286; D8S551; D8S548; D8S1805; D8S1705 and D8S1757 demonstrated a common disease haplotype for markers D8S286; D8S551, D8S548, and D8S1805. The association of the mutation with a common haplotype suggested a founder effect.
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P0688. Coffin-Lowry syndrome - two novel mutations in the *RSK2* gene in Polish patients

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Coffin-Lowry syndrome (CLS) is an X-linked semidominant disorder characterized by mental retardation, facial dysmorphism and skeletal abnormalities. CLS is caused by mutations of the *RSK2* gene (*RPS6KA3*) located in Xp22.2 region and split into 22 exons. The gene encodes for a serine/threonine kinase RSK2, composed of two functional kinase catalytic domains. RSK2 acts at the distal end of the mitogen induced Ras-MAPK signaling pathway involved in regulating wide range of cellular functions.

The aim of the study was to identify *RSK2* mutations in a group of Polish patients with clinically recognized Coffin-Lowry syndrome assembled in Department of Medical Genetics of the Children's Memorial Health Institute in Warsaw. Mutation screening of the entire *RSK2* gene was performed in seven patients. Twenty-two exons were amplified by PCR and subsequently analyzed by SSCP and sequencing technics. Molecular analysis revealed the presence of two novel mutations in the *RSK2* gene. One of them was a one-nucleotide deletion (c.896delT) in exon 11. The deletion creates a stop codon at codon 311, resulting in a truncated RSK2 protein lacking a part of N-terminal domain and the whole C-terminal domain. The other mutation was a substitution G to A in the first position of intron 10 (c.845+1G>A), affecting the splice site. In both cases mutations appeared *de novo*. Further studies are planned to evaluate the role of the *RSK2* gene in the pathogenesis of CLS in Polish population.

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P0689. Mutational spectrum in steroid 21-hydroxylase gene (CYP21A2) in Congenital Adrenal Hyperplasia patients of Russia

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More than 90% of congenital adrenal hyperplasia (CAH) of cases result from 21-hydroxylase deficiency caused by mutations in the CYP21A2 gene. By means of PCR analysis the spectrum of mutations in the steroid 21-hydroxylase gene (CYP21A2) was analyzed in 72 Russian patients with salt-wasting (SW) and simple virilizing (SV) forms of CAH. Specific patterns of diagnostically important mutations were identified for each clinically different CAH forms. The salt-wasting form of the disease is most frequently associated with gene deletion (48%) and the 655A/C→G mutation in the second intron (24%) of CYP21A2 gene. 1172N mutation in exon 4 (18%), 655A/C→G mutation (24%) and gene deletion (14%) were most frequent in the patients of simple virilizing CAH form. Altogether mutation detection rates were equal to 86% and 66% of affected chromosomes from the patients with the SW and SV forms respectively.

P30L, del8, delA2, 655A/C→G, I172N, V237G, V281L, Q318X, R356W, P453S mutations were studied in 26 patients affected with the non-classical form (NC) of CAH. Mutations were detected in 60% (15/26) of the chromosomes from the patients with NC form altogether. Novel type of mutation - CYP21gene/CYP21pseudogene hybrid gene was identified in 31% (8/26) of patients. Hybrid gene resulted from the fusion of 5' part of CYP21A2 gene with 3' part of the neighboring CYP21A1P pseudogene with the common junction site located somewhere after exon 8 of CYP21A2 These data are of substantial practical value for genetic counseling and prenatal diagnostics of CAH in Russia.

P0690. Congenital heart disease: a genealogical and genetic study in São Miguel Island, Azores

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Congenital heart defects are among the most common birth defects, and the leading cause of birth defect-related deaths. Recently, we demonstrated that in Sao Miguel Island the CHD prevalence is relatively high: 9.16 per 1000 live births. Considering that half of the population lives in small rural localities and the internal migration is reduced, aspects that increase endogamy and inbreeding, we performed a structured family questionnaire which includes: 1. queries for CHD risk factors (maternal diabetes mellitus, alcohol and drug abuse by the mother during pregnancy, viral infections of the fetus and genetic conditions), and 2. a detailed family history to construct the ascending genealogy until the 3rd generation. To that end, 195 CHD families were contacted by phone and/or letter, 109 (55.9%) of which accepted to participate. We identified 39 (35.8%) multiplex families (with 2 to 5 patients), 5 (4.6%) consanguineous families, and 5 (4.6%) multiplex families with consanguinity. In order to carry out molecular genetic analysis, a biobank consisting of DNA and RNA from patients and parents was built after written informed consent. The first mutation analyzed was the C677T of the *MTHFR* gene in 469 healthy individuals from Sao Miguel, being 84 (17.2%) homozygous for the mutation (TT) and 221 (45.5%) heterozygous (CT). From these observed genotypes, the C677T allele frequency in Sao Miguel population is 41.5%, the second highest value in Europe. A comparative analysis will be performed for this mutation in the CHD patients, as well as for other candidate genes (NKX2.5, TBX5 and GATA4).

P0691. The analysis of a mouse mutant that models aspects of human Cri du Chat syndrome.

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Human Cri Du Chat Syndrome (CDCS) is a common deletion disorder with an incidence of 1 in 20,000 live births. Hallmarks of CDCS include a cat-like cry, mental retardation, hypotonia, microcephaly, micrognathia, and a spectrum of behavioural abnormalities. Ultimately, identification of the key genes involved and analysis of their functions should aid in the design of better diagnoses and therapies for CDCS patients. To identify novel mouse mutations that affect cranial nerve development we performed a small-scale, recessive mutagenesis screen using the chemical mutagen *N*-ethyl-*N*-nitrosourea (ENU). In one of the mutants, anti-neurofilament staining of 10.5dpc revealed an abnormally sprouting facial nerve (cranial nerve VII). At embryonic days 11.5-12.5dpc, homozygotes have severely reduced lower jaws (micrognathia), abnormally shaped heads, and various vascular defects. These mutants are lethal at day 13.5dpc. Using meiotic recombination I mapped this phenotype to a 1.4Mb region on mouse chromosome 15, which is syntenic to the critical interval associated with craniofacial abnormalities and mental retardation of human CDCS. By sequencing the genes within this interval and by expression analyses, I have identified an excellent candidate gene and am currently employing further molecular and genetic approaches to understand its role in normal craniofacial development and in Cri Du Chat syndrome.

P0692. Novel CARD15/NOD2 mutations in Finnish patients with Crohn's disease and their relation to phenotypic expression

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Crohn's disease (CD) is a multifactorial trait with significant genetic predisposition. R702W, G908R, and 1007*fs* mutations of the CARD15/NOD2 gene associate with CD and account for 80% of disease susceptibility caused by this gene. In Finland, only 16% of the CD patients carry one of these mutations even though CD prevalence and incidence are comparable to other Western populations. Our aim was to explore whether Finnish type of CARD15 mutations exist that would explain the residual linkage to chromosome 16. We screened the CARD15 gene in 240 CD patients. For *in vitro* functional studies, blood mononuclear cells were cultured alone or with MDP, and IL8 levels were measured. We identified 30 sequence variations, including 12 new ones. Allele frequencies for the R702W, G908R, and 1007*fs* muta-

tions were 3.3%, 0.4%, and 4.8%, respectively. Five novel amino acid substitutions (R38M, W355X, P727L, W907R, R1019X) were found in five CD patients; four of these patients also carried another CARD15 variant. The biochemical nature of these mutations, cross-species comparisons, and low IL8 production all favour their pathogenic role. These mutation carriers had a complicated form of ileal or ileocolonic disease. We were unable to identify any novel population-prevalent CD susceptibility allele, and only the 1007*fs* mutation associated with CD susceptibility. In conclusion, our data suggest that there may be yet undetectable pathogenic mutations in the flanking or intronic regions of the CARD15 gene. Compound heterozygosity or homozygosity for the CARD15 mutations should be considered in complicated CD patients.

P0693. Molecular analysis of the CUL7 gene in 3 M syndrome

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3M syndrome is an autosomal recessive condition characterized by pre- and post-natal growth retardation, facial dysmorphism, large head circumference, normal intelligence and skeletal changes including long slender tubular bones and tall vertebral bodies. Studying a series of 29 families, we have mapped the disease locus gene on chromosome 6p21.1, and then identified mutations in the CUL7 gene.

Following this initial study, we have collected the samples of 29 additional 3 M families and identified CUL7 mutations in 16/29 comprising 18 novel mutations. This series included one terminated pregnancy at 33 weeks of gestation. The fetus presented with severe growth retardation, normal head circumference, facial features, prominent heels and long slender tubular bones suggestive of 3 M syndrome. By direct sequencing, we first identified a CUL7 splice site mutation (c.1215+1G>A), inherited from the father. In addition, we found a large de novo deletion (> 3.84 Mb) encompassing the CUL7 gene. Histological study of the femoral growth plate from this case showed an increase in the chondrocyte density and size in the resting and proliferative zones but no major abnormalities in the prehypertrophic and hypertrophic zones, suggesting that CUL7 is mainly involved in the chondrocyte proliferation but not in their differentiation.

We conclude that CUL7 is the major gene responsible for 3 M syndrome accounting for 77.6 % of our cases (45/ 58). The absence of any mutation in 13 patients and the exclusion of the 6p21.1 locus in 5 consanguineous families argue in favor of a genetic heterogeneity.

P0694. Mutational analysis of *CYP 21* gene in patients with non-classical and heterozygous forms of 21-Hydroxylase deficiency

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Congenital adrenal hyperplasia (CAH) is a common autosomal recessive disorder and more than 90% of CAH cases are caused by mutations in the *CYP 21* gene. Variable degrees of 21-hydroxylase enzyme impairment caused by different mutations are correlated with the clinical severity of the disease. Two forms of CAH exist depending on the clinical symptoms. The classical, severe form consists of the simple virilizing and the salt-wasting types, whereas the non-classical (NC) form is milder and later onset. In this study, we screened *CYP 21* gene in forty-one NC and heterozygote form of patients to detect the most common mutations, other rare mutations, and possible novel mutations. At first, we screened all cases for the V281L mutation by PCR-RFLP. We detected V281L mutation in three patients, one of whom was homozygote, and two were heterozygote for the mutation. Using sequencing technique, we detected two different partial gene conversions comprising the promoter and the first exon of the *CYP 21* gene. Some alterations associated with 21-hydroxylase deficiency were detected, such as C89T, C/A655G, C2108T, and C2578T. We also described three novel non-synonymous exonic alterations (T2555A, A2094T, and C2700A) that could be considered as potential mutations affecting the enzymatic activity. In addition, we identified a new genomic change (G-47A) in the promoter region of the gene. The other alterations detected in this study were intronic or at the 3' UTR of the *CYP 21*. This is the first study characterizing the molecular basis of NC and heterozygous forms of CAH in Turkey.

P0695. No evidence for a founder effect of CYP21A1P/CYP21A2 chimeric genes causing 21-hydroxylase deficiency in Brazil

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CYP21A2 gene which encodes 21-hydroxylase enzyme, as well as the class III histocompatibility genes, is located between HLA-B and HLA-DR loci. In the same loci there is a cluster of overlap genes including *RP*, *C4*, *CYP21* and *TNX* which is known as RCCX module. Almost 70% healthy individuals present a duplicated RCCX module arranged in tandem (bimodular), but mono- or trimodular arrangements are also observed in normal populations. The variability of RCCX number is considered to be a result of unequal crossovers. Mutations on *CYP21A2* gene cause 21-hydroxylase deficiency which is responsible for 95% Congenital Adrenal Hyperplasia, an inborn error of metabolism that impairs cortisol synthesis in the steroidogenesis. In Brazil, after studying the molecular basis of 21-hydroxylase deficiency in 112 unrelated patients, a total of 44 disease-causing alleles were found to carry a chimeric *CYP21A1P/CYP21A2* gene (20%). In the present study we describe different compositions of chimeric genes observed in mono-, bi- and trimodular alleles (50%, 41% and 9%, respectively). Seven different chimeric *CYP21A1P/CYP21A2* haplotypes were observed in momomodular alleles whereas five and four chimeric haplotypes were identified in bi- and trimodular alleles, respectively. In conclusion, the results described here indicated that there is no evidence for a founder effect of alleles bearing chimeric genes causing 21-hydroxylase deficiency in Brazil.

P0696. Cystic fibrosis studies: an individual spectrum of CFTR gene mutations in Lithuania

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Mutational testing of cystic fibrosis transmembrane conductance regulator (CFTR) gene is in action in Lithuania since 1993. However, molecular genetic diagnostics of cystic fibrosis (CF) in is still a considerable problem due to mutational heterogeneity of this gene in the Lithuanian population. 68 unrelated CF patients (136 CF chromosomes) were tested for CFTR gene mutations combining different methods. Initial testing for F508del using PCR with oligonucleotide primers bridging the deletion was followed by the DGGE-based screening of all CFTR gene exons and subsequent identification of the mutations by direct sequencing. Duplex PCR was applied to identify the CFTRdele2,3(21kb) mutation. The CF patients with one or two CFTR gene mutations unidentified were tested for 33 most common mutations using Cystic Fibrosis v.3 Genotyping Assay (Abbott, Germany). 18 different CFTR gene mutations were identified in Lithuania. Out of them, five appear to be relatively common: F508del (61.76%), CFTRdele2,3(21kb) (5.15%), R553X, N1303K, 3849+10kbC>T (each 2.94%). Other mutations were identified in single cases (0.74%). Some of them are relatively common in other populations (W1282X, G542X, 394delTT), but the majority are rare (G314R, R1066H, 3667insTCAA, 574delA, L138ins, 4006-4A>G, E217G, V754M) or even not yet described elsewhere (4171insCCTA, 794delC). Mutations on 20 CF chromosomes (14.71%) have not yet been identified. In conclusion, the applied PCR-based approach resulted in 18 different CFTR gene mutations in CF patients from Lithuania with the overall identification rate of 85.3%. F508del prevails (61.76%), but the spectrum of CF mutations in Lithuania appears to be individual in comparison with other European populations.

P0697. Cystic fibrosis and modifier genes in mexican population

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Cystic fibrosis (CF) is the most common monogenic disease in Caucasian population, its frequency is about one in 2500 live born. The

estimated frequency in Mexican population is about 1/8500. The mutation frequency differs specially for low frequent alleles. Heterogeneity in pulmonary manifestations cannot be explained by the genotype. Recent studies are focused to the study of genes modifiers, which are different genes from the primary gene cause of the disease that influences the clinical manifestations. This work shows some advances in CF diagnosis and modifier genes analysis in Mexican population. DNA was extracted from peripheral blood of CF patients. Thirty six frequent mutations in CFTR gene were analyzed by PCR and reverse hybridization with Allele Specific Oligonucleotides probes. The polymorphism analyses of modifier genes were made by PCR-RFLP. Mutation was detected in one or both alleles in 15 out of 19 patients (79%). Delta (D) F508 frequency was 45%, lower than the reported for Caucasian population (60-70%), and in agree of a published paper for our population. Twenty nine percent was different mutant alleles of DF508, and 26% of the mutant alleles remain undetected. This work is in progress of selection and recruitment of patients and controls, as well as standardization of the other genes modifiers, along with some other biomarkers that will allow to understand the physiopathology of CF and to define a possible genetic risk profile of severe pulmonary disease, and the pursuit of patients with risk profile with the purpose of improving the quality of life.

P0698. CFTR gene study in African children with cystic fibrosis-like phenotypes: identification of a novel p.A204T mutation

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Cystic fibrosis (CF) is one of the most common autosomal recessive disease in Caucasians with an incidence of 1 in 2500 births. However, very little is known about CF in Black populations from Africa where this disease has been considered to be rare. Nevertheless, CF is still under-diagnosed in this population. Indeed, CF clinical features are often similar to those of other frequent diseases in Africa such as malnutrition, tuberculosis, chronic pulmonary infections and HIV/AIDS. Moreover, molecular analyses remain inaccessible in many African countries. We investigated 60 unrelated Rwandan children with CF clinical features. We applied a gene scanning approach using DHPLC and MLPA techniques to analyse all exons and flanking intron sequences of the CFTR gene, in order to characterise CF mutations, sequence variations and gross genomic rearrangements. Four different CF-mutations, including one previously undescribed missense mutation (p.A204T in exon 6a), and 9 polymorphisms were identified. A study of CFTR-p.A204T mutation by immunoblotting in transiently transfected HeLa cells, revealed a lower level of p.A204T CFTR mature protein relative to CFTR-wild type. The frequencies of coding single-nucleotide polymorphisms (c.2694T>G, c.4521G>A and c.1540A>G) were similar in a control group. Our study identified five African patients with CF clinical features, a positive sweat test and a single CFTR mutation. One of them carried a novel mutation. Despite of multiple phenocopies due to environment-linked diseases, we consider that a sweat chloride test and, if positive, a specific CFTR mutation screening could be useful for these patients with CF-like symptoms.

P0699. A new splicing mutation in CFTR gene

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We describe 2 unrelated Italian patients affected by Cystic Fibrosis whose CFTR mRNA contains an insertion of 100 nt between exons 6b and 7 corresponding to a portion of intron 6b.this sequence resembles a cryptic exon because it is characterized by an upstream *ag* and a downstream *gt* sequence which is most probably recognized as 5' and 3' splice site by the spliceosome. At DNA level, sequencing analysis of the 2 patients showed a short deletion in intron 6b. We hypothesized that the mechanism involved in the aberrant splicing may be that the mutation creates a new sequence identified as a target sequence of an SR protein. In fact, the inserted sequence at transcript level was analyzed by ESEfinder web site (http://exon.chsl.edu/ESE/) that identifies exonic splicing enhancer (ESE) consensus motifs. The analysis showed that the short deletion creates a new sequence that is the target site of an SR protein allowing the definition of the new exon. In order to elucidate the mechanisms involved in the aberrant splicing, we carried out hybrid minigene experiments. With these experiments, we demonstrate that the short nucleotide deletion in intron 6b is the cause of altered splicing as we found in the 2 CF patients at RNA level.
RNA-protein interaction studies are in progress to detect which protein interacts with the mutated allele.

P0700. Mapping and sequencing of the mouse Dac2j mutation, a model for human ectrodactyly SHFM3

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SHFM3 is a congenital limb malformation affecting the hands and feet. It is caused by an ~500kb duplication at 10q24. Dactylaplasia is a similar inherited limb malformation in mice and is thus a model for human SHFM3. Sidow et al reported two alleles, Dac1J and Dac2J, mapping in the region syntenic with the duplication in SHFM3. Dac1J is an insertion of a transposon 10kb upstream of Fbxw4, while the exact molecular lesion in Dac2J has not yet been characterized. Here, we report mapping and sequencing of Dac2j by inverse PCR and show that it is caused by insertion of an transposon, similar to the Dac1j allele. Complete sequencing of the Dac2j and Dac1j insertions demonstrated that both elements belong to the MusD family of retroelements. We constructed a phylogenetic tree including our sequences and all Blast matches. These MusD elements are close to each other, and they branch within the clade of other active young MusD elements from the mouse genome, suggesting that active MusD elements group together. Interestingly, Dac2j occurs within a highly conserved element that may represent a regulatory sequence. We tested for duplication of the region in Dac mice, since this is the mutation mechanism in human SHFM3. qPCR on genomic DNA failed to identify any copy number differences between either Dac mutant and wild-type littermates. Both the human and mouse phenotypes seem to be caused by a disruption in the normal gene expression patterning in limb bud development, but the mechanisms are not known.

P0701. Mutational analysis of the ATP2A2 gene in Darier's Tunisian families

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Darier's disease (DD, MIM 124200) also known as keratosis follicularis, is a rare autosomal dominant genodermatosis characterized by altered keratinisation of the epidermis, nails and mucous membrane. The typical clinical presentation includes keratotic papules and plaques in seborrheic regions, palmoplantar pits and nails dystrophy, with histological acantholysis and dyskeratosis. The disorder has an estimated prevalence of 1: 55 000. Several mutations within *ATP2A2* gene have been identified as the cause of the disease. This gene encodes the sarco(endo)plasmic reticulum Ca^{2+}-ATPase type 2 (SERCA2) involving in intracellular calcuim signalling.
We report here the first molecular investigation of DD in Maghrebian population. Mutations analyses were performed by direct sequencing of five Tunisian patients belonging to two unrelated families. Two previously reported heterozygous mutations were identified (R677X) and (D702N), within exon 14 and exon 15 of *ATP2A2* gene respectively. Previous studies showed that R677X mutation resulted in the synthesis of truncated protein and D702N completely abolished ATPase activity. We compared clinical data of examined patients with phenotypic features described among patients from the literature, and found no obvious genotype-phenotype correlation. Phenotype variability was observed even among Tunisian patients sharing the same mutation suggesting that additional factors are important contributors to the clinical phenotype.

P0702. Identification of reduced copy number of DAZ genes in a group of Romanian idiopathic infertile men

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Male infertility is now a major reproductive health problem and the field has attracted considerable attention from scientists and clinicians. Approximately 15% of men with idiopathic infertility have microdeletions of the Y chromosome. The DAZ genes are candidate fertility factors that lie within the human Y chromosome's AZFc region. AZFc deletions including all four members of DAZ gene family represent the most frequent molecular cause of spermatogenic impairment. Besides complete DAZ family deletions, partial deletions are also associated with impaired spermatogenesis. PCR digest assay is one of the methods that can distinguish among the different copies of the four DAZ genes using subtle sequence differences located in introns and among members of the DAZ genes. These differences are termed sequence family variants (SFVs). We use this approach to determine the exact number of genes in a group of idiopathic infertile men. We screened the genomic DNA of 54 infertile males and 40 healthy fertile controls. Using PCR digest assay we determined that 2 patients of 54 had a deletion of two copies of DAZ (DAZ1 and DAZ2) showing that these deletions can be the cause for spermatogenic failure No deletions were detected in the control group. Initial molecular investigation by multiplex PCR was conducted according to European guidelines for the molecular diagnosis of Y chromosome microdeletions and all patients presented no deletions for DAZ genes. Our study, the first in a Romanian population, suggest a cause and effect relationship between partial DAZ gene microdeletion and idiopathic infertility.

P0703. Growth factor-induced stem cell differentiation into inner ear hair cell precursors on two- and three- dimensional surfaces

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Sensorieural hearing loss is associated with damage and loss of inner ear hair cells. Although hair cells within the avian and reptile cochlea are able to undergo spontaneous regeneration following damage to the auditory system, mammalian hair cells do not possess this ability. The availability of stem cells has presented the opportunity to establish therapies based on replacing or regenerating damaged cells within the inner ear. To explore these therapies it is essential to understand the normal hair cell differentiation pathway. Our investigations focused on the ability of mouse embryonic stem (ES) cells to commit to hair cell lineage by identifying key cytokines involved in hair cell determination. ES cells were differentiated into embryoid and neurectodermal embryoid bodies and treated with different combinations of cytokines. Commitment to hair cell lineage was assessed by expression of hair cell markers via semi-quantitative RT-PCR and flow cytometry. Since terminally differentiated hair cells were not generated with high efficiency on 2D tissue culture plates, a tissue engineering approach was undertaken using biodegradable polymers. The copolymer poly(lactic-co-glyoclic acid) PLGA was formed into 3D constructs using thermally induced phase separation. Morphology of polymers mimicked key architectural features of the inner ear sensory epithelium to encourage growth and differentiation of cells into inner ear types. Taking the most promising culture conditions from 2D work, partially differentiated ES cells were successfully grown on PLGA scaffolds with cells co-expressing hair cell markers. These approaches provide encouraging results for the possibility of differentiating inner ear hair cells *in vitro*.

P0704. Diamond-Blackfan Anemia - Haploinsuffiency or RNA binding?

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Diamond-Blackfan-Anemia (DBA) is a congenital disorder with absence/decrease of erythroid precursors in the bone marrow. ~25% of DBA patients carry a mutation in the ribosomal protein S19 (*RPS19*), ~2% in the *RPS24* gene. The molecular basis underlying DBA is yet unclear.

We investigated fibroblast from patients with RPS19 and RPS24 mutations and found a significantly prolonged ($P<0.05$) generation time (41h and 37h) compared to controls (~25h). These results might reflect haploinsuffiency as a suggested mechanism in DBA. To investigate whether these mutations have an effect on ribosomal proteins (RPs), we measured relative amounts of several RPs in mutant fibroblasts. No consistent reduction of RP-levels could be detected. To clarify the regulation of RPS19 transcript and protein levels, we analyzed the RPS19 transcript and its interaction with protein more intensively. Using 5'RACE, we identified a surprising variation in the transcription start site. Over 10 previously unknown 5'UTR variants could be detected (up to 95 additional nucleotides at 5' end compared to NM_0010122). The longer variants seem to be less abundant but highly structured. qRT/PCR revealed tissue specific expression patterns of these variants. Furthermore, we discovered that the RPS19 protein binds to the 5'UTR of its own mRNA with a K_d of 28.5±2.9nM. Preliminary mapping of the binding site indicates binding of RPS19 protein close to the 5'TOP-sequence in the 5'UTR. Taken together, our data suggest a regulatory mechanism of *RPS19* mRNA by the interaction with RPS19 itself and, by a variety of transcript variants which differ in the highly structured 5'UTR.

P0705. A novel Titin mutation causing dilated cardiomyopathy was found in family from Galilee

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Idiopathic dilated cardiomyopathy (DCM) is a major cause of heart failure and heart transplantation in young adults. In about 30% of the cases DCM is a familial disease. We studied a large Muslim-Arab kindred from Galilee with familial DCM inherited as an autosomal dominant trait.

We identified 13 affected individuals: 8 males and 5 females. Two were cardiac transplant recipients and 7 had symptomatic heart failure. None had skeletal myopathy.

Linkage analyses to candidate loci excluded the following established causes of DCM: MYH7, MYH6, SCN5A, DES, TNNT2, LMNA and SGCD. ACTC and PLN were excluded by direct sequencing.

Haplotype surrounding the titin (TTN) gene, was found in all of the affected individuals. LOD score calculation yielded a maximal score of 3.44.

During sequence analysis we identified an Adenine insertion at position 59014 creating a stop codon 4 bp down stream, predicting a truncated protein. The mutation was not found in 400 normal chromosomes but was identified in 6 non-affected family members. The novel mutation was not found in 2 other Muslim-Arab families with DCM.

TTN, mapped to chromosome 2q31, encodes to a giant muscle protein with a key role in muscle assembly, force transmission and maintenance of resting tension.

TTN is a structural protein required for sarcomere function, which has been considered a rare cause of DCM. Truncation at about 3/4 of the protein's length is predicted to compromise titin's binding to myosin (at the A-band) and its anchoring to the M-disk, thereby impairing the sarcomere's structure and passive tension.

Identification of the mutation in asymptomatic family members should facilitate early diagnosis and therapy in pre-clinically affected individuals.

P0706. MLPA assay as a screening tool for mutations in DMD/BMD patients and their female relatives

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Our study, part of a current national research program, aims to evaluate the dystrophin gene mutational pattern for the Duchenne/Becker muscular dystrophies (DMD/BMD). Knowing the mutational pattern of these diseases is proved to be of prognostic value and major importance for genetic counseling. For this purpose we recently introduced the multiplex ligation-dependent probe amplification (MLPA) method to identify mutations on the dystrophin gene in a series of DMD/BMD patients and their female relatives. We report our one year results and experience with the MLPA DMD commercial kits, which allow scanning of all 79 dystrophin exons in two PCRs. The amplicons were analysed on a 3100 Avant ABI Genetic Analyzer. We investigated 102 DNA samples from: 52 male patients with clinical and muscular biopsy picture of DMD, 43 female relatives, one amniotic fluid sample from a possible affected fetus. We assessed the extent and location of deletions and duplication and ascertained frequency of de novo or familial mutations with major importance for genetic counselling. The MLPA screen revealed mutations in 75% of cases: 85.72% deletions, 14.28% duplications and one non-contiguous deletion. The largest deletion detected involved almost half of the gene (exons 3-42), while the others were restricted to 2-5 exons, especially the exons 45 - 50. Because most the detected mutations encompassed more exons, no additional analysis was performed. The method proves to be easy to handle, accurate, highly reproducible. Our results recommend the MLPA assay as a routine screening tool for dystrophin mutations.

P0707. Identification of deletions and duplications of the DMD gene in Macedonian patients using Multiplex Ligation-dependent Probe Amplification (MLPA)

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Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are caused in the majority of cases by deletions of the DMD gene. Mutational analysis is complicated by the large size of the gene, which consists of 79 exons and 8 promoters spread over 2.2 million base pairs of genomic DNA. The majority of recognized mutations are however, copy number changes of individual exons, which traditionally have been identified by three common multiplex polymerase chain reaction. Here we report the use of the newly developed quantitative assay multiplex ligation-dependent probe amplification (MLPA) to determine the copy number of each of the 79 DMD exons. The sensitivity and accuracy of MLPA were assessed and compared with multiplex PCR in total of 92 subjects with DMD or BMD. MLPA was able to detect all previously detected deletions. In addition, we detected five new deletion and four duplications. The extend of the deletions and duplications could be more accurately defined which in turn facilitated a genotype - phenotype correlation.

P0708. Reduction of sperm DNA-fragmentation via Magnetic Assisted Cell Sorter (MACS)-system

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In the course of an ART and especially an ICSI the used spermatozoa are meticulously examined according to WHO-criteria, like concentration, motility and morphology. So a fragmentation or other deterioration of the DNA cannot be detected. The aim of our study is to determine and, if required, reduce the amount of spermatozoa with DNA-fragmentation (DNA-Fragmentation Index, DFI) within the ejaculate.
Ejaculate samples of 55 patients were purified via the MACS-system. The native and the purified part of the sample were then measured in a cytometer. The purification was carried out with the help of magnetic marked Annexin V and an appropriate column (the MACS-System). The DNA was stained by 4´-6-diamidino-2-phenylindole (DAPI). In each sample 20,000 cells were measured and the percentage of cells with DNA-fragmentation was determined. In comparison, samples of 37 additional patients were analyzed as described above, both natively and according to density gradient centrifugation.
The average DFI was 17.6% in native ejaculate, while, after purification by MACS, this percentage was reduced to 11.29%. By purification via density gradient centrifugation a percentage of 11.83% was obtained, while the percentage in native ejaculate was 15.96%. So we obtained a reduction via MACS of 35.85% and via density gradient centrifugation of 25.88%.
On the basis of our results it has been found that by means of MACS the DFI can be reduced distinctly (35.85% vs. 25.88%). Furthermore, the high DFI in the samples purified by density gradient shows that DNA-fragmentation cannot be reduced by this common method.

P0709. Folate gene alteration: Dose it influence the chromosome 21 nondisjunction in Down syndrome

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Common polymorphisms in the MTHFR (C677T and A1298C) and MTRR (A66G) genes have been reported to be a maternal genetic risk factor for Down syndrome in some populations. However, considering parental origin of trisomy 21 in mother of Down syndromes has drawn less attention in previous studies. This may cause reduction in degree of associations of those maternal genetic risk factors and occurrences of chromosome 21 trisomy. In this study parental origin of chromosome 21 were tested in 260 families of Down syndromes using five STR markers related to chromosome 21 (D21S11, D21S1414, D21S1440, D21S1411, D21S1412). Parental origins were determined successfully for 226 of cases. Mothers have been categorized in maternal (198) and paternal (28) groups. All mothers of Down patients totalled 226 individuals, and a normal control group contaned 176 mothers with halthy children. These were tested for common polymorphisms C677T, A1298C in the MTHFR and C66G in the MTRR gene. A significant association was detected between maternal derived chromosome 21 mothers and A1298C of the MTHFR gene ($P<0.002$, $\chi^2>12.06$). This study has showed for the first time the importance of parental origin determination in the study of maternal genetic risk factor of Down syndrome. The significance of A1298C polymorphism of MTHFR gene as maternal genetic risk factor of Down syndrome in Iranian population increases the knowledge about etiology of Down syndrome and helps in better prevention of Down syndrome.

P0710. Homozygous Dubin-Johnson Syndrome. A Novel Mutation in MRP2 Gene in a Large Family from Slovakia

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Hepatobiliary excretion of conjugated bilirubin is mediated by an ATP dependent canalicular transporter, the multidrug resistence associated protein 2 (MRP2/cMOAT). MRP2 is responsible for biliary excretion of glucuronide and glutathione conjugates of endogenous and exogenous compounds.
Dubin-Johnson syndrome (DJS) is an inherited autosomal recessive disorder characterised by the absence of functional protein MRP2 at the canalicular membrane of hepatocytes. Known mutations in this gene cause impaired maturation and trafficking of the mutated protein from the endoplasmic reticulum to the Golgi complex.
As a result, conjugated hyperbilirubinemia, increased urinary excrection of coproporphyrinogen isomer I, and deposition of melanin-like pigment are found. Otherwise liver function is normal.
Here we present a study of a large Slovak family with unconjugated hyperbilirubinemia Dubin-Johnson type. These subjects were found to be homozygous for the novel mutation 1012delGT, which causes a frameshift.
Dubin-Johnson syndrome is very rare disorder, and the homozygous form of mutations has been reported only in unique cases.
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P0711. Investigation of mitochondrial tRNA^{Lys} and ATPase 6/8 genes mutation in Duchenne Muscular Dystrophy (DMD) patients

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Duchenne muscular dystrophy (DMD) is an X-linked recessive genetic disorder resulting from mutations in the dystrophin gene. About two-thirds of the affected patients have large deletions or duplications, which occur in the 5′ and central region of the gene. The mitochondrial tRNA^{lys} and ATPase6/8 genes mutations are the hot spots in mitochondrial disorders. We performed mutations screening of tRNA^{Lys} gene and also ATPase6/8 genes in 20 patients who referred as DMD to evaluate probable role of mitochondrial mutations in DMD. Mitochondrial tRNA^{Lys} gene and ATPase 6/8 genes were studied by PCR and automated DNA sequencing methods to find out any possible mitochondrial DNA (mtDNA) damage. We found 7 different mtDNA mutations in 8 patients (40%) out of 20 cases including T8503C, G8697A, A8850G, G8697A, C8684T, G8584A, A8701G and C8574T, meanwhile 87.5% of the mutations were observed on MT-ATP6.
Also, our study showed that 4 patients (20%) had an 8.9kb deletion in mtDNA. This study suggests that mitochondrial mutations may be important in pathogenesis of DMD.

P0712. A case of Smith-McCort Syndrome caused by a new mutation in the *Dymeclin* (*FLJ20071*) gene

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Dyggve-Melchior-Clausen (DMC) and Smith-McCort (SMC) are autosomal recessive allelic spondyloepimetaphyseal disorders caused by mutations in the *Dymeclin / FLG20071* gene (Cohn et al., *2003;* El Ghouzzi et al., *2003*). Most DMC and SMC rare families described in the literature are from Middle East and North Africa. Clinical identical features of both disorders are short limbs and trunk, a barrel shaped chest, progressive kyphoscoliosis, varus and valgus deformity of the knees, limitation in joint movement and brachydactyly. The two osteochondrodysplasias are only distinguished by the presence in the DMC patients of important development delay or mental retardation (Spranger et al, 1976). Radiological features include platyspondyly, metaphyseal and epiphyseal irregularities and a patognomonic lacy appearance of the iliac crests. Here we describe a case of Smith-McCort of a 6 years girl from a consanguineous family from Madeira Island. This affected girl presents the main clinical and radiological features of this disorder not revealing any development delay. Molecular analysis of the all entire coding region of *Dymeclin* gene was performed. A novel missense variant was identified in exon 15, C542R, in homozygosity. In order to clarify its clinical significance, additional studies were carried out. The comparison of the *Dymeclin* protein sequence and homologous proteins in other species showed that this amino acid residue is evolutionary conserved. This variant was also excluded in 50 Portuguese healthy individuals screened (100 chromossomes), which strongly supporting a pathogenic role. Therefore, we believe that this homozygous C542R mutation is responsible for the Smith-McCort phenotype.
Study supported by FLAD.

P0713. Mutational spectrum of the *DYSF* gene based on a large cohort of dysferlin deficient patients.

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Dysferlinopathies belong to the heterogeneous group of autosomal recessive muscular dystrophies. Mutations in the gene encoding dysferlin (*DYSF*) lead to distinct phenotypes, mainly Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM).
Due to the clinical heterogeneity, initial dysferlin protein analysis on muscle biopsy samples is essential to orientate diagnosis. However, diagnosis should be confirmed by molecular analysis of the *DYSF* gene.
The large size of the *DYSF* gene (> 150 kbp, 55 exons) makes this a challenging task on a routine basis. Methods for mutation screening are particularly useful in this regard.
Here, we report the results of mutational screening in the largest cohort reported to date. Altogether, 144 patients presenting dysferlin protein deficiency identified on muscle biopsy samples using immunohistochemical or Western-blot analysis were included.
Genomic DNA was screened for *DYSF* mutations using DHPLC analysis, and subsequent sequencing of detected variants, in a routine diagnostic setting.
In 100 patients (70%), molecular analysis identified two disease-causing mutations, confirming the diagnosis of primary Dysferlinopathy on a genetic basis. Furthermore, one mutation was identified in 17 (12%) patients, without identification of a second deleterious allele. Among the identified mutations, 37 have not been reported before, which corresponds to more than 10% of all *DYSF* mutations reported to date. Most of the identified mutations are predicted to produce a truncated protein or one amino-acid substitution, but we a report a high proportion of nonsense mutations as compared to previous series.

P0714. A dominant negative form of the transcriptional factor C-Jun alters the expression of two lipid metabolism related genes that result in opposite effects on mouse plasma lipid levels

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c-Jun (cellular Jun) is a transcription factor activated by phosphorylation by the SAPK (Stress Activated Protein Kinase) / JNK (c-Jun-N-terminal-Kinase) pathway in response to extracellular signals and cytokines. We show that adenovirus mediated gene transfer of dn-cJun (dominant negative form of c-Jun) in C57BL/6 mice increased greatly apoE (apolipoprotein-E) hepatic mRNA and plasma levels, which resulted in dyslipidemia characterized by elevated plasma lipid levels and accumualtion of discoidal HDL (High-Density-Lipoprotein). A similar but more severe phenotype was generated by overexpression of the mouse apoE in C57BL/6 mice, suggesting that the dyslipidemia induced by the dn-cJun can be attributed to the overexpression of apoE.
Unexpectedly, infection of apoE-/- mice with adenovirus expressing dn-cJun reduced by 70% plasma cholesterol, suggesting that the dn-cJun influenced the expression of other genes that control plasma cholesterol levels. To identify these genes we performed whole genome expression analysis of livers from two groups of 5 apoE-/- mice, infected with adenoviruses expressing either the dn-cJun or the Green Fluorescence Protein. Bioinformatical analysis and Northern Blotting validation, revealed that dn-cJun increased greatly the apoE mRNA and reduced by 70% the Scd1 (Stearoyl-CoA-Desaturase 1) mRNA. The involvement of Scd-1 in lowering plasma cholesterol was confirmed by restoration of the high cholesterol levels of apoE-/- mice following coinfection with adenoviruses expressing dn-cJun and Scd-1.
Conclusively dn-cJun appears to trigger two opposing events in mice that affect plasma cholesterol and triglyceride levels: One results in apoE overexpression and triggers dyslipidemia and the other results in inhibition of Scd-1 and offsets dyslipidemia.

P0715. Functional studies of in silico predicted splicing regulatory elements in the dystrophin gene

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The splicing of pre-mRNA is accomplished by the combinatorial recognition of multiple degenerate signals, resulting in a network of RNA-protein interactions across an exon and/or intron. In addition to canonical sequence elements adjacent to splice junctions, more distant auxiliary regulatory elements such as exonic splicing enhancers (ESEs) and exonic splicing silencers (ESSs) play a crucial role in many RNAs for correct splicing. Identifying functional ESE or ESS sites is rather difficult due to the short and degenerate nature of these elements and the lack of understanding of their sequence context. Besides the identification of naturally occurring exonic mutations that cause splicing defects in patients and provide valuable tools to identify such auxiliary RNA *cis*-elements, we have developed a new strategy to identify splicing signals in the dystrophin gene. This strategy is based on two complementary approaches: (1) the bioinformatics prediction of putative splicing signals using the Splice Site Finder software (http://www.umd.be/SSF), and (2) the experimental validation of the *in silico* predicted sequences. Functional assay relies on an easy-to-use and reliable system based on fluorescent splicing reporter minigenes, allowing flow cytometry analyses, in which the level of fluorescence depends on the splicing of a tested exon. The characterization of functional *cis*-acting splicing sequences such as branch points and ESEs will contribute to the comprehensive knowledge of multiple signals that regulate splicing of this huge gene. Also, the identified sequences could constitute therapeutic targets of the exon-skipping strategy to reverse the severe DMD phenotype into the milder BMD one.

P0716. Different profiles of SNPs association with high arterial pressure syndromes

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There are a number of syndromes connected with high arterial pressure that have genetic predisposition. This circimstanse arise a question concerning of molecular bases of genetic predisposition and differentiation of the syndromes on association with different genes. We investigated a role of AGT, ACE, ADRB2 and APOE genes in essential arterial hypertension (EAH), gestantional hypertension (GH) and preeclampsia. Different profiles of SNPs association with the syndromes were revealed. The ADRB2 Arg16Arg variant was associated with EAH (OR=3,84, P=0,005). The association of APOE PI-22M/Q polymorphism with EAH was not significant. However an association of APOE 22QQ with ADRB2 Arg16Arg among patients with EAH that significantly increases a risk was found (OR=6,82, P=0,01). This may be due to a contribition of dysbolism of plasmatic lipids in EAH onset. Three genes (AGT, ACE, ADRB2) were significantly associated with GH (OR=2,87-3,91; P=0,01-0,005). An addition GAH was characterisized by genetic interaction between ACE D/D and ADRB2 Arg16Arg with a risk increasing (OR=5,80, P=0,01). These results may be explained by increasing of RAAS system activity during a pregnancy. The AGT T235T variant was associated with preeclampsia (OR=3,2; P=0,01). The data suggest a possibility distinguish predisposition to the syndromes with high arterial pressure by different profiles of SNPs association.

P0717. Inventory of TOR1A mutation carriers in France.

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Dystonia is a neurological movement disorder characterized by involuntary movements or postures. The only currently identified primary Early-Onset Torsion Dystonia (EOTD) gene is *TOR1A* that encodes the torsinA chaperon protein. The majority of cases from various ethnic groups are caused by a unique and recurrent autosomal dominantly inherited deletion: c.907delGAG. EOTD prevalence in the general European population is estimated between 0.3 to 0.5:100,000, that would correspond to a calculated number of symptomatic carriers ranging from 184 to 307 in the French population. Collaborations have been established between the four French laboratories in charge of this diagnostic: 52 probands with EOTD of which 26 cases in a familial context of the disease have been diagnosed with the mutation. Whenever possible, the presence of the deletion in each parent, the number of relatives carrying the mutation, and their phenotypic status (symptomatic or asymptomatic) has been investigated. Overall, 108 individuals were found to carry the mutation of which 20 were asymptomatic. This number of *TOR1A* carriers, lower than expected, may be related to non exhaustive testing of these families and/or lower prevalence of *TOR1A*-linked dystonia in France.
Haplotypes with *TOR1A* flanking microsatelites could be constructed in 31 families. The common Ashkenazi Jewish (AJ) haplotype was found in two families and the second AJ haplotype previously reported (Lebre 1999) has been found in two other families. Only one other recurrent haplotype has been characterized in two other families confirming the quasi absence of founder effect for the *TOR1A* mutation in France.

P0718. Novel splice mutation in the *EDAR* gene in a Lebanese female with an atypical form of anhidrotic ectodermal dysplasia

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More than 170 different pathological clinical conditions have been defined as ectodermal dysplasia (ED). They all share as common feature abnormalities in hair, teeth, nails and sweat glands, but must be associated with anomalies in other organs and systems. EDA is characterized by the triad of signs comprising sparse hair, abnormal or missing teeth and inability to sweat due to lack of sweat glands. Here we present a 18-year-old woman, born to first cousin parents that belong to the Lebanese Shiite Muslim community. Behind the triad of features characteristics of EDA she presents an atypical association with various clinical manifestations. Lacrimation was nearly absent and salivary secretions reduced. The lips were thick and everted. The skin was dry and velvet. Absence of breasts and a rudimentary extranumerary areola and nipple on the left side were noted as well. She also had marked palmar and plantar hyperkeratosis. Light microscopy of skin biopsies showed orthokeratotic hyperkeratosis and absence of sweat glands. We identified a novel homozygous mutation (IVS9+1 G>A) in *EDAR* gene. RT-PCR performed on patient skin biopsy RNA, showed that the mutation results in total absence of *EDAR* transcript and consequently of the EDAR protein, which likely results in total abolition of Ectodysplasin mediated NF-kB signaling. NF-kB signaling is involved principally in early development of ectodermal appendages and in tooth development. It's recent involvement in embryonic salivary gland development as well as EDAR expression in lacrimal, and mammary glands may explain the complex phenotype observed in our patient carrying *EDAR* mutation.

P0719. Functional consequences of *EDAR* mutations on NF-kB and Lef-1/β-catenin signalling

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France.
Anhidrotic ectodermal dysplasia (EDA) is an ectodermal differentiation disorder characterized by sparse hair, abnormal or missing teeth and inability to sweat. X-linked EDA is caused by mutations in *EDA* gene, encoding ectodysplasin, a member of the TNF family. Autosomal forms of EDA, have been also described and are accounted for by two genes. *EDAR,* encoding a TNF receptor and *EDARADD* encoding EDARADD (for EDAR-Associated Death Domain). Recently, 17 novels mutations were described in *EDAR* gene in both familial and sporadic cases of EDA. The six truncating mutations result probably in the absence of EDAR and consequently in total abolition of NF-kB signalling. Among the 11 missense mutations, 5 are located in EDAR Death Domain, and are responsible for both dominant and recessive EDA. In order to determine the effect of these mutations on EDAR function and signalling involving EDAR, site-directed mutagenesis was performed and NF-kB activation was first assessed after HEK293T cells transfection. The recessive I418T, T403M and R375H mutations affect NF-kB activation without impairing the expression of the mutated proteins. Interestingly, the dominant L377F and T413P mutations resulted in the absence of EDAR expression leading to total abolition of NF-kB signalling. Behind the central role of the Lef-1/β-catenin pathway in normal embryonic development and carcinogenesis, it also play important roles in ectodermal differentiation and hair follicle development and it was interestingly shown that EDAR represses the Lef-1/β-catenin-dependent transcriptional activity, independently of its effect on the NF-kB pathway. We are currently investigating the effect of our mutations on Lef-1/β-catenin pathway.

P0720. Polymorphism of IL4RA gene is associated with endometriosis

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Endometriosis is a common multifactorial disease. Typical endometrioid cells are characterized by increased cytokine activity. Cytokine genes are highly polymorphic that results in synthesis of proteins with various functional activities. The goal of the study focuses on the role of allelic variants of IL4 and IL4RA genes in pathogenesis of endometriosis.
DNA samples from the patients with endometriosis (n=36) and control group of women without any gynecologic complications (n=69) were included in the study. Polymorphisms of IL4 (-590T>C) and IL4RA (1902A>G or Gln551Arg) were defined by PCR-RFLP assay.
The distribution of IL4 and IL4RA genotypes was in agreement with the HWE law (p>0.05). The frequencies of alleles and genotypes of IL4 gene did not differ between studied groups (p>0.05). However the frequency of Arg/Arg genotype of IL4RA gene was significantly higher in endometriosis patients (16.7%) if compared to the control group (1.4%, p<0.01).
The results of this pilot study demonstrate an association of IL4RA gene polymorphism with endometriosis. According to odds ratio value (OR=13.60; CI: 2.43-76.26) Arg551/Arg551 genotype could be responsible for 13-fold increase in the risk of endometriosis . Our data substantiate the conclusion that Gln551Arg polymorphism could be treated as prognostic geneic marker of endometriosis.

P0721. Genetic investigation of epidermodysplasia verruciformis in Tunisian patients: exclusion of Algerian mutations

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Epidermodysplasia verruciformis (EV: MIM#226400) is a rare genodermatosis associated with a high risk of skin cancer. EV is inherited as an autosomal recessive trait. To date, 2 loci (EV1 and EV2) have been reported and 2 genes in EV1 locus, EVER1 and EVER2, have been identified. Mutations in these genes, encoding TMC6 (Transmembrane channel-like) and TMC8 respectively, are responsible for EV. Few populations have been studied and for the two genes, so far six mutations have been reported. As for several genodermatosis, similar mutational spectrum has been identified among Algerian and Tunisian patients, EV Tunisian patients were screened for the "Algerian mutations" (280 CàT and 754 or 755 del T).
This study was carried out in four EV independent nuclear Tunisian families. Mutation analysis was performed by direct DNA sequencing. None of the two Algerian explored mutations was found, suggesting that Tunisian patients do not share the same mutations as those described among Algerians. Three SNPs were identified: 1049C/T, IVS5+47A/G, and IVS5+26A/T, the last one being a novel sequence variation.

P0722. Autosomal recessive erythropoietic protoporphyria

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Erythropoietic protoporphyria (MIM 177000) results from deficiency of ferrochelatase (FECH)(EC 4.99.1.1.). It is a disorder of heme biosynthesis in which most cases are autosomal dominant inherited. However its penetrance is reduced. Overt disease requires the presence of one of the almost one hundred mutations in the *FECH* gene reported so far and the common allele in some populations IVS3-48C in trans. Less than 5% of the cases are classified as autosomal recessive forms. We describe one of such cases where the family studies also corroborate the relationship with disease manifestation of FECH mutations and the IVS3-48C allele.
The proband had skin photosensitivity since the age of 3 years old and colelithiasis at the age of 28. Her parents, brother and sister were all healthy adults. The family concern was the care of the first son of the proband, then 6 months old. In order to be able to assess the health care required genetic counselling was requested.
The molecular characterization of the *FECH* gene identified two deleterious mutations in the proband: IVS1-23C>T and C411G. All the other five family members were classified as heterozygous, three for the mutation C411G and two for the IVS1-23C>T. Although the allele IVS3-48C was also present in two of the five heterozygous it was so in cis.
This family is one of the few examples reported of autosomal recessive inheritance of erythropoietic protoporphyria and is yet another example of how a common allele modulates the penetrance of a dominant mutation.

P0723. Analysis of *p63* as a candidate gene for bladder exstrophy

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The Bladder-Exstrophy-Epispadias-Complex (BEEC) represents a spectrum of urologic abnormalities in which part or all of the distal urinary tract fail to close and are exposed on the outer abdominal wall. This rare congenital anomaly is thought to be a clinical spectrum ranging from isolated epispadias (EP) to classic bladder exstrophy (CBE), to its most severe form - cloacal exstrophy (CE). *P63*, a homolog of the *p53* tumor-suppressor gene, encodes multiple tissue-specific isoforms that act as transcription factors essential for proper embryologic development. Null *p63* mice exhibit severe craniofacial, limb, and skin anomalies. In addition to these, bladder exstrophy was recently reported in *ΔNp63*$^{-/-}$ mice. Human *p63* mutations have been associated with at least five developmental phenotypes, some with anomalies of the urogenital system, but not BEEC. We have initiated *p63* analysis in a cohort of BEEC patients. Direct sequencing of the entire coding region of *p63* did not identify obvious mutations in genomic DNA of 15 CBE and five CE patients. RT-PCR of cDNAs derived from normal and exstrophic human bladder and lymphoblast RNA is in progress. Several previously unreported *p63* isoforms were observed in both control and exstrophic samples. Furthermore, unusual RT-PCR band patterns were observed in some patients' samples and are being currently characterized. These data indicate that while genomic mutations of *p63* cannot explain BEEC in our study group, further studies of this BEEC candidate gene are warranted.

P0724. Hereditary Multiple Exostoses: report of a case and a novel mutation in *EXT1* in Iran

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Hereditary multiple exostoses is an autosomal dominant disorder characterized by multiple benign cartilaginous tumors at the juxta-epiphyseal regions growing outward from the metaphyses of long bones. We report a 28 years old man with hereditary multiple exostoses. He and his fiancée wanted to know the risk of transmission of theses bony overgrowth to their future infants. Clinical examination revealed multiple exostoses in humerus, femur, scapular and knee joints and shortening of metacarpals.
Molecular analysis of the genes EXT1 and EXT2 showed a novel mutation in exon 8 of EXT1. This mutation occurred in nucleotide 1701 (1701ins5) and produced a truncated protein in aminocid 620(L620X). Two genes have been identified so far mapping to 8q23-q24 and 11p11-p12, respectively. A third gene is supposed to be located on the short arm of chromosome 19 . About 90% of cases of Hereditary Multiple Exostoses are linked to these loci. The detection of this mutation has enabled us to provide appropriate genetic counseling concerning this complex situation. There has been no study on Iranian patients with HME. A national program to detect the genetic causes of HME in Iran can help to find novel mutations and to define proper mutation detection strategy.

P0725. Extralysosomal Localization of Globotriaosylceramide in Fabry Disease

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OBJECTIVE: To describe cellular and subcellular localization of globotriaosylceramide in order to assess potential abnormal interactions of globotriaosylceramide leading to disease in Fabry patients. METHODS: We used an anti-globotriaosylceramide monoclonal antibody for immunogold electron microscopy, immunohistochemistry, and immunofluorescence studies of tissues from patients on long-term enzyme replacement therapy (ERT) and controls. RESULTS: Immunoreactivity for globotriaosylceramide was present in heart, kidney, brain, intestines, adrenal gland, aorta, skin, liver and spleen in a variable pattern. In the brain, positive neuronal immunoreactivity was found only in the parahippocampal region. In all organs examined, globotriaosylceramide immunostaining was present in the cell membranes and cytoplasm of endothelial cells, even in the absence of lysosomal inclusions. Immunofluorescence immunolabeling of heart and kidney tissues from a Fabry patient showed colocalization of globotriaosylceramide with lysosomal, ER, and nuclear markers. Immunogold electron microscopy confirmed the presence of globotriaosylceramide in the cell membrane, lysosomes, ER, nuclear membrane and nucleus of vascular endothelial cells and fibroblasts even in the absence of lysosomal inclusions. Cultured fibroblasts from patients showed similar findings. Immunolabeling of organ tissues and cultured fibroblasts from 3 unaffected controls was uniformly negative for globotriaosylceramide by immunohistochemistry and electron microscopy. CONCLUSIONS: A substantial amount globotriaosylceramide immunoreactivity remains in cells and tissues even after years of ERT in Fabry disease. For the first time we demonstrate the presence of accumulated globotriaosylceramide in extralysosomal cellular regions. These findings are crucial for the understanding of disease mechanism and suggest the use of immunostaining for globotriaosylceramide to assess response to novel specific therapies.

P0726. Diagnosis in Facio Scapulo Humeral dystrophy remains challenging: atypical molecular patterns in two families

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Facio scapulo humeral muscular dystrophy (FSHD) is an autosomal dominant disease associated with a contraction of the repeated D4Z4 sequence at the 4q35 subtelomeric region. Routine diagnosis of FSHD is based on pulsed-field-gel-electrophoresis using the D4Z4 adjacent p13e11 probe. However, sequences homologous to D4Z4 exist in other chromosomal regions and cross hybridization might appear. Moreover, 4q subtelomeric region is subject to frequent translocations with the subtelomeric region 10q26, while D4Z4 contraction on 10q is not pathogenic. Towards clearing diagnosis procedures in FSHD, allele characterization is improved by the use of BlnI, cleaving only canonical 10q26 unit.
Here, we report two unrelated patients presenting typical FSHD features (Asymetric weakness, wasting of the upper limb girdle and face) with a particular molecular pattern. While simple digestion by EcoRI evidenced only normal-sized, supposedly non-pathogenic, D4Z4 alleles, EcoRI/BlnI double digestion revealed a short D4Z4 repeat array on chromosome 4, suggesting a complex rearrangement between 4q35 and 10q26 within the D4Z4 repeat. This feature is shared by several relatives, including one of the unaffected parents in each family.
Our knowledge of the FSHD pathophysiological processes is still incomplete, and these observations raise questions on the validity of usual molecular diagnosis tests in FSHD as well as their use for genetic counselling. Developing novel and/or complementary technical approaches, as for example competitive dosage analysis, is thus an absolute necessity to improve molecular diagnosis as well as for a better knowledge of the molecular structure of the region in affected patients.

P0727. Molecular characterization of hemophilia A in South East Bulgaria

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Hemophilia A is a common X-linked bleeding disorder affecting 1 in 5,000 males worldwide. The most common molecular defect is intron 22 inversion, while the rest of the mutations are single nucleotide substitutions in different parts of the gene. The knowledge of the causative gene defect has become an important tool in hemophilia care with respect to prediction of the patients' clinical course and safe genetic counseling of relatives. The aim of this study was to determine the molecular defects underlying hemophilia A patients from South-East Bulgaria. The molecular characterization of fVIII gene was performed in 50 unrelated hemophilia A patients. Southern blot analysis was used for detection of inversions in intron 22, while PCR followed by SSCP or DGGE was performed for mutation screening. The molecular defect was found in 33, or 66%, of analyzed patients. The most frequent molecular defect was inversion in intron 22, found in 18 (36%) of all studied patients. In one severely affected patient an Alu insert was found, in exon 14 at Cd.1224. Nucleotide substitutions were found in 14 (28%) patients. Using SSCP as a screening method, we have identified an Arg531→Cys missence mutation in exon 11 in six (12%) unrelated patients with mild to moderate hemophilia A. In two unrelated patients the same nonsense mutation was found (Cd-5; CGA→TGA). Six missense mutations (Asn90→Thr; Arg372→His; Tyr473→His; Glu456→Val; Arg1689→Cys; Arg2163→His) were private. Two of them were found for the first time.

P0728. Angelman syndrome caused by an identical familial 1487-kb deletion

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Angelman syndrome (AS, OMIM#105830) is a neurodevelopmental disorder characterized by mental retardation, ataxia, hypotonia, epilepsy, absence of speech, and specific facial features. At least four major mechanisms causing AS were validated: i) an interstitial deletion of 15q11-q13 (70-75%), ii) uniparental disomy (2-3%), iii) imprinting defects (3-5%), iv) *UBE3A* mutations (20%). Most deletions are similar in size (approximate 4 Mb) and occur de novo through maternal unequal

crossing over between low copy repeats (LCRs). Paternal occurrence of similar deletions, instead, results in Prader-Willi syndrome (PWS, OMIM#176270). In PWS no coding mutations have been found in contrast with *UBE3A* mutations in AS, suggesting that PWS is caused by loss of function of multiple genes.
Different sized deletions associated with AS are very rare. To our knowledge, at least two familial atypical deletions were reported, and the microdeletions caused AS in maternal inheritance but no PWS features in paternal inheritance, enabling differentiation of the PWS critical region (PWSCR) from the AS critical region (ASCR).
We encountered a similar family with an atypical microdeletion, consisting of an AS boy later confirmed, and asymptomatic mother and maternal grandfather. All three had an atypical microdeletion. We could successfully determine deletion breakpoints of the family, and will discuss about genes responsible for PWS.

P0729. Familial Defective apo B-100 (FDB): founder effect in a population of the South of Europe and comparison with the Familial Hypercholestolaemia (FH).

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We have identified a large number of subjects (n=19) with FDB (OMIM = 144010) due to the R3500Q mutation at APOB gene in a population of the South of Europe and the 7th FDB homozygote in the world. This was possible through identification of the first FDB heterozygous in Spain. The study of his family and the population of the same geographic area allowed us to identify the rest of the FDB cohort.
We studied the lipoprotein phenotype of 19 FDBs, and compared it with the one of subjects with classic FH of the same geographic origin, taking in account the type of mutations at LDLR gene: null-mutations TC= 343.7 (69.6), missense mutations affecting binding 3-5 repeat region TC= 394.4 (47.9), and missense mutations not affecting that binding region TC= 335.2 (60.2). FDB subjects showed a milder clinical and lipoprotein phenotype TC= 286.5 (57.9).
We have also studied the founder effect of our FDBs and the history of that geographic area of the Valencian Region. The analysis of the haplotype of the gene APOB of our FDBs and historical records of that geographic zone leads us to conclude that their mutation originated 7000 years ago in the Southwestern region of Germany, between Rhin and Main rivers.
TC = Total Cholesterol; data expressed in mg/dl with P<0.05 ANOVA.

P0730. A novel missense mutation in GALNT3 disrupts O-glycosylation activity and causes familial tumoral calcinosis

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Familial tumoral calcinosis (FTC) is an autosomal recessive disorder characterized by ectopic calcifications and elevated serum phosphate levels. FTC is caused by mutations in the *GALNT3* gene, which encodes the UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3), or by mutations in the *FGF23* gene, which encodes the fibroblast growth factor 23 (FGF23). GalNAc-T3 controls the initiation step of mucin-type O-glycosylation whereas FGF23 is a secreted phosphaturic factor involved in the regulation of phosphate homeostasis. Our previous studies demonstrated that FGF23 O-glycosylation by GalNAc-T3 is required for the secretion of functional intact FGF23. We performed a mutation analysis in a family with two affected individuals who presented the clinical features of FTC. Sequencing of *GALNT3* revealed a homozygous missense mutation (c.985G>A) at the amino acid position p.G329R which is highly conserved within the 15 members of the human GalNAc transferase family and among species. This residue is situated in the linker region between the catalytic and the ricin-like domain of GalNAc-T3. In vitro analysis of FGF23 O-glycosylation by GalNAc-T3(G329R), performed by MALDI-TOF mass spectrometry, showed lack of GalNAc-T3(G329R) activity. Previously reported *GALNT3* mutations in FTC have been null mutations or compound heterozygous missense mutations in the catalytic domain. We conclude that the novel missense mutation of GalNAc-T3 causes FTC.

P0731. A study of FGFR3 gene mutations in brazilian patients with achondroplasia, hypochondroplasia and thanatophoric dysplasia

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We studied the FGFR3 mutations in 80 brazilian patients with achondroplasia , 22 with hypochondroplasia and 9 with thanatophoric dysplasia. The DNA methodology used was first the RFLP with restrition enzymes and, if the mutation was not detected, we used direct sequencing of all gene.
In Achondroplasia we detected 75 cases with the G1138A mutation, 2 cases with the G1138C mutation, 1 case with the G1123T and 1 case with a C1150T mutation. When we studied the C1150T mutation in 50 normal Brazilians from different ethinic origins we detected 1 person with this mutation, and concluded that this mutation is a polymorphism .We didn't detected the pathologic mutation in two typical achondroplasia patients.
In Hypochondroplasia we found 14 patients with the C1620G mutation and 7 cases with the C1620A mutation . We didn't detected the mutation in one typical hypochondroplasia patient.
In Thanatophoric Dysplasia we had 7 patients with the type I, all of them presented the C742T mutation and, 2 cases of the type II, both presented the A1948G mutation.
This is the first study of a brazilian patients sample for FGFR3 mutations and we could concluded that the types and frequencies of FGFR3 mutations detected are similar to that described in other regions studied. We could define that the C1150T mutation (Trujillo et al., 2004) and detected by us in one achondroplasia patient is a polymorphic variant not related to diseases determined by mutations in FGFR3 gene.

P0732. MEFV Mutations in Turkish Patients Suffering From Familial Mediterranean Fever

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Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory periodic disorder characterized by febrile and painful attacks due to inflammation involving the serosal membranes in the abdomen, chest or joints. Over 50 mutations have been identified in the MEFV gene responsible for FMF. AIM: To identify the distribution and the frequency of the MEFV gene mutations in Turkish FMF patients PATIENTS AND METHODS: The study was carried out on 354 clinically diagnosed Turkish FMF patients. Mutation screening of the MEFV gene was performed by DNA sequencing of exon 10 in all patients and by FMF specific StripAssay (this assay is based on a Polymerase Chain Reaction-Reverse Hybridization technique) for E148Q, P369S and F479L mutations of exons 2,3, and 5, respectively in 88 patients. RESULTS: Of the 354 unrelated patients investigated, 179 (50.6%) had one or two mutations : 38 patients (10.7%) were homozygous; 44 (12.4%) were compound-heterozygous; 97 (27.4%) heterozygote mutations. Of the mutations, M694V (A>G), M680I (G>C), V726A, R761H accounted for 73.7, 18.4, 5.3, and 2.6 %, respectively. The E148Q, P369S and F479L mutations of exons 2, 3 and 5 were seen in one case each. One case was E148Q/M680I compound-heterozygous. CONCLUSION: Exon 10 is the most common site for FMF mutations in Turkish population is exon 10 whereas exons 2,3 and 5 accounts for about 4.5% of the cases. The commonest mutation among Turks is M694V (A>G). Morever, because of confirmed results, StripAssay for 12 common mutations might be used in routine mutation screening analysis.

P0733. Identification of two new alternatively spliced *MEFV* transcripts in human peripheral blood leukocytes

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Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever and serositis. FMF is

caused by mutations in the *MEFV* gene. *MEFV*, expressed in granulocytes and monocytes, encodes marenostrin/pyrin (M/P), a presumed regulator of inflammation.
Recently, multiple alternatively spliced *MEFV* transcripts were identified suggesting the existence of several proteins *in vivo*. The expression pattern of the native protein has poorly been investigated especially in cells physiologically expressing *MEFV*.
To address this issue, we performed RT-PCR analysis of *MEFV* mRNA from human control peripheral blood leucocytes using exonic primers spanning exon 1 to 10. The PCR products were cloned and directly sequenced.
We identified two new transcripts due to alternative splicing events.
A complex transcript, *MEFV*-d2-9ext, resulted in an in-frame deletion of exon 2 and a 5' 117 bp extension of exon 9 introducing a premature stop codon. The second transcript, *MEFV*-d2-3-4 was generated by removal of exon 2, 3 and 4, resulting in a frameshift that predicts a short protein corresponding to exon 1 only.
We raised polyclonal antibodies against 6 peptides designed from the *MEFV* coding sequence, and detected by Western-blotting protein isoforms that we are currently characterizing. Previous data and ours strongly support that numerous *MEFV* transcripts are produced by alternative splicing. Because alteration of *MEFV* expression regulation may be involved in FMF pathophysiology, we will analyze the pattern splicing of *MEFV* transcripts, in FMF patients.

P0734. Heteregenous clinical profile of familial mediterranean fever associated to M680I (G/C) mutation in a Tunisian arab muslim family from Sfax

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Familial Mediterranean fever (FMF) is an autosomal recessive disorder particularly common in Mediterranean population,characterised by recurring attacks of fever and serositis.
The cloning of the FMF gene on 16p13 and the subsequent finding that its tissue expression is limited to granulocytes, has helped to explain the dramatic accumulation of neutrophils at the symptomatic serosal sites. The most frequent sequence alterations in MEFV gene are M694V, V726A, M680I, M694I and E148Q. The wide clinical variability of the disease has been related to MEFV allelic heterogeneity.
In this study, we report clinical profiles and molecular findings in a Tunisian Arab Muslim family from Sfax town. Only two members of this large consanguineous family fulfilled the diagnostic criteria for FMF with typical acute and recurrent crises of fever and serosal inflammation, leading improperly to abdominal surgery. Other subjects manifested a mild or atypical phenotype. All members underwent molecular genetic exploration to confirm diagnosis for some ones and establish carrier status for others. Mutations were investigated by PCR amplification and digestion with appropriate enzymes made to distinguish the wild type from the mutant allele. Genetic analysis revealed a M680I (G/C) mutation in all members of the family. Heterozygote members were healthy but homozygotes were heterogeneously affected.
We discuss clinical presentations of FMF disease in this Arab Muslim family and emphasize the role of genetic analysis.We conclude that specific MEFV mutations are probably not the sole determinants of phenotype, and that unknown environmental factors or modifying genes act as accomplices in this disease.

P0735. Different expression of the P369S mutation in the MEFV gene in the Armenian population

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Familial Mediterranean Fever (FMF) is the most prevalent hereditary inflammatory disorder with diverse clinical presentation. The identification of MEFV gene mutations causing the disease have provided an important laboratory tool that helps in the diagnosis of FMF and perhaps in understanding its heterogeneous clinical presentation.
Screening of healthy controls in Armenian population for MEFV mutations compared with the distribution of the mutations in the patients, has shown that P369S mutation is the most common in the normal population (4.9%) but is less frequently represented in the patients (0.1%). This suggests a reduced penetrance of P369S.
Among 250 controls we detected 10 genotypes with P369S complex alleles, 7 were not associated with clinical FMF, and 3 manifested only mild disease, suggesting that P369S might ameliorate the phenotypic effect of exon 10 mutations.
We detected 12 mutations in 2400 individuals with clinical signs suggestive of FMF and found P369S rare mutation in 14 of them: 9 heterozygotes, 3 compound heterozygotes (1 with P369S/F479L and 2 with P369S/E148Q), and 2 displayed complex alleles (P369S/E148Q/R761H; P369S/E148Q/M694V). Evaluation of the phenotypic features of the patients with P369S mutation showed the presence of 8 asymptomatic individuals. Four patients with P369S mutation, one - with P369S/E148Q and one - with P369S/E148Q/M694V had the FMF clinical picture.
On the basis of our results and recent data, we suggest that in some cases other factors along with MEFV genotype, such as environment or possibly other genetic factors play a role in the determination of the severity of the inflammatory attacks in FMF.

P0736. Disruption of conserved non-coding elements in a t(2;3)(q12;p13)*de novo*

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It was recently discovered that not only can disruption of important developmental genes cause disease, but so can disruption of the regulatory landscapes surrounding these genes, e.g. by translocations that remove regulatory elements from the gene they regulate. Here we present a balanced t(2;3)(q12.3;p13)*de novo* in a patient with mental retardation, speech defect and strabismus. The breakpoints were mapped by FISH using BAC clones to a gene empty region upstream of *FOXP1* on chromosome 3p13. The breakpoint on chromosome 3p13 disrupts a cluster of conserved non-coding elements (CNEs) associated with *FOXP1*. It has recently been shown that similar conserved elements probably function as tissue-specific enhancers and that they are important for the diverse spatio-temporal functions of the associated key developmental genes. The disrupted elements upstream of the breakpoint on chromosome 3p13 have been studied *in vivo* by a functional assay using zebrafish. Several of these elements revealed GFP expression in the zebrafish central nervous system. Recently, *FOXP2*, which encodes another member of the Forkhead box (Fox) family of proteins, has been identified as the gene underlying a human developmental language abnormality. Since *FOXP1* is specifically co-localized with *FOXP2* in the bird and human brain, it is predicted to be related to speech disorders as well. The objectives of this study are to identify regulatory sequences related to the candidate gene associated with the 3p13 breakpoint in this translocation and contribute to the understanding of the etiology of speech defect associated with mental retardation and strabismus.

P0737. Frequency of FRAXA and FRAXE and polymorphism at FMR1 and FMR2 genes in Indian population

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Fragile X syndrome (FRAX) is one of the main causes of mental retardation. It is caused mainly by dynamic expansions at FMR1 (FRAXA) and FMR2 (FRAXE) genes at Xq27.3 and Xq 28. In agreement with the polymorphism of the CGG/CCG repeats and the methylation status of the gene, the FRAXA and FRAXE alleles can be divided into three categories; normal (2-60), premutation (60-200) and full mutation (>200).
A total of 203 individuals (194 males; 9 females) with MR of unknown etiology, were analyzed for the expansion and also for the polymorphism at FMR1 and FMR2 genes. Radioactive-PCR and southern blotting using Stb12.3 (for FRAXA) and Oxe20.0 (for FRAXE) were employed in analyzing the patients. 5 males did not show amplification for FRAXA allele because of the full mutation. These were again

confirmed by southern blot analysis. Therefore, a total of 207 and 212 X-chromosomes were analyzed for the CGG repeat number in FRAXA and GCC repeats in FRAXE respectively. The most frequent FRAXA allele size was 29 CGG repeats (25.6%). 28 repeat containing alleles were the next frequent (16.9 %) allele. A total of 25 FRAXA allelic variants from 11-45 CGG repeats were observed. The most frequent FRAXE allele size had 15 GCC repeats (24.1%) followed by allele containing 18 repeats (18.3%). A total of 20 FRAXE allelic variants from 4-31 GCC repeats were observed. Our study revealed frequency of FRAXA to be 2.5% and FRAXE to be 0% indicating the absence or rarity of FRAXE mutation in our population.

P0738. *FRAXA* locus investigation of mentally retarded patients

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Mutations at *FRAXA* locus on distal Xq may cause mental impairment. Most common mutation at *FRAXA* locus is expansion of CGG triplet repeats located in the 5'-untranslated region of the *fragile X mental retardation-1* (*FMR1*) gene. The expanded CGG triplet repeats are hypermethylated and the expression of the *FMR1* gene is repressed in patients with fragile X syndrome (FXS), which leads to the absence of FMR1 protein (FMRP) and subsequent mental retardation (MR). Normal alleles vary from 6 to 50 CGG repeats. Intermediate alleles 45 - 55 repeats, premutation alleles 59 - 200 repeats, full mutation greater than approximately 200 repeats (methylated).

The group of 292 unrelated patients with MR referred from clinical geneticists was screened by PCR for a normal allele. For 179 chromosomes CGG repeats number was detected by Applied Biosystems protocol on ABI Prism 310. The prevalence of 29, 30 and 31 CGG repeats were found. Five affected patients were detected (1.71%). The final diagnosis of FXS confirmed by Southern blotting. In four FXS families we found 4 females permutation carriers, 3 females with full mutation, 3 affected males with full mutation, 1 affected mosaic male. All permutated and mutated alleles in FXS families were associated with single nucleotide polymorphism (SNP) ATL1 allele G.

105 chromosomes of patients with normal CGG repeats number were analyzed for ATL1 SNP. For 62% of analyzed chromosomes ATL1 allele A was found.

The estimation of STR-based haplotype structure for further investigation of Latvian FXS patients and their families are in progress.

P0739. GAA repeat expansion-associated DNA methylation changes in Friedreich ataxia

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Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disorder that is primarily caused by a GAA repeat expansion mutation within intron 1 of the *FXN* gene, leading to a decreased level of frataxin protein expression. The mechanism by which this mutation acts is currently unknown, but two models have been put forward. Firstly, it has been suggested that the GAA repeat expansion may adopt an abnormal triplex structure that interferes with *FXN* gene transcription. Secondly, there is evidence that the GAA repeat expansion is associated with epigenetic changes, such as DNA methylation and modification of histones, producing a heterochromatin-mediated gene silencing effect.

In support of this second hypothesis, we have recently obtained data that shows increased DNA methylation of specific CpG sites immediately upstream of the expanded GAA repeat sequence in FRDA patient autopsied brain tissue, compared with non-GAA repeat expansion containing brain tissue. In contrast, no such changes were identified in the *FXN* promoter region. We have also identified similar DNA methylation increases in brain and heart tissues from our recently established GAA repeat expansion-containing *FXN* YAC transgenic mouse model, compared with similar non-GAA repeat expansion *FXN* YAC transgenic mice. These studies will be detailed, together with our more recent investigations to identify potential GAA repeat expansion-associated changes in methylation and acetylation of histones at the *FXN* locus. Such epigenetic studies to identify the potential GAA repeat expansion mechanism of action will provide valuable information for novel FRDA therapies.

P0740. Investigation for point mutations on different parts of Mitochondrial DNA, relating to adjunct of pathogenesis of FRDA, on 20 Iranian patients with Friedreich's ataxia

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Friedreich's ataxia (FA,FRDA) is the most common inherited ataxia. Clinically, FRDA is characterized by multiple symptoms including progressive gait and limb ataxia, dysarthria, diabetes mellitus, and hypertrophic cardiomyopathy. The gene defective in FRDA, encodes a mitochondrial protein known as frataxin. A triplet repeat expansion within intron 1 of the FRDA gene results in a marked decrease in frataxin expression.There is much evidence to suggest that FRDA results from mitochondrial iron accumulation leading to cellular damage and death by the production of toxic free radicals by Fenton chemistry. Due to the important role of the mitochondria and considering the clinical symptoms of FRDA, failure in ATP production and presence of free radicals in mitochondria of patients with FRDA we are analyzing different parts of mtDNA; MT-ATP8 , MT-ATP6, and highly mutative genes like; MT-LTI , MT-NDI, MT-COII, MT-TK, in 20 Iranian FRDA patients to find any probable point mutation by PCR method and automated DNA sequence that can be involved as an adjunct in the pathogenesis of FRDA

P0741. Hypomethylation is restricted to the D4Z4 repeat array in phenotypic FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy affecting predominantly the muscles of the face, shoulder and upper arm. Most FSHD patients show a contraction of the D4Z4 repeat array in the subtelomere of chromosome 4q. This contraction is associated with significant allele-specific hypomethylation of the repeat, suggestive for a chromatin restructuring at 4qter in FSHD. Hypomethylation of D4Z4 is also observed in phenotypic FSHD patients without D4Z4 contraction and in patients suffering from the immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome, an unrelated disorder that does not present with muscular dystrophy and is in part caused by mutations in the *DNMT3B* gene. In order to identify the gene defect in phenotypic FSHD and to unravel the pathogenic epigenetic pathway in FSHD, we have aimed to identify the differences and commonalities in phenotypic FSHD and the ICF syndrome by (1) investigation of DNA methylation of non-D4Z4 repeat arrays, (2) analysis of mitogen-stimulated lymphocytes to detect pericentromeric abnormalities involving chromosomes 1, 9 and 16, (3) determination of IgA, IgG and IgM levels and (4) mutational analysis of candidate genes to identify a second disease locus involved in the pathogenesis of phenotypic FSHD. Our results do not show epigenetic or phenotypic commonalities between phenotypic FSHD and ICF other than the earlier observed D4Z4 hypomethylation, suggesting that phenotypic FSHD is not caused by a defect in the same molecular pathway as ICF. We neither could identify any mutations in the candidate genes tested for.

P0742. Comprehensive mutation analysis in a clinically well defined cohort of patients with exudative vitreoretinopathy.

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Familial exudative vitreoretinopathy (FEVR) is a hereditary disorder characterized by vitroretinal pathology. Retinal exudates, retinal neovascularisation, retinal folds and retinal detachment as well as vitreous haemorrhage have been described to occur in the first decade of life. The disease is slowly progressive, with in the terminal stages of severely affected eyes a chronic retinal detachment, leading to total blindness. To date, 3 genes and 1 locus have been described. *FZD4* mutations were found in patients with autosomal dominant EVR. X-linked EVR is caused by mutations in *NDP,* and *LRP5* mutations can be inherited both automal dominant as well as autosomal recessive. The EVR3 (605750) locus maps to 11p12-p13. In a series of 30 sporadic patients and dominant families, we analysed the involvement of the *NDP*, *FZD4* and *LRP5* genes by sequence analysis. We found in one patient a mutation in the *NDP* gene. Mutations in the *FZD4* gene were found in approximately 30% of the patients and families, (9/30), with the c.957G>A (p.Trp319X) mutation as the most frequent mutation (5/30). The analysis of *LRP5* revealed a mutation in one family, and an alteration of unknown pathogenicity in another. Careful clinical evaluation of the patients will lead to determination of a possible relationship between phenotype and genotype in this cohort. Furthermore, families without a mutation in one of the known genes, will help to identify new loci for FEVR.

P0743. Molecular Identification of Mutations in G6PD Gene in Patients in Kerman and Yazd Provinces of Iran.

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Glucose- 6-phosphate dehydrogenase (G6PD) is a housekeeping enzyme and its gene is located on the Xq28 region of X chromosome. G6PD deficiency is one of the most common inherited disorders of mankind affecting more than 400 million people worldwide. The main clinical manifestations are neonatal jaundice and acute hemolytic. More than 130 mutations and 400 biochemical variants have been reported. Since, the variants including Mediterranean, G6PDA$^-$ and Chatham are reported in all over the world, and among them Mediterranean variant is the most common, also in Arabic countries and Persian Gulf, in this study, we investigated Mediterranean, Chatham, Cozenza and A$^-$ mutations in the central areas of Iran. The extracted DNA from 119 patients with history of favism was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) for above mutations. The results determined that, from the total 119 samples, 41 samples from state of Kerman (63%) and 35 samples from state of Yazd (64%) hence a total of 63.86% had G6PD Mediterranean and 2 from each states had G6PD Chatham (3.36%). Cosenza and A$^-$ mutation were not observed. G6PD Mediterranean was the commonest mutation in Iran and the most of other countries in tropical and subtropical areas. The frequency of Chatham was the second minimum in Central provinces in comparison with others in Iran. The Major ethnic groups in this region are Fars, Arab Turk and minorities of Kurd. For unknown samples other Methods such as SSCP and DNA sequencing are using to determine other mutations.

P0744. A new Vohwinkel-like disease is caused by a novel missense mutation in GJB2

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Gap junctions are intercellular channels that mediate rapid intercellular communication. They consist of connexins, small transmembrane proteins that belong to a large family found throughout the animal kingdom. In the skin, several connexins are expressed and are involved in the regulation of epidermal growth and differentiation. The gap junction gene GJB2, that codes for the protein connexin26, is highly expressed in the epidermis and is associated with a wide variety of diseases, showing a strong genotype-phenotype correlation. Despite extensive research in the past few years, this phenomenon is poorly understood. The identification of novel diseases caused by mutations in GJB2 may help to shed light on its strong genotype-phenotype correlation and help elucidate the function of different parts of the protein. Here, we report on a novel GJB2 mutation that causes a syndrome of focal palmoplantar keratoderma with sensorineural deafness, a phenotype that is reminiscent of Vohwinkel's disease. Using fluorescent fusion proteins, we show that the mutation causes a transport defect similar to that found for the Vohwinkel syndrome mutation D66H.

P0745. Genetic polymorphism of glutathione-S-transferase T1 and M1 in the newborns with neonatal syndromes in Ukrainian population

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The more recent discovery suggested the physiological role glutathione-S-transferases (GSTs) in protecting against chronic diseases that arise from oxidative tissue damage. The development of the neonatal syndromes are connected with perinatal hypoxia. The functional allele of these genes may protect from hypoxia and the risk of neonatal syndromes.
Material and methods: We conducted a case-control study of 117 cases of severe perinatal pathology with neonatal syndromes, such as: perinatal damage of the central nervous system, respiratory distress syndrome, necrotizing enterocolitis, neonatal jaundice and 70 control group (clinical healthy newborns). Genetic polymorphism of *GSTT1* and *GSTM1* were detected by multiplex polymerase chain reaction (PCR). Differences in these groups were assessed by χ^2 analyses.
Results: In our investigation an increased significant frequency of *GSTT1"-"* genotypes among neonates with perinatal pathologies (29,91%) compared to newborns in the control group (12,86%), χ^2 =6.17, P<0.05 was observed. There as no significant difference between the polymorphism of *GSTM1* genotype in two groups. The rate of *GSTT1"-" / GSTM1"-"* allelic combination was also significantly increased in the neonates with severe perinatal pathologies and neonatal syndromes (17,09%) compared to newborns in the control group (2,86%), χ^2 =7.24, P<0.01. Conclusion: Our investigations have shown associations between *GSTT1 „-"* genotype, combination of *GSTT1"-" / GSTM1"-"* genotypes in newborns and increased risk of perinatal pathologies in newborns. Thus determining the factors of inheritance predisposition in the neonates can allow to predict the risk of neonatal syndromes.

P0746. The -3440 C->A variant in the gtPBREM region of the UGT1A1 gene enhances gene expression.

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Gilbert, Crigler-Najjar Type I and II Syndromes are characterized by unconjugated hyperbilirubinaemia caused by molecular defects of the UGT1A1 gene codifying for the UGT1A1 enzyme. CN1 syndrome is the most severe form characterized by complete absence of UGT1A1 enzyme activity and serum bilirubin levels of 540-850 µm/l. CN2 syndrome, less severe, presents intermediate levels of hyperbilirubinaemia and the UGT1A1 enzyme can be induced by Phenobarbital administration. Mild hyperbilirubinaemia is associated with Gilbert Syndrome due to the presence of the TA7 or TA8 repeat at the promoter sequence A(TA)6TAA. Recently, the gtPBREM enhancer module of the human UGT1A1 was characterized, which is activated by nuclear receptors such as CAR and PXR in response to xenobiotics. A -3279 T->G mutation in the gtPBREM region led to hyperbilirubinaemia. In this study, we analyzed a polymorphic variant located at position -3440 C->A of the gtPBREM flanking the PXR binding site which is not common in the general population but highly frequent in Sardinia. To test the functional consequences of the -3440 variant, we quantified the transactivation activity of the variant in epatic cells. We also evaluated if the PXR receptor could bind to the consensus sequence after Rifampicine treatment. These experiments demonstrated that the -3440 variant does not modify PXR's binding capacity and shows a prominent transactivation activity compared to the wild type sequence. The -3440 variant could balance the reduced activity of UGT1A1 due to presence of the TA7 allele and would explain the asymptomatic phenotype of patients with the (TA)7/(TA)7 genotype.

P0747. Functional analysis of 14 *GLB1* mutant alleles found in GM1-gangliosidosis and Morquio B patients

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GM1-gangliosidosis and Morquio B disease are lysosomal storage disorders caused by β-galactosidase deficiency due to mutations in the *GLB1* gene. Three major clinical forms of GM1-gangliosidosis have been established on the basis of age of onset and severity of symptoms: infantile, late infantile/juvenile and adult. All these subtypes share a different degree of psychomotor retardation with neurological involvement and high amounts of ganglioside GM1 storage. On the other hand, Morquio B disease is characterized by progressive, generalized skeletal dysplasia without central nervous system involvement and no clinical signs of storage disease in neuronal tissues. β-galactosidase is a lysosomal enzyme that cleaves β-galactoses from different substrates such as ganglioside GM1, keratan sulfate or glycopeptides.

Our group had previously identified different *GLB1* mutations in GM1-gangliosidosis and Morquio B patients. In the present work some of these mutations were heterologously expressed and the resulting mutated proteins characterized by residual enzyme activity and Western blot analysis. *In vitro* expression was performed by transfecting COS-7 cells with plasmids bearing the different mutant *GLB1* cDNAs. Twelve mutations and 2 functional polymorphisms (p.R521C and p.S532G) were studied. Ten of these changes had not been expressed before. Mutations causing the infantile form of GM1-gangliosidosis resulted in the complete absence of the enzyme activity, whereas mutations associated with the milder forms of the disease resulted in a great reduction of the enzyme activity. The two polymorphisms showed only a partial reduction of the activity. Therefore, in most cases a good correlation between residual activity and phenotype could be established.

P0748. *PATCHED* mutations and the Nevoid Basal Cell Carcinoma Syndrome: clues to an active participation of dermal fibroblasts in the Basal Cell Carcinoma development

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The Nevoid Basal Cell Carcinoma Syndrome (NBCCS) or Gorlin syndrome is a rare autosomal dominant disorder due to germinal mutations in the *PATCHED* tumor suppressor gene. NBCCS patients present developmental defects and a high predisposition to Basal Cell Carcinoma (BCC). BCC is the commonest cancer in adult human. Increasing evidences have revealed the contribution of the stroma in tumorigenesis. Stromal dependency of BCC cells seems of particular interest provided the virtually null metastatic potential of this tumor and the incapacity of BCC cells to grow *in vitro*. In order to define the influence of the stroma cells in the BCC proneness phenotype of NBBCS patients, we cultured primary fibroblasts and keratinocytes from NBCCS patients. Using these cells we set up a system of organotypic skin cultures, allowing us to study the consequences of *PATCHED* mutations in mesenchyme-epithelium interactions. The presence of NBCCS fibroblasts induced a delayed and reduced expression of epidermal differentiation markers, while paradoxically, a decrease of the Ki67 proliferation marker was observed in overlaying normal and NBCCS keratinocytes. These observations strongly suggest that NBCCS fibroblasts alter the proliferation/differentiation balance of keratinocytes. To further document the implication of NBCCS dermal fibroblasts in mechanisms leading to BCC development, we performed a whole genome microarray assay using mRNA from NBCCS and normal fibroblasts cultured in a tri-dimensional collagen dermal equivalent. Results of this screen, sustained by Q-RTPCR, WB and ELISA analyses, show that NBCCS fibroblasts over-express proteins involved in the tumorigenesis process such as the pro-tumoral Matrix Metalloproteinase 3.

P0749. The GP IIb gene expression, Ile843Ser GP IIb gene polymorphism, glycoprotein IIb-IIIa number and platelet aggregation in healthy donors

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Platelet hyperaggregation is a risk factor for coronary thrombosis and platelet GPIIb-IIIa receptor plays a key role in this process. It's known that the Leu33Pro GPIIIa gene mutation and quantity of the GPIIb-IIIa receptors are associated with high aggregation. In our study the GPIIb gene expression, Ile843Ser GPIIb gene polymorphism and their effect on the GPIIb-IIIa number or platelet activity were investigated. We formed a group of 57 volunteers (21-54 years) and analyzed the Ile843Ser GPIIb by PCR-RFLP technique, the GPIIb gene expression (level of mRNA from 10 donors) by RT-PCR using the TaqMan assay in ABI Prism 7000 Sequence detection System, cDNA prepared from total RNA isolated from platelets, ADP-induced platelet aggregation by photometric method and the GPIIb-IIIa number per platelet by flow cytometry with mAB. We revealed 17 carriers of IleIle genotype, 30 carriers of IleSer genotype and 10 - SerSer genotype. The GPIIb-IIIa numbers varied from 40500 to 82700 per platelet and the GPIIb mRNA levels were widely varied too - from 1,6 to 15,6. However no correlation between these parameters was found may be because small analyzing group. The Ile843Ser GPIIb gene polymorphism didn't influence the GPIIb mRNA level. But the 843Ser allele was associated with higher velocity of aggregation - 56.8±6.4%/min vr. 39.2±3.2%/min in IleIle carriers ($p=0.04$). The platelet activity also depended on the GPIIb-IIIa number (Spearman correlation R from 0.4 to 0.5 for ADP 1.2μM and 2.5μM, $p<0.005$). In conclusion, the impact of the GPIIb gene expression on platelet function deserves further investigation.

P0750. Functional characterization and proteome analysis of synaptic endings in the *crv4* mouse, a spontaneous model for hereditary ataxia affecting metabotropic glutamate receptor 1.

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A mouse mutant recently described by our group, *crv4*, carries an LTR intronic insertion which disrupts splicing of type 1 metabotropic glutamate receptor (*Grm1*) gene and causes absence of the protein. *crv4* homozygous mice present mainly with an ataxic phenotype. Hereditary ataxias may be caused by mutations in different genes and, although no *Grm1* mutations have been reported in human so far, emerging evidence indicate as glutamate signaling from *Grm1* plays an important role in ataxia pathophysiology.

Grm1 is involved in synaptic plasticity, indeed it has been suggested to control presynaptic glutamate release thus modulating synaptic function.

We characterized the functional effects of *Grm1* mutation in the *crv4* mice at presynaptic level and investigated expression profiles of proteins in tissues (cerebral cortex, hippocampus and cerebellar cortex) critical for pathways involving *Grm1*.

By using cortical synaptosomes in experimental conditions known to permit the selective activation of *Grm1,* we have confirmed the existence of presynaptic *Grm1* in mouse cortical nerve endings whose activation potentiates the release of glutamate evoked by depolarizing stimuli.

Then, we have demonstrated that the *crv4* mutation abolishes this presynaptic positive control of glutamate release from synaptosomes. The loss of the auto−regulatory mechanism in *crv4* mice may affect the glutamate amount in the biophase and glutamate−mediated regulation of synaptic events contributing to the development of the affected phenotype.

Finally, we compared total synaptic proteins (proteome) obtained from synaptosomes of *crv4* homozygous and control mice by the 2D-DIGE

technique to define key molecules involved in *Grm1* mediated synaptic activity.

P0751. Molecular studies on chromosome 9p haploinsufficiency and *DMRT1* gene mutations in 46,XY patients with gonadal dysgenesis

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SRY gene is responsible for testis determining in mammals. Among patients with gonadal dysgenesis (GD), only 15-20% presents an inactivating mutation in *SRY* coding region. It is well known that 9p chromosomal deletion can cause sex reversal in 46,XY individuals. In the present study, 9p haploinsufficiency was invstigated using five different 9p microsatellites: D9S1779, D9S1858, D9S143, D9S54, D9S1813. Were included thirty-three *SRY*-normal non-related patients and seven 46,XY individuals of a family, three with GD (one complete and two partial), the father and three normal siblings all of them bearing the R30I *SRY* mutation. Homo- or hemizygosis for five and four loci was observed in one and two patients, respectively. The three affected individuals of the family bore identical 9p genotype, which was different from normal individuals in the sibling. *DMRT1* gene maps to 9p24.3 within the sex reversal region on 9p. The *DMRT1* g.133T>A nucleotide change was observed in 24 T/T homozygous and in 9 T/A heterozygous patients. This results in S45T mutation but is considered a neutral SNP (rs3739583). Interestingly, the A allele is the most frequent in four out of five populations deposited in database, whereas this study showed frequencies of 76% and 23% for T and A, respectively. The g.52198T>C and g.52308C>T variations in exon 3 were detected in one patient; both are rare SNPs. Finally, a new 3'UTR g.126313insT was found in a heterozygous patient. The involvement of those SNPs on either function or expression of *DMRT1* must be investigated to confirm them as causing DG.

P0752. Borate transporter SLC4A11 mutations cause both Harboyan syndrome and non-syndromic corneal endothelial dystrophy

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Harboyan syndrome (CDPD) consists of congenital corneal endothelial dystrophy and progressive perceptive deafness and is transmitted as an autosomal recessive trait. CDPD and autosomal recessive, non-syndromic congenital hereditary endothelial corneal dystrophy (CHED2) both map at overlapping loci at 20p13, and mutations of SLC4A11 were recently reported in CHED2. We here report genotype studies in six families with CDPD and one family with either CHED or CDPD, from various ethnic backgrounds (in the seventh families hearing loss could not be assessed because of the proband's young age). We found novel SLC4A11 mutations in all patients. Why some mutations cause hearing loss in addition to corneal dystrophy is presently unclear. Our findings extend the implication of the SLC4A11 borate transporter beyond corneal dystrophy to perceptive deafness.

P0753. HAX-1 is a new interacting protein of phospholamban and a novel regulator of calcium homeostasis and cardiac cell survival

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Phospholamban (PLN), a 52 amino acid transmembrane protein of the sarcoplasmic reticulum (SR), is a key regulator of Ca^{2+}-homeostasis and contractility in the heart. Its regulatory effects are mediated through its interaction with the sarcoplasmic reticulum Ca^{2+}-ATPase, (SERCA2a), resulting in alterations of its Ca^{2+}-affinity. Impaired SR Ca^{2+} cycling is a characteristic of human heart failure and mutations in PLN have been associated with dilated cardiomyopathy. To identify additional proteins that may interact and thus regulate PLN, we screened an adult human cardiac cDNA library using the yeast two-hybrid system.

We identified HS-1 associated protein X-1 (HAX-1), a ubiquitously expressed mitochondrial protein with anti-apoptotic function, as a new PLN-binding partner. The minimal binding regions were mapped to amino acids 203-245 for HAX-1 and 16-22 for PLN. GST pull-down and *in vitro* binding assays confirmed the direct interaction between PLN and HAX-1, while kinetic studies determined a K_D of ~1μM as the binding affinity of the protein complex. The interaction was found to be modulated by PLN phosphorylation and changes in Ca^{2+} concentration. Moreover, SERCA2a was also detected in the pull down samples, suggesting that HAX-1 forms part of the PLN/SERCA2a complex. Immunofluorescence studies localized HAX-1 to the mitochondria. However, in the presence of PLN, HAX-1 redistributed and co-localized at the endoplasmic reticulum. Importantly, the anti-apoptotic function of HAX-1 was found to be enhanced in the presence of PLN, indicating an important role of the PLN/HAX-1 interaction in cell survival, possibly through the regulation of SR Ca^{2+}-homeostasis and consequent mitochondrial Ca^{2+} redistribution.

P0754. Mutation analysis of the GJB2 gene in Latvian patients with nonsyndromic sensorineural hearing loss

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Mutations in the GJB2 gene at the DFNB1 locus on chromosome 13q11-q12 have been established to be the major cause of nonsyndromic sensorineural hearing impairment (NSHI) in different populations. Up to date there are reported 110 different GJB2 mutations.One particular frame shift mutation named 35delG is the most prevalent in the populations of the Caucasian origin.

The aim of our work was to investigate the prevalence of the GJB2 gene mutations within Latvian NSHI individuals from 41 families. All samples were screened for 35delG mutation using RFLP method. Remaining GJB2 mutations were identified by the direct sequencing.

Four different mutations in the GJB2 gene have been identified in Latvian patients with nonsyndromic sensorineural hearing loss: 35delG, 311-324del14, 235delC and M34T.

The most prevalent mutation in patients with NSHI in Latvia is 35delG (62% of all probands are homozygotes for this mutation). Frequency of 311-324del14 mutation is 7%, but 235delC and M34T mutations were found in 2% of patients.

P0755. Integrated approach to the study of Congenital Heart Diseases

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This study is part of a research project financed by the European Community (Health-e-Child, Contract n°: IST-2004-027749), that involves three Pediatric European Centers. It foresees vertical integration of epidemiological, clinical, radiological, laboratory, biological, and genetic data.

The study includes congenital heart diseases that lead to Right Ventricular Overload (RVO): Atrial Septal Defects (ASD), Post-operative Tetralogy of Fallot (ToF) and Anomalous Pulmonary Venous Return (APVR). The study aims to collecting the following data :

-Clinical Evaluation: demographic information, personal and family history, family pedigree, and detailed physical examination.

-Instrumental Evaluation: Electrocardiogram (ECG), Holter ECG, Signal-Averaged ECG, Chest X-ray, 2D and 3D echocardiography, tissue doppler imaging, acoustic boundary detection, integrated backscatter, color kinesis, transesophageal echocardiography and magnetic resonance imaging, cardiac catheterisation.

-Chromosomal analysis by standard karyotyping;

-In case of normal karyotype, screening of three candidate genes (GATA4, TBX5 e NKX2.5), known to be fundamental for normal cardiac development and function.

-The negativity of the previously mentioned tests make the patient can-

didate for CGH array.
During this initial phase of application of the diagnostic protocol, we have identified a mutation in the TBX5 gene in a patient who was previously considered to be affected with isolated ToF. The mutation leads to aminoacid substitution of arginine into a stop codon (R279X). Careful physical examination of the patient showed subtle hand malformation in form of long first metacarpal, that can coincide with Holt Oram syndrome diagnosis. To the best of our knowledge this mutation has never been associated with ToF phenotype.

P0756. Purple and Yellow. Hereditary Coproporphyria and Gilbert's Syndrome in Czech Family.

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Hereditary coproporphyria is a rare acute hepatic porphyria with autosomal dominant inheritance characterized by deficient activity of coproporphyrinogen III oxidase (CPO). The gene encoding human CPO has been cloned and localized to chromosome 3q11.2. The CPO gene spans 14 kb and has a single promoter and seven exons. The cDNA encodes a protein containing 354 amino acids. The enzyme is active as a homodimer of 76 kDa.
Gilbert´s syndrome (GS) is a hereditary, autosomal recessive disorder with mild, unconjugated hyperbilirubinemia. In Caucasian patients, Gilbert's syndrome is linked primarily with a TA insertion in the TATA box promoter of the UGT1A1 gene, most frequently present as A(TA)7TAA rather than A(TA)6TAA.
In this study, we report on patient with hereditary coproporphyria associated with Gilbert´s syndrome. Molecular diagnosis of the regulation region of the UGT1A1 gene showed a TA insertion in the promoter. Analysis of the CPO gene revealed a small deletion of 3 bp at nucleotide 1168 (390delGly) in exon 5. This deletion removes glycine without altering the coding frame. Mutational analyses were carried out on sixteen members of proband's family.
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P0757. Analysis of connexin 32 gene (GJB1, Cx32) in the hereditary motor and sensory neuropathy (HMSN) patients from Bashkortostan

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Hereditary motor and sensory neuropathy (HMSN) or Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous disorder of peripheral nervous system. The HMSN frequency in Bashkortostan Republic (BR , Russia, the territory of the South Urals) is 10,3:100000. HMSN type I is prevalent in BR. In 104 unrelated HMSN families gene PMP22 duplication screening was carried previously and obtained that this mutation frequency in BR is 20,63% for all types of HMSN and 45,0% - for HMSN type I.
In this investigation the searching of mutations in GJB1 gene in patients without PMP duplication (114 affected members from 77 unrelated families) was carried.Two mutations and one polymorphism - Pro87Ala, Arg22Gln and 45G>A we determined using SSCP analysis followed by sequencing of shifted samples. It turned out, that Pro87Ala mutation is frequent among HMSN patients from Bashkortostan, it was revealed in 13 unrelated families, that amount 12,03% for all types of HMSN and 20,63% - for HMSN type I. This mutation was the most frequent in patients of Bashkir ethnic origin (35% in Basckir patients), less frequent - in Russian (10,8%) and Tatars (3,20%). Arg22Gln mutation was defined in three patients from two families of Tatar ethnic origin (2,77% for all patients from BR and 6,45% for Tatar patients without HMSN type differentiation. Both found mutations were described previously, they cause the dominant form of HMSN type I with relatively mild clinical features. The polymorphism 45G>A revealed in two unrelated patients of Mordvinian ethnic origin, it was not describe earlier.

P0758. High throughput Genotyping: New Diagnostic Resequencing Microarray for Hereditary Spastic Paraplegia

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Hereditary Spastic Paraplegia (HSP) is a heterogenous group of inherited neurological disorders. Insidiously progressive spastic weakness of the lower extremities is the common criteria in all 33 forms described so far. Autosomal dominant, autosomal recessive and X-linked mode of inheritance have been described. Clinically HSP is differentiated into pure (uncomplicated) and complex (complicated) forms, depending on isolated impairment of corticospinal tracts or more wide spread affection of neuronal circuits or systemic involvement.
For proper diagnosis molecular genetic analysis is fundamental since clinical parameters alone are not reliable in distinguishing HSP forms. In order to establish high throughput genotyping, we designed a HSP resequencing microarray (Affymetrix platform) covering the coding exons and flanking intronic sequences of the HSP genes L1CAM (SPG1), PLP1 (SPG2), Atlastin (SPG3A),Spastin (SPG4), NIPA1 (SPG6), Paraplegin (SPG7), KIF5A (SPG10), HSP60 (SPG13), BSCL2 (SPG17), Spartin (SPG20), Maspardin (SPG21),
(13 - 94kb, 5 - 28 coding exons), as well as the 59 most frequent small deletions, insertions and insertion/deletions in these genes.
We present data on microarray analysis of 20 autosomal dominant HSP index patients.

P0759. Elucidating the molecular function of Zfyve27, the gene mutated in hereditary spastic paraplegia (SPG33)

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Hereditary spastic paraplegias (HSP) are a group of neurodegenerative disorders, which are clinically characterized by progressive spastic paralysis of the legs, usually caused by a length-dependent distal degeneration of the corticospinal tract axons. HSP are genetically heterogeneous and till now 35 loci have been identified. Recently, we reported a mutation in a novel endosomal protein ZFYVE27 (SPG33) in a German family with autosomal dominant HSP. ZFYVE27 was identified as a spastin interacting protein and we have characterized the interaction between these two proteins in mammalian cells. Moreover, our studies revealed that the mutated ZFYVE27 protein shows aberrant intracellular pattern in tubular structure of cells and its interaction with spastin is severely affected. Intracellular distribution studies revealed that ZFYVE27 is expressed in punctate vesicles which were both of endosomal and endoplasmic reticulum origin. Furthermore, overexpression of GFP-ZFYVE27 in fibroblast (NIH3T3) cell line, promoted neurite formation through directional membrane trafficking process. A comprehensive expression analysis of Zfyve27 through Western blot revealed high level of Zfyve27 primarily in the HSP affected tissues in mouse such as brain, cerebellum and spinal cord. To gain mechanistic insights in murine Zfyve27 function, currently, generation of loss of function mouse models by using both gene-trap and knockout strategy are in progress.Conceivably the phenotype of these mouse models might mimic the pathological features of HSP, therefore will provide us with a valuable model system to elucidate the underlying cause for HSP etiology.

P0760. MLH1 promoter methylation in peripheral blood cells of 12 patients clinically presenting HNPCC features

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Germline mutations in mismatch repair genes, tumors with high microsatellite instability (MSI-H) and loss of protein expression are the hallmarks of HNPCC or Lynch-Syndrome respectively. While involvement of somatic MLH1 promoter hypermethylation is accepted in tumorigenesis of defferent sporadic tumors, abnormal germline MLH1 promoter methylation is controversly discussed as a disease-predisposing mechanism for HNPCC.
To clarify the MLH1 deficiency of 94 HNPCC suspected individuals with immunohistochemically MLH1-negative and MSI-H tumors but without germline mutation in MLH1 or other mismatch repair genes, we studied the methylation pattern in the MLH1 promoter region by bisulphite conversion, methylation-specific PCR, and sequencing.
MLH1 promoter methylation in peripheral blood cells was found for twelve patients displaying a HNPCC-phenotype with early-onset colorectal cancer and/or multiple neoplasias. The aberrant methylation was complete in all CpG dinucleotides analysed and displayed allele-specifity in seven cases with a heterozygous SNP. The causality between a new promoter mutation and methylation in-cis in one case can not be ruled out. Peripheral blood cells as well as normal colonic tissue, buccal mucosa, and tumor tissue available from three patients presented the epigenetic MLH1 defect. Expression analysis revealed monoallelic MLH1 expression and complete silencing in one case, in another case only partial silencing was found by signal reduction of one allele.
Our findings confirm that abnormal MLH1 promoter methylation in normal body cells mimics HNPCC. The identification of hypermethylation as a pathogenic pre-lesion has definitive implications on surveillance recommendations, while the heritability of methylation is questionable and was not found in offspring of methylation-carriers.

P0761. Hereditary Polyneuropathy with Liability to Pressure (HNPP). Report of a case.

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An 11 year old girl was referred for a neuropediatric consultation by an orthopedist because of an acute right hand paresis after a minor traumatism while playing. The severity of the traumatism could not justify very well the paresis. The clinical evaluation revealed right ulnar and median nerve paresis and a milder left median nerve paresis as well. The rest of the neurological evaluation was normal. The girl was admitted in our pediatric department for further investigations. Standard hematological and biochemical control, VDRL, coagulation and immunological investigations were all normal. Antibodies for Borrelia, Mycoplasma, Rickettsias, and Hepatitis were normal. Only the title of IgGs antibodies of Bartonella was elevated. Cerebral and Cervical MRI were also normal, but the neurophysiological investigations were compatible for a polyneuropathy, especially for HNPP. HNPP is an autosomal dominant disorder characterized by recurrent entrapment neuropathies usually after minor traumatism. Her personal medical history was marked from an uncomplicated prematurity and asthma well controlled but her mother suffered from a polyneuropathy the last 10 years. The clinical presentation of the patient, the positive family history and the results of the neurophysiological investigations were compatible for the diagnosis of HNPP. PCR analysis confirmed the diagnosis by showing loss of PMP-22 gene. PMP-22 gene is the hallmark of HNPP. The child has completely recovered with physiotherapy in a month. The point of this case report is that a first peripheral nerve paresis after a minor traumatism could hide a much more complicated diagnosis than a trivial traumatism.

P0762. Holoprosencephaly: 10 years of genetic study on 400 patients

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Holoprosencephaly (HPE) is the most common brain malformation resulting from incomplete cleavage of the prosencephalon (1 out of 16.000 live births; 1 out of 250 conceptuses). HPE is associated with a wide spectrum of craniofacial malformations ranging from lethal forms (alobar HPE with cyclopia) to less severe forms such as lobar HPE and normal face. The aetiology is very heterogeneous involving environmental factors, chromosomal abnormalities and at least 7 genes. Since 1996, our team has started to work on the wide clinical and genetic variability of holoprosencephaly. Patient samples (foetuses or children with normal karyotype) and clinical data (from HPE to microforms) were collected from medical teams in France and Europe. Systematic mutation analysis and genomic rearrangements of the five main genes (SHH, ZIC2, SIX3, TGIF, GLI2) were performed. About 19%sequence changes were identified among 350 DNA samples. The familial cases confirmed an extreme clinical variability. Then, microrearrangements were detected by QMPSF, MLPA and CGH array (particularly in foetuses) improving the rate of molecular defects to a total of 30%. Several patients samples with 2 microdeletions and/or duplications were identified supporting multiple-hit hypothesis involving others genetics and/or environmental factors. We performed molecular prenatal diagnosis four times with foetal US scan and cerebral MRI screening. At last, we showed involvement of cerebral malformations as Aprosencephaly/Atelencephaly linked to SIX3 mutations and cerebellar hypoplasia to SHH gene. Pan-hypopituitarism and cleft lip/palate were associated with GLI2. This work has improved molecular diagnosis in Holoprosencephaly and therefore genetic counselling.

P0763. Extented neonatal screening for homocystinuria in the Qatari population

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Homocystinuria is an autosomal recessive disorder of methionine and homocysteine metabolism caused by a deficiency of cystathionine β-synthase . We previously reported that homocystinuria due to cystathionine β-synthase deficiency is common in Qatar with an incidence of approx. 1:3000. As determinations of methionine in dried blood spots (DBS) proved insensitive for neonatal screening, we developed a novel two-tear strategy. To measure total homocysteine (Hcy) in DBS, a robust, stable HPLC method with tandem mass spectrometry detection is described, including all practical details and analytical performance results. For mutation analysis, DNA was extracted from DBS and common mutations R336C and D234N in the CBS gene were tested for only native Qatari. Both methods are suitable for processing a large number of samples.
We have analyzed 6597 newborns from Qatar, of which 2586 were of Qatari origin. A total of 4 neonates with homocystinuria were identified. All showed highly elevated Hcy concentrations in DBS whilst methionine was elevated in two neonates only. Three children were homozygous for the common mutation R336C; follow-up sequence analysis in the fourth patient revealed homozygosity for mutation G347S, not previously observed in the Qatari population. Metabolic screening of t-HCY in DBS appears to have 100 % sensitivity for the detection of classical homocystinuria and support a very high incidence of homocystinuria in Qatar, possibly reaching up to 1:600 and caused by a high degree of consanguinity. Molecular neonatal screening is feasible but metabolic screening appears to have a higher sensitivity for the detection of homocystinuria in Qatar.

P0764. Partial hypoxanthine-guanine phosphoribosyltransferase deficiency as Lesch-Nyhan syndrome variant - the first case detected in Lithuania

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Lesch-Nyhan syndrome is a rare, X-linked recessive inheritable disorder caused by a deficiency of the enzyme *hypoxanthine-guanine phosphoribosyltransferase* (HPRT) (OMIM #300322). HPRT gene has been mapped to Xq26-q27 and more than 200 mutations responsible for this disease have been characterised. Depending on the amount of residual enzyme activity the spectrum of the disease expression

is wide: from isolated hyperuricemia and gout, to hyperuricemia with profound neurobehavioral dysfunction. Diagnosis could be made according to clinical symptoms, biochemical blood and urine test results, enzyme activity and molecular genetic testing.
We present a patient with severe neurological symptoms and mild dysmorphism of phenotype. Our patient is a one year and eight months old boy, first child of healthy non consanguineous parents. Pregnancy has been complicated .The genealogy of this family is uncomplicated. Patient's development was normal till first five months. The phenotype is characterised by macrocephaly, thin upper lip and cryptorchism. Muscle hypotonia with psychomotoric development delay and indifference to pain are observed. Clinical follow-up showed next findings: symptoms of frontal lobes atrophy and internal hydrocephaly (CT scan), kidney ultrasound results without pathology. Laboratory investigations revealed increased serum uric acid, 0.5 mmol/l (normal range 0.13-0.23 mmol/l) and urinary uric acid, 6.3 mmol/mmol creat. (ref. < 2.1 mmol/mmol creat.). Moreover, increased amounts of urinary hypoxanthine, xanthine and inosine (195; 109; 10 µmol/mmol creat. were found, respectively. Subsequently severely decreased of HPRT activity - 0.10 µmol/mmol Hb/hr (ref range 0.92-4.37) was detected in lysed erythrocytes. This led to diagnosis of HPRT deficiency. Allopurinol therapy is started.

P0765. Investigation of tRNA$^{Leu/Lys}$ and ATPase 6 , 8 genes mutations in Iranian Huntington's Disease

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Huntington disease (HD) is a genetically dominant condition caused by expanded CAG repeats coding for glutamine in the HD gene product huntingtin. Huntingtin is expressed in almost all tissues, so abnormalities outside the brain might be expected. Mitochondria dysfunction is reported in HD brains. Mitochondria are organelles that among other functions regulate apoptotic cell death. Involvement of nuclei and mitochondria in HD pathophysiology has been suggested. The tRNA gene mutations are one of hot spots that cause mitochondrial disorders. We performed mutation screenings of tRNA $^{leu/lys}$ genes and also ATPase 6 genes in 20 patients with HD.
Mitochondrial tRNA $^{leu/lys}$ genes and ATPase 6,8 genes were studied by PCR method and automated DNA sequencing to evaluate any possible mtDNA damage. We found some mutations including an A8656G mutation in one patient. We propose that it may causal to the disease. Understanding the role of mitochondria in the pathogenesis of neurodegenerative diseases could potentially be important for the development of therapeutic strategies in HD.

P0766. Pathogenic significance of the homozygous LMNA missense mutation p.Lys542Asn in patients with autosomal recessive Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder with children displaying features reminiscent of premature senescence. Recurrent heterozygous de novo point mutations in the LMNA gene encoding lamin A/C, a component of the filamentous meshwork of the nuclear lamina, have been shown to cause sporadic, non-familial HGPS. Recently, we have provided molecular evidence for autosomal recessive inheritance of HGPS in a consanguineous Indian family. In this family all 4 affected children carry the homozygous missense mutation c.1626G>C (p.Lys542Asn) in LMNA, whereas their parents as well as a sister are healthy heterozygous mutation carriers. To assess the pathogenic consequences of the p.Lys542Asn mutation, we investigated primary cultured skin fibroblasts from affected homozygous and healthy heterozygous mutation carriers for a) morphological changes in the nuclear envelope, b) telomere length alterations, and c) differences in gene expression using GeneChip® Human Genome U133 Plus 2.0 2 arrays. Here we present initial results on the molecular pathogenesis of the p.Lys542Asn LMNA mutation.

P0767. A Fourth Phenotype for Autosomal Dominant Hypercholesterolemia

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Autosomal Dominant Hypercholesterolemia (ADH), characterized by isolated elevation of LDL-cholesterol, is associated with high risk of premature cardiovascular disease. Three genes have already been implicated : LDLR (low density lipoprotein receptor), APOB (apolipoprotein B-100) and PCSK9 (proprotein convertase subtilysin kexin-like 9). We now report a large French ADH family in which involvement of these three genes was excluded and named the pathology HCHOLA4. Our aim is to identify the new disease gene and to define the associated pathophysiology. A whole-genome scan, using 232 polymorphic microsatellite markers, located the HCHOLA4 gene at 16q22.1. Functional candidate genes in the critical interval were tested by sequencing but no causal mutation was detected. In vivo kinetics of apolipoprotein B-100-containing lipoproteins, conducted in 2 affected members, mainly showed a decrease in LDL catabolism. Q-PCR analysis of LDLR expression in EBV-transformed lymphoblasts showed that cells of two affected subjects do not reply to cholesterol deprivation by activating LDLR expression contrary to controls in whom the expression rate increases 2-fold. These results suggest that this novel form of ADH is due to an alteration, direct or not, in LDL receptor endocytosis or intracellular traffic. Furthermore, we performed two-dimensional electrophoresis for cytosolic and membrane proteins from lymphoblasts and fibroblasts and observed different profiles for affected subjects when compared to non-affected relatives. Mass spectrometry for twenty of the more significatively different proteins is in process. We expect to identify one or more proteins for wich the coding gene is localized in the 16q22.1 interval of interest.

P0768. PCSK9, from gene to protein: a new protagonist implicated in autosomal dominant hypercholesterolemia

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Autosomal Dominant Hypercholesterolemia (ADH) is one of the most frequent human inherited disorders. Until 2003, mutations in two major genes had been clearly implicated : LDLR and APOB. We were the first to identify a third gene involved in ADH by analysis of non-LDLR/non-APOB French families : PCSK9 (Proprotein Convertase Subtilisin Kexin 9). Several hypercholesterolemic mutations of PCSK9 have been reported: S127R, F216L, D374Y, R218S and R357H. Two non sense variations Y142X and C679X were associated with a reduction of LDL-cholesterol levels and of CHD. The R46L variation is associated with a reduction of LDL-cholesterol of 15% and 47% of CHD.
We studied the frequency of 2 variations in 600 Caucasians. R46L was found only in controls with a frequency of 2%. A443T was identified in a woman with mild hypercholesterolemia and was not found in 340 French Caucasians controls. This variation turns out to be a rare polymorphism in whites, more frequent in blacks, associated with lower plasma levels of LDL-C but which has been found in both low and high LDL-C subjects in the Dallas Heart Study. Finally, the R237W that we had first described in a Canadian woman with hypercholesterolemia, but has been reported to be a hypocholesterolemic variation by Berge et al., seems to be found only in high-LDL-cholesterol subjects in the Dallas Study.
PCSK9 is an attractive therapeutic target for LDL-C lowering but further investigations are required to understand its precise role in cholesterol homeostasis and to identify its substrates and inhibitors.

P0769. Identification of a novel mutation of troponin-T gene causing malignant form of hypertrophic cardiomyopathy

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Objectives: Genetic origin is important in the development of hypertrophic cardiomyopathy (HCM) which is characterized by myocardial hypertrophy and rhythm disorders. The third most common mutated gene leading to HCM is the troponin-T (*TNNT2*) gene with an incidence of 2%. Our aim was to analyze mutations of the *TNNT2* gene in children and young adults.
Patients and methods: DNA was isolated from peripheral blood of 26 patients followed by PCR (*TNNT2* gene, exons 8,11,14,15,16). Mutation analysis was performed using dHPLC and positive chromatograms were sequenced.
Results: One mutation was found in the patient cohort (3,8%). It is a novel mutation, a deletion of a glutamic acid in exon 11, at one of the amino acid positions between 165-168. This mutation has not been published in the literature so far. The patient is a 19 year-old girl, who had endured aborted sudden cardiac death (SCD) twice, so implantation of an implantable cardioverter defibrillator was necessary. Family screening revealed that the patient's mother also had HCM. Her disease was characterised by mild septal hypertrophy, pronounced fibrosis and an uncommon restrictive diastolic dysfunction. She had suffered malignant arrhythmias several times and died of progressive heart failure at the age of 30. In another patient, two polymorphisms were found in intron 14 (base 18497C>G, base 18585C>T).
Conclusion: We have identified a novel *TNNT2* gene mutation which resulted in malignant ventricular arrhythmias and aborted SCD despite a mild myocardial hypertrophy. The phenotype was consistent with the previous data of *TNNT2* gene mutations causing HCM published in the literature earlier.

P0770. An A8296G mutation in mitochondrial tRNA^{Lys} gene in a patient with epilepsy; Pathogen or rare polymorphism?!

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Mitochondrial DNA (mtDNA) mutations are important cause of human diseases. A homoplasmic A8296G mutation was detected in a 24yr-old man with idiopathic generalized epilepsy. He experienced his first seizure at age 13. He suffers from deafness. His seizures begin suddenly and without warning. He loses consciousness and experiences typical generalized tonic or tonic-clonic seizures listing 1-5 minute each. The A8296G mutation in the mitochondrial DNA tRNA^{Lys} gene has been associated with severe mitochondrial diseases in a number of reports. The pathogenesis of this mutation or its association with a specific disease is unclear. This mutation has been reported alone as well as together with other mutations in trials in mtDNA. As in this case the mutation was homoplasmic and there were no clinical finding in other family members, we suggest that this mutation is rare polymorphism or it acts as pathogen in combination with other mutations inside or outside of the tRNA$^{Lys.}$

P0771. Evaluation of MLPA in routine diagnostics for the detection of subtelomeric rearrangements in 1040 patients with idiopathic mental retardation

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We screened 1041 patients with idiopathic Mental Retardation for subtelomeric aberrations by a multi-step strategy consisting in 1) analysis with the P036 MLPA kit assay, 2) confirmation with the P070 kit, 3) verification by conventional FISH analysis.
79 rearrangements were detected by the P036 kit (7.6%). 48 (61%) of them were confirmed by the P070 probes panel. 30 of this confirmed rearrangements were verified by FISH analysis and 10 are still under cytogenetic investigations.
Then, we focused on discrepancies between the P036 and P070 or, MLPA and FISH results.
From the 31 rearrangements detected by the P036 kit, 13 could not be found in a second experiment with the same kit, showing non-reproducibility of the technique in about 1% of cases. When the 2 kits mapped the same locus and gave different results (11/31), the imbalance detected was considered as a false positive reflecting the sensitivity of the probe to a polymorphism. From the 48 imbalances detected by the P036 kit and confirmed by P070, 7 were not verified by FISH analysis. From the 7 FISH/MLPA and 7 P036/P070 discrepancies, 6 imbalances were detected in one parent of the patient and therefore were not considered to be phenotype related. Real time PCR was performed on the 8 discordant samples left. Aberrant copy number detected by quantitative PCR confirmed MLPA analysis against FISH findings for 6 of them.
MLPA is a sensitive, cost-effective technique for screening mentally retarded patients which results must be validate by another cytogenetic or molecular method.

P0772. Molecular Diagnosis of Immunodeficiencies Syndromes

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Fourteen children with primary immunodeficiency diseases were investigated. Clinical and family history data was recorded. A molecular investigation, tailored to match the clinical diagnosis, was devised for each patient independently. Five candidate genes, namely, BTK, ITGB2, SLC35C1, UNG and WASP were screened in a multistep analysis, including, PCR, RFLPs, DHPLC (denaturating high performance liquid chromatography) and sequencing technology. Altogether, eleven mutations were identified in ten patients, including five new unreported mutations and six previously described.
Missense mutations detected in patients with LADI and LADII were restricted to conserved sequences, in line with those previously reported. A deletion encompassing two exons in BTK, no doubt disrupting the structure of the protein caused a mild disease in our patient with XLA but was otherwise lethal to other family members who died in infancy. Two splicing mutations, which majorly disrupt the protein, were identified in two WAS patients. In two of the four patients in whom no mutations were detected, the diagnosis of HIGM syndrome was subsequently revised. Genotype-phenotype correlation analyses in our patients support several concepts. The notion that genetic diseases and the immune response in particular, involve a complex interplay between environmental and genetic factors is sustained. The mutations contributing to LADI and LADII were restricted to conserved regions thereby implicating that variations located in other regions do not cause a disease. The view that the WAS phenotype, which may vary from isolated thrombocytopenia to severe classic lethal immunodeficiency is "mutation" dependent is confirmed.

P0773. The functional polymorphism -703T/C in the promoter of the human *IL5* gene is associated with atopic and non-atopic bronchial asthma, but not with atopic dermatitis

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Analysis of association between -703T/C polymorphism in promoter of *IL5* gene and atopic bronchial asthma (BA), non-atopic BA, and atopic dermatitis (AD) was carried out by case-control study. It was shown that -703C allele is associated with overexpression of the *IL5* gene, probably, because of moving off the binding site for negative regulator CLOX and therefore may predispose to atopic disease. Using logistic regression analysis we found significant association of the -703C allele both with atopic and non-atopic BA ($p<0.0001$), but not with AD ($p=0.686$). When AD patients were divided into two groups in respect to atopic BA developing within three years after first diagnosis (AD), we found a weak association between the -703C allele and BA in patients with AD ($p=0.016$), but again with not AD alone. Therefore, we found that -703T/C exhange in *IL5* gene is a risk factor for bronchoobstructive syndrome irrelatively of risk of atopy. This confirms that end-organ and systemic atopy-related genetic predisposing factors of atopic disease are different. This also suggests that the *IL5* gene is an end-organ specificity candidate gene associated with BA itself rather than with atopy.

P0774. *IRF6* gene's nucleotide sequence changes in patients with nonsyndromic orofacial clefting from Lithuania

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The hunt for the causes of nonsyndromic orofacial clefing (NS-OFC) is extremely complex, involving multiple techniques that have been used to identify numerous candidate genes in which disruption results in increased risk of clefting. In recent years, a number of independent groups have targeted for investigation the involvement of *IRF6* (interferon regulatory factor 6 gene). *IRF6* is related to syndromic OFC - Van der Woude syndrome.
Our study was aimed to investigate whether mutations in the *IRF6* gene contribute to NS-OFC in the population of Lithuania.
Patients with NS-OFCs from Lithuania were tested for nucleotide sequence changes in the *IRF6* gene (206 patients). DNA fragments covering exonic parts of the *IRF6* gene were PCR-amplified and direct sequenced.
23 different nucleotide sequence changes were revealed in the *IRF6* gene by comparison of sequencing results with reference DNA sequences of the genes. Scanning *IRF6* gene resulted in ten novel nucleotide sequence variants. Out of them, four were missense mutations (p.S212I, p.L295P, p. Q340K, p.R400L), which, together with the p.A61G mutation found in the case of Van der Woude syndrome, might be related to the NS-OFC phenotype in the population of Lithuania.
Our study highlights the *IRF6* gene sequence variability and supports the hypothesis that variation in this gene contributes to NS-OFC phenotype encouraging further investigations to test if *IRF6* gene mutations identified in the individuals from Lithuania are rare alleles causative for NS-OFC.

P0775. The R176C amino-acid change in hemojuvelin as a novel haemochromatosis mutation: phenotypic and functionanl evidences

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Background: Juvenile haemochromatosis (JH) is an early-onset autosomal recessive condition of iron metabolism caused by mutations in either the hemojuvelin (*HJV*) or the hepcidin-encoding gene (*HAMP*). The two JH gene products are implicated in a same physiologic process, where hepcidin controls iron flow into plasma and hemojuvelin enhances hepcidin expression at the transcriptional level via the classical bone morphogenetic protein (BMP) cell signalling pathway. Aim of the study and results: In this study, we report a novel missense *HJV* mutation that leads to the replacement of arginine by a cysteine residue at position 176 (R176C). We associate homozygosity for this novel mutation with the iron overload phenotype observed in a 17-year-old girl. We also show that the HJV 176C mutated protein fails to up-regulate the hepcidin promoter activity. Lastly, we suggest that, due to its nature and position, the R176C amino-acid change prevents an autocatalytic cleavage that normally occurs during HJV intracellular processing. Conclusion: Our results definitively demonstrate that the R176C substitution is a novel HJV loss-of-function mutation. They also highlight that rapid advances in comprehension of the HJV intracellular processing and function have paved the way for functional characterizations.

P0776. Early age at onset is the major clinical feature in Parkinson disease related to *PARK2* gene mutations

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Autosomal recessive juvenile Parkinson disease (JPD) is characterized by rigidity, bradykinesia, rest tremor, age at onset before 40 years, good response to levodopa treatment, and slowly progressive disease. Mutations in the parkin gene (*PARK2*) are the major genetic cause of JPD.
To refine the clinical characteristics of the *PARK2*-associated JPD, we investigated 77 patients referred to our diagnostic lab for *PARK2* analysis.
Searching for point mutations by sequencing and for large rearrangements by Multiplex Ligation-dependent Probe Amplification (MLPA kits P051/P052, MRC-Holland) gave the following results:

Number of *PARK2* mutations	Presence of *LRRK2* p.Gly2019Ser mutation (detected by the MLPA kits)	Number of patients	Type of *PARK2* mutations (P: point mutation, R: rearrangement)	Age at onset : mean ± 2SD (range) in years	Molecular diagnosis of JPD
2	no	15	19 R + 11P	24 ± 11 (17-35)	yes
1	no	2	2 P	(35-51)	no
0	no	55		36 ± 21 (3-53)	no
1	yes	2	2 P	(43-not available)	no
0	yes	3		37-54	no

The distribution of *PARK2* genotypes (2, 1 or 0 mutation) differed dramatically from Hardy-Weinberg equilibrium, strongly suggesting that, for most patients with no identified *PARK2* mutation, the phenotype is independent from *PARK2* gene rather than due to mutation in *PARK2* unexplored regions.
Onset of disease was younger than 36 years in all the patients with 2 *PARK2* mutations. However, *PARK2*-related JPD was established in only 40% of patients with age at onset less than 36 years old. The *LRRK2* G2019S mutation was found in cases significantly older than *parkin* cases. Patients presenting with 2 *PARK2* mutations were clinically indistinguishable from the others, according to the frequency of rigidity, bradykinesia, tremor, positive response to levodopa, and asymmetric onset.
Altogether, these results show that early age at onset is the major clinical criteria for *PARK2* molecular diagnosis of Parkinson disease.

P0777. Lamellar ichtyosis caused by TGM1 DNA mutation in a Family from Azerbaijan

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A case of autosomal recessive lamellar ichthyosis in two newborn male sibs from consanguineous Azerbaijan family is presented. The parents of the children are the first cousins. The first child was born in the 34-th week of the pregnancy and died on the third day. The second child was born in 37-th week and died on the first day. Both of them had got the same clinical sign : tight shiny covering, described as collodion membrane and erythroderma. During the first hour of life the membrane is disrupted. They had got severe ectropion and eclabiun too. There is no any biological material from the sibs, but the pariens is caused by heterozygous mutations in 3-d exon of TGM1 gene P.142 Arg>His. The mutation of TGM1 gene is known to be responsible for phenotypes of lamellar ichthyosis.

P0778. Spectrum of NPHP6 (CEP290) Mutations in Leber Congenital Amaurosis and Delineation of the Associated Phenotype

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Mutations in the NPHP6 gene were shown to account for Joubert syndrome and Senior-Loken syndrome as well as Leber congenital amaurosis (LCA). All reported patients affected with LCA carried an intronic mutation resulting in an aberrantly spliced transcript and low levels of wild-type transcript that was believed to explain the absence of cerebellar and renal involvement in LCA patients. The aim of the present study was to give the survey of NPHP6 mutations in our series. 192 unrelated LCA cases were screened for mutations. The natural history and ophthalmologic data were reviewed for all patients har-

bouring NPHP6 mutations NPHP6 mutations were identified in 38/192 LCA families of our series. The common NPHP6 intronic mutation accounted for 33/76 of all disease alleles in our series. Twelve unrelated LCA cases did not carry this common intronic mutation, ten of which, at least, harboured two mutations expected to truncate the protein.
We confirm the high frequency of NPHP6 mutations in LCA (19.8%) as well as that of the c.2991+1655A>G mutation (43% of disease alleles). We also suggest that a significant fraction of LCA families segregate two NPHP6 null alleles questioning the relevance of the assumption according to which the retinal-restricted phenotype in LCA patient could be due to a residual NPHP6 activity. Indeed, Joubert syndrome was excluded by cerebral MRI in all patients presenting with developmental delay. Finally, we show that all patients of our series are affected with the cone-rod subtype of the disease whatever their NPHP6 genotype.

P0779. The mutation p.Phe128Ser (c.383T>C) in the *TAZ* (*G4.5*) gene in a patient with left ventricular non-compaction

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Left ventricular non-compaction (LVNC) is a rare cadiomyopathy of genetic origin, characterized by deep trabeculations in the ventricular wall. Clinically, is characterized by systolic and diastolic dysfunction, and associated at times with arrhythmias and systemic embolic events, and has a high mortality rate.
Mutations in the *TAZ* (*G4.5*) gene are mainly associated with Barth Syndrome, but may also be the cause of LVNC.
The finding of the mutation p.Phe128Ser (c.383T>C) of the *TAZ* (*G4.5*) gene in a 17 years old male, that suffered from LVNC, is presented. This mutation was previously referenced on a case of a child with Barth Syndrome [1].
The subject presented class III-IV progressive congestive heart failure according to the New York Heart Association functional classification system. The diagnosis was obtained by echocardiography and magnetic resonance imaging. Complete sequencing of the *TAZ* (*G4.5*) gene was performed on an Applied Biosystems 3700 system.
The mutation c.383T>C is located within exon 5. Tazfarin, the protein encoded by *TAZ*, presents itself in 5 isoforms, 2 of witch containing the amino acid sequence encoded by exon 5. Our results are in accordance with other observations that indicate a relevant role for the isoforms containing exon 5.
Genetic analysis of the *TAZ* (*G4.5*) gene was helpful for establishing the precise diagnosis of LVNC and for adequate genetic counselling. This finding also underlines the variability of phenotype associated even with one sole mutation in the *TAZ* (*G4.5*) gene [2].
[1] http://www.barthsyndrome.org/english/View.asp?x=1357
[2] Gonzalez IL. *Am J Med Genet.* 2005, 134:409-14.

P0780. *LMNA* mutations and phenotypes in Russian families

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LMNA gene (1q21.2-21.3) encodes two nuclear envelope proteins _ lamins A and C. Numerous mutations of the gene cause a wide spectrum of disorders called laminopathies. We performed a search of *LMNA* mutations in a group of families with characteristic phenotypes. In 11 families, ten different mutations were found, namely Arg249Gln, Asp47His, Gly232Arg, del Lys261+ins15bp in 4 families with autosomal dominant Emery_Dreifuss muscular dystrophy (AD EDMD); Arg-249Gln, Arg377His in two families with limb girdle MD type 1B (LGMD 1B); Arg541His, Ala350Pro, Gly635Asp, Leu52Pro in 4 families with autosomal dominant arrhythmic dilated cardiomyopathy (DCMP 1A), heterozygous Asn459Tyr in a family with autosomal recessive polyneuropathy (second mutation in this families was not detected). Mutations Arg249Gln and Arg377His were reported previously, eight mutations are novel. Seven cases are familial, four cases present mutations de novo, among them both Arg249Gln mutations. Since this mutation occurred de novo also in other reported cases, a mutational “hot point” is supposed. Three families show an overlap between DCMP 1A and MD’s. Along with common phenotypes, atypical variants were found out, i.e. (1) a severe Duchenne-like EDMD, (2) an infantile DCMP 1A, (3) a combination of LGMD 1B and histologically proven localized scleroderma (morphea), and (4) a severe infantile polyneuropathy in two sibs distinct from HMSN 2B1. The latter two may present novel phenotypes. In a subset of families with EDMD-like phenotypes we found neither emerin nor *LMNA* mutations, which may indicate the existence of other genes producing similar disorders.

P0781. Bone remodeling in MADA disease: involvement of matrix metalloproteinases secretion

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Mandibuloacral dysplasia type A [MADA; OMIM # 248370] is a rare multysistem disorder belonging to a heterogeneous group of diseases, collectively called Laminopathies. These disorders are caused by mutations in the *LMNA* gene. Bone is one of the most involved tissues. The MADA patients are characterized by postnatal growth retardation along with typical skeletal abnormalities such as hypoplasia of the mandible and clavicles, acroosteolysis, delayed closure of the cranial sutures and joint contractures. Bone alterations are mediated by extracellular matrix (ECM) dysregulation which involve modification in the expression profile and activities of the matrix metalloproteinases (MMPs). We investigated the MMP production by TNF-α stimulation in MADA fibroblasts, as model *in vitro*, in order to mime the changing that could be occurred in an alterate homeostasis of bone and in the extracellular matrix remodeling.
Dermal fibroblasts from MADA and control patients were seeded in multi-well plates in complete media. Ninety percent confluent cells were treated with TNF- α (15ng/ml) for 48h in serum free media. Supernatants were assayed for some MMPs by western blot and zymography analysis. A significant decrease of some MMP protein levels in stimulated MADA fibroblasts conditioned media was observed, suggesting a modification of the MMPs pathway that occurs in MADA disease.

P0782. Molecular diagnosis of congenital long-QT syndrome in Polish patients

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Long QT syndrome (LQTS) is a cardiac disorder that causes sudden death from ventricular tachyarrhythmias, specifically *torsade de pointes*. The molecular basis of LQTS is associated with delayed repolarization of the myocardium increasing the QT interval measured on electrocardiogram. LQTS can be inherited as a more common autosomal-dominant disorder (Romano-Ward syndrome, R-W type 1-6) or a rarer autosomal-recessive disorder associated with congenital neuronal deafness (Jervell and Lange-Nielsen syndrome, JL-N type 1-2). Mutations in a group of genes encoding cardiac ion channels (*KCNQ1, KCNH2, KCNE1, KCNE2 and SCN5A*) are involved in the LQTS pathogenesis.
The aim of the study was to investigate the molecular basis of long QT syndrome, inheritance traits and type of the disease in Polish population. Thirty-three patients suspected of having LQTS from 29 unrelated families treated at the Cardiology Department of the Children’s Memorial Health Institute in Warsaw were screened. Genomic DNA was extracted from lymphocytes, and coding regions of three genes (*KCNQ1, KCNH2, KCNE1)* were amplified and analyzed by SSCP and sequencing analyses. In 8 families pathogenic mutations in gene *KCNQ1* (p.Y171X, p.R243H, p.V254M, p.A341V, p.G306R, p.V416fsX462 and p.C445X) and gene *KCNH2* (p.C108R, p.N633S and p.P1122L) were identified. Among them five families presented R-W syndrome type 1 or 2, and three families JL-N syndrome (one family without hearing loss). In many patients several polymorphic nucleotide substitutions (p.G38S and p.D85N in gene *KCNE1,* p.F485F and p.S546S in gene *KCNQ1,* and p.I489I and p.L564L in gene *KCNH2*) were found.
The study was supported by KBN Project 6P05E15021.

P0783. A new diagnostic service for lymphoedema: Screening of *VEGFR3 (FLT4)* and *FOXC2*

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Lymphoedema is a chronic tissue swelling usually of the lower extremities caused by abnormal lymph drainage. There are 3 main clinical subtypes of primary lymphoedema - Milroy disease (MD), Lymphoedema Distichiasis (LD) and Meige disease, all of these conditions exhibit autosomal dominant inheritance. MD patients present with lymphoedema at birth or in early infancy. The penetrance of MD is estimated to be about 88%. LD presents with distichiasis (aberrant eyelashes arising on the inner eyelid) and lymphoedema in late childhood or puberty and is about 90% penetrant.

To date two genes have been identified; *VEGFR3 (or FLT4)*, associated with MD and *FOXC2*, associated with LD. Mutations reported in the *VEGFR3* gene are primarily missense and have to date only been found in exons 17-26 which encodes the highly conserved tyrosine kinase domain. Diagnostic testing has been developed for the 10 exons in the *VEGFR3* gene using dHPLC on the Transgenomic WAVE Analyser and any variants observed are sequenced in both directions. The *FOXC2* gene consists of a single 1.5kb exon encoding a forkhead transcription factor. Bi-directional sequencing of 4 overlapping fragments of *FOXC2* is employed as a screening technique. To date *FOXC2* mutations found in the laboratory consisted only of small insertions and deletions. However, missense and stop mutations have been previously reported. Screening of both these genes is offered as a clinical molecular diagnostic service.

P0784. Genetic screening for patients with non-obstructive infertility from Ukraine

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Microdeletions of the long arm of the human Y-chromosome are associated with spermatogenic failure and have been used to define three regions of Yq (AZFa, AZFb, AZFc) that are recurrently deleted in infertile males. It was supposed that apart from cystic fibrosis, mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are involved in male infertility. Mutations in the CFTR gene cause congenital bilateral absence of the vas deferens (CBAVD) in approximately 1% of the infertile males.

We have screened Y-chromosome microdeletions, mutations and poly-T sequence of CFTR gene in 680 patiens (azoospermia and oligospermia) and in 621 healthy valantiers by PCR-based methods. For each patient, multiplex PCR analyses were performed on DNA isolated from leukocytes derived from peripheral blood to screen 18 sY-sequences on Yq. Cytogenetic investigation was performed according to standard methods (GTG-banding). No chromosomal anomalies were found in men.

Microdeletions were detected in 2,79% infertile men. During patients research we have made a conclusions: a) damage of genes from AZFb region results in impairments of last stage spermatogenesis; b) deletions in AZFc region are critical for early stage spermatogenesis.

The frequency of CFTR gene mutations carriers detected in 630 patients was statisticaly significant higer than in control group. 5T allele of CFTR gene associated with CBAVD was detected in 1,93% of patients. The obtained data produce the evidence of the possible involment of CFTR protein in spermatogenesis. The genetic consultation and preimplantation analysis were recommended for the couples with identified Y-chromosome deletions and CFTR gene mutations.

P0785. Expression profiling of human normal testis by microarray technology

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Spermatogenesis is a complex process that demands several genes to action but the biological mechanisms underlying sperm production are still largely unknown. In this view, it is crucial to obtain a full picture of the global expression profile of normal testis.

In order to identify the list of testis up and down regulated genes directly linked to testis metabolism and function towards the human general metabolism, we start to analyse, using an 21,329 spotted oligonucleotides microarray, global gene expression pattern of healthy testis biopsies versus an home made RNA universal reference.

Our preliminary research revealed a group of 2.718 up-regulated and 2.654 down-regulated annotated UniGene in testis as compared to the reference RNA pool. These annotated genes were grouped together using the EASE software, which allowed us to identify a list of biological process. Among these the most abundant categories were those related to physiological processes and metabolism, which are very unspecific classes, while less represented but more specific were those containing genes involved in fuction related to human testis, such as reproduction, meiosis, imprinting. Among these, of interest is the presence of genes mapped within the AZF loci of the Y chromosome such as DAZ, BPY, DBY, SMCY as well as DAZL autosomal homolog gene.

Our results clearly demostrates that expression profiling using microarray technology is able to evidence genes specifically involved in testicular fuction, and to provide a general pattern of the human normal testis trascriptome, which could be certainly useful for understanding also the pathological pathway.

P0786. Gene expression studies in testicular tissue. Suitability of PBGD and HPRT as internal references genes for normalization

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Semiquantitative gene expression experiments require normalization to compensate for differences in the amount and quality of biological material and reverse transcription reaction efficiency in the tested samples. An extended strategy is to normalize to internal reference genes, which should show similar expression in the samples investigated.

We have assessed the suitability of *PBGD* and *HPRT* genes as candidates for gene normalization in both pathological and normal testicular tissue. We have analysed their expression levels in testicular biopsies of 13 non-obstructive infertile men, who showed either severe hypospermatogenesis or maturation arrest (patient group 1), 7 men diagnosed with germ-cell tumour (patient group 2) and 10 infertile men with obstructive azoospermia (control group). The quantitative real-time PCR reactions were performed in a LightCycler® 1.5 Instrument (Roche), using SYBR Green I fluorescence dye. The Mann-Whitney U test was used to analyse gene expression differences in case and control patients.

Non-significant differences were observed in *PBGD* expression levels in both patient groups 1 and 2 compared to controls (P= 0.077 and P= 0.262, respectively) and in *HPRT* expression profile between patient group 1 and control group (P= 0.515). Interestingly, *HPRT* was found to be differently expressed in patient group 2 compared to controls (P= 0.001) suggesting that *HPRT* would not be a suitable reference gene to be used for normalization of target genes expression data in testicular malignant phenotype. Hence, while choosing reference genes, the testicular phenotype under study should be considered to avoid erroneous normalizations.

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P0787. Microdeletions in AZF region of Y chromosome in infertile males, a study from Latvian population

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Male factor infertility accounts for about half the cases of couple infertility in Latvia. The most common genetic cause of human Y-linked male infertility is partial or complete deletions of the AZF region on the Y chromosome. AZF region consists of three intervals, AZFa, AZFb, and AZFc, which are associated with different spermatogenic failure.

The aims were to introduce the molecular screening method of Y-

chromosomal microdeletions in Latvia and to detect microdeletions in *AZFa, AZFb,* and *AZFc* gene families.
Objects for Y-chromosomal microdeletions and male infertility association study were 55 individuals with different spermatogenic arrest such as azoospermia, severe oligozoospermia, oligo-astheno-terato-zoospermia. All cases of spermatogenic failure resulting from endocrine or obstructive causes or with a cytogenetic abnormality were excluded from our study.
Microdeletions in AZF region were determined by two multiplex PCR amplifications using ten primer pairs. Two non-polymorphic STS loci were analyzed in each AZF region (sY84, sY86, sY127, sY134, s134, sY254, sY255). Internal PCR control (ZFX/ZFY) was used as well as DNA sample from a fertile male and from a female.
Out of 55 analyzed samples we have found three cases (5.5%) with microdeletions that were observed only in AZFc region at SY254 and SY255 STS loci in DAZ gene.
The frequency of Y-chromosomal microdeletions is low in Latvian population, however, Y chromosome microdeletion screening is important not only to define the aetiology of spermatogenic failure but also because it gives precious information for more appropriate clinical management of both the infertile male and his future male child.

P0788. Analysis of three Glycine substitutions in loop-regions of calcium-binding Epidermal Growth Factor-like domains of fibrillin-1: possible key positions for domain folding.

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Among the patients refered to our centre for molecular diagnosis of Marfan syndrome (MFS) we identified several novel heterozygous missense mutations of the FBN1 gene. Interestingly, three of them were predicted to result in Glycine substitutions that occur in a loop-region of the corresponding calcium-binding Epidermal Growth Factor-like (cb-EGF-like) domains of fibrillin-1 : c.1753G>C (p.Gly585Arg in cb-EGF-like#5), c.4981G>A (p.Gly1661Arg in cb-EGF-like#24) and c.6418G>A (p.Gly2140Arg in cb-EGF-like#32). These mutations were identified in three probands with MFS (Ghent criteria fullfilled) or suspected MFS (a criterium is missing). Familial investigations, molecular studies (DNA/RNA), in silico analyses (conservation, 3D modeling) coupled with data of the literature provide positive arguments for a crucial role of the corresponding Glycine position in cb-EGF-like type 1 domain structure maintenance.

P0789. SIL1 and SARA2 mutations in a family with Marinesco-Sjogren and Chylomicron Retention Diseases

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Marinesco-Sjogren Syndrome (MSS) is an autosomal recessive disorder, characterised by cataracts, ataxia, and mental retardation.Recently, Anthonen identified the linkage of the MSS phenotype to 5q31 chromosome in a Finnish family, and identified a mutation of the SIL1 gene in all investigated MSS patients. Senderek using homozygosity mapping in three small consanguineous families with typical MSS narrowed a critical region on 5q31. In the current study, we further investigated a small Italian pedigree. In this family, two brothers had MSS syndrome, with a very low serum concentration of vitamin E and absence of postprandial chylomicrons, a finding consistent with chylomicron retention disease (CMRD). On a subsequent study , we demonstrated that these two brothers carried a mutation in the SARA2 gene belonging to the Sar1-ADP-ribosylation. On the basis of the recent data on SIL1 in MSS, we analyzed the SIL1 gene in our patients with MSS and CMRD, in their healthy parents. Sequencing of the SIL1 gene revealed a homozygous mutation at position 331 in exon 4 resulting in a premature stop codon at amino acid 111 (R111X) in the affected brothers, while both parents were heterozygous for the mutation . In our previous study , we hypothesized that both MSS and CRMD found in the two Calabrian brothers could be due to defects in a gene crucial to the assembly of the chylomicron particle.The present results, however, demonstrate that CMRD and MSS observed in our patients are distinct diseases due to defects in two different genes, SARA2 and SIL1.

P0790. Sequence analysis of multidrug resistance protein 3 (MDR3) in Italian patients with intrahepatic cholestasis of pregnancy with raised serum γ-GT

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Intrahepatic cholestasis of pregnancy (ICP) is characterized by intense pruritus and abnormalities of liver function tests and bile acids. Usually, serum gamma-glutamyl transpeptidase (γ-GT) activity remains within normal limits. However, in some cases γ-GT is increased. Although the prognosis is generally favourable for the mother, ICP is associated with increased fetal risks, such as preterm delivery and fetal distress. Intrauterine fetal death has been reported in 0.8-1.6% of ICP cases. The cause of ICP is still unknown, but there are evidences that genetically disfunction MDR3 is associated to the development of ICP. MDR3 P-glycoprotein is a canalicular phopholipid translocator involved in the biliary secretion of phospholipids. The aim of this study was to identify new disease-causing mutations in a specific group of Italian women suffering from ICP with raised serum γ-GT (>40 mg/dL). DNA sequence analysis of the MDR3 promoter and the 27 coding exons with their exon-intron boundaries was performed in 11 ICP patients with raised γ-GT levels and in 43 control women. Two heterozygous mutations were found in the ICP patients. One is a novel mutation consisting of A to G transition at the 3' acceptor splice site of MDR3 intron 7 (IVS7[-2]A→G); the second one is R590Q, a mutation previously reported in a patient with primary sclerosing cholangitis. The identification of MDR3 mutations in 18% of the affected women supports the hypothesis that genomic variants of this gene play a pathogenetic role in the subset of ICP patients presenting with raised γ-GT values.

P0791. The first laboratory expierence of diagnostics of MEN2 syndrome in KMUH (Lithuania)

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MEN2 syndrome is a rare autosomal dominant syndrome with medullary thyroid carcinoma and other tumours of endocrinological system. MEN2 syndrome has three subtypes: MEN2A (medullary thyroid carcinoma, pheochromocitoma and primary hyperparathyroidism), MEN2B (medulary thyroid carcinoma, pheochromocitoma and typical phenotype - marphanoid constitution with ganglioneuromatosis) and FMTC (familiar medullary thyroid carcinoma). *RET* is the only gene known to be associated with MEN 2. In 2006 year the diagnostics of MEN2 syndrome was performed for members of two families in Kaunas Medical University Hospital. The molecular genetics diagnostics was performed using two restrictions enzymes *Fok I* and *Pag I.* The first family was of medullary carcinoma patient and her 4 year old son. The mutation was pC618R in *RET* gene codon 618. The presymptomatic diagnostics was performed for the child of patient and results showed, that he has no mutation. Another family has the girl with medullary carcinoma. The girl was 8 years old when operation was performed. She had phenotypic signs of MEN2B syndrome: ganglioneuromatosis of tongue and lips. The mutation analysis using restriction enzymes confirmed the most typical mutation of MEN2B syndrome :p.M918T in exon 16 of RET gene.
The molecular diagnostics using restriction enzymes is cheap, fast and easy performed. This laboratory diagnosis can be used for confirmatory of diagnosis, presymptomatic diagnosis and corelation between mutation and disease prognosis.

P0792. Serine-arginine repressor protein, SRrp35, is disrupted by an inv(6)(p21.3q15) in a patient with mental retardation and obesity

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Mental retardation (MR) is defined by an overall IQ score below 70 and deficits in adaptive behavior that are recognized in childhood. Genetic abnormalities frequently give rise to a MR phenotype.
Here, we present an obese, mentally retarded patient with a *de novo* pericentric inversion of chromosome 6, inv(6)(p21.3q15). FISH performed with BAC and PAC clones revealed that RP11-355M6 (6p21.2) and RP11-63L7 (6q15) span the breakpoints. We could show that both breakpoints disrupt a gene, on 6p21.2 the *C6orf128* gene and on 6q15 the *SRrp35* gene. Mutation analysis for both genes, in 150 Prader-Willi like MR patients, did not reveal any mutations. There are no strong indications for *C6orf128* being a candidate gene for MR. No other 6p21 rearrangements in MR patients are known from the literature, the gene is ubiquitously expressed and its function is unknown. *SRrp35*, however, is more indicative for being a good candidate, since 12 MR patients have been reported with deletions of 6q15, the region *SRrp35* is mapping. Furthermore, *SRrp35* is predominantly expressed in fetal and adult brain and the SRrp35 protein is member of the group of proteins that are SR (serine-arginine) protein-like alternative splicing regulators. Prader-Willi syndrome (PWS) also has the phenotypic characteristics of obesity and MR. The PWS candidate gene *SNRPN* (*SNURF-SNRPN*) is highly expressed in the brain and also has a role in mRNA processing. Therefore it has been postulated that mental retardation may be caused by defects in mRNA processing.

P0793. Common allelic variants of APOA5 gene in the metabolic syndrome

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The metabolic syndrome affects the 15-30 percent of the European population. It is characterized by hypertriglyceridaemia, hypertension, diabetes mellitus and obesity. Naturally occuring variants of the APOA5 gene associated with elevated triglyceride levels. The T-1131C variant has been found to associate with cardio- and cerebrovascular disorders like coronary artery disease and stroke.Relationship of the common four SNPs was examined using PCR/RFLP tests. 220 patients affected with metabolic syndrome and 140 apparently healthy controls were genotyped for T-1131C; IVS3 +G476A, T1259C and C56G variants of the APOA5 gene.The allele frequencies for metabolic syndrome patients/controls were as follows: 10.4/4.1 % for -1131C, 8/2.5% for IVS3+ G476A, 8.2/7% for 1259C and 7.25/4% for 56G. We found increased serum triglyceride levels in carriers compared with non-carriers for three polymorphisms (T-1131C; IVS3+ G476A, T1259C, p<0.05 carriers vs. non carriers). The serum cholesterol levels measured were similar in all subjects. The multiple logistic regression analyses adjusted for age, gender, total serum cholesterol, acut myocardial infarction and stroke revealed that -1131C and IVS3+ 476A variants confer a significantly increased risk for development of metabolic syndrome (OR 2,874; 95% CI: 1.241-5.123; p=0.010; and OR 3.529; 95% CI: 1.308-9.029; p=0.009). Further association studies are required to delineate the significance of the naturally existing haplotypes in the development of metabolic syndrome.

P0794. Methylenetetrahydrofolate reductase gene and the neural tube defects in Kazakh population

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This study determined the frequencies of the C677T and A1298C 5,10-methylenetetrahydrofolate reductase (MTHFR) gene's mutations for 30 neural tube defects (NTD) cases, 84 mothers, 39 fathers of NTD cases and 50 males and 100 females for the controls of Kazakh population.
The frequency of homozygous TT genotype in mothers of NTD cases (8,3%) was statistically higher than their respective controls (1,0%, $\chi 2=4,3$; p<0,05). The fathers of NTD patients were heterozygous for the C677T variant allele (59,9%) at a higher rate than the males of control group (38,0%, $\chi^2 =3,8$; p<0,05). Kazakh NTD cases were homozygous (13,3%) and heterozygous (66,7%) for the C677T variant allele at a higher rate than their respective controls (42,0% and 1,3%, p<0,05).
The frequencies of genotypes of A1298C mutation were not significantly different between parents of NTD cases and controls. The patients with NTD were homozygous (16,7%) and heterozygous (56,7%) for A1298C variant allele at a higher rate than the health control (34,0% and 8,0%, p<0,05). Our study provides evidence that the maternal C677T homozygous mutant genotype, the paternal heterozygous for the C677T variant allele and the patient's TT, CT genotypes of C677T mutation, ac and cc genotypes of A1298C mutation are a possible risk factor for NTD in Kazakh population.

P0795. Mitochondrial abnormalities in murine models of Mut class methylmalonic acidemia

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Mut class methylmalonic acidemia (mut-MMA) is an autosomal recessive inborn error of metabolism caused by a defective activity of methylmalonyl-CoA mutase. Affected patients suffer from life-threatening intermittent metabolic decompensation, pancreatitis, and renal failure; the etiology of these alterations remains unknown. Current treatments include dietary restriction of precursors, alkali supplementation, and liver transplantation in the most severely affected patients. Mitochondrial dysfunction has been suggested as contributory to symptoms in the affected patients but never convincingly demonstrated. To assess the role of mitochondria in this disorder, murine models of mut-MMA were created and used to examine ultrastructural and energetic aspects of mitochondrial function. Megamitochondria in the hepatocytes of the mut-MMA animals were observed. Liver extracts displayed electron transport chain dysfunction, with a substantial decrease in cytochrome c oxidase activity in the mutant animals. Over time, the proximal tubules of the kidney and exocrine pancreas also developed abnormal mitochondria, which were enlarged with distorted cristae and contained inclusions and unusual lamellar structures whereas mitochondria were normal in the skeletal muscle and heart. Our findings indicate a tissue specific response inherent to mut-MMA and for the first time, connect mitochondrial function to the etiology of organ system pathology in this disorder. Furthermore, these observations explain the protective effects of liver transplantation in methylmalonic acidemia and should guide the development and testing of new therapies for affected patients.

P0796. Feasible contribution of inherited thrombophilia factors in pregnancy miscarriage

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To determine whether the inherited thrombophilia risk factors are associated with spontaneous abortion (SA) of unexplained etiology, genotypes distributions of the 2 mutations (*F*V Leiden, *PROT* G20210A) and two gene polymorphisms (*FGB*G-455A and *MTHFR* C677T) were compared in 172 women with at least 1 SA before the 20th week of gestation and 108 women with the live birth and without a history of pregnancy loss. The analysis of anti-PL antibodies (APA) for 94/172 patients with SA was provided retrospectively. No significant differences were observed between distributions of any studied genotypes both in the miscarriage and control groups. Meanwhile, in the subgroup women with with ≥3 SA the frequency of *MTHFR*677 T/T genotype was significantly higher when compared to the control group (9/57, 15,8% versus 5/108, 4,6%; p<0,05). It was especially pronounced in the women with ≥3 SA and no live births in anamnesis (9/46, 19,6%; p<0,01). Patients with APA had "thrombophilic genotypes" (*MTHFR*677 T/T, *FGB*G-455 A/A and/or *F*V Leiden and *PROT* G20210A in heterozygotes) more often than non-APA patients (17/49, 34,7% versus 7/45, 15,5%; p<0.05). CONCLUSIONS: 1. Genotype *MTHFR*677 T/T is associated with recurrent spontaneous abortions with ≥3 fetal loss. 2. Presence of inherited thrombophilia factors correlates with presence of APA in 69,6% (16/23) of cases. It could be speculated that both APA and inherited thrombophilia could be treated as cumulative risk-factors

initiating SA or that thrombophilia itself plays a role of initial predisposition factor in production of APA.

P0797. Mitochondrial DNA haplogroups in infertile males

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A variety of mtDNA mutations responsible for human diseases have been associated with molecular defects in the OXPHOS system. It has been proposed that mtDNA genetic alterations can also be responsible for sperm dysfunction. To investigate any possible association between infertility and mtDNA haplogroups (hg), the nucleotide sequence of the Hypervariable Segment I (HVS-I) of mtDNA was determined in 99 unrelated Kuwaiti patients with infertility and 54 normal controls with the same ethnicity. DNA was extracted from the peripheral blood after having obtained informed consent. The nucleotide sequence of HVS-I (np 16,024-16,383) was directly determined. High-resolution RFLP analysis and control-region sequencing revealed high proportion of haplogroup J and M in normal controls (64% and 20.3%) compared to infertile men (26.2% and 8.1%) respectively. (P= 0.002 for J and 0.03 for M) Therefore, we hypothesize that individuals classified as haplogroup J and M demonstrate a significant decrease in risk of infertility in Kuwaiti population.

P0798. Mutational analysis of the mitochondrial tRNA$^{Leu(UUR)}$ gene in Tunisian patients with mitochondrial diseases

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The mitochondrial tRNA$^{Leu(UUR)}$ gene (MTTL) is a hot spot for pathogenic mutations that are associated with mitochondrial diseases with various clinical features. Among these mutations, the A3243G mutation was associated with various types of mitochondrial multisystem disorder, such as Mitochondrial Inherited Diabetes and Deafness (MIDD), Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke like episodes (MELAS), Myoclonus Epilepsy with Ragged Red Fibres (MERRF), maternally inherited Progressive External Ophthalmoplegia (PEO), hypertrophic cardiomyopathy, and a subtype of Leigh syndrome.
We screened 128 Tunisian patients presenting various mitochondrial diseases (MIDD, diabetes, hearing loss, Leigh Syndrome, cardiomyopathy, metabolic encephalopathy…) for the A3243G mutation in the mitochondrial tRNA$^{Leu(UUR)}$ gene. This screening was carried out using PCR-RFLP with the restriction endonuclease *ApaI*. None of the 128 patients or the 100 controls tested were found to carry the mitochondrial A3243G mutation in the tRNA$^{Leu(UUR)}$ gene in homoplasmic or heteroplasmic form. After direct sequencing of the entire mitochondrial tRNA$^{Leu(UUR)}$ gene and a part of the mitochondrial NADH dehydrogenase 1, we found no mutations or polymorphisms in the MTTL1 gene in the tested patients and controls and we confirmed the absence of the A3243G mutation in this gene. We also found a T3396C mutations in the mitochondrial ND1 gene in one family with nonsyndromic hearing loss. This substitution was absent in the other patients and in 100 normal individuals. No polymorphisms or other mutations were found in the mitochondrial tRNA$^{Leu(UUR)}$ gene in the tested patients.

P0799. Aberrant splicing is a common mutational mechanism in MKS1, a key player in Meckel Gruber syndrome

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Meckel-Gruber syndrome (MKS) is an autosomal recessive, usually lethal multisystemic disorder characterized by early developmental anomalies of the central nervous system, cystic kidney dysplasia, hepatobiliary ductal plate malformation and postaxial polydactyly. Three MKS loci have been mapped and recently, two genes were identified: MKS1 on 17q22 in Caucasian kindreds and MKS3 on 8q22 in Omani and Pakistani families, putting MKS on the growing list of ciliary disorders ("ciliopathies"). We performed linkage analysis for MKS1-3 in 14 consanguineous and/or multiplex families of different ethnic origins with histologic diagnosis and at least three classic MKS manifestations in each kindred. Unexpectedly, only five families were linked to any of the known MKS loci, clearly indicating further locus heterogeneity. All five families showed homozygosity for MKS1 and, intriguingly, were of non-Caucasian origin. MKS1 sequencing revealed no mutation in two of these pedigrees, whereas different, novel splicing defects were identified in the three other families and an additional sporadic German patient. Given that all of our mutations and two of the in total four known MKS1 changes cause aberrant splicing (while the other two known mutations were frameshift mutations), we hypothesize that splicing defects are a crucial mutational mechanism in MKS1 which apparently is one of the main loci and key players in MKS. Our results indicate that MKS1 mutations are not restricted to the Caucasian gene pool and suggest further genetic heterogeneity for MKS. Overall, our data have immediate implications for genetic counselling and testing approaches in MKS.

P0800. Multiple ligation-dependent probe amplification (MLPA) analysis of the dystrophin gene in Bulgarian patients with Duchenne/Becker muscular dystrophy (DMD/BMD)

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MLPA is a quantitative method for detection of deletions/duplications of one or more exons of a gene.In more than 70% of the DMD/BMD cases the disease causing mutations are deletions or duplications in the dystrophin gene. The MLPA method provides a cheap and powerful tool to screen the whole dystrophin gene in two multiplex reactions. We analyzed 24 unrelated DMD/BMD Bulgarian families without deletions in the dystrophin gene after multiplex PCR. We used SALSA PO34/PO35 kit specific for the dystrophin gene. In 19 families we found deletions or duplications. We found 12 deletions distributed in the main deletion hotspot of the gene. These deletions include exons 42-45, 44 (2 times detected), 45-47, 45-50, 46-50, 46-52, 49-50, 53-54, 55, 58, 79. In addition, 7 different duplications were detected, all but two starting in the 5'-end of the gene. Duplications include exons 2-10, 2-33, 8-11, 8-13, 13-40, 48-50, 51. Carrier status was clarified in all females at risk. MLPA failed to detect deletions or duplications in five families where the disease causing mutation could be a point mutation or the clinical diagnosis might need reevaluation.The present work proved that MLPA is a powerful tool in clarifying the molecular defects along the dystrophin gene. MLPA permits to detect carrier status of female relatives of affected boys. Moreover, this analysis is the only one which allows DNA testing in families where the index patient is no more available. MLPA became a method of choice for genetic analysis of Bulgarian DMD/BMD families.

P0801. Point mutation p.Tyr997X in exon 23 of the dystrophin gene detected by MLPA analysis in Bulgarian DMD family (case report)

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We introduced and optimized for diagnostic purposes the new MLPA method. The SALSA PO34/PO35 kit specific for deletions/duplications detection along the dystrophin gene was applied to screen Bulgarian DMD/BMD patients. The mathematical calculations were performed with Excel program. In 19 Bulgarian families we found 11 different deletions and 7 different duplications in the dystrophin gene.In one of

the tested families MLPA showed no pick for exon 23 on the electrophoregrams, whish supposed deletion of this exon. A single PCR amplification of exon 23 showed the exon presence in the patient. Further sequencing analysis revealed a point mutation generating stop codon in exon 23 - c.2991C>G, p.Tyr997X. The nucleotide change affects the place of hybridization of the exon 23 specific MLPA probe, which is the reason for absence of exon 23 on the electrophoregrams. The patient was severely affected by Duchenne-type muscular dystrophy. Both patient's mother and sister were found to be carriers of the mutation p.Tyr997X. Our experience in MLPA analysis stressed upon the fact that single exon deletions should be interpreted with caution and should be proved by alternative methods. In some cases it is possible to detect point mutations and polymorphisms by MLPA analysis.

P0802. Screening for subtelomeric abnormalities in patients with idiopahtic mental retardation by MLPA

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Recent studies ascertained that 5 to 10% of mental retardations are due to mutations in the subtelomeric regions. Knowing the mutational pattern is of major importance for genetic counseling. We have studied the subtelomeric rearrangements using a MLPA assay in a series of clinically selected patients with mental retardation and negative chromosomal analysis. The study was initiated in the framework of a multicentric national research program. The MLPA probes included in the commercial kits allowed us to scan all the subtelomeric regions in two PCR reactions. The amplicons were analyzed on a 3100 Avant ABI Genetic Analyzer. We investigated 123 DNA samples and assessed the location of deletions and duplications. The MLPA screen revealed mutations in 6% of cases (on the short arms of chromosomes 1, 7 and 18). Except one, all the mutations were deletions and no multiple deletions were detected. One case revealed a double subtelomeric mutational pattern, consisting of a 7q deletion associated with 9q duplication. The MLPA method proved to be easy to handle, accurate and highly reproducible. For the patients carrying subtelomeric rearrangements, further investigations (FISH) are planned in order to confirm the mutation and DNA samples from their relative are collected.

P0803. First case of Gamma-Thalassemia

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Numerous deletional Thalassemia have been reported, involving one or several globin genes in combination. However, deletions specifically targetted on the fetal β-like genes have never been reported so far, likely due to a fetal lethal phenotype induced by homozygous mutations in these genes. By contrast, heterozygosity is likely clinically silent. Here, we report on the observation of a fetal β-like genes deletion that we detected because it was located in Cis of a Sickle Cell β-globin gene.

The proband is a newborn diagnosed at birth as having Sickle Cell Anemia (SCA) in the course of neonatal screening. During the first consultation, at 6 weeks of age, haemoglobin analysis revealed the following Hb rates : HbF 55%, HbS 27% and HbA 15% . Molecular analysis of the β-globin gene showed, as a single defect, heterozygosity for the prevalent sickle cell mutation. During the course of evolution, the level of HbA raised slowly to a normal value and, finally, a typical profile for HbS carrier was observed at 8 month of age.

In order to further explore this peculiar phenotype, the whole β-globin locus was explored by means of MLPA technical procedures and allowed to evidence a heterozygous deletion of the fetal genes, A γ and G γ-globin. We conclude that a deletion of the fetal genes have led to a premature switch on the adult βS-gene and to SCA profile at birth. This is the first case of γ-thalassemia ever reported, representing a pitfall in neonatal screening of SCA.

P0804. A Retrospective Analysis of Patients Previously Tested For Gene Polymorphisms Implicated In Influencing Susceptibility To Thrombosis In A Reference Center in Izmir/ Turkey

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Arterial and venous thromboembolism is one of the most common diseases affecting populations throughout the world. Recently the polymorphisms found in a number of genes which play a role in the thromboembolic processes have been reported to be risk factors for thromboembolism.

For two years our department has made use of a strip test (CVD StripAssay, ViennaLab, Austria) detecting the following gene polymorphisms. These polymorphisms**;** FV R506Q(Leiden), FV H1299R, Prothrombin G20210A, Factor XIII V34L, ß-Fibrinogen -455 G-A, PAI-1 4G/5G, GPIIIa L33P(HPA-1), MTHFR C677T, MTHFR A1298C, ACE I/D, Apo B R3500Q,Apo E2/E3/E4.

In this study we retrospectively investigated the referral indications and the frequencies of the polymorphisms in the children and adult patients tested using this CVD stripassay at the Ege University Medical Genetics Department. The frequencies of the polymorphisms found in patients were compared to the frequencies of the liver transplantation donors who were routinely tested before transplantation.

During the two year period**,** 426 patients (146 children, 280 adults) were tested using the CVD strip test. The majority of patients in the adult group were referred to our department with indications of cardiovascular and/or cerebrovascular diseases from the cardiology, neurology and internal medicine departments. The frequencies of Factor V (H1299R) beta fibrinogen(455G-A) and MTHFR (C677T) were found to be significantly higher in patients having cardiovascular and/or cerebrovascular diseases compared to the frequencies found in control group ($p<0.05$).

As a conclusion Factor V (H1299R) β-fibrinogen (455G-A) and MTHFR (C677T) polymorphisms might play a role in susceptibility to thrombosis in our population.

P0805. Targeted mutation reveals essential functions of the homeodomain transcription factor Shox2 in sinoatrial and pacemaking development.

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Background: The identification of molecular pathways regulating the development of pacemaking and coordinated heartbeat is crucial for a comprehensive mechanistic understanding of arrhythmia related diseases. Elucidation of these pathways has mainly been complicated by an insufficient definition of the developmental structures involved in these processes and the unavailability of animal models specifically targeting the relevant tissues. We here report on a highly restricted expression pattern of the homeodomain transcription factor *Shox2* in the sinus venosus myocardium, including the sinoatrial nodal region and the venous valves. Methods: To investigate its function *in vivo*, we have generated mouse lines carrying a targeted mutation of the *Shox2* gene. While heterozygous animals did not exhibit obvious defects, homozygosity of the targeted allele led to embryonic lethality at 11.5 to 13.5 dpc. Results: *Shox2*$^{-/-}$ embryos exhibited severe hypoplasia of the sinus venosus myocardium in the posterior heart field including the sinoatrial nodal region and venous valves. We furthermore demonstrate

aberrant expression of Connexin40 and Connexin43 and the transcription factor *Nkx2.5 in vivo* specifically within the sinoatrial nodal region, and show that *Shox2* deficiency interferes with pacemaking function in Zebrafish embryos. Conclusion: From these results, we postulate a critical function of *Shox2* in the recruitment of sinus venosus myocardium comprising the sinoatrial nodal region.

P0806. Multiple sulfatase deficiency is due to hypomorphic mutations of the SUMF1 gene

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Sulfatases catalyze the hydrolysis of sulfate esters bonds from a wide variety of substrates. Several human inherited diseases are caused by the deficiency of individual sulfatases. Multiple sulfatase deficiency (MSD) is a rare autosomal recessive disorder characterized by the simultaneous deficiency of all known sulfatases. MSD patients carry mutations of the Sulfatase Modifying Factor 1 (*SUMF1*) gene encoding the α-formylglycin generating enzyme (FGE) which is required for the post-translational modification of sulfatases. Residual sulfatase activities are detectable, at variable levels, in all MSD patients. We have used a recently developed *Sumf1* KO mouse line which completely devoid of all sulfatase activities to investigate on the nature of the residual sulfatase activities detected in MSD patients. Four mutations (i.e. S155P, R224W, R345C, R349W) found in homozygosis in MSD patients were over-expressed, using viral-mediated gene delivery, in *Sumf1-/-* mouse embryonic fibroblasts (MEFs). The results obtained indicate that mutant *SUMF1* cDNAs encode stable SUMF1 proteins which are of the appropriate molecular weight and are properly localized in the endoplasmic reticulum. Expression of these cDNAs in *Sumf1-/-* MEFs results in low, albeit significant, FGE activity, and in partial rescue of sulfatase activities. These data indicate that MSD is due to hypomorphic *SUMF1* mutations and suggest that a complete loss of SUMF1 function is likely to be lethal in humans.

P0807. Frequency of LHON mutations in mitochondrial disease phenotypes from Centre Portugal

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INTRODUCTION: Leber's hereditary optic neuropathy (LHON) is a maternally mitochondrial disorder, characterized by bilateral visual loss, most frequently found in young males. Classical LHON is mainly associated to mitochondrial DNA (mtDNA) mutations G11778A, G3460A and T14484C, localized in the coding regions for ND4, ND1 and ND6 (complex I subunits of mitochondrial respiratory chain), respectively. Other mutations (secondary) have also been described in LHON cases.

METHODS: We have studied a total of 648 subjects, counting with 46 healthy family members. The techniques employed included PCR, RFLP and sequencing analysis. We investigated 7 point mutations, described as primary (G3460A, G11778A, T14484C) or secondary (T4216C, A4917G, G13708A, G15257A), associated previously to LHON.

RESULTS: We have found 40 positive cases (6.2%), corresponding to the analysis of 52 tissues. Considering the total number of cases studied, the frequencies found were: 0.002; 0.012; 0.003; 0.034; 0.026; 0.014 and 0.012, for mutations G3460A, G11778A, T14484C; T4216C, A4917G, G13708A and G15257A, respectively.

DISCUSSION: The most frequent mtDNA alterations found, among primary and secondary mutations studied, were G11778A and T4216C, respectively. The primary mutations were found only in LHON or LHON-plus cases. Secondary mutations were observed in a wide variety of phenotypes, as expected. On the other hand, the primary mutations were found isolated, whereas secondary mutations were frequently encountered in combinations.

P0808. Mutations in mtDNA in Children with manifestations of mitochondrial encephalomyopaties

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Mitochondrial encephalomyopaties are composed disorders with wide range of clinical manifestations. Lack of specific correlation between genotype and phenotype makes the genetic diagnostic even more difficult. Particularly tedious is identification of mutations in mitochondrial DNA. In years 2004 to 2006 in Department of Child Neurology muscle biopsies were performed in a group of sick children with manifestations of mitochondrial encephalomyopaties. In the biopsies the biochemical, histopathological, and ultrastructural analyses have been conducted. The cellular respiratory chain enzymes activities were assayed in muscles and skin fibroblasts. Based on clinical manifestations and results of enzymatic assays a group of 21 sick children was selected for further molecular studies.

Aim of the study: The goal was to identify mutations in mitochondrial DNA isolated from muscle biopsies of affected children.

Material and methods: Molecular analysis performed in two steps for entire mitochondrial DNA. First step was to screen the mitochondrial DNA for any mismatches by heteroduplex analysis using endonuclease SURVEYOR kit (Trangenomic, Inc., Omaha, NE, USA). DNA fragments showing mismatches were subjected to DNA sequencing using ABI PRISM 310 DNA analyzer.

Results: The most common changes found in all analyzed fragments of mitochondrial DNA were polymorphisms. Mutations were detected in few genes e.g.: a) 16S rRNA (in 8 children); b) subunits ND2, ND4L, ND5, and ND6 of complex I (in 6); c) tRNA for leucine (in 1); and d) tRNA for proline (in 1). Further analysis is necessary for comparing genotypes with clinical phenotypes to final confirmation of pathogenic character of detected mutations.

P0809. Mutational analysis of the mitochondrial 12SrRNA and tRNASer(UCN) genes in patients with aminoglycoside-induced and nonsyndromic hearing loss from Volga-Ural region and Siberia (Russia)

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Several mtDNA mutations have been found to be associated with nonsyndromic sensorineural hearing loss (NSHL). The m.1555G>A (12SrRNA) was confirmed as the main cause of aminoglycoside induced deafness in different populations. The m.7445A>G, m.7472_7473insC, m.7510T>C, m.7511T>C (tRNASer(UCN)) were reported to be associated with NSHL. Pathogenicity of some other variations in 12SrRNA and tRNASer(UCN) genes associated with NSHL has not yet been confirmed (http://www.mitomap.org). We report here the results of mutational screening for 12S rRNA and tRNASer(UCN) genes among Cx26- and Cx30-negative deaf individuals of different ethnicity from some regions of Russia. Previously, 301 unrelated patients (70 with aminoglycoside-induced deafness, and 231 with NSHL) from Volga-Ural region, 78 unrelated patients with NSHL from the Republic Sakha (Yakutia, northeastern Siberia), and 119 deaf patients (75 unrelated families) from the Republic Altai (south Siberia) were analyzed for Cx26 and Cx30 mutations. Different variations at the 961 position in 12S rRNA gene have been found among deaf individuals from Volga-Ural region. Four patients of Tatar ethnicity, one with m.961delTinsCn, and three with m.961delTinsC, two Russian patients, one with m.961T>G, and another with m.961T>A, were detected. Moreover, the m.7444G>A (tRNASer(UCN)) was found in one Russian patient with NSHL. The m.1555G>A (12S rRNA) was detected in one Yakut patient (Republic Sakha). Finally, m.7445G>C (tRNASer(UCN)) was found in two sibs of one Kazakh family (Altai region) in whom moderate sen-

sorineural hearing loss was co-existed with goiter. Further studies are needed to confirm pathogenicity of some mtDNA variations associated with deafness in patients from some regions of Russia.

P0810. Common polymorphism of A1298C in MTHFR gene is associated with maternal meiosis I chromosome 21 nondisjunction in Down syndrome

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Parental origin and stage of meiotic error of chromosome 21 nondisjunction has been determined in 226 families with Down syndrome. Their mothers hav e been genotyped for common polymorphisms in the MTHFR (C677T and A1298C) and MTRR (A66G) genes to identify any specific association of these previously described maternal genetic risk factor to the etiology of chromosome 21 nondisjunction. Five STR markers related to chromosome 21 (D21S11, D21S1414, D21S1440, D21S1411, D21S1412) were used to determine the parental origins and stage of meiotic error successfully for 226 cases. In total, 140 cases showed a meiotic error in meiosis stage I, 58 in meiosis maternal meiosis stage II, 20 in paternal meiosis I and 8 in paternal meiosis II. All four groups of mother cases and a normal control group consisted of 176 normal mothers with at least one healthy child were tested for common polymorphisms of C677T, A1298C in the MTHFR and C66G in the MTRR genes. Significant association was detected between maternal meiosis I chromosome 21 nondisjunction and the A1298C polymorphism of the MTHFR gene ($P<0.0014$, $\chi^2>11.13$). This study has showed for the first time the importance of stage of meiotic nondisjunction in the study of maternal genetic risk factor of Down syndrome. The significant association of stage I meiotic nondisjunction and A1298C polymorphism of MTHFR gene in mother of Down syndrome presents the relationship of acid folic metabolism in the etiology of Down syndrome in Iranian cases.

P0811. Is the MTHFR C677T gene polymorphism a modifier factor in SMA?

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The methylation process is essential for the regulation of genes expression controlling the development and function of the neurons. The formation of SMN complex, responsible for motoneurons survive, is dependent by the symmetric dimethylation of some component proteins. We speculated that this interaction could be affected by the MTHFR gene although any data regarding this association are not available. The MTHFR C677T polymorphism is strongly associated with the reduction of MTHFR activity. Based on this observation, we analyze a possible association of this polymorphism with the SMA severity in the Romanian patients.

Our study was carried out on 63 SMA clinically diagnosed patients, classified by *ISMAC* criteria, 60 parents and 100 controls. The blood samples were obtained from Bucharest pediatric hospital. Informed oral consent was obtained from all subjects. The MTHFR C677T gene polymorphism was analyzed by PCR-RFLP method.

The highest frequency of TT genotype was observed in the SMA III group (21,05%) compared with SMA II (17,6%) and I (14,8%), and with parents (13,3%) and control (7%) groups. This genotype may increases the risk for disease in SMA type I (OR = 2,31) and II (OR =2,84), while in SMA type III, only the presence of one T allele was associated with the disease.

We can not exclude the contribution of mutated allele to the SMA phenotypes because our lots are not very large.

	MTHFR677CC	MTHFR677CT	MTHFR677TT
SMA	19	33	11
Parents	22	30	8
Control	48	45	7

P0812. A66G polymorphism of methionine synthase reductase (MTRR) gene is associated with spontaneous abortions

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Spontaneous abortion (SA) is a frequent health problem of women of the reproductive age. A number of causes including uterine anomalies, maternal\paternal balanced translocations, luteal phase defect, hyperhomocysteinaemia have been attributed to the origin of SA. MTRR is an enzyme essential for normal folate metabolism. A common polymorphism in this gene was recently found to be associated with increased risk of neural tube defects. It also might contribute to increased risk for SA.

The 66A\G polymorphism of the MTRR gene was studied by PCR-RFLP analysis in 108 women with one and more normal pregnancies and no SA in anamnesis (control group I) , in 78 nonpregnant women with a history of at least one SA (II group), in 75 patients with two recurrent SA (III group) and in 67 patients with numerous (>- 3) recurrent SA(IV group).

The distribution of genotype frequencies for MTRR gene was significantly different ($p<0,01$: df2) between 3 groups with SA and the control group (Table.I).

Table I. The frequencies of genotypes MTRR gene in 4 studied groups

genotype	Igroup(n=108)	IIgroup(n=78)	IIIgroup(n=75)	IVgroup(n=67)
A\A	(23)21,3%	(10)12,8%	(10)13,3%	(7)10,5%
A\G	(61)56,5%	(39)50%	(38)50,7%	(35)52,2%
G\G	(24)22,2%	**(29)37,2%**	**(27)36%**	**(25)37,3%**

Conclusions: Thus, the polymorphism A66G of MTRR gene might be considered as a risk factor for spontaneous abortions.

P0813. A report of three cases affected with Mucolipidosis type II

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Mucolipidosis type II is a Hurler-like condition with severe clinical and radiologic features, peculiar fibroblast inclusions, and no excessive mucopolysaccharidurie. It first was described in 1967 by Leroy and DeMars and named it I-cell disease. This disease is inherited as an autosomal recessive trait and both sexes are affected equally.

Here we report three cases affected with Mucolipidosis type II, who had short stature and microcephaly at birth. Growth and developmental retardation and hypotonia developed in the first year of life. They suffered from coarse facial features, prominent and thick upper lip, depressed nasal bridge, thick eyebrows, bulbous nose, sparse hair and died in the first year of life. CT scan revealed brain atrophy. Enzyme analysis showed decreasing in fibroblast cultures and in contrast increases in plasma in all cases and confirmed Mucolipidosis type II.

P0814. Seventeen years experience of prenatal diagnosis of Mucopolysaccharidoses (MPS) in Iran

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Most of the metabolic disorders due to lysosomal storage disease including MPS are fairly frequent in Iran, because of high frequency of intermarriage. Due to the lack of curable or beneficial symptomatic treatment, upon a scientific collaboration, families having affected offspring suspected for MPS disease have been enzymatically analyzed. In 82 families the deficit enzymes were detected. Seventy prenatal diagnoses for pareous at risk were performed, revealing 53 unaffected and 17 affected fetuses. All families with affected fetuses opted for pregnancy termination. The postnatal results of unaffected newborns confirmed the prenatal diagnosis findings. The summary of clinical findings and epidemiological distribution of MPS disorders and PND results are shown in the table.

P0815. Multiple Sclerosis: Defect on GD3 synthase as a factor determining B-cell response

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Cell surface gangliosides represent the major class of glycoconjugates on neurons and bear the majority of sialic acid within the central nervous system (CNS). In a previous study we found that a variant in GD3 synthase (GD3S) was associated with a disruption of the ganglioside pathway in a unique pedigree segregating Multiple Sclerosis. The patients show a reduced expression of GD3 synthase enzyme which leads to a different co-localization of GD3 ganglioside (GD3G) on peripheral blood mononuclear cells (PBMC) and these could represent a foundation of the disease in this family. Here we present evidence that GD3S is mainly expressed on the B cell surface thus providing evidence of a potential role of B cells in this autoimmune disease

The ability to recognize self from non self components it is crucial for the immune system as this can lead to the disruption of the body's own tissue. MS is the result of a complex interaction of genes and environment and the need to understand this complex mechanism is essential to establish the correct therapies. Although MS is considered to be mediated by T-helper 1 (Th1) cells, emerging evidence implicates B lymphocytes and their products in the pathogenesis of this disorder. Evidence from recent pathology studies has led to a renewed interest in the role that chronically activated B cells may play in the pathogenesis of MS, independent of antibody production.

P0816. Microsatellite Expansion in Myotonic Dystrophy Patients: The Search for Underlying Pattern

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Microsatellite expansion is the cause of a number of severe diseases including fragile X, Huntington disease and myotonic dystrophy. An interesting common feature of these disorders is the instability of the mutation: once expanded, the number of repeat units continues to change in patient's cells. An understanding of the mechanism and dynamics of microsatellite expansion will provide more informative genotype to phenotype relationships, which will in turn be of considerable utility in allowing patients and families to make more informed life and reproductive choices, and facilitate the clinical management of disease. Recently (J. Theor. Biol., 242, 401-408 (2006)), a mathematical model was proposed to describe the dynamics of microsatellite expansion and the resulting distribution of repeat lengths in patient's DNA. In this work, we compare the theoretical predictions with observed repeat length distributions in a large cohort of myotonic dystrophy type 1 patients. We find that the theoretical predictions agree fairly well with the clinical data with the observed distributions close to those predicted by the mathematical model. We also used the clinical data to estimate the theoretical parameters underlying the model: the rate of increase of the number of repeats and the rate of widening the distribution. We find that while these parameters have large individual variations, the average values give reasonable predictions for the development of mutations. These values can be used to estimate the initial mutation (the number of repeats in the progenitor allele) and to predict the development of the disease.

P0817. Molecular-genetic diagnostic of Juvenile nephronophthisis.

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Juvenile nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease, which leads to end-stage renal failure at an average age of 13 years following the initial symptoms of polyuria, polydipsia, renal salt loss, and secondary enuresis.

Juvenile nephronophtisis is most often caused by mutations in the NPHP1 gene, encoding nephrocystin. About 70% of cases of Juvenile nephronophtisis are caused by large deletions in the 2q13 region.

Two previously described markers (NPHP804/6 and NPHPC11) localised within the common NPHP1 deletion interval were amplified in multiplex PCR with control markers - exon 13 of the PAH gene, mapping outside the deleted region.

We have studied 6 unrelated patients. A homozygous deletion of the NPHP1 gene was found in 3 of 6 probands.

All patients with deletion had polyuria, polydipsia, isosthenuria and diffusive ostepenia.

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P0818. Missense alterations in the *NF1* gene: how to make sense of them?

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Neurofibromatosis type 1 (NF1), notorious for its phenotypic variability, is characterized by neurofibromas, skinfold freckling and café-au-lait spots. Recently, a relationship between a 3-bp inframe deletion in the *NF1* gene and a specific phenotype, i.e. absence of cutaneous neurofibromas, was found. This finding holds promise that other missense or 1-2 AA-deletions may exist that correlate a specific missense (or 1-2 AA-deletion) to the expression of a specific clinical phenotype. As a first step, all *putative* missense alterations need to be classified correctly.

We developed a comprehensive *NF1* assay using RNA- and DNA-based techniques including long-range RT-PCR, cDNA sequencing, FISH and MLPA and use this approach in a clinical setting. For all patients, the phenotype is recorded using a standardized checklist.

So far, we identified a pathogenic mutation in 1400 unrelated patients. All 172 different missense alterations, found in 299 patients, were further analyzed for effect on splicing; presence/absence of another possible deleterious *NF1* mutation; presence/absence in >1000 control alleles; evolutionary conservation; *in silico* predicted effect by PolyPhen, SIFT and AGVGD; familial clinical and genetic assessment. Using this approach, we classified 17 different "missense" alterations, present in 46 patients, as splicing mutations, 19 novel alterations, residing in cis or trans of a clearly deleterious mutation, as rare benign variants and 2 as variants of still unknown significance. The remaining 136 different missense alterations were classified as pathogenic mutations. As 30 of these mutations are recurrent in unrelated families, collection of phenotypic data of affected family members may provide additional genotype-phenotype correlations.

P0819. Functional analysis of splicing mutations in exon 7 of *NF1* gene

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Background. Neurofibromatosis type 1 is one of the most common autosomal dominant disorders, affecting about 1:3,500 individuals. NF1 exon 7 displays weakly defined exon-intron boundaries, and is particularly prone to missplicing.

Methods. In this study we investigated the expression of exon 7 transcripts using bioinformatic identification of splicing regulatory sequences, and functional minigene analysis of four sequence changes [c.910C>T (R304X), c.945G>A/c.946C>A (Q315Q/L316M), c.1005T>C (N335N)] identified in exon 7 of three different NF1 patients.

Results. We detected three exonic splicing enhancers (ESEs) and one putative exonic splicing silencer (ESS) element. The wild type minigene assay resulted in three alternative isoforms, including a transcript lacking NF1 exon 7 (NF1ΔE7). Both the wild type and the mutated constructs shared NF1ΔE7 in addition to the complete messenger, but displayed a different ratio between the two transcripts. In the presence of R304X and Q315Q/L316M mutations, the relative proportion between the different isoforms is shifted toward the expression of NF1ΔE7, while in the presence of N335N variant, the NF1ΔE7 expression is abolished.Conclusions. It appears mandatory to investigate the

role of each nucleotide change within the NF1 coding sequence, since a significant proportion of NF1 exon 7 mutations affects pre-mRNA splicing, by disrupting exonic splicing motifs and modifying the delicate balance between aberrantly and correctly spliced transcripts.

P0820. The role of Nonsence-mediated RNA decay (NMD) mechanism in modifying clinical course and mode of inheritance of cardiac arrhythmias

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Background: Nonsense-mediated mRNA decay (NMD) is an mRNA quality-control mechanism that degrades aberrant mRNAs containing premature translation termination codons (PTCs). The PTC mutations in cardiac ion channels genes can realize through haploinsufficiency due to elimination of the mutant mRNA. Thus, these mutations lead to the presence only normal ion channel proteins on surface of the cardiomyocyte but in depressed amount.
Methods: Blood samples were collected from 85 unrelated Russian families with Long QT syndrome (LQTS) and from 14 Brugada syndrome (BrS) patients. Genomic DNA and total RNA were extracted from blood using standard methods. Mutation screening was performed using PCR-SSCP method and direct sequencing. Results: We identified 4 PTC mutations in 18 LQT1 patients (fifteen heterozygous and 3 homozygous mutations), 1 PTC mutation in 5 related LQT2 patients and 3 PTC mutations in 3 BrS patients. All heterozygous carriers of PTC mutations in *KCNQ1* had favorable clinical course of disease. Homozygous carriers of PTC mutations in *KCNQ1* had extremely malignant forms. Heterozygous patients with PTC mutations in KCNH2 and SCN5A had characterized by moderate clinical sings but SCD were recorded in these families. Conclusions: All heterozygous carriers of PTC mutations in *KCNQ1* had mild clinical course of disease. Such mutations in KCNH2 and SCN5A were not so propitious. Homozygous LQT1 probands with two “favorable” mutations had unexpectedly fatal manifestations. We speculate that haploinsufficiency of IKr- and INa-channel subunits have more serious consequences for repolarization than the same for IKs.
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P0821. Biochemical and structural characterization of Noonan syndrome-causing mutations affecting SHP-2’s phosphotyrosyl-binding pockets

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Missense mutations in PTPN11 cause Noonan syndrome (NS), a genetically heterogeneous developmental disorder with a pleiomorphic phenotype. PTPN11 encodes SHP-2, an SH2 domain-containing protein tyrosine phosphatase that relays signals from activated cell-surface receptors to RAS. NS-causing mutations promote SHP-2’s gain of function by either destabilizing its catalytically inactive conformation or increasing the affinity and/or specificity of the SH2 domains for phosphotyrosyl ligands. While the identity of substitution does not seem to be critical in some cases, suggesting a crucial role in SHP-2’s function for the residue being replaced, an invariant amino acid change is frequently observed, indicating a specific role for the introduced residue. Here, we characterized functionally and structurally two invariant NS-causing mutations, T42A and E139D, affecting residues placed in the N- and C-SH2 pockets which mediate SHP-2 binding to phosphotyrosyl-containing signaling partners. By analyzing in vitro biochemical behavior (basal and ligand-stimulated phosphatase activity, and ligand-binding properties assayed by surface plasmon resonance) of all possible substitutions arising from a single base change affecting codons 42 and 139, we show that T42A and E139D SHP-2 proteins are the only mutants exhibiting a significant increase in ligand-induced phosphatase activity and enhanced phophopeptide binding affinity. Molecular dynamics simulations performed on selected mutants provide structural insights of the effects generated by individual mutations on protein function. In conclusion, this study provides functional explanation for the invariant occurrence of the T42A and E139D mutaions in NS as well as the molecular mechanism of their pathogenicity.

P0822. Cancer or not cancer : differential impact of mutations in the XPD helicase in xeroderma pigmentosum and trichothiosdystrophy ?

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Genetic alteration of the repair of UV-induced DNA lesions (cyclobutane pyrimidine dimers, CPD, and 6-4 photoproducts, 6-4 PP) by nucleotide excision (NER) may result in photo genodermatoses, such as the cancer prone xeroderma pigmentosum (XP) syndrome or, the cancer free trichothiodystrophie (TTD) syndrome. We aimed at comparing the impact of mutations in the XPD gene resulting in either the XP-D (R683W) or the TTD/XP-D (R112H) syndrome. We cultured primary fibroblasts and keratinocytes from small skin biopsies of XP-D and of TTD/XP-D patients. Comparative study of DNA repair kinetics demonstrated faster and better DNA repair capacity of TTD/XP-D cells compared to XP-D cells and hence, a shorter P53 stabilisation in the former. In order to reproduce the 3D cutaneous architecture, we elaborated organotypic TTD/XP-D and XP-D skin cultures. Response to UVB (290_320 nm) irradiation in term of DNA repair and apoptosis, was also better in TTD/XP-D XP-D than in XP-D organotypic skins. We also assessed expression of markers of epidermal homeostatis and revealled alteration or extinction of some epidermal differentiation markers such as filaggrin in XP-D, but not in TTD/XP_D organotypic skin cultures.
Altogether these results indicated that the R112H XPD mutation resulting in the cancer-free TTD/XP-D syndrome are associated to a better vital prognostic of cutaneous cells compared to the XPD R683W mutation characteristic of XP-D cancer-prone syndrome.

P0823. Investigation of plasma carnitine ester profiles in a family with homozygous and heterozygous OCTN2 deficiency

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OCTN2 is the high affinity transporter protein for carnitine uptake into the cells. In the gene coding for this protein, *slc22a5*, a homozygous 844C deletion causing a V295X nonsense mutation was found in a three-year old male Roma patient with hepatopathy and cardiomyopathy. This kind of mutation has already been described in another Hungarian Roma subject with primary systemic carnitine deficiency. We measured 20 short-, medium- and long-chain carnitine esters by ESI tandem mass spectrometry from plasma samples of the patient, his consanguineous parents (first cousins) and siblings. The free carnitine and all circulating carnitine esters were severely decreased in the proband. The three heterozygous pediatric siblings in the family (2 males and 1 female) showed also reduced carnitines levels between the normal controls and the proband. The parents, who were also heterozygotes, exhibited a highly similar pattern. Oral supplementation with 50 mg/kg/day dose of L-carnitine normalized the hepatomegaly, elevated transaminases and the previous pathologic cardiac ultrasound parameters of the proband. In the plasma samples 2 and 13 months after the onset of carnitine treatment the free carnitine and many of the carnitine esters increased to about half-normal level in response to the therapy; however, some individual esters remained still much below the controls. The data presented here show severely affected carnitine ester metabolism besides the dramatic decrease of the free carnitine in primary systemic carnitine deficiency caused by deleterious *slc22a5* mutations. The possible functional consequences of the reduced carnitine esters associated with the heterozygous genotype require further investigations.

P0824. Mutational analysis of OCA patients in Denmark

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Oculocutaneous albinism (OCA) is a genetic heterogeneous disorder caused by hypopigmentation of the eyes, hair and skin. The hypopigmentation results from defects in melanin production. Lack of melanin in the eyes causes misrouting of the optic nerve fibers, resulting in nystagmus, foveal hypoplasia, strabismus, photophobia and greatly decreased visual acuity. Hypopigmentation of the skin results in enhanced sensitivity to light and increased risk of skin cancers.

Clinical diagnosis of subtypes of OCA is difficult due to phenotypic variation and overlap between the different types of OCA, and genetic analysis is often necessary to establish the diagnosis. We investigated 58 patients with OCA for mutations in four genes known to cause OCA, namely TYR causing OCA1 (OCA1A and OCA1B), OCA2 causing OCA2, TYRP1 causing OCA3 and MATP causing OCA4. Overall, we found two mutations (homozygous or compound heterozygous) explaining the OCA in 44 % of the patients (26 patients of 58), and one mutation in 29 % (17 of 58). In the remaining 26 % (15 of 58) we found no mutations in any of the four genes. Of the 26 patients with two mutations, 62 % (16 of 26) had two mutations in TYR, 31 % (8 of 26) had two mutations in OCA2 and 7 % (2 of 26) had two mutations in MATP. No mutations were found in TYRP1.

P0825. Skin changes in oculo-dento-digital dysplasia are correlated with truncating mutations in GJA1

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Oculo-dento-digital dysplasia (ODDD, OMIM no.164210) is a pleiotropic disorder caused by mutations in the GJA1 gene that codes for the gap junction protein connexin43. While the gene is highly expressed in skin, ODDD is usually not associated with skin symptoms. We recently described a family with ODDD and palmoplantar keratoderma. Interestingly, mutation carriers had a novel dinucleotide deletion in the GJA1 gene that resulted in truncation of part of the C-terminus. We speculated, that truncation of the C-terminus may be uniquely associated with skin disease in ODDD. Here, we describe a patient with ODDD and palmar hyperkeratosis caused by a novel dinucleotide deletion that truncates most of the connexin43 C-terminus. Thus, our findings support the notion that such mutations are associated with the occurrence of skin symptoms in ODDD and provide the first evidence for the existence of a genotype-phenotype correlation.

P0826. Genotype-phenotype correlations in mitochondrial optic neuropathies

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Mitochondrial optic neuropathies include Autosomal Dominant Optic Atrophy (ADOA), Leber's Hereditary Optic Neuropathy (LHON) and Autosomal Dominant Optic Atrophy and Cataract (ADOAC). ADOA generally starts in childhood, is characterized by progressive decrease in visual acuity and optic atrophy. Mutations in the optic atrophy 1 (OPA1) gene are implicated in about 40% of the cases of ADOA. LHON, caused by mutations in mitochondrial DNA, is characterized by severe bilateral optic atrophy usually starting between the ages of 18 and 35. ADOAC, a rare phenotype of optic atrophy and cataract, is due to mutations in a mitochondrial protein nuclear encoded (OPA3).

We report here a molecular study of 600 patients with optic atrophy referred to our center during the last three years. Of these, 60% were familial cases and 40% sporadic. The mutation responsible for the disease was identified in 290 patients (49%). Mitochondrial DNA mutations were found in 90 patients (15%), OPA1 mutations in 186 patients (31%) and OPA3 mutations in 14 patients (2%). Several ADOA patients were affected by neurosensorial deafness or peripheral axonal neuropathy. A specific genotype-phenotype correlation was observed in patients harboring the R445H OPA1 mutation and optic atrophy associated to deafness. 10% of sporadic patients carried pathogenic mutation in the OPA1 gene.Mitochondrial DNA and OPA1/3 gene analyses allow diagnosing about 50% of the patients. Our results suggest that patients with hereditary optic neuropathies could greatly benefit from neurological investigations and that sporadic cases of optic atrophy need to be explored at the molecular level.

P0827. Morphological abnormalities of the mitochondrial network in fibroblasts of patients affected with autosomal dominant and recessive optic neuropathies

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Inherited optic atrophies (OPA) are frequently inherited as an autosomal dominant trait. Most of them are related to mutations in the OPA1 gene. An other uncommon loci have been reported on 22q12.3 (OPA5). Besides, autosomal recessive non syndromic OPA are rare. Only one locus has been reported on 8q23 (ROA1).

OPA1 and genes involved in syndromic forms of OA were shown to play a crucial role in the mitochondrial function. The purpose of this study was to investigate a possible involvement of mitochondria in autosomal dominant and recessive isolated OAs of unknown genetic origin.

Fibroblasts of 1 OPA1, 2 OPA5 and 2 ROA1 patients were labelled using MitoTracker RedCMXRos dye and compared to control fibroblasts.

Morphological abnormalities of the mitochondrial network were observed in the five patients compared to the respective controls. The cellular distribution of mitochondria in fibroblasts of patients related to OPA5 and OPA1 was superimposable: the labelling of the MitoTracker appeared fragmented and highly concentrated around the nucleus of cells (ring-like aspect).

Interestingly, the distribution of mitochondria in the fibroblasts of the two ROA1 patients was also superimposable but different from that of other patients. The labelling of the MitoTracker was highly heterogeneous and testified a fragmentation of the mitochondrial network but no nuclear ring-like aspect was noted.

This study suggests that a selective vulnerability of the optic nerve to perturbations in mitochondrial function may help selecting candidate genes as genes encoding proteins with high mitochondrial targeting probability for both the OPA5 and ROA1 loci.

P0828. Molecular Analyses of OPN Gene in Urolithiasis Patients

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Urolithiasis, which is largely composed of calcium oxalate, is a common disease and urinary tract stone development has not been elucidated at the molecular level yet. Accumulating evidences suggest that urolithiasisis a complex process including crystal nucleation, growth, aggregation, and crystal retention within the renal tubules. Several model studies suggest that the protein constituents of stone matrix are the stone formation modulators. One of these proteins is osteopontin, an aspartic acid rich urinary protein and a potent inhibitor of CaOx stone formation. OPN gene is located on chromosome 4q21-25 and consists of 7 exons. OPN gene has a large promoter region site. We think that OPN gene promoter region may play an important role in the CaOx stone formation process. The upstream nucleotide sequence of OPN promoter region is between 111-2268 bp (D14813). In this study, three families having familial transmission (n=21), and sporadic urolithiasis patients (n=20), and control cases (n=20) were analyzed for OPN gene. OPN gene was analyzed by PCR-SSCP and DNA sequencing, respectively. We found two SNP's in exon 7 (C6983A, T8274C) and three SNP's in promoter (G-1748A, A-592T, G-155T). In this study, we demonstrated that new five SNP's in OPN gene. Our preliminary work is the first study for investigating the role of OPN gene promoter region in urolithiasis. The results showed that SNP's in promoter region of the OPN gene may be related to familial urolithiasis. We concluded that these new SNP's need to be analyzed in large patients groups with urolithiasis in different populations.

P0829. Estrogen-related receptor gamma genotype influences bone mass in a healthy population of French-Canadian women.

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Background: The relationship between osteoporosis and decreasing estrogen levels at menopause has been known for 60 years. Some genes involved in the estrogen pathway, mainly estrogen receptor 1 (ESR1), have been widely studied in association with bone density. Estrogen-related receptors (ESRRs) are orphan receptors identified on the basis of their sequence similarity to ESR1 and for which no natural ligand is identified to date. All three (ESRRalpha, ESRRbeta and ESRRgamma) were shown to interfere with estrogen signaling.
Methods: Given the cross-talk between ERs and ESRRs, we studied the association between their sequence variants and bone measurements (heel QUS and DXA at femoral neck(FN) and lumbar spine(LS)) in 2481 French-Canadian women. Sixteen women were sequenced to identify polymorphisms that were then genotyped by high-throughput ASO-PCR.
Results: We report statistically-significant associations between a silent coding variant of ESRRgamma and LS, SOS, BUA and SI in women (p-values from 0.006-0.04). The association with FN was not significant (p=0.05). Given that this variant might be in linkage disequilibrium with another unidentified polymorphism, we analyzed the linkage block containing this variant using TagSNPs selected from HapMap data. Eight SNPs were selected in a block of 20kb and were genotyped in a sample of 1340 premenopausal women with the Sequenom platform. Among those, two SNPs were more strongly associated than Ser319Ser with bone phenotypes in a large group of 5021 women aged from 25 to 91 years, pre and postmenopausal. Conclusion: ESRRgamma appears to be associated with bone density in women.

P0830. Further delineation of OTX2 as a causative gene for microphthalmia: use of LightScanner and MLPA for rapid mutation detection.

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Developmental eye anomalies, including anophthalmia and microphthalmia (AM), are a major cause of childhood visual impairment and affect 1/3-4,000. We previously identified heterozygous mutations in the bicoid-type homeodomain gene, OTX2 (MIM 600037) in eight families with developmental eye anomalies, including twins. The principal features were AM and retinal dystrophy in association with developmental delay, seizures and brain malformation. There was a high incidence of gonosomal mosaicism and complex inheritance patterns. We screened a further cohort of 60 individuals with developmental eye anomalies and identified six patients with heterozygous mutations or deletions of OTX2. One patient had a visible cytogenetic deletion of 14q22-q23 that included the OTX2 gene, four had novel mutations detected by High-Res Melting™ in a Lightscanner™ machine, confirmed on sequencing, and one had a small deletion of the OTX2 gene detected by MLPA. The first mutation was c.85G>A (p.V29M) (unlikely to be causative since patient has distinctive Donnai-Barrow syndrome, and patient's unaffected father also has mutation). The other mutations were: (1) c.93C>G (p.Y31X); (2) c.313C>T (p.Q105X) (patient also has a twin with a less severe phenotype); (3) c.395delAG (p.E132fs143X); and all cause premature truncation of the protein. The findings support a model of loss-of-function mutations in OTX2 causing microphthalmia. Furthermore, the presence of the mutations in some unaffected family members supports our previous finding of incomplete penetrance of OTX2 mutations. The use of LightScanner™, MLPA and direct sequencing in tandem allowed rapid identification of these novel mutations and their inheritance pattern.

P0831. The association of mutation in FSHR, INHα and FMR1 genes with ovarian dysfunction.

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Premature ovarian failure (POF) is a secondary gonadotrophic amenorrhoea affecting 1-3% of females. FSH (follicle stimulation hormone) and its receptor (FSHR) play a major role in the development of follicles and regulation of steriogenesis in ovary. Mutations in FSHR might theoretically lead to an impaired signal transduction and diminished ovarian reserve. Genes encoding three inhibin subunits can be proposed as candidates for POF due to its role in negative feedback control of FSH. POF was first noted as an unexpected phenotype among FMR1 gene premutation carriers. The association of Asn680Ser transition in FSHR, Ala257Thr transition in INHα1 and CGG-repeats in 5'UTR region of FMR1 genes with POF and individual susceptibility to exogenocal GT was studied. The frequencies of INHa1 Ala257Thr transition (7,8%) and mutant FSHR genotype Ser680Ser (23%) in women with clinical POF diagnosis (n=77) were higher (4,4% and 16%) than in control (n=183). The high risk alleles of FMR1 gene (> 40 CGG-repeats) were revealed in 3,9% of POF patients. 2,6% of patients, but nobody in control group, had Ala257Thr transition and high risk alleles of FMR1 gene. The quantity of oocytes after stimulation were significantly lower in Ala257Thr transition carriers versus women without Ala257Thr transition. The frequency of Ser680Ser FSHR (32%) mutant genotype was statistically higher (16%) in "poor responders" group (n=28) than do normal-ovulatory controls (n=122). Our data demonstrated that investigated mutation in genes encoding FSHR, INHa1 and FMR1 proteins involved in POF-pathogenesis and altered ovarian sensitivity to exogenous FSH.

P0832. Mutational analysis of the p63 gene in EEC syndrome and related disorders

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The p63 (TP73L) gene codes for a protein belonging to the p53 family. Coding sequence consists of 16 exons generating several different transcripts. The protein contains two transactivation domains, a central DNA binding domain (DBD), an isomerization domain, a protein-protein interaction and an inhibitory domain. p63 plays a crucial role in the formation and maintenance of stratified epithelia, skin and its appendages, and limb development. Heterozygous mutations have been associated with syndromes affecting skin, ectodermal derivatives, limbs and cranio-facial district. Prototype of this family of syndromes is EEC, characterized by Ectrodactyly, Ectodermal displasia, Cleft lip-palate. p63 is mutated in the 98% of cases of EEC syndrome type 3, in other related disorders (AEC, LMS, ADULT, Rapp-Hodgkin) and in isolated ectrodactyly.
We describe 11 patients, 9 sporadic cases and a family with two affected members. 4 individuals affected by EEC syndrome, 6 individuals with isolated ectrodactyly, and a single case with ectrodactyly/cleft lip-palate associated with complex phenotype. Mutational analysis of p63 coding exons allowed identification of 5 heterozygous variants: 4 different mutations in the EEC patients and a polymorphic variant in a case with isolated ectrodactyly. In the EEC patients, we have found three different missense mutation leading to residue substitution in the DBD of the protein: R204W, S272T, R279C for which we propose functional speculations. Moreover, we have found an intronic substitution resulting in aberrant splicing, as assessed on patient's cDNA. Experiments aimed to clarify the impact of this mutation on protein function will be described.

P0833. Paraoxonase *PON1* gene polymorphisms, enzyme activity and lipid profile relationship in healthy volunteers

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Human serum paraoxonase 1 (PON1), a high-density lipoprotein (HDL)-associated enzyme, has been shown to reduce the oxidation of low-density lipoprotein (LDL) and HDL by degrading lipid peroxides. This property of PON1 accounts for its ability to protect against atherosclerosis. The gene encoding this enzyme PON1 shows two polymorphisms Q192R and L55M. Q/R alleles show high and low lipid peroxides hydrolysis ability, respectively. The aim of the present study

was to investigate in healthy male volunteers (N= 89, 18-55 years) from Zaragoza (north centre of Spain) the relationship of the PON1 Q192R and L55M polymorphisms with lipid profile parameters. *PON1* gene polymorphisms were checked by PCR/RFLPs, and serum paraoxonase/arylesterse activities were measured spectrophotometrically by hydrolysis of paraoxon and phenylacetate respectively. Plasma lipids: Triglycerides (TG), Cholesterol (Chol), HDLc, LDL were measured by routine enzymatic methods, and atherogenic indice (AI) was determined: Chol/HDLc.
The allelic frequencies of PON1 192/55 were: p(Q)=0.63, p(R)=0.37, p(L)=0.66, P(M)=0.34 and were in Hardy-Weinberg equilibrium. Results of activities paraoxonase and arylesterase were respectively 118.9 ± 72.5 U/l and 95.4 ± 20.5 U/l; and ratio paraoxonase/arylesterase showed a good correlation with genotype as described in Caucasian population (p<0.0001). Also, this ratio was corrolationated significatively with the AI which was higher in individual aged more than 40 years and specially who's carrying R allele (ANOVA P<0.0001). These results allow us to conclude that R allele from 192 PON1 polymorphism could represent an atherogenic risk factor associate to the age and lipid profile.

P0834. LRRK2 mutations and alpha-synuclein level in patients with familial Parkinson's disease.

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Parkinson disease (PD) is the second most common neurodegenerative disease in humans; its etiology is largely unknown. The increased level of alpha-synuclein (SNCA) is implicated in PD pathogenesis. The identification of causative mutations in several genes demonstrates the existence of monogenic forms of PD. PD caused by mutations in the leucine-rich repeat kinase 2 (LRRK2) gene is the most frequent. We screened common mutations in the LRRK2 gene (G2019S, I2012T, I2020T, R1441C/G/H) in 222 PD patients (mean age 62,7±9,8 years) from the North-Western region of Russia. Three families bearing the G2019S mutation (3/63; 4.8%) were found. In addition, we found one patient with the G2019S mutation and one patient with the R1441C mutation among 159 patients with sporadic PD. The levels of SNCA mRNA in lymphocytes were estimated in nine familial PD patients (mean age 62.7±11.4 years) with unknown etiology of the disease and eighteen controls (mean age 62.6±16.3 years) using quantitative real-time PCR with TagMan probes. The level of G protein (GNB2L1) mRNA was used as internal control. The level of SNCA mRNA was higher in PD patients (2.1±0.67) than in controls (1.0±0.53) (p<0.0001). Our study showed the high frequency of the LRRK2 mutations among PD patients in Russia and demonstrated further support that elevated level of SNCA expression might be implicated in PD pathogenesis.

P0835. Analysis of the parkin gene (PARK2) exon 1-12 dosage in patients with sporadic Parkinson's disease from Russia

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Most patients with Parkinson's disease (PD) have sporadic form of the disease with a multifactorial etiology due to interactions between environmental conditions and the genetic constitution of the individuals. One of the causes of PD is homozygous or compound heterozygous mutations in the PARK2 gene. Deletions and duplications of single exons or exon groups account for a large proportion of the gene mutations, therefore it becomes necessary to assess the frequency of these mutations in patients with sporadic PD. To detect these mutations, we developed and applied an effective technique based on the real-time TaqMan PCR system. We analyzed rearrangements in exons 1-12 of the PARK2 gene in 140 patients with early-onset Parkinson's disease (EOPD, age of onset less than 50 years) and in 100 patients with classical sporadic Parkinson's disease (age of onset more than 50 years). All the patients were from Russia. The frequency of these mutations in EOPD patients was 12%, in classical sporadic PD patients - 3%. Most frequent rearrangements were detected in exons 3 and 4. The results of our work let us conclude that exon rearrangements in the PARK2 gene have a significant role in the pathogenesis of sporadic PD in patients from Russia and it is rational to start detection of these mutations with the analysis of exons 3 and 4. Detection of exon rearrangements in the PARK2 gene can be used for the diagnosis of Parkinson's disease, symptomless stage included, which is very important for the effective treatment.

P0836. Analysis of the human periostin promoter and its polymorphic variants

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Periostin is a secreted protein that is highly expressed in early osteoblastic cells in vitro and periosteum, periodontal ligament tissues in vivo, as well as in the endocardial cushions and valves of the developing heart. It is known to support cellular adhesion and spreading in vitro of chondrocytes, fibroblasts, and a number of cancer cell lines. The mechanisms of transcriptional regulation of human periostin are poorly understood. With the objective to clarify the molecular basis of gene regulation, we have undertaken a characterization of the human Periostin regulatory region by transfection of promoter fragments, from -1790, -1000 and -500 to +1, fused to the luciferase reporter gene into the U20S osteosarcoma cell line. Transcriptional activity of all promoter constructs was well appreciable: the promoter construct containing region -1000/+1 showed the highest luciferase activity compared to the other constructs. DNA elements with negative effect on transcriptional activity were detectable between -1000 and -1790 and positive effect between -1000 and -500. Analysis of the periostin 5' UTR revealed the presence of variants inside the promoter region. Genotyping of 100 caucasian blood donors showed that there are three polymorphic sites in the first 2000 bp upstream of the ATG start codon: -1945 (MAF 0.22), -1658 (MAF 0.16), -941 (MAF 0.22). The effect and characterization of different combinantions of the above variants are discussed.

P0837. Based on allele-specific ligation reaction PCR-analysis for registration of the most frequent mutations in the *PAH* gene.

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Phenylketonuria (PKU) is the most common inborn error of amino acid metabolism in Europeans. It is caused by mutations in the phenylalanine hydroxylase (*PAH*) gene (MIM# 261600). Up to date it is known more than 500 mutations. This disease is associated with a severe mental retardation.
For most frequent in Russian PKU-patients mutation screening (p.Arg408Trp, IVS12+1g>a, IVS10-11g>a, IVS4+5g>t, p.Arg252Trp, p.Arg261Gln, p.Arg158Gln, p.Pro281Leu) in exons 4, 5, 7, 11 and 12 of the *PAH* gene the multiplex system based on allele-specific ligation reaction PCR-analysis was created. This system applied to detect 81,1% mutations in our patients.
The PAH probemix contains 24 different probes with amplification products between 83 and 139 bp. Length difference between consecutive amplification products is 3 or 4 bp.
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P0838. Phenotype-genotype correlations for patients with phenyketonuria in Latvia

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Deficiency of phenylalanine hydroxylase (PAH) impairs hepatic hydroxylation of phenylalanine to tyrosine and is the most common inborn error of amino-acid metabolism in Caucasians. Mutations of the PAH gene coding the hepatic enzyme result in various degrees of enzyme impairment, resulting in different metabolic and clinical outcome of hyperphenylketonuria (HPA). For diagnostic and therapeutic purposes is used the following phenotype classification: ,,classic", ,,moderate" and ,,mild" PKU and ,,mild hyperphenylalaninemia".
The prevalence of the R408W mutation among Latvian PKU patients determines the severe clinical form for the disease. The R408W mutation (c.1222C->T), a C to T transition in exon 12 of the PAH gene, results in the substitution of tryptophan for arginine at amino-acid residue 408 and is a null mutation associated with < 0,3% of normal enzyme

activity. In Latvian population the R408W mutation is associated with RFLP haplotype 2, the VNTR-3 allele, and the 238bp-STR allele with frequency 77% from all mutant chromosomes. Almost 56% of patients are homozygous for the R408W, 40% are compound heterozygous and 3,3% have no R408W mutation. According to the pre-treatment Phe blood level and Phe tolerance, 90,2% (55/61) patients are classified as having a classical PKU phenotype, 4,9% (3/61) patients - moderate, and 4,9% (3/61) patients - mild.
Patients with moderate and mild PKU phenotype are compaund heterozygotes, five of them have R408W in one allele and G272X, A104D and E178G in other allele, two patients have an unidentified other allele and one patient has no R408W mutation. Final results are in progress.

P0839. PLP1 overexpression in PMD and PMLD patients: interest of fibroblasts analysis

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The myelin proteolipid protein 1 (PLP1), which encodes for the main proteins of the CNS myelin, is implicated in the X-linked disorders of CNS myelination, Pelizaeus-Merzbacher Disease (PMD) and Spastic Paraplegia 2 (SPG2). Large duplications including the whole gene account for 60% of gene alterations. Transgenic mice with additional Plp gene copies are identically hypomyelinated and have demonstrated that Plp overexpression may act in a dominant negative effect on myelinating oligodendrocytes that enter into apoptosis.
By real time quantitative RT-PCR, we quantified PLP/DM20 mRNA levels in nerve biopsies (n=6) and primary cultured fibroblasts (n=14), from PMD patients carrying a PLP1 gene duplication. An overexpression of the PLP/DM20 transcripts was observed in all samples compared to controls, suggesting that PLP1 expression in fibroblasts and in PNS may reflect PLP1 expression level in the CNS. Therefore, fibroblasts from 17 male PMD/SPG2 patients without identified PLP1 or GJA12 abnormality (PMD Like, PMLD), were analyzed. Among them, 8 present with a PLP/DM20 mRNAs overexpression, implicating a PLP1 dysregulation due to mechanism(s) alternative to the gene duplication. All have a mild form of dysmyelination. Neither genomic mutations nor rearrangements have been found in the PLP1 promoter and in the ASE cis regulating elements. A mutation was observed for one patient in the 3'UTR. Its functional relevance on the mRNAs stability is under evaluation.
In conclusion, fibroblasts represent a useful tool to quantify the PLP1 gene expression level and have allowed us to demonstrate that the PLP1 gene may remain a candidate gene for PMLD patients.

P0840. Splicing abnormalities associated with different types of PLP1 mutations.

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The myelin proteolipid protein 1 (PLP1) gene encodes, by alternative splicing of its exon 3, for the main proteins of the CNS myelin: PLP and DM20. Duplications of the gene and point mutations are implicated in the X-linked disorder of CNS myelination: Pelizaeus-Merzbacher Disease and Spastic Paraplegia 2. To assess the functional consequences of different types of PLP1 mutations (7 exonic punctual mutations, 3 deletions of 1 nucleotide, 3 consensus splicing site and 1 nucleotide substitution in intron 3 of unknown significance), PLP/DM20 transcripts were sequenced from peripheral nerves and/or primary cultured fibroblasts.
Transcripts analysis has shown that the intron 3 mutation leads to the intron retention, demonstrating its implication in the phenotype and suggesting the potential existence of an intron splicing regulatory element in the surrounding sequence. Unexpectedly, one of the 7 exonic punctual mutations, initially thought to be a missense, was demonstrated to impair the splicing by creating a new donor site exclusively used, at least in the fibroblasts, instead of the classical site. In addition, transcripts analysis has allowed to assess that mutations in the consensus splicing sites are deleterious for PLP1 gene expression and to identify the different cryptic sites that are alternatively used.
This study demonstrates that splicing abnormalities can result from various types of mutations. Therefore, such approaches are useful to better evaluate the genotype-phenotype correlations and to identify intronic or exonic splicing regulatory elements.

P0841. Polycystin-2 regulates cellular proliferation in a p21/Cdk2-independent manner

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Autosomal dominant polycystic kidney disease (ADPKD) is a common inherited disorder, characterized by progressive cyst formation and loss of kidney function. ADPKD is principally linked to two genes, PKD1 and PKD2. The pathogenesis is currently thought to involve dysregulated epithelial cell proliferation and differentiation, alteration in membrane proteins polarity and abnormal fluid accumulation. The molecular mechanism of cystogenesis has not been fully elucidated. A recent report implicated polycystin-2 in the regulation of epithelial cell proliferation. Specifically, the authors suggested that PC-2 overexpression suppresses cell proliferation through inhibition of the p21/Cdk2 pathway. To better understand the role of PC-2 in epithelial cell proliferation, we utilized various cellular models generated by stable expression of mutated (R742X and 1-702) and wild-type PKD2. In contrast to the previous data, over-expression of wild-type or mutated polycystin-2 in two different cell-lines does not affect cellular growth. On the contrary, electrophysiology experiments demonstrated that overexpression of wild-type PKD2 increases both inwards and outwards K+ currents in these cells. Interestingly, primary renal epithelial cells from transgenic rats generated by expression of the 1-702 PKD2 have elevated levels of the proliferation marker, PCNA. However, in both primary cells and stable cell lines, wild -type or mutated PC-2 do not alter p21 levels and Cdk2 activity. Collectively, these data suggest that in our models, PC-2 regulates epithelial cell proliferation in a p21/Cdk2-independent manner and that PC-2 inactivation by itself is not sufficient for abnormal cell proliferation. Experiments are underway to verify these data by genome-wide expression and pathway analysis.

P0842. Digenic/Triallelic inheritance in polycystic kidney disease suggests a new mutational mechanism with mutations in a transcription factor and its regulated gene

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The transcription factor HNF1ß has been recently shown to be crucial for kidney development, as demonstrated by in vivo experiments where Xenopus embryos microinjected with mutant HNF1ß RNA transcripts had impaired pronephros development. HNF1ß binds specifically as homo- or heterodimer in concert with HNF1α to the proximal PKHD1 promoter and stimulates transcription of the gene responsible for autosomal recessive polycystic kidney disease (ARPKD). Recent studies revealed that mutations of the HNF1 binding site, expression of a dominant-negative HNF1ß mutant and mice with renal-specific inactivation of Hnf1ß exhibit a drastic defect in PKHD1 expression. Here we present a patient with polycystic kidney disease heterozygote for the novel PKHD1 1-bp deletion c.1151delA (p.P383fs), while a second PKHD1 change was not identified. HNF1ß mutation analysis revealed the novel, non-conservative missense mutation c.244G>A (p.D82N) that affects an evolutionarily highly conserved residue and considerably disturbs the protein's DNA binding domain. Further arguments of its pathogenic character are the conservation of p.D82 in its counterpart HNF1α and that it was not present among 500 tested chromosomes. Reporter gene analysis is currently underway to further corroborate its functional significance. Beyond the first description of digenic and/or triallelic inheritance in polycystic kidney disease, our study is of general interest as it demonstrates an intriguing new regulatory mutational mechanism with mutations in a transcription factor and its activated gene.

P0843. Classic Rett syndrome (RTT) and Preserved Speech Variant: new approaches to understand the genetic differences

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In classic RTT, the III stage is characterized by mild improvement of eye contact. At the same stage the Preserved Speech Variants (PSV) recover the ability to speak and to use hands. Both phenotypes are due to similar or identical *de novo* mutations in *MECP2* (http://www.biobank.unisi.it and Sampieri, Hum Mut 2007). In order to understand the genetic differences between the two phenotypes we have: i) searched for statistically significant differences in allele frequencies of polymorphisms in genes associated to a similar phenotype (*CDKL5*), target genes (*BDNF*) or genes involved in neurodegeneration (*APOE*); ii) analysed differences in genomic variations by array-CGH in two sisters with discordant phenotypes, balanced XCI, and the same *MECP2* partial deletion absent in parents. We have established that the p.Q791P *CDKL5* polymorphism is not involved in the modulation of RTT phenotype. Array-CGH analysis on the above described RTT sisters showed a duplication of 390 Kb on chromosome 16 in the classic RTT girl inherited from the healthy father and absent in the PSV sister. The duplication includes 10 genes. Two are disease-genes: one related to a known myopathy and the other to a CNS disease. Our hypothesis is that one or more duplicated genes could be dosage sensitive and could act as modifiers of the phenotype generated by *MECP2*. Understanding the genetic differences between classic RTT and PSV will help in designing therapeutic strategies.

P0844. Primary Ciliary Dyskinesia with normal axoneme ultrastructure caused by DNAH11 mutations

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Primary Ciliary Dyskinesia (PCD) is a disorder characterized by perturbed or absent beating of motile cilia and often associated with random organ lateralization (Kartagener Syndrome, KS). We present a German family with five individuals affected by PCD and one with KS. Ciliary beating was reduced or absent in native respiratory cilia of the majority of affected individuals. Analysis using high-speed video microscopy revealed an abnormal hyperkinetic beating of respiratory cilia with a reduced bending capacity of the axonemes. The axonemal ultrastructure was normal and outer dynein arms were intact, as analyzed by electron microscopy and immunohistochemistry. Microsatellite analysis indicated linkage to the dynein heavy chain DNAH11 on chromosome 7p15. All affected individuals were found to be compound heterozygotes for mutations c.12415C>G and c.13583_13639del. Both mutations are located in the C-terminal domain and result in a truncated DNAH11 protein (p.4128Y>X, p.A4518_A4523delinsQ). Our findings indicate that mutations in the DNAH11 gene indeed cause PCD and KS, and that the reported DNAH11 nonsense mutations are associated with a normal axonemal ultrastructure and are compatible with normal fertility.

P0845. Investigation of *PROX1* expression during human cardiogenesis and it's potential role in congenital heart defects.

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Hypoplastic left heart syndrome (HLHS) is a severe congenital heart defect characterised by hypoplasia of the left ventricle with aortic and mitral valve stenosis or atresia. HLHS is frequently associated with lymphoedema and maldevelopment of the lymphatic system.
The homeobox transcription factor, *PROX1*, is located within 1q32, deletions of which are a cause of congenital heart defects and therefore a region critical to cardiac development. PROX1 has a role in organogenesis and cell type specification including budding and sprouting of the lymphatic system. Knockout mice lacking *Prox1* die mid-gestation with multiple developmental anomalies. *Prox1* is expressed in many organs including the heart.
This study assessed the candidacy of *PROX1* as a cause for HLHS including expression in the human heart during cardiogenesis and investigation of children with HLHS for mutations within *PROX1*.
Human embryonic and fetal tissue was obtained with informed consent and ethical permission. Hearts were available from fetuses aged between 7-11 weeks post conception (wpc). Using an antibody raised to the Prospero and homeodomains (Abcam), PROX1 immunoreactivity was detected in critical areas of the heart. These included the aortic valve, aortic wall, atria and lymphatic vessels. We therefore investigated patients with HLHS for changes in the nucleotide sequence of coding exons and splice sites of *PROX1*. The DNA from 12 patients was directly sequenced but no nucleotide changes were detected.
The localisation of PROX1 within the heart during cardiogenesis supports a role for PROX1 in normal development and as a strong candidate gene for HLHS.

P0846. Mutations in the gamma-carboxylase gene *GGCX* cause a novel Pseudoxanthoma Elasticum-like disorder

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Aims: We recently described a novel autosomal recessive PXE-like disorder, characterized by a generalized increase of excessive, leathery skin folds, a mild retinopathy with limited angioid streaks and preserved visual acuity and a deficiency of the vitamin K-dependent clotting factors. Ultrastructural analysis showed distinct differences in mineralization and fragmentation of dermal elastic fibres compared to PXE. Furthermore, no mutations in the *ABCC6* gene, responsible for PXE, could be detected. This study aimed to unravel the molecular pathogenesis of this phenotype.
Methods: Using a candidate gene approach, we performed molecular analysis of the *VKORC1* and *GGCX* genes in 7 patients. Both candidate genes cause hereditary deficiency of the vitamin K-dependent clotting factors. Using conformation-specific ELISAs, the balance between carboxylated (functional) and non-carboxylated (inactive) serum osteocalcin and matrix gla protein (MGP) was assessed.
Results: Direct sequencing revealed missense and nonsense mutations in *GGCX* in 6 patients. The GGCX-enzyme is important for activation (γ-carboxylation) of vitamin K-dependent proteins such as clotting factors but also known inhibitors of calcification (e.g. MGP, osteocalcin), thus explaining the combined phenotype. ELISA experiments revealed imbalances in the ratios of active and inactive serum gla proteins, supporting the causality of the mutations and hence our pathogenetic hypothesis.
Conclusion: We have identified *GGCX* as being the defective gene in a novel PXE-like syndrome. Interestingly, this enzyme has a critical function in pathways involved in inhibition of ectopic mineralization. Because of the clinical and histopathological resemblance with PXE, this rare disorder may give us valuable insights in the pathogenesis of PXE.

P0847. Pyridoxamine 5'-phosphate oxidase deficiency: A new inherited lethal disorder causing neonatal seizures, treatable with pyridoxal phosphate.

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Neonatal seizures present a serious diagnostic dilemma. A comprehensive workup and immediate treatment are essential.
We present a female infant born who suffered severe seizures few

hours after birth, did not respond to a wide range of anticonvulsant drugs, and died at the age of 6 weeks.
Metabolic investigations showed elevated glycine and threonine concentrations in plasma and CSF, and elevated Homovanillic acid and Vanillactic acid in the urine and CSF. This biochemical profile indicates a possible deficiency of pyridoxine 5'-phosphate oxidase (PNPO), the enzyme that converts pyridoxamine to its active form pyridoxal phosphate (PLP). PLP acts as an important coenzyme for more than 100 enzymes.
The biochemical findings in our patient were suggestive of a defect in PNPO activity.
Sequence analysis of the *PNPO* gene revealed a homozygous G>A transition at nucleotide 284 in exon 3 (c.284 G>A) resulting in a R95H missense mutation. Expression studies in CHO cells, using mutated *PNPO,* showed that the c.284 G>A mutation reduced the enzymatic activity of PNPO to 18% relative to wild-type. PNPO deficiency is a recently described rare and lethal autosomal recessive disorder that has been reported in less then 15 patients worldwide. This potentially treatable entity should be considered in the differential diagnosis of neonatal seizures. Molecular and biochemical diagnosis is feasible and will provide tools for comprehensive genetic counseling and PND to interested couples, as well as screening of couples at risk for this devastating disorder.

P0848. Q6NUR6 gene is overexpressed in an autistic patient with a translocation (7,16) de novo.

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Based on the hypothesis that the autism has an important genetic component, our goal is to map and identify the gene (or genes) that predispose to autism. The proposed strategy is to test candidate regions identified by cytogenetic studies.
We describe a 10 years old child with autistic disorder and a de novo balanced (7,16) (p22.1,p16.2) translocation. G-banded chromosomes and fluorescent in situ hybridization (FISH) were used to examine the patient's karyotype as well as his parents'. FISH with specific RP11-BAC clones mapping near 7p22.1 and 16p11.2 were used to refine the location of the breakpoint
The breakpoint's spanning clone on 7p22.1 fully overlaps a human known protein coding sequence corresponding to the Q6NUR6 gene which was analyzed by TaqMan. The study demonstrated a significant overexpression of this gene in the patient. We postulate that the rearrangement is responsible for the patient's phenotype and for his Q6NUR6 gene overexpression.

P0849. The study of CYP1A1, GSTM1, GSTT1, GSTP1 and NAT2 polymorphisms genes and possible role in recurrent pregnancy loss

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The aetiology of recurrent pregnancy loss (RPL) remains largely unclear. Epidemiological studies have suggested that the risk of spontaneous abortions associated with exposure to endogenous or exogenous substances may be modified by the genetic variation in individual metabolic detoxification activities, thus in the phase I/phase II balance. Glutathione S-transferase (GST) catalyses the binding of a large variety of electrophils to the sulphydryl group of glutathione, they are involved in the detoxification of free radicals and have main function in binding and transport of a wide variety of harmful compounds. GST are present in large amounts in many tissues including those of the genital tract and placenta. The aim of this study was to investigate the role of CYP1A1, GSTM1, GSTT1, GSTP1 and NAT2 polymorphisms in the pathogenesis of RPL. After informed consent we have studied the polymorphic variants of genes encoding enzymes in 49 women (case group) with recurrent pregnancy loss and in 169 women (control group) with an uncomplicated obstetric history. The frequency of GSTM1 0/0 genotype in case group (0.77) was significantly (p=0.018) higher than in control group (0.50). The frequency of GSTT1 0/0 genotype in the case group (0.25) was higher than in control group (0.19) but this difference was not significant. The frequencies of NAT2, CYP1A1 and GSTP1 "slow alleles" were practically identical in both analysed groups. It had been shown that GSTM 0/0 variant really can be involved in process recurrent pregnancy loss, which may be connected with changes in steroid hormones level.

P0850. Mechanisms of apoptosis mediated by dependence receptors: The model of Ret

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Dependence Receptors constitute a newly described family of functionally-related receptors which transduce a positive signal leading to survival, differentiation or migration in the presence of their ligand, whilst in its absence, a proapoptotic fragment which initiates a negative signal for apoptosis, is generated by caspases cleavage.
One member of this family is the proto-oncogene RET (rearranged during transfection), a classical tyrosine kinase receptor spliced in two main alternative forms: RET9 and RET51.
Mutations in RET proto-oncogene have been associated with both neoplasia (MEN2A, MEN2B and FMTC) and a congenital neural crest defect (Hirschsprung disease).
Hitherto, the positive signal transduced by RET has been well studied, while less is known about the signal transduction leading to apoptosis.
To shed light on new proteins involved in this latter pathway, we planned to use a modified yeast two hybrid method named "split ubiquitin system" employing as baits RET51 against a human brain expression library.
We identified ten proteins potentially interacting with RET51. The interaction between four of these and RET51 has been confirmed through co-immunoprecipitation analysis and co-localization assay, while one of these proteins (GSTP1, an enzyme belonging to the family of the glutathione-S-transferase) appears to be a false positive interactor.
Currently, we are verifying the role of such interactors (as modifiers of RET associated diseases in patients) and the interaction between RET-51 and the remaining proteins.
Furthermore, the "split ubiquitin system" is going to be used employing also RET9 and RET (708-1017) as baits.

P0851. An oncogenic mutation in exon 27 of the RB1 gene

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Retinoblastoma (Rb) is initiated by mutational inactivation of the RB1 gene, a tumour suppressor composed of 27 exons. The spectrum of point mutations (1900 mutation events) is dominated by nonsense (44%) and frameshift (23%) mutations, which result in premature termination codons located > 55bp upstream of the last exon-exon junction. Therefore, the mutant mRNA is likely to be subject to nonsense mediated decay. No mutation was identified in exons 26 and 27 so far. We have performed mutational analysis of the RB1 gene in more than 900 patients. In view of the mutation spectrum, we suspended direct sequencing of exons 26 and 27 recently. However, we now identified a frameshift mutation in exon 27 of the RB1 gene (RBg.177098_177099dup) by quantitative multiplex PCR in a boy with sporadic unilateral Rb. His father is a heterozygous carrier of this mutation. We showed that the mutation occurred de novo. The mutant +2bp transcript was detected by RT-PCR on RNA prepared from peripheral blood cells. We performed sequencing of exons 26 and 27 in patients (8, 13, and 8 patients with familial, isolated unilateral, and bilateral Rb, respectively) in whom we had not identified an oncogenic mutation previously. None of these 29 patients showed a mutation.Our results indicate that a frameshift mutation in the terminal exon of the RB1 gene can be oncogenic. In view of the phenotypes associated with the mutant allele in the reported family it can be expected that mutations in this region will be associated with milder phenotypic expression.

P0852. Mutations in the MECP2 gene in patients of Slavonic origin with Rett syndrome

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Rett syndrome is an X-linked dominant neurodevelopmental disorder affecting almost exclusively females. It has an incidence of 1:10,000 female births and is characterized by apparently normal development for the first 6-18 months of life followed by the loss of acquired motor and language skills, autistic features, and development of stereotypic hand movements. Rett syndrome is caused primarily by de novo mutations in the methyl-CpG-binding protein 2 gene (MECP2). Methyl-CpG-binding protein 2 plays important roles in the regulation of gene expression and RNA splicing. Here we report the mutational analysis of the MECP2 gene in 95 patients from the Czech and Slovak Republics and the Ukraine with classical Rett syndrome. The patients, all girls, were screened for mutations by DNA sequencing of the entire coding sequence and the exon/intron boundaries of the MECP2 gene. RFLP analysis was performed to confirm the mutations that cause the creation or abolition of the restriction site. Mutation-negative cases were subsequently examined by multiple ligation-dependent probe amplification (MLPA) to identify large deletions. Sequencing analysis revealed 33 different mutations in 74 patients, and MLPA analysis revealed large deletions in two patients. The detection rate was 78%. One mutation in exon 1 (c.48_55del8) has not been published yet. Our results confirm the high frequency of MECP2 mutations in females with Rett syndrome and provide data concerning the mutation heterogeneity in the Slavonic population. The project was supported by grants MSM0021620849 and IGA 8355-3.

P0853. Large genomic rearrangements in the *MECP2* gene in Polish patients with Rett syndrome

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Rett syndrome (RTT, MIM#312750) is a neurodevelopmental disorder affecting almost exclusively females with prevalence estimated to be 1 in 10000-15000 live births. In affected girls a period of apparently normal development is followed by mental retardation, loss of acquired skills, stereotypical hand movements, seizures, scoliosis and autonomic dysfunction. The disease is caused by mutations in the X-linked *MECP2* gene encoding methyl CpG binding protein (MeCP2). The gene contains four exons and produces two transcripts consisting of exons 2, 3 and 4 (MeCP2A isoform) or exons 1, 3 and 4 (MeCP2B isoform). To date, more than 370 various *MECP2* mutations have been found. Of 140 Polish patients with classic form and variants of RTT, 57 harbor point mutations and small deletions or insertions in *MECP2* exons 3 and 4. Recently, several patients with mutations involving exon 1 have been reported.

The aim of our study was to identify large genomic rearrangements in the *MECP2* gene in Polish RTT patients. Molecular analysis was performed in forty-six patients, who had been tested negative for mutations by SSCP and sequencing of *MECP2* exons 2, 3 and 4. Patients' genomic DNAs were screened for large deletions/duplications by multiplex ligation-dependent probe amplification (MLPA) using SALSA P015C kit (MRC-Holland). Large deletions including promoter region and exon 1 were identified in three patients, and deletions covering exon 4 and, probably, exon 3 in two patients. Besides, partial deletion of exon 4 was observed in one RTT girl.

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P0854. Monosomy of the X chromosome in primary and overlapping autoimmune diseases.

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The significant female predominance of autoimmune diseases (AID) has been associated to acquired X-chromosome monosomy (XCM) and foetal cell microchimerism (MC). However, this characteristic was not investigated in overlapping AID like Reynolds syndrome (RS). Rationale: RS show higher rate of XCM than single AID. To address this hypothesis we studied women with RS (12), CREST syndrome (13), Primary Biliary Cirrhosis (PBC) (12) and a control group paired by sex and age (24).

Methods: after signed consent, information on male pregnancies, transfusions, abortions and older brothers in the sibship of index cases, 5 ml of whole blood was obtained for lymphocytes cultures, FISH studies in interphase nuclei (IN) and DNA extraction for Q-PCR of *SRY* gene sequences. CEP-X spectrum green probe was used for MX analysis, and CEP-15 spectrum orange as control. Samples were blindly assessed and 500 IN were analysed in each subject.

Results: Median ages in the different groups were quite similar (59-61). Proportion of MX IN were 14% (13.1-14.8); 11.5% (10.5-12.1); 11.3% (10.7-12.3) and 7.2% (6.8-7.7) for RS, CREST, PBC and controls respectively. Chi-square comparisons of the amount of MX cells, showed significant statistical differences (SSD) between each AID and controls, between RS and CREST, and RS and CBP. Q-PCR of *SRY* sequences discarded MQ as a confounding factor.

Conclusions: Our results confirm that MX is significantly increased in AID respect healthy controls, that MQ is not an associated cause and that probably an additive effect could explain the SSD between RS and the other two AID.

P0855. A real time PCR assay to detect causative mutations of the ryanodine receptor gene

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The ryanodine receptor (RYR1) is an essential component of the calcium homeostasis of the muscle in mammals. Defects in the RYR1 gene in humans (chromosome 19q13.1) are associated with malignant hyperthermia (MH), life-threatening and frequently fatal disorder triggered by commonly used anesthetics. Susceptibility to MH (MHS), dominantly inherited predisposition to MH, is diagnosed by using an invasive diagnostics test on excised muscle bundles, the in vitro contracture test (IVCT). This test is based on the differential contractile response of normal and MH muscle to caffeine and halothane.

Molecular genetic screening plays an integral role in the diagnosis of MHS. Genetic data provide additional diagnostic information or contribute information independent of IVCT. Genetic screening is very difficult due to low incidence of each mutation and the vatness of the RYR1. Until now, 23 RYR1 mutations causing MH have been listed by European MH Group. A detection MH causative RYR1 mutations can be used in predictive genetic testing. We describe an assay that allows analysis of RYR1 genotypes using real time PCR. We provide a test based on melting point analysis of fluorscently labelled probes after high speed PCR amplification. After nucleic acid extraction from the white blood cells, or muscle cells RYR1 mutation can be detected in less than 1hour. This assay is cheaper and faster than traditional one used to date. Our RYR1 mutation analysis protocol currently screens for the most common RYR1 mutations in Czech population. Our aim is to develop analysis test for all published MH causative mutations.

P0856. Characterization of COX16, a novel human COX assembly gene

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COX16 is a gene essential for cytochrome c oxidase (COX) biosynthesis in yeast.. We have now cloned and characterised its human homologue.

The human gene was identified in the expressed sequence tags (EST) database using a cyberscreening method.. Human COX16 is comprised of 4 exons on chromosome 14 and encodes a 106 aa protein with a N-terminal putative transmembrane domain and a C-terminal hydrophilic domain, with no homology to any known family of proteins. The gene is expressed ubiquitously with highest levels in muscle, heart, and liver. We found multiple transcription initiation sites, the most upstream of which is located 100 bp from the ATG.

Cox16 protein is located in mitochondria, presumably in the mitochondrial inner membrane.

Silencing of the COX16 transcript by siRNA caused a significant reduction of COX activity after 48 hours in transfected cells.

The precise function of COX16 is unknown. Its small size suggests that it doesn't have a catalytic role, but rather that it could act by stabilizing nascent COX subcomplexes in analogy to PET100. The effect of COX16 silencing on COX activity is striking, suggesting that COX16 may play an important role in the regulation of the COX assembly process.
The role of COX16 in COX deficiency has yet to be determined. A previous study failed to detect mutations in patients with isolated COX deficiency, we are now screening a novel cohort of patients for mutations in this gene.

P0857. Investigation of the Spinocerebellar ataxia type 10 mutation in the Cypriot population

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Spinocerebellar ataxia type 10 belongs to the group of neurodegenerative diseases known as autosomal dominant cerebellar ataxias. Genetic studies in patients with SCA so far revealed 12 genes responsible for ADSCA and 12 mapped loci without gene identification. SCA10 is characterized by progressive ataxia and seizures. The underlying mutation is a large expansion of an ATTCT repeat in intron 9 of the SCA10 gene. Our aim is to determine the relative frequency of SCA10 in Cyprus, which constitutes part of our wider effort to identify the genetic defects of the Cypriot SCA sporadic patients and families, which prove to be exceptional in comparison with other populations. We analyzed the ATTCT repeats in 37 SCA patients, previously excluded from other genes (FRDA, SCA1-3, SCA6-8, SCA12, SCA17 and DRPLA). We also determined the size of repeats in 57 normal controls from the Cypriot polulation. The repeat lengths were analyzed by polymerase chain reaction followed by fragment analysis. Southern blot analysis is currently performed for samples with one allele detected, in order to confirm homozygosity or presence of a SCA10 expansion. Normal control sample repeat lengths ranged from 11 to 20 with 82,5% heterozygosity and the 14 repeats allele is more frequent (38%) in the Cypriot population. In the patient group, repeats ranged from 12 to18 with 73% heterozygosity. Three patients have already been confirmed to be homozygous for a normal range allele. In conclusion, our results agree with other studies demonstrating that SCA10 is rare in polulations other than the Mexican.

P0858. SCA17 transgenic mice exhibit a neurodegenerative phenotype

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SCA17 is a progressive neurodegenerative disease leading to cerebellar ataxia and dementia. Several accessorial symptoms such as Parkinsonism, dystonia, and psychiatric disturbances commonly aggravate the disease course. Genetically, a CAG/CAA expansion in the TATA binding protein (TBP) is expanded in SCA17 patients, leading to an expanded polyglutamine chain in this ubiquitously expressed transcription factor.
We have generated transgenic mice overexpressing a 64 CAG/CAA repeats containing human TBP (Q64TBP) gene under the control of the truncated human prion protein promoter (PrP).
Transgene protein expression throughout different brain regions (cortex, basal ganglia, cerebellum, and brain stem) was clearly demonstrable. Onset of motor dysfunction (Accellerod) started by 20 weeks and the life span of transgenic animals was reduced.
We present detailed morphological and phenotypical data for this rodent model of SCA17, which enables us to further study the pathogenesis of this progressive neurodegenerative disease.

P0859. SOST gene mutation in two Brazilian families with sclerosteosis

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Sclerosteosis is a rare, autosomal recessive disease characterized by a progressive craniotubular hyperostosis. The main features are: high stature, nail dysplasia, cutaneous syndactyly of some fingers, significant sclerosis of the long bones, ribs, pelvis and skull, leading to facial distortion and entrapment of cranial nerves. The sclerosteosis gene has been mapped to 17q12-21 and is known as the SOST gene encoding sclerostin protein. We report on one familial and one sporadic case with clinical and molecular diagnosis of sclerosteosis.
Case 1: 34-year-old male, with difficulty in opening mouth, hemifacial and tongue pain, gustatory hyperlacrimation and hypoacusis. Four sisters had similar clinical complaints, three of whom had cutaneous syndactyly of some fingers. Physical examination: macrocephaly, long face and widely spaced teeth. Radiological studies: poor development of the mandible angle, asymmetric face, sclerosis of the skull and craniofacial bones, hyperostosis of the ribs, pelvis and long bones.
Case 2: 6-year-old girl, presented with recurrent facial paralysis. There was no significant facial dysmorfism. Cutaneous syndactyly of the index and middle fingers was present. Radiological studies: sclerosis of the skull, spine, pelvis and long bones.
The clinical diagnosis of sclerosteosis in both cases was confirmed by analysis of the SOST gene showing the same mutation (Trp124X). We reported this mutation previously in other Brazilian patients. Curiously, both families were from the same state in Brazil, but they denied familial relationship.
In conclusion, these patients confirm the clinical picture as found in other cases with a loss of function mutation in the SOST gene.

P0860. Determining the expression of the human *SPP2* in lymphocytes, endothelial cells and monocytes and the effect of TNF-α and LPS on the expression using RT-PCR method

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Secreted phosphoprotein 24 (spp24) is a member of the cystatin superfamily and was first identified in cattle as a minor component of cortical bone. Subsequently it was recognised as a component of the fetuin-mineral complex. In the original study, the expression of the gene encoding spp24 (*Spp2*) was demonstrated in bovine bone periosteum and liver and it was later demonstrated that *Spp2* is expressed in chicken and mouse T-cells and also in the mouse thymus. Hence, it was decided to investigate the expression pattern of the human spp24 gene (*SPP2*) in peripheral white blood cells.
Mononuclear lymphocytes and monocytes were isolated from whole fresh human blood and endothelial cells were prepared from the umbilical cord of a neonate. PCR amplification of reverse-transcribed mRNA indicated that *SPP2* is expressed in human lymphocytes and endothelial cells, but not in monocytes.
The effects of lipopolysaccharide (LPS) and tumour necrosis factor alpha (TNFα) on the expression of the human *SPP2* gene were studied by their addition to cultures of monocytes, mononuclear cells and endothelial cells for up to 24 hours. Under standard culture conditions, which included bovine serum albumin (BSA) in the culture medium, there is expression of *SPP2* in mononuclear lymphocytes and in endothelial cells at 16 hours, but not in monocytes. The addition of either LPS or TNFα down-regulates *SPP2* expression in lymphocytes in a time-dependent fashion. However, the down-regulation effect of TNFα on endothelial cells is, by comparison, only modest. These findings may help us understand the function of spp24.

P0861. Mitochondrial DNA instability and recessive *POLG* mutations in patients with isolated adult-onset sensory ataxic neuropathy

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Nuclear gene defects affecting mtDNA stability include the mtDNA polymerase (*POLG*), the adenine nucleotide transporter (ANT1) and the Twinkle helicase. There is a considerable variability in the phenotype associated with *POLG* mutations which are responsible for autosomal dominant and recessive progressive external ophtalmoplegia (PEO), Alpers syndrome, a sensory ataxic neuropathy, dysarthria and ophtalmoparesis (SANDO) and a mitochondrial recessive ataxic syndrome. Nevertheless, patients with *POLG* mutations and sensory

ataxic neuropathy always presented with associated muscular and/or central neurological system features. The aim of our study was to test whether *POLG* mutations can be responsible for isolated sensory ataxic neuropathy. We screened 15 patients by direct sequencing. Seven patients were men and the median age of the population was 57 years. The presenting and only feature was ataxia caused by axonal sensory neuropathy. A 50 year-old woman was found to be a compound heterozygous carrying the c.1391T>C mutation (M464T) in combination with the c.2302A>G substitution (K768E). No *POLG* mutation was found in other patients. Nevertheless, a muscle biopsy was performed in two cases. Ragged-red and COX negative fibers were found in one patient, with multiple mtDNA deletions by both long range PCR and Southern blot analysis. The patient was a 67 year-old man who developped ataxic symptoms at the age of 47. ENMG revealed normal motor potentials and absent sensory potentials in the four limbs. No mutations were detected in *ANT1* or Twinkle.
In conclusion, mitochondrial disease has to be considered as a cause of isolated adult-onset sensory ataxic neuropathy.

P0862. Sepsis and mitochondrial DNA

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Sepsis-induced multiple organ failure is the major cause of mortality and morbidity in critically ill patients. However, the precise mechanisms of this dysfunction are largely unknown. Genetic studies suggest a strong genetic component to both the risk of developing sepsis and the subsequent outcome in terms of survival.
The failure of cellular oxygen use appears to be important in the development of multiple organ dysfunction in severe sepsis. Mitochondria are small membrane-bound intracellular organelles. They are involved in multiple cellular processes such as energy metabolism, apoptosis and generation of reactive oxygen species (ROS).
Impaired mitochondrial function in septic patients is associated with poor clinical outcome. Epidemiological studies indicate that premature death from infection has a strong inherited component and natural genetic variation in mitochondrial DNA (mtDNA) provides a potential explanation.
Human mtDNA is maternally inherited. The population can be divided into several mtDNA haplogroups on the basis of specific single nucleotide polymorphisms (SNPs) scattered throughout the mitochondrial genome, indicating that mutations accumulated by a discrete maternal lineage during evolution. The SNPs that define the mtDNA haplogroups are markers of a particular maternal lineage of mtDNA. The majority of Europeans belong to one of nine haplogroups with haplogroup H being the most common (44%). Our group found an improved survival in septic patients with haplogroup H. MtDNA copy number was measured in 59 haplogroup H patients and 34 control samples. Differences in mtDNA copy number were observed between survivors and non-survivors.

P0863. Histidine-Rich Calcium Binding Protein Interacts with SERCA2 in a Ca-Dependent Manner

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Deregulation of Ca cycling by the sarcoplasmic reticulum (SR) in the cardiomyocyte has been associated with depressed cardiac function which may progress to heart failure. The histidine-rich calcium binding protein (HRC) is a low affinity, high capacity Ca-handling SR protein that binds to triadin. Through this interaction HRC may affect Ca release by the ryanodine receptor. HRC overexpression in transgenic mouse hearts was associated with decreased rates of SR Ca uptake and delayed relaxation, which progressed to hypertrophy upon aging. Using a combination of *in vivo* co-immunoprecipitations and pull-down assays in human and mouse cardiac homogenates, and *in vitro* blot overlay experiments with GST and MBP recombinant proteins, we identified the direct binding of HRC to SERCA2 in cardiac muscle. This interaction involves the histidine and glutamic acid-rich domain of HRC (320-460 aa) and part of the N-terminal cation transporter domain of SERCA2 (74-90 aa), which projects into the SR lumen. The SERCA2 binding domain is upstream from the triadin binding region in human HRC (609-699 aa). Ca-titration experiments indicated that the binding of HRC to SERCA2 was reduced by 75%, when the Ca concentration as raised from 0.1 to 100 μM. Increasing Ca-concentrations had opposite effects on the HRC binding to triadin. Collectively, our data suggest that HRC may be involved in the regulation of SR Ca-cycling by its direct interaction with SERCA2 and triadin, which may be affected by local Ca changes. Thus, HRC may mediate a fine cross-talk between SR Ca uptake and release.

P0864. Mutation spectrum of IL2RG gene in Russian families with X-linked combined immunodeficiency.

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X chromosome-linked severe combined immunodeficiency (X-SCID) is a rare disease of serious imbalance cellular and humoral immune function. X-SCID results from a mutation in the gene encoding the gamma subunit of the interleukin-2 receptor (*IL2RG*), a component of several IL receptors. *IL2RG* gene contains 8 exons and has been mapped to the Xq13.1 region.
Direct DNA sequencing of all exons and exon-intron junctions and PCR-RFLP performed a search of *IL2RG* gene mutations.
We have detected 8 mutations in 9 unrelated families with X-SCID. In this molecular investigation we identified 6 novel mutations, the others have been reported.
In exon 2 and exon 3 of *IL2RG* gene we show three missence mutations: reported - p.Glu68Lys, p.Tyr105Cys and novel - p.Cys72Trp. We identified a new missense mutations (p.Cys182Ser) and (p.Cys182Tyr) in exon 4 of *IL2RG* gene in a two affected probands with X-SCID. In a two patients with X-linked combined immunodeficiency in our Center we found two unicue splice mutations (IVS 5 as-2nt a->g) and (IVS 5 as-1nt g->a) in intron 5 of *IL2RG* gene. One insertion (c.837ins9bp) we identified in a boy with X-SCID in exon 6 of *IL2RG* gene.
The mothers of all patients were heterozygous for the mutation. Also, three prenatal diagnostics of X-SCID were made.
In this molecular investigation of nine familys with X-linked combined immunodeficiency we have detected different mutations in exon 2, 3, 4, 6 and intron 5. We supposed two "hot spots" in exon 4 and in intron 5 of *IL2RG* gene.

P0865. Identification of small genomic deletions flanking the human SHOX gene

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In addition to point mutations and gene deletions, haploinsufficiency of the human SHOX gene can also result from deletions 3' of the gene. These are generally 80 to 500 kb in size, with a 29kb minimal deleted region, that exert a position effect. These classes of mutation account for only 70% of Leri-Weill dyschondrosteosis (LWD) patients. Therefore we have used the MRC-Holland SHOX MLPA kit supplemented with additional probes to screen for novel types of SHOX mutation in 73 cases in whom no mutation was previously identified (16 with LWD, 12 with short stature and 45 with undefined skeletal dysplasia). Upstream of SHOX we identified two deletions, of maximum size 73kb and 160kb, each in a single patient. Neither deletion was present in 88 normal controls. Downstream (3') of SHOX we identified two deletion classes: 12 of the 73 patients (16%) carried an approximately 10kb deletion and two LWD patients (3%) were compound heterozygotes for the 10kb deletion and a larger overlapping deletion of up to 60kb. The larger deletion was absent among the 88 controls, but there were 13 carriers of the 10kb deletion (15%). Among 12 LWD mutation positive cases, there were two 10kb deletions (17%) and one larger deletion (8%). In summary additional types of SHOX mutation are likely to exist, although it will be difficult to assess their functional significance. The deletions identified in this study do not appear to cause classical SHOX haploinsufficiency, however it is possible they may act to modify phenotypic expression.

P0866. Bisulfite based methylation analysis of the 6th CTCF target site within the IGF2/H19 imprinting centre region 1 (ICR1) has a higher sensitivity compared to Southernblot analysis of the 3rd CTCF target site

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Silver-Russell syndrome (SRS) is a clinically and genetically heterogeneous syndrome characterized by severe pre- and postnatal growth retardation, body asymmetry and a typical facial phenotype including triangular face and relative macrocephaly. Apart from rare families, most patients are sporadic. Approximately 10 % of patients have maternal uniparental disomy of chromosome 7. In another 30 % of patients, the differentially methylated *IGF2/H19* imprinting centre region (ICR) at 11p15 is hypomethylated. This region contains seven CTCF target sites (CTSs). In previous studies methylation of a HpaII restriction site close to *CTS3* was analysed by Southern-blotting (Gicquel et al., 2005). Using bisulfite treatment and a real time PCR based methylation assay (QAMA), we determined the methylation at *CTS6* in 15 patients who showed a normal *CTS3* methylation by Southern analysis. We observed *CTS6* hypomethylation in 6 patients. The degree of methylation ranged from 4 - 16 %. By Multiplex Ligation-dependent Probe Amplification (MLPA) we extended the methylation analysis to four additional ICR loci located between 300bp upstream and 2150bp downstream of *CTS6* and found similar degrees of hypomethylation in all of the six patients. As transmission of SRS has been observed, we analysed the degree of methylation in spermatozoa from one patient and found complete methylation of *CTS6*, which is consistent with a normal methylation pattern.

P0867. Heterogeneity of *NSD1* alterations in 116 patients with Sotos syndrome

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Sotos syndrome is an overgrowth syndrome characterized by distinctive facial features, learning difficulties and macrocephaly with frequent pre- and postnatal overgrowth with advanced bone age. Here we report on our experience in the molecular diagnosis of Sotos syndrome on 116 patients. Using direct sequencing and a QMPSF-based assay allowing accurate detection of both total and partial *NSD1* deletions, we identified *NSD1* abnormalities in 104 patients corresponding to 102 Sotos families (90 %). *NSD1* point mutations were detected in 80 % of the index cases, large deletions removing entirely the *NSD1* gene in 14 % and intragenic *NSD1* rearrangements in 6 %. Among the 69 detected distinct point mutations, 48 were novel. The QMPSF assay detected an exonic duplication and a mosaic partial deletion. QMPSF mapping of the 15 large deletions revealed the heterogeneity of the deletions which size varies from 1 to 4.5 Mb. Clinical features of *NSD1*-positive Sotos patients revealed that the phenotype in patients with non-truncating mutations was less severe that in patients with truncating mutations. This study confirms the heterogeneity of *NSD1* alterations in Sotos syndrome and therefore the need to complete sequencing analysis by the screening for partial deletions and duplications in order to ensure an accurate molecular diagnosis of this syndrome.

P0868. Molecular analysis of *SPG4* and *SPG3A* mutations in families with hereditary spastic paraplegia

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Autosomal dominant hereditary spastic paraplegias (AD-HSPs) are a genetically and clinically heterogeneous group of neurodegenerative disorders, characterized mainly by progressive weakness and spasticity of the lower limbs. Overall, *SPG4* and *SPG3A* mutations account for about half of all AD-HSP cases. *SPG4* encodes spastin, and is associated with a pure phenotype, with no predictable age-at-onset, whereas *SPG3A* encodes atlastin, being also associated with a pure phenotype, but particularly distinguishable by its early onset and a slower progression of symptoms. Recent data suggested that spastin, an ATPase from the AAA protein family, and atlastin, an oligomeric GTPase, are binding partners and function in the same biochemical pathway. To genetically characterize Portuguese families with HSP, 80 unrelated patients, 61 with AD-HSP and 19 isolated cases were analysed. Mutational analysis was performed by DHPLC followed by sequencing. Twelve mutations in *SPG4,* nine of which novel ones, as well as one novel mutation in *SPG3A* have been identified, so far. The *SPG3A* mutation was a novel frameshift causing early onset (< 10 years) and a pure phenotype; all the patients presenting *SPG4* mutations had a pure phenotype; p.G370R was the only recurrent mutation in Portuguese HSP patients. In conclusion, we genetically identified 21% of all AD-HSP in our group of Portuguese families. *SPG4* mutations had a frequency of 20%; together with the five other *SPG4* families previously described, *SPG4* accounts for approximately 28% of known AD-HSP Portuguese cases.

P0869. SMN1 mutation analysis in 803 Spanish spinal muscular atrophy patients.

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Spinal muscular atrophy (SMA) is an autosomal recessive disease characterised by degeneration of the anterior horn cells of the spinal cord and caused by mutations in the SMN1 gene. We present the results of a systematic SMN1 gene analysis in 803 unrelated Spanish SMA patients.

In 729 patients (91%) the molecular defect was absence of the SMN1 gene and in 41 cases (5%) it was the presence of SMN hybrid genes. We detected 24 subtle mutations (3%), 14 of which were c.399delAGAG (representing 1.7% of the total Spanish SMA cases). Two of these cases were homozygous for the mutation. By quantitative PCR techniques, the remaining patients were heterozygous for the SMN1 gene deletion. In the other allele we identified the following mutations: p.Y272C (one case), p.T274I (one case) and 813ins/dup11 (two cases) described in other populations and p.I116F, p.Q136E, c.738-740insC and c.867+2T>G described to date only in Spanish SMA patients. Furthermore, two novel mutations, c.311-312insA (exon 3) and p.W190X (exon 4) were identified. In the remaining 9 patients no changes were detected in the sequence of the codifying regions and exon intron boundaries. mRNA was available in two of these patients and no SMN1 transcripts were detected. Novel variants were found in the promoter region of these patients. However, it is not possible to ascribe these variants to the SMN1 or SMN2 promoter.

Employing this systematic approach, the SMN1 genotypes of the vast majority of the Spanish SMA patients were characterised, including the existence of non-functional SMN genes. FIS 05-2416.

P0870. Brain-derived neurotrophic factor (BDNF): mRNA expression in rat brain focal ischemia under the treatment with neuropeptides Semax and PGP

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Consisting of a fragment of ACTH4-7 and C-terminal PGP tripeptide neuroprotective polypeptide Semax is used for acute therapy of stroke. To investigate *Bdnf* mRNA expression after treatment either with saline, Semax or PGP the brains of male Wistar rats were analyzed at three time points following permanent middle cerebral artery occlusion (pMCAO): 3, 24 and 72 hours. The intraperitoneal injection of solutions were done at 15 min, 1 h, 4 h after the occlusion and then after every 4 hours. The last injection was done at 56 h after operation. Real-time reverse transcription and polimerase chain reaction has been used to measure changes in *Bdnf* expression in the ipsilateral and contralateral frontoparietal cortex and subcortex of rat brains. *Gapdh* was used as the internal control.
In the lesioned cortex *Bdnf* mRNA expression was increased at 24 h post-MCAO and returned to control level after 72 h. Decreasing of *Bdnf* mRNA expression in contralateral hemisphere 3 h after operation is probably concerned with depolarization expansion and neuroplasticity. Under Semax treatment in ischemic cortex such increasing of *Bdnf* mRNA expression was detected at 3 h after operation and the level of *Bdnf* mRNA was high at 24 and 72 h post-MCAO. Thus Semax supports earlier increasing of *Bdnf* expression in the ischemic tissue and helps to retain high level of *Bdnf* mRNA to the point of 72 h after occlusion. C-terminal PGP tripeptide also increases *Bdnf* mRNA expression 3 h after ischemia but then *Bdnf* expression returned to control level.

P0871. A microdeletion on chromosome 1q21 is required but not sufficient for development of thrombocytopenia-absent radii (TAR) syndrome

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Thrombocytopenia-absent-radius (TAR) syndrome is a rare congenital disorder with an incident of 1-2 in a million. TAR syndrome is characterized by hypomegakaryocytic thrombocytopenia and bilateral radial aplasia in the presence of both thumbs. Other frequent associations are congenital heart disease and a high incidence of cow's milk intolerance. The molecular basis as well as the inheritance pattern for this disorder is still ill-defined.
Here, we present evidence that a microdeletion of about 200 kb on chromosome 1q21 encompassing about 11 annotated genes is essential for developing TAR syndrome. We tested 32 individuals affected by TAR syndrome from 30 unrelated families by submegabase microarray-based comparative genomic hybridization (array-CGH), fluorescence in situ-hybridization (FISH), or quantitative RT-PCR and detected the microdeletion in all samples tested. The absence of this deletion in a cohort of control individuals argues for a specific role of the microdeletion in the pathogenesis of TAR syndrome. The microdeletion occurred *de novo* in about 25% of pedigrees analyzed. Intriguingly, in the other pedigrees inheritance of the deletion along the maternal as well as the paternal line was observed. The deletion was also present in additional unaffected family members spanning up to three generations. Thus, it is obvious that the occurrence of the microdeletion is required but not sufficient to cause TAR syndrome. We therefore conclude that TAR-syndrome has to be considered a genetically complex disorder rather than a monogenic disorder, suggesting the presence of a yet to be identified modifier of TAR (mTAR).

P0872. The *tau* H2 haplotype contribute to susceptibility to Parkinson disease in a Southern Italy population.

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There are many evidences that tau protein is involved in common neurodegenerative pathways, and a number of association studies have been conducted to clarify the role of the *tau* gene in neurodegenerative diseases, including Parkinson's disease (PD). A contribution of the H1 haplotype to PD susceptibility was suggested. Several polymorphisms localized along the entire *tau* gene length are inherited in complete linkage disequilibrium as 2 distinct extended haplotypes designated H1 and H2, respectively. Here, we investigated the distribution of *tau* haplotypes in a group of 262 sporadic PD patients from Southern Italy compared with 197 healthy controls from the same area.
We reconstructed *tau* haplotypes genotyping 3 SNPs (BanII in exon 3, MspI in exon 9, AluI in exon 11) and a dinucleotide polymorphism in intron 9. Of interest, we found a significant overrepresentation of the H2 haplotype in PD patients (OD 2.26 - 95% CI 1.64-3.18), suggesting that the H2 haplotype is a risk factor for parkinsonism in our sample. Southern Italy population appears different from most of other Caucasian populations in which, on the contrary, the *tau* H1 haplotype contribute to susceptibility to Parkinson's disease.

P0873. Genetic screening for beta-thalassemia point mutations using two-steps effective approach

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The occurrence of β-thalassemia in Romania is not so common. Taking into account that thalassemia demography is in continuous changing, a developing country should handle with its prevention. A first stage to this objective is to characterize the molecular spectrum of β-globin gene mutation in the respective population. One hundred and twenty eight β-thalassemic chromosomes derived from ninety-four β-thalassemia carrier and seventeen homozygous patients were investigated for their β-globin mutations. Molecular screening approach was performed in two steps: indirect scanning by DGGE followed by direct PCR based methods - ARMS and PCR-RFLP. Applying this effective approach, we were able to find a total of 12 mutations: IVS I-110 (G-A)-43 chr., IVS I-6 (T-C)-21 chr., Cd 39 (C-T)-17 chr., IVS II-745 (C-G)-15 chr., IVS I-1 (G-A)-9 chr., Cd 6 (-A)-5 chr., -87 (C-G)-4 chr., Cd 8 (-AA)-4 chr., Cd 5 (-CT)-3 chr., +22(G-A)-1 chr., Poly A (A-G)-1 chr., Cd 51 (-C)-1 chr. Besides these mutations, DGGE reveals four uncharacterized mutations which remain to be sequenced. All the identified mutations, less cd 51 (-C), are of Mediterranean origin which demonstrates a gene flow from that area.
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P0874. Genetic Polymorphism in Deep Vein Thrombosis

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A deep vein thrombosis (DVT) is a blood clot (thrombus) that develops in a deep vein, usually in the lower leg. Factor V Leiden (FV Leiden) (G1691A) mutation is one of the most important genetic factors causes deep vein thrombosis. Another genetic thrombosis reason thought to cause venous thrombosis tendency is Prothrombin Factor II (FII) (G20210A) mutation. Some study reports on hyperhomosisteinemia being a risk factor in DVT, made us think searching Methylenetetrahydrofolate reductase (MTHFR) (C677T) gene polymorphism in this patient group might be significant.
In our study 27 patients diagnosed to be DVT by Cardiovascular Surgery experts and 31 healthy controls were investigated. In all cases FV Leiden, FII and MTHFR gene polymorphism investigations were performed by PCR-ELISA method.
Even though the result of genetic analysis performed for the frequency of FV Leiden mutation and MTHFR gene polymorphism were detected to be high in the patient group, it was not found to be statistically meaningful ($p>0,05$). FII mutation was found to be significantly high in the patient group ($p<0,05$).
Generally genetic predisposition for thrombosis is clinically silent unless additional environmental factors interfere. In this study it was aimed to evaluate distrubution of genetic venous thrombosis in patients with DVT. In the performed genetic investigation, no significant association were detected between DVT and FV Leiden mutation and MTHFR gene polymorphism, whereas prothrombin mutation detected to show a positive association.These data are the first results of the planned comprehensive study, and were evaluated with the similar studies in the literature.

P0875. Frequency and forms of thrombophilies at patients with syndrome of loss of fetus in Kazakh population

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The aim of this research is the determine the frequency and forms of the genetically forms of thrombophilies at patients with syndrome of loss of fetus in Kazakh population.

We investigated the genotype's frequencies of C677T mutation of MTHFR gene, G20210A mutation of prothrombin gene and Leiden mutation at 100 kazakh patients with loss of fetus in anamnesis and 100 healthy female for controls. The genetically forms of thrombophilies was founded at 53% patients. Thus C677T mutation was founded at 41% patients, from them a 37% women has a heterozygous forms and 4% patients has a homozygous forms. The isolated form of this mutation was founded at 28% patients and in combination with other mutations -at 13%. The C677T mutation was founded at 24% patients of control group, from them at 23% are heterozygous forms and at 1% patients are homozygous forms. The Leiden mutation was founded at 9% patients and all causes were heterozygous of these mutations. The isolated form was founded at 4% patients and in combination with other mutations -at 5%. Leiden mutation was founded at 3% patients of controls. The G20210A mutation of prothrombin gene was founded at 4% patients, and all causes were heterozygous. The isolated form was founded at 2% patients and in combination with other mutations -at 2%. In controls this mutation was not founded. The combinations two and even of three defects of thrombophilies was founded at 18% patients and in control group was founded only isolated forms.

P0876. Functional characterization of a novel mutation in TITF-1 in a patient with Benign Hereditary Chorea

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Benign hereditary chorea (BHC) is an autosomal dominant disorder of early onset characterised by non progressive choreic movements with normal cognitive function occasionally associated with hypothyroidism and respiratory problems. Numerous pieces of evidence links BHC with TITF-1/NKX2.1 gene mutations. We studied a patient with a familial Benign Hereditary Chorea and normal thyroid and respiratory function. Sequence analysis of TITF-1 exon 1, 2 and 3 of proband's DNA revealed the presence of a heterozygous C>T variation resulting in a substitution of a highly conserved aminoacid for a stop codon in the protein homeodomain. A functional analysis shows that the mutated TTF-1 neither binds DNA, nor activates a canonical thyroid target gene promoter and does not interfere with the ability of wildtype TTF-1 to activate transcription. In addition, the mutated protein is predominantly cytoplasmic, rather than nuclear as occurs with the wildtype TTF-1. The results show that the mutation leads to a haploinsufficiency of TITF-1 and opens the question of genotype/phenotype correlation.

P0877. Molecular studies on the titinopathies TMD/LGMD2J

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Tibial muscular dystrophy (TMD) is a dominant late onset distal myopathy, caused by mutations in the C-terminal (M-line) part of the sarcomeric protein titin. In homozygotes, the same mutations lead to the different, more severe limb-girdle muscular dystrophy 2J (LGMD2J). In Finland, TMD/LGMD2J is caused by the FINmaj mutation, exchanging 4 amino acids in the titin M10 domain. Other missense and nonsense mutations in M-line titin cause TMD/LGMD2J outside the Finnish population.

The molecular pathways behind TMD/LGMD2J are unknown, but muscle selectivity and normal sarcomere ultrastructure suggest a defect in signalling functions of titin rather than structural disruption of the sarcomeres. Loss of protein interactions of C-terminal titin is likely, caused by direct disruption of the binding or by cleavage of the entire titin C-terminus. Our aim is to elucidate the molecular pathomechanism of TMD/LGMD2J by identifying the protein interactions disrupted and by determining the effect of the mutations on the stability of C-terminal titin.

In a yeast two-hybrid (Y2H) screen, myospryn (CMYA5) and phosphoglucomutase 1 (PGM1) among others were identified as potential ligands of the M10 domain. Further two-hybrid and protein chemical studies have been performed to confirm the interactions. At least the titin-myospryn interaction seems genuine, as it is disrupted in the Y2H system by the FINmaj mutation, but conclusive evidence from protein chemical studies is still pending.

Several lines of evidence support that proteolytic processing of C-terminal titin is altered in TMD/LGMD2J, potentially extending the effect of the disease mutations to a larger region in M-line titin.

P0878. Investigating Factor V Leiden, Prothrombin mutations and Methylenetetrahydrofolate Reductase gene polymorphism in newborns

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Among childhood age groups, the term trombosis most frequently seen is newborn term. Trombosis is multifactorial. In this study it is aimed to evaluate distrubution of genetic venous thrombosis risk factors in newborns with Intrauterine Growth Retardation and in premature newborns. Due to this, Factor V Leiden (FV Leiden) (G1691A), Prothrombin (FII) (G20210A) mutations and Methylenetetrahydrofolate reductase (MTHFR) (C677T) gene polymorphism were investigated.

96 newborn babies were included in our study. Babies included in the study were divided into four groups. These groups were as following: 1.Term and IUGR (n=10), 2. Preterm and IUGR (n=15), 3. Preterm and AGA (n=29), 4. Term and AGA (control group) (n=42). In all groups, FV Leiden, FII mutations and MTHFR gene polymorphism investigations were performed by PCR-ELISA method.

When the genetic investigation results of the groups were evaluated, highest muation frequencies were detected to be for FV Leiden mutation in group III, for FII mutation in group IV and for MTHFR (C677T) gene polymorphism in group I.

The control group and the three case groups were compared for FV Leiden and FII gene mutations and no significant differences were found ($p>0.05$), whereas among the control group and the three case groups statistically significant difference was found for MTHFR gene polymorphism ($p<0.05$). These data are the first results of the planned comprehensive study, and were evaluated with the similar studies in the literature.

P0879. Sequence analysis of TSPY repetitive gene in male with impaired fertility and patients with seminoma

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The human TSPY gene family is situated in the MSY region on the Y chromosome. The role of the TSPY gene in spermatogenesis and tumourogenesis has recently being intensively investigated.

The aim of this study (supported by IGA MZ CR NR/7821-3) was TSPY gene sequence comparison in DNA from patients with testicular tumour, infertile patients and controls.

Genomic DNA samples from 51 infertile patients, 6 seminomas and 10 controls were sequenced in whole gene.

There were found statistically significant differences in both the infertile group and the patients with tumour.

Comparision of infertile patients to controls: $p < 0.001$

Breakdown Table of Descriptive Statistics N=61 (No missing data in dep. var. list)			
Infertility	Mean	N	SE
0	3.412669	10	0.251166
1	2.064455	51	0.149818
All Grps	2.285473	61	0.146088

Comparision of patients with seminoma to controls: $p < 0.001$

Breakdown Table of Descriptive Statistics N=16 (No missing data in dep. var. list)			
Seminoma	Mean	N	SE
0	3.412669	10	0.251166
1	1.666862	6	0.249400
All Grps	2.757991	16	0.281166

Sequence analyses surprisingly revealed generally higher occurrence of SNPs in

controls, which supports the hypothesis on possible emergence of particular lineages with reduced function, which affects fertility and tumour genesis. The number of SNPs alone has obviously no influence on pathogenesis. Surely there could play a great role particular representation and combination of SNPs. It is important to realize, that the male lineage haplotype structure is changing rarely except for natural genome mutability.

P0880. Abnormal cortical lamination with brain stem and vermis hypoplasia resulting from *TUBA3* mutations

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Developmental defects of cortical lamination are severe brain malformations characterized by abnormal gyral pattern ranging from focal cortical dysgenesia to agyria-pachygyria. These malformations can result from abnormal neuronal migration related to mutations within several genes, namely *LIS1, DCX, ARX, RELN* and *VLDLR*. The *TUBA3* gene encoding the α-Tubulin has recently been shown to be involved in two patients presenting with agyria-pachygyria associated to partial *corpus callosum* agenesis and pontocerebellar hypoplasia. We report here two additional unrelated cases with *TUBA3* mutations. The first case is a 30 months old girl presenting with severe mental retardation, early onset seizures and bilateral ptosis. MRI revealed partial *corpus callosum* agenesis, periventricular laminar heterotopia, brain stem and vermis hypoplasia. In the second case, foetal ultrasonography at 34 WG showed microcephaly with complete agyria, severe ventricular dilatation in addition to brain stem and vermis hypoplasia. Medical termination of the pregnancy was performed at 36 WG according to the French law. Neuropathological examination revealed absence of cortical lamination combined with severe hypoplasia and disorganisation of basal ganglia. Brain stem nuclei were absent. The cerebellum was strongly dysplastic with failure of Purkinje cells migration. Screening of the entire coding region of the *TUBA3* gene lead to the identification of two *de novo* missense mutations, c.562A>C (p.Ile188Leu) and c.908T>G (p.Val303Gly) in case 1 and case 2, respectively. These two new cases suggest that the presence of *TUBA3* mutations should be considered in abnormal cortical lamination associated with brain stem and cerebellum hypo-dysplasia.

P0881. Evaluation of p16^{INK4A}, Cytokeratins and HPV-mRNA as potential markers for lymph node micrometastases and occult tumor cells

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In patients with cervical cancer, lymph nodes metastases represent the most important prognostic factor for recurrence. However, 15% of patients suffer from recurrent disease although their lymph nodes were free from histologically evident metastases (pN0). The role of occult tumour cells in cervical lymph nodes is still unknown and is currently being addressed in several studies. We have assessed an immunhistochemical approach (IHC) and reverse transcription nested PCR (RT-PCR) for the detection of metastatic tumour cells in sentinel lymph nodes (SLN). IHC was done with a pan-reactive antibody against cytokeratins (AE1/3), an antibody against CK19 and an antibody against p16^{INK4A}. The latter protein is invariably upregulated in cervical cancers and is a surrogate marker of viral oncogene activity. Viral oncogene activity (HPV-mRNA) was also directly detected by RT-PCR. A total of 120 SLN from 48 patients were analysed. There was perfect agreement among all IHC markers, except CK19, for the detection of metastases and micrometastases. However, considerable discordance was observed for all markers for the detection of tumour cell clusters (<0.2mm), multiple tumour cells and isolated tumour cells. As expected, disagreement was most evident at the single cell level. Discordance was also observed when comparing IHC with RT-PCR. This pilot study clearly shows that a single marker is not specific enough for the reliable detection of occult tumour cells. Moreover, the non-random distribution of tumour cells in lymph nodes requires multiple sectioning to achieve high sensitivity. In this regard molecular markers detected at the RNA level provide an obvious advantage.

P0882. Tumor necrosis factor-α promotor polymorphisms and type 1 diabetes mellitus

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Aim: Type 1 diabetes mellitus (T1DM) is complex, heterogenous disease initiated by an autoimmune response against pancreatic β cells and followed by progressive defect of insulin secretion from β cells. Tumor necrosis factor (TNF-α) is a potent cytokine, with a wide range of proinflamatory activities that has been implicated in the pathogenesis of many kinds of inflammatory or autoimmune diseases. Therefore we tested an association of two single nucleotide polymorphisms (SNP) in promoter region of TNF-α gene with the susceptibility to T1DM in the Dalmatian population.
Materials and methods: 229 T1DM patients and 445 controls were genotyped for G-238A and G-308A promoter variants of TNF-α gene. The PCR products were digested with *MspI* to detect TNF-α -238A allele and *NcoI* to detect TNF-α -308A allele. Haplotype analysis of these two SNPs was made using the statistical program EH+. Genotype and haplotype association and susceptibility to T1DM was further analyzed using chi-square test.
Results: A genotype distribution of G-238A and G-308A TNF-α promoter variants did not differ between the T1DM patients and the controls (p=0.147, p=0.119, respectively). However the TNF-α -238A minor allele was significantly more frequent in the controls (p=0.046). Furthermore, a specific haplotype (-238G, -308A) was observed more often but not quite significantly in the T1DM patients (p=0.069) indicating its possible role as a risk factor.
Conclusions: Our findings indicate that haplotype combination (-238G, -308A) of TNF-α gene may contribute to the susceptibility to T1DM in the Dalmatian population.

P0883. Determination of the pathogenicity of USH2A and MYO7A genes variations.

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Abnormalities of pre-mRNA splicing are increasingly recognized as an important mechanism through which gene mutations cause disease. It has been shown that intronic or exonic enhancer (ISE/ESE) or silencer (ISS/ESS) *cis*-regulatory elements are required for splice site (ss) recognition. Consequently, nonsense, missense and even silent mutations could be a novel form of splicing mutations. Numerous intronic and exonic variations for which the pathogenic effect is not well established were found in the *MYO7A* and *USH2A* genes responsible for Usher syndrome. In this study, the putative effect on splicing of some of these variations was determined. As specific transcripts analysis was not possible in these patients, we use a splicing reporter minigene system. We first concentrated on intronic variations localized inside or outside the canonical splice sites. Several mutations revealed a splicing alteration. For instance, a mutation localized in position -14 and predicted to create a novel 3'ss, was found to activate a downstream exonic cryptic splice site. Another one, localized in position +3 and not predicted to alter the splicing by *in silico* tools, induces an exon skipping. Secondly, we evaluated the effect of missense or silent mutations predicted to alter ESE sequences, in particular SF2/ASF sites, by measuring the ability of these predicted ESE to rescue splicing of an enhancer-dependent exon using a fluorescent reporter minigene. These results are of great help to assess the pathogenicity of unclassified variants and will also contribute to a better understanding of the splicing regulatory mechanisms in genes involved in Usher syndrome.

P0884. Genetic screening of 1500 warfarin receiving patients

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Warfarin (Coumadin, Marevan, Waran) is the most widely prescribed anticoagulant drug but is very difficult to administer because of its high inter-individual dose variation and its narrow therapeutic range. Moreover, warfarin treatment may lead to bleeding, an adverse drug response in around 5% patients.

In a cohort study with 201 Swedish patients, we examined 850 single nucleotide polymorphisms (SNPs) in 30 genes involving warfarin action and metabolism. Common variants in *VKORC1, CYP2C9, PROC,* age, bodyweight, diagnosis and other medications were used in a regression model that could explain 62% of the variance in warfarin dose. However, our results suggested that *GGCX*, *EPHX1*, and *ORM1* may be additional predictors but their contribution needed replication in an independent as well as larger cohort.

We selected to test 29 genes on 1518 warfarin receiving patients who were recruited in the National WARG (Warfarin Genetics) project in Sweden (http://www.druggene.org/), and type a total of 201 tag SNPs. Most patients had been monitored for at least 3 months, and the average weekly warfarin dose ranged from 7mg to 82mg.

Among the 29 genes tested, *VKORC1* demonstrated the strongest correlation with warfarin dose with the most significant SNP located in the extended promoter region. The study also replicated the effect of the *CYP2C9**2 and *CYP2C9**3 alleles and their role in poor metabolising patients. We are currently accessing the impact from the other genetic and non-genetic factors which will be presented at the meeting.

P0885. Molecular pathology of Menkes and Wilson diseases in Czech Republic

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Copper plays an essential role in biology as a cofactor for many enzymes. There are two intracellular copper binding P-ATPases in humans: ATP7A, connected with X linked Menkes disease, and ATP7B, connected with autosomally recessive inherited Wilson disease. Here we report the mutational analysis of the ATP7A and ATP7B genes of 3 patients with Menkes disease and 101 patients with Wilson disease from the Czech Republic.

Genomic DNA was used to amplify 23 exons of ATP7A gene and 21 exons of ATP7B gene. PCR products were examined by RFLP and automatically sequenced.

Molecular analysis revealed 3 mutations in ATP7A gene, two of which have not been previously published (Q724X and E1249X), and 14 mutations in the ATP7B gene (including the H1069Q mutation, prevalent in Central Europe, and the newly found A1135T mutation).

Molecular analysis of the ATP7A gene allows for genetic counseling and prenatal diagnosis in families affected by Menkes disease. Screening of the prevalent H1069Q mutation in ATP7B gene shows, that the frequency- 36,6% of analysed alleles- is in accordance with its occurrence in Central Europe.

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P0886. Another side on Wilson disease

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Wilson disease is an inherited disorder of copper metabolism, characterized by liver cirrhosis and CNS involvement. DNA analysis of Wilson disease (WD) gene *ATP7B* had numerous advantages to clinical confirmation of the diagnosis. Since 2004 sixty four patients were referred for the DNA analysis with WD suggestive symptoms. The most common mutation H1069Q of *ATP7B* gene was tested for all of them. Gene sequencing was performed for ten patients, who were heterozygous carriers for mutation H1069Q or WD point score exceeded 3. Twelve patients were confirmed homozygous carriers of H1069Q mutation, one was compound heterozygous H1069Q/ 3106G>A. Cascade screening revealed 11 heterozygous carriers of H1069Q mutation. In a very short period of time diagnosis of Wilson disease was confirmed in 13 patients of Latvia, thus indirectly leading to conclusion about more common H1069Q mutation presence in Eastern Europe.

From the identified patients, pre-symptomatic diagnosis was confirmed in one patient, thus preserving the patient from irreversible liver of brain damage.

On the basis of clinical information from patients with confirmed diagnosis by DNA analysis, Wilson disease diagnostics criteria were re-evaluated and more impact was addressed towards diagnosing disease at an early stage for the better therapeutic effect. From WD scoring system the most valuable information comes from ceruplasmin level in blood and copper level in 24 hour urine.

There were no patients observed with Kayser-Flesischer ring and neurological symptoms were not suggestive.

P0887. Extremely skewed X-chromosome inactivation in juvenile idiopathic arthritis

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Juvenile idiopathic arthritis (JIA) is the most common childhood rheumatic disease with an incidence between 7-21/100,000. The genetic basis of JIA is unknown. It rarely manifests familial recurrence. But the monozygotic twin data suggest that there is a considerable genetic basis, which is likely to involve multiple epigenetic events (*Autoimmun Rev*, 5:279, 2006). We identified an association between extremely skewed X-chromosome inactivation (XCI) and female predisposition to autoimmunity (*Arth&Rheum*, 52:1564, 2005*; Eur J Hum Gene*t 14:791, 2006). Since JIA is thought to have an autoimmune etiology, we studied the XCI patterns of 72 female patients diagnosed with JIA and 183 female controls. The control group comprised of newborns (n=91) and children with no history of an autoimmune condition (n=92). To determine XCI status, androgen receptor locus was analyzed by methylation sensitive *Hpa* II digestion followed by PCR. A male control (46, XY) was used for complete digestion. Extremely skewed (>90% skewing) XCI was observed in DNA from the peripheral blood cells in 9 of 56 informative patients (16.1%), and in 4 of 124 informative controls (3.2%, P=0.0039). When patients and controls with 80-89 percent skewing were also included in the analysis, 14 patients (25.0%) and 12 controls (9.7%, P=0.0108) displayed skewed XCI. These results show that there is a significant association (odds ration 16.9, 95% confidence interval 6.2-45.8) between extremely skewed XCI and JIA. "Loss of mosaicism" for X-linked gene expression could be considered as a potential mechanism in break-down of self tolerance. Supported by grants from TUBITAK-SBAG 3334, ICGEB-CRP/TUR04-01.

P0888. Transcriptional effects of mutations in the XLMR gene JARID1C

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Moderate to severe X-linked mental retardation (XLMR) affects approximately 2 in 10.000 males. Non-syndromic XLMR (NS-XLMR) in which mental retardation is the only clinically consistent feature is genetically heterogeneous and mutations have been found in >20 different genes. One of these is the transcription factor JARID1C, where mutations have been found in 13 families, 12 of which belong to the cohort of the Euro-MRX Consortium. In order to investigate transcriptional effects of JARID1C deficiency we first performed genome-wide expression analysis of mRNA from a patient cell line with a premature termination codon in JARID1C and 3 controls, using a Sentrix Human-6 Expression Beadchip together with the BeadStudio analysis software (Illumina). This investigation revealed consistent differential expression between the JARID1C deficient cell line and each of the 3 controls in 55 genes. In a second set of experiments we were able to verify the expression pattern for 25 genes by quantitative RT-PCR or Northern blot analysis using RNA from all 12 Euro-MRX patient cell lines and 5 controls. Our results revealed among others up-regulation

of MYC and MKNK2 and down-regulation of TNFSF4, and show that JARID1C missense changes and nonsense mutations had an equal impact on transcription. Therefore our results suggest that RNA expression profiling in XLMR mutation carriers might be a tool for the identification of putative pathways which play a role in cognition. Furthermore they show that this approach could in general be useful for refining the molecular diagnosis of patients with disorders where mRNA expression is affected.

P0889. Comprehensive survey of mutations in RP2 and RPGR in patients affected with distinct retinal dystrophies: Genotype-phenotype correlations and impact for genetic counselling

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X-linked forms of RP (XLRP) account for 10-20 % of families with RP and are mainly accounted for by mutations in the RP2 or RPGR genes. The purpose of this study was to give a comprehensive survey of mutations in these two genes in 93 familial cases of RP suggesting X-linked inheritance (48/93 families with expression in females but no male to male transmission), 7 male sibships of RP, 25 sporadic male cases of RP and 2 cone dystrophies (COD).

RP2 and RPGR mutations were identified in 15.9% and 74.8% of familial cases of RP suggestive of X-linked transmission, respectively. These frequencies are in accordance to that reported previously (RP2: 6-20 %; RPGR: 55-90%). Interestingly, while more than 99% of women harbouring RP2 mutations do not develop RP, in 40.6% of families segregating RPGR mutations, at least one carrier woman was affected with a severe form of the disease.

RP2 or RPGR mutationsWe show that about 30% of male sporadic cases and 30% of male sibships of RP carried , confirming the pertinence of the genetic screening of XLRP genes in male patients affected with RP commencing in the first decade and leading to profound visual impairment before the age of 30.

These data along with *i)* the respective frequencies of mutations in the RP2 and RPGR (exon1-14 and ORF15) genes and *ii)* the identification of highly recurrent mutations, allowed the drawing of a decision-making flowchart for the molecular diagnosis in families segregating RP.

P0890. Duplication of the *MECP2* gene region is a frequent cause of mental retardation in males.

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X-linked mental retardation (XLMR) is a very heterogenous disorder. Until recently mutations in large number of genes have been identified to cause XLMR. One of these genes is *MECP2,* its mutations and large deletions are responsible for most of the Rett syndrome cases among females. The phenotypes resulting from *MECP2* mutations in males are variable and depend on the mutation severity (from lethal neonatal encephalopathy to mild MR). The search for microdeletions and microduplications on X chromosome by array-CGH led to identification of the whole *MECP2* region duplications in males with severe MR and neurological symptomes.

We decided to screen for *MECP2* duplications in mentaly retarded patients with additonal clinical findings. Dosage analysis of the *MECP2* gene and flanking regions was performed by multiplex ligation-dependent probe amplification (MLPA) method.

In a preliminary study we have analysed 33 patients and identified 2 duplications. In patient 1 the duplication involves also *IRAK1* gene. In patient 2 the duplication is larger and encompasses *IRAK1*, *L1CAM, IDH3G* and *SLC6A8* (11Kb, 160 Kb, 236Kb and 507 Kb upstream to *MECP2* respectively). We also included in the group of *MECP2* duplicated patients a third patient identified by array-CGH. In patient 3 the duplication involves *IRAK1* and *L1CAM*.

Our results suggest the high frequency of *MECP2* duplication among mentally retarded males. However, the clinical phenotype is not similar. The only common feature is severe MR. After finishing the studies in a larger group of patients we are planning to include this analysis in routine diagnostics of MR.

Po06. Genetic analysis, linkage, and association

P0892. Application of the 2-locus TDT for testing NOD1 and NOD2 effects on Crohn's disease

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Crohn's disease (CD) is a multifactorial disease caused by the interplay of multiple genetic and environment factors. The NOD proteins: NOD1 (encoded by *CARD4*) located on 7p14 and NOD2 (encoded by *CARD15*) located on 16q21, are very similar in composition and both activate the nuclear factor kappa B pathway and cell death in response to bacterial lipopolysaccharide.

Up to date, only NOD2 has been definitively associated with CD. However, the involvement of NOD1 in CD remains controversial and uncertain. In the absence of noticeable main effect of NOD1 and considering the fact that the two genes are recognized as key elements of the metabolic pathway involved in the CD pathogenesis, we propose to apply the 2-locus TDT for NOD1 and NOD2 genes.

In fact, we have recently developed a new method: the 2-locus TDT for detecting susceptibility genes with weak or no marginal effect. In such a situation, we have shown that our method is particularly powerful for detecting the effect of two genes through their interaction.

A total of 1252 individuals (390 CD families composed of 308 trios and 82 ASP) were genotyped for NOD1 rs 2075822 and 3 SNPs (R675W, G881R and 980fs) on NOD2 by TaqMan and direct sequencing. The 2-locus TDT is achieved in two steps. First, we estimate the penetrances using the transmitted and non-transmitted parent gametes. Secondly, we test the fit of these estimates to an independent effect of the two loci. Results will be presented at the meeting.

P0893. Novel mutation in *CUL7* in Yakut patients with 3-M syndrome

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We identified 37 families with short stature prevalent in the Republic of Sakha (Yakutia) in Russia. Clinical features are resembles a rare autosomal recessive disorder 3-M syndrome characterized by sever pre-and postnatal growth retardation, facial dysmorphism, normal intelligence, and characteristic radiological findings (MIM 273750). We compared with clinical presentation of 3-M syndrome described so far, Yakut families had a distinct feature, that is 41.9% of patients had severe respiratory distress at birth. A genome-wide linkage analysis for these families revealed linkage to region 6p21.1 with the highest multipoint LOD score of 24.6 at D6S282. In the critical region of 3.1 cM between D6S1552 and D6S451, we found *CUL7*, that has just been identified as a causative gene for 3-M syndrome and 25 mutations in *CUL7* have been identified in 29 families with 3-M syndrome originated from Tunisia, Morocco, France, Algeria, Syria, Portugal, Sri Lanka, Turkey, Germany, Austria, Italy, Surinam, India, and Brazil (Huber et al., 2005). Mutational analysis revealed a novel homozygous mutation 4582 ins T in exon 25 in *CUL7*, resulting in a frameshift and subsequent premature stop codon at 1533 (Q1533X) in all 43 patients. Haplotype analysis revealed a strong linkage disequilibrium spanning 623kb, suggesting a strong founder effect in Yakut patients with 3-M syndrome.

P0894. The influence of the ACE gene polymorphism on personality traits of athletes

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It is well known that a I/D polymorphism of the human angiotensin-1-converting enzyme (ACE) gene is associated with ACE activity and endurance. The I allele is low active and traditionally is connected with high endurance during physical performance. Recently it is occurred publications about influence of the ACE polymorphism on other physiological features, and -the most intriguing- on emotional spheres. It was shown that ACE has influence on personality characteristics (novelty seeking) in females.

The present study examined the possibility ACE I/D functional polymorphism might be associated with particular personality traits of athletes. In current investigation 204 athletes (synchronized swimming, swimmers, footballers, hockey players, and skiers) participated. All persons have the sports category from the first sports up to the master of the international class. Control group consisting of non-trained persons (N=105). Firstly we study I -and D-alleles distribution in various groups and discovered the considerable differences in allele frequency. Part of persons was tested with psychological questioner (Buss-Durkee Hostility Inventory) to determine the level of different forms of aggression. Our findings are that the ACE I/D polymorphism of synchronized swimming (females) is clearly associated with the physical aggression.

P0895. No Association Between the DAT1 10-Repeat Allele and ADHD in the Iranian Population

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Association studies between attention deficit hyperactivity disorder (ADHD) and the 10-repeat allele of a polymorphism (a 40 bp variable number of tandem repeats) in the dopamine transporter gene (DAT1) have resulted in mixed findings in different populations. We performed a case/control study to clarify the contribution of this allele with ADHD in the Iranian population. No association was observed between the 10-allele and disease (Chi 2 = 0.081, p<0.9). Furthermore, no significant difference was observed in the homozygosity of this allele between the case and control groups (Chi 2 = 0.022, p<0.9). Implication of the dopamine transporter gene in the pathophysiology of ADHD warrants investigation of other functional polymorphisms within this gene in the Iranian ADHD patients.

P0896. Increasing the mutation detection rate for Autosomal Dominant Hereditary Spastic Paraplegia: screening *SPG3a*, *NIPA1*, and *REEP1*.

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Hereditary spastic paraplegia (HSP) is a heterogeneous group of disorders characterised by progressive lower limb spasticity, hyperreflexia and weakness. The autosomal dominant form of the disease (AD-HSP) accounts for approximately 70-80% of HSP families and by sequencing *SPG4* we currently detect a mutation in approximately 29% of families (58 in 202). Mutations detected include novel missense, nonsense, small insertions, deletions and duplications. One mutation has been found in 6 apparently unrelated individuals, suggesting a possible founder effect, and in one case a modifying mutation acted in concert with a truncating mutation to lead to early onset in a patient.

To increase the mutation detection rate, we have implemented MLPA for *SPG4* and *SPG3a* and have screened 36 patients, finding an exon 1 deletion and three aberrations due to mutations in the probe binding sites. We are now offering sequencing of the Atlastin (*SPG3a*), *NIPA1* (SPG6) and *REEP1* (SPG31) genes. Atlastin has been reported to account for 20-30% of AD-HSP cases and a high percentage of early onset cases (we have detected *SPG3a* mutations in two families). *NIPA1* and *REEP1* have not been extensively studied to date and both are small genes with 5 and 7 exons respectively. A UK family has been shown to harbour a *NIPA1* mutation (Reed *et al* 2005) and mutations in *REEP1* have been shown to account for around 6.5% of cases of AD-HSP (Zuchner *et al*., 2006).

After implementation of these services, we hope to increase our detection rate for AD-HSP to around 70%.

P0897. Role of HLA in Anti-Endothelial Cell antibody positive Indian SLE patients

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Anti-endothelial cell antibodies (AECA) are heterogeneous group of antibodies against a variety of antigenic determinants of endothelial cells (EC). AECA plays an immunopathogenic role in triggering EC activation leading to vascular damage. The presence and the strength of AECA have been found to correlate with disease activity in various systemic vasculitic diseases like systemic lupus erythematosus. To assess the involvement of HLA alleles in AECA production, 45 clinically and histopathologically proven cases of class IV lupus nephritis were studied for their HLA A and HLA B alleles by standard NIH microlymphocytotoxicity assay. All patients fulfilled ARA classification criteria for SLE. AECA were detected by indirect immunofluorescence using cultured human umbilical vein endothelial cells (HUVEC). Forty percent of the SLE patients possessed the AECA antibodies. The HLA alleles A9 (24) (OR=2.90, EF=0.29,p value 0.08) and B21 (OR=74, EF= 0.11, p value 0.038) were significantly increased while HLA A1 (OR=0.27, PF= 0.35, p value 0.039) and B40 (OR= 0.29, PF= 0.25, p value 0.076) were significantly reduced among AECA positive SLE patients when compared with AECA Negative patients. Further two-locus haplotype analysis revealed that A19-B35, A3-B21, and A28-B21 were observed with significant T value among AECA positive patients. The common clinical symptoms among the AECA positive patients observed were lupus nephritis (84%), involvement of skin (22%), involvement of joints (17%) and CNS as well as hematological involvement (11%). Our findings suggest that immunogenetic mechanism may be involved in AECA antibody production leading to the immunopathogeneis in a subset of SLE patients.

P0898. The association of regulatory genes' polymorphisms with aerobic and anaerobic performance of athletes

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The aim of the study was to investigate an allelic distribution of *PPARA* (G/C polymorphism), *PPARG* (Pro/Ala), *PPARD* (+294T/C) and *PGC1A* (Gly482Ser) genes in rowers (*n*=205) and controls (*n*=659), and to find correlation between genotypes and physiological parameters. Genotyping was performed by restriction fragment length polymorphism analysis. Physiological parameters were evaluated by PM 3 Rower Ergometer and MetaMax 3B Gas Analyzer. The frequencies of *PPARA* G (90.1% vs 83.6%) and *PPARG* Ala (23.1% vs 16.2%) alleles in elite athletes, and of *PPARD* C (19.1% vs 10.5%) and *PGC1A* Gly (75.4% vs 66.5%) alleles in sub-elite athletes were significantly higher than in controls. Furthermore, *PPARA* G (when oxygen pulse was measured) and *PGC1A* Gly (when maximal aerobic power and anaerobic threshold (%) of VO_{2max} were measured) alleles were associated with high values of aerobic performance. Thus, *PPARA* G, *PPARG* Ala, *PPARD* C and *PGC1A* Gly alleles can be considered as genetic markers associated with enhanced physical performance.

P0899. Polymorphism of paraoxonases and catalase genes in connection with age gradation in Tatars from Russia.

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It was generally recognized that toxic effect of lipid metabolism and respiration by-products contributes to aging. Gene polymorphic structure may influence composition and activity of protein production. The aim was to investigate genes polymorphisms of paraoxonase 1 (PON1, Q192R), paraoxonase 2 (PON2, C311S) and catalase (CAT, -262C/T) in different age groups in Tatars from Bashkortostan (Russia).
We examined 1627 healthy persons: young (1-20 years), maturity (21-55 years), elderly (56-74 years), senile (75-89 years) and long-livers (90-109 years). Genotyping was performed using PCR-RFLP. Fisher's two-tailed exact rest (Statistica v. 6.0) was used for age groups comparison.
PON1*Q/*Q genotype frequency was lower in group of long-livers then among maturity (P=0.039), elderly (P=0.035) and senile (P=0.021). PON1*R/*R genotype frequency was raised among long-livers than in senile (P=0.014). PON1*R allele frequency was higher among long-livers in comparison with other age groups, excluding young (P<0.05). PON2*C/*C genotype frequency was lower among senile in comparison with young (P=0.006) and elderly (P=0.011). Using CHAID algorithm from SPSS (v. 13.0) we found that in group of 43-52 years old persons frequencies of CAT*C/*T genotype (P<0.001) and CAT*T allele (P<0.05) were reduced in comparison with groups of 1-42 and 53-109 years old persons.
Thus, we have demonstrated diversity of PON1, PON2 and CAT genes polymorphisms genotypes and alleles frequencies between different age groups. Possibly, the same polymorphic variant plays a protective role for an organism at its different age stages.

P0900. Alcoholism candidate gene polymorphisms and Alexithymia in alcoholic outpatients

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Candidate gene polymorphisms that changes alcoholism-related intermediate phenotypes include dopaminergic system polymorphic variants (DRD2 and DAT), 5-HTT gene promoter polymorphisms, Catechol-O-Methyltransferase (COMT), and monoamine oxidase A (MAO A). A recent study indicates a possible association between the COMT Val108/158Met gene polymorphism and alexithymia (Ham et al., 2005).
Alexithymia (disability to identify and to describe emotions) is an useful construct in the understanding and treatment of alcohol dependent patients. Patients with alexithymia show significant differences in psychological, behavioral and biochemical features. Alexithymia associated with depression and work problems delimited an alcoholic thymophatic subtype (Cardoso et al., 2006) in our alcoholism research unit (NETER).
Males at high genetic risk for alcoholism are more alexithymic and data suggest that MAO-A polymorphism moderates the development of psychopathology and influences vulnerability to environmental stress (Kim-Cohen et al., 2006)
The aim of the study is to investigate the relationship between alcoholism candidate gene polymorphisms and alexithymia.
Our sample (N=95) of sequential alcohol-dependent patients submitted to an outpatient therapeutical program integrated in the alcoholism unit of Santa Maria General Hospital were tested using TAS-20 scale. They were genotyped using polymerase chain reaction for 5HTLPR, 5-HTVNTR, COMT, DRD2, DAT and MAO-A polymorphisms.
We found an significant association between MAO-A polymorphism and alexithymia (factor 1 and 2 of TAS-20) indicating that alcoholic patients carrying the 4 allele tend to have a more disability in the affective-emotional process.
This data may suggest a linkage between MAO-A polymorphism and the thymophatic subtype of our clinical typology of alcohol dependent patients.

P0901. Detection of novel mutations in Alexander disease using DHPLC analysis of *GFAP* gene.

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Alexander Disease (AXD) is a severe neurological disorder, characterized by a leukodystrophy associated with Rosenthal Fibers (RFs, eosinophilic inclusions within astrocytes). This autosomal dominant disease is caused by *de novo* mutations in *Glial Fibrillary Acidic Protein* (*GFAP*) gene. Mutations seem to induce a toxic gain of function resulting in GFAP aggregation in astrocytes (RFs) and subsequent white matter suffering.
In infantile and juvenile classical forms of AXD, brain MRI criteria have been defined in order to screen patients for *GFAP* mutations. This molecular screening is usually performed by exons amplification and direct sequencing. However, numerous *GFAP* mutated patients with atypical brain imaging and unusual clinical symptoms have been described. To improve the rapidity of this screening for a larger number of patients, we developed a new protocol based on DHPLC analysis of PCR products and selective sequencing of abnormal profiles.

During the last 2 years, 67 patients (29 index cases, sporadic and some familial forms) were entirely tested by DHPLC. 20 index cases were found mutated with 13 different mutations, 8 of which being previously reported. DHPLC detected 5 novel mutations believed to generate AXD as: mutations arose *de novo*, were absent from healthy relatives and from 100 control chromosomes, affected a conserved amino-acid and a functionally important domain, and no other change was detected. Several prenatal diagnosis have been already carried out. DHPLC screening for *GFAP* mutations is confirmed to be a rapid, sensitive and reliable, cost-effective and high-throughput method for AXD diagnosis.

P0902. The analysis of Eotaxin (CCL11) gene single nucleotide polymorphisms in allergic rhinitis patients and healthy donors from Volga-Ural region of Russia

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Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa that is characterized by sneezing, nasal congestion, and watery rhinorrhea. It is the most common form of atopic disease, affecting about 20% population in the world. Eotaxin is believed to play an important role in AR as a potent chemoattractant and activator of eosinophils and Th2 lymphocytes.

The purpose of this study was to investigate the allele and genotype frequencies of two polymorphisms (−384A>G and 67G>A) of the gene in AR patients and in controls.

The patient group consisted of 285 individuals with allergic rhinitis, the control group included 169 unrelated non-allergic individuals. Genomic DNA was extracted from peripheral blood leucocytes by standard phenol/chloroform method. Genotyping was performed by PCR followed by restriction digestion.

No significant difference was observed in allele or genotype frequencies of any SNP between AR patients and controls. The most common genotype of −384A>G polymorphism was AG, revealed in 48,16% of patients and in 53,13% of control group . The frequency of GG genotype was insignificant higher in patient (29%) than in healthy donors (23%). The GG genotype of 67G>A polymorphism was prevalent in both groups (65,96% - in the patient, 69,82% - in the control).

Thus, the data of this study has revealed negligible differences in the distribution of polymorphic alleles and genotypes of the CCL11 gene between compared groups, but not detected significantly association of single nucleotide polymorphisms of Eotaxin gene with AR in Volga-Ural region of Russia .

P0903. Whole-genome association study of Alzheimer's Disease using DNA pooling with microarrays

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Late-onset Alzheimer's Disease (LOAD) has a strong genetic component but the only susceptibility gene definitively identified to date is *APOE*. We wanted to identify further susceptibility genes by performing a whole-genome association study. In order to reduce the cost, we used a DNA pooling approach. We constructed pools of 1000 LOAD cases and 1200 age- and gender-matched controls. We used Affymetrix 250K Sty and Nsp SNP genotyping arrays, as well as Illumina HH300 and HH240S arrays. These assay a total of over 1 million SNPs, of which 80,000 are represented on both platforms. Depending on the cost and quality of the hybridisations, we replicated each pool between 4 and 15 times on each array, and used between 3 and 8 high quality replicates of each array for analysis. In order to prove that our experiment is capable of detecting true genetic association, we examined whether we were able to detect the known association at *APOE*. 7 SNPs close to *APOE* showed differences in allele frequencies of >6%, of which 4 were >10%. This work demonstrated the accuracy of our pooling method and indicates that we would have easily detected the association with *APOE*, in spite of the fact that neither of the two SNPs that determine the ApoE4 genotype is on the arrays. Two of the four arrays detected the effect, probably due to the different SNP coverage and low LD in this region, showing the potential benefit of using all arrays. We are individually genotyping the other promising loci.

P0904. Association of Long Polyglycine Tracts (GGN repeats) in Exon 1 of the Androgen Receptor Gene with Cryptorchidism and Penile Hypospadias in Iranian Patients

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Hypospadias and cryptorchidism are the two most common congenital malformations in males affecting 0.3-0.7% and 2-4%, respectively, at birth. To study the association of CAG/GGN trinucleotide repeats in the androgen receptor gene (AR gene) with cryptorchidism and hypospadias in Iranian population, we performed a comprehensive case-control study of 76 cryptorchid and 92 hypospadiac (divided into subgroups of glanular, penile, and penoscrotal hypospadias) Iranian males. The length of the CAG/GGN repeat segment was evaluated by using PCR-sequencing in exon 1 of AR gene. To eliminate other mutations in the AR gene, exons 2-8 of AR gene were screened by PCR-SSCP. Exclusion of known causes of male infertility was done by karyotype analysis, Y chromosome microdeletion analysis, cystic fibrosis transmembrane regulator gene (CFTR) mutation analysis, and INSL3/LGR8 gene mutation analysis. There were no significant differences in CAG lengths between the cases and controls but GGN numbers were found to be significantly higher (median 24 *vs.* 22) among both subjects with penile hypospadias (P = 0.018) and those with a history of cryptorchidism (P = 0.001), compared with controls. In addition, the GGN numbers among subjects with penile hypospadias were significantly different, compared with the two other subgroups of hypospadias (P = 0.001). We were able to identify 12 different CAG alleles and 8 different GGN alleles in the cryptorchid group. Although further studies are needed to elucidate the possible role of specific CAG/GGC combinations, our data suggested the possible association between polyglycin tract polymorphism in androgen receptor gene and cryptorchidism.

P0905. Allelic variants of APOA5 and its transcription factor USF1 predispose to atherogenic dyslipidemia

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Atherogenic dyslipidemia (AD) is a component of the metabolic syndrome, a well-known cluster of risk-factors for type 2 diabetes. AD is characterized by abnormally high serum triglyceride and low HDL-cholesterol levels.

The USF1 transcription factor and apolipoprotein A5 (APOA5) are key players in lipid metabolism. The two genes operate on the same pathways with USF1 known to be a major regulator of APOA5. We recently reported the association of USF1 with FCHL, a common dyslipidemia predisposing to cardiovascular disease (Pajukanta 2004) and also established the significance of USF1 in prospective population cohorts (Komulainen 2006). Multiple studies link plasma levels of APOA5 and the allelic variants of this gene with elevated plasma triglycerides.

Here we show for the first time that allelic variants of APOA5 (S19W, -1131T>C, -1464T>C) associate with AD (p=0.009) as well as its component traits, changes in plasma triglycerides (p=0.00001) and HDL-cholesterol (p=0.0001) in 130 Australian AD families (n=533). We observed a dose-dependent association of a common APOA5 haplotype with elevated triglycerides and decreased HDL-cholesterol. A protective haplotype was also observed. Conditioning for the carriership of the APOA5 risk allele, a risk haplotype of USF1 was identified, in agreement with previous studies. Importantly, individuals carrying risk haplotypes for both genes had even higher plasma triglyceride levels than those carrying either APOA5 or USF1 risk haplotype alone (p=0.001).

We thus hypothesize that the joint effect of risk alleles of APOA5 and its regulator, USF1, contribute to the pathogenesis of AD and potentially also to the metabolic syndrome.

P0906. Apolipoprotein A5 gene T1259C polymorphism associated with elevated circulating triglyceride levels but does not confer susceptibility for ischaemic stroke

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Objectives: The common apolipoprotein A5 (ApoA5) gene variants, the T1131C, T1259C, IVS3+476G→A and S19W are subjects of extensive investigations due to the central role of the ApoA5 in the triglyceride metabolism. We examined the possible pathogenic role of the T1259C in triglyceride metabolism and in development of ischaemic stroke. *Methods and results*: A total of 399 patients who had stroke events were categorized into three stroke subgroups (small-vessel, large-vessel and mixed groups), 182 healthy subjects served as controls. The ApoA5 T1259C variant was determined by PCR-RFLP test. We found elevated triglyceride levels in patients bearing 1259C allele in each subgroup. However, the serum cholesterol levels showed no difference between subjects carrying C allele and subjects with TT genotype in either group. The 1259C allele frequencies were similar in each stroke subgroups and in controls (small-vessel: 11.6; mixed: 13.4; large-vessel: 11.5; overall: 12 vs. 10.2%; $p<0.05$). Using logistic regression analyses adjusted for differences in age, gender, total serum cholesterol levels, ischaemic heart disease, bmi, hypertension, diabetes, smoke, drink and triglyceride levels we did not find direct association between carrying C allele and development of stroke. *Conclusions*: Our results suggest that bearing 1259C allele elevates triglyceride levels, but does not confer risk factor for stroke. Further investigations are needed to clarify the possible significance of the 1259C and the associated haplotypes in the development of ischaemic stroke.

P0907. Autozygosity mapping in a large Iranian pedigree with autosomal recessive mental retardation reveals linkage to the CC2D1A locus on Chromosome 19

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Little is known about the molecular basis of non-syndromic autosomal recessive mental retardation (NS-ARMR). This is partly due to insufficient family sizes and lack of consanguinity, which hinder successful mapping and identification of candidate loci in western societies. Up to now, only three genes have been found for NS-ARMR: PRSS12 (neurotrypsin1), CRBN (Cereblon) and CC2D1A (Coiled-coil and C2 Domain-containing 1A). By identifying 8 new loci for NS-ARMR we have recently provided evidence for the considerable genetic heterogeneity of this disorder, which is in keeping with the fact that so far only one mutation was found for each known gene.

During the course of our ongoing efforts in elucidating the genetic causes of ARMR we have now identified the first locus overlapping a known NS-ARMR gene. Through array based SNP genotyping (Illumina IVb panel) and linkage analysis in a large consanguineous family from Iran we observed a 14 MB linkage interval with a parametric LOD score of 3.4 on Chr19 (Chr19p12-p13.3). This locus encompasses the previously described CC2D1A gene, which encodes a putative signal transducer involved in the NF-κB pathway, and we are presently screening the coding region and exon-intron boundaries for mutations.

The possible finding of a sequence change in CC2D1A would enable us to report the first recurrence of an ARMR gene, while the detection of sequence integrity for this gene would indicate the presence of a causative mutation in a different gene and thus provide further evidence for the genetic heterogeneity of NS-ARMR.

P0908. Association study of SNPs in the PHF11 gene in Italian families with allergic asthma

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In an our previous genome wide scan for asthma in 123 Italian families, phenotyped for clinical asthma and rhinitis, skin prick test positivity to common aeroallergens, total serum IgE levels (IgE), bronchial hyperresponsiveness to methacholine, linkage on chromosome 13q14 has been detected for elevated IgE. Association of the PHF11 gene with IgE and atopic dermatitis (AD) was found in two recent studies (Nat Genet 34:181;2003; Genes Immun 6:264;2005).

We have now performed a linkage and association study of the PHF11 gene polymorphisms in a subset of 24 families (144 subjects) which have shown positive linkage for IgE. The following SNPs located inside the gene and reported to be associated with IgE and AD in the above mentioned studies were selected and analysed: b7_2 (intron1); rs2031532 (ex2); rs2247119 (intron3); rs2274276 (intron4); b4_2 (intron5); b5_2 (intron9); rs1046295 (3'UTR). These SNPs were genotyped by minisequencing (SNaPShot Multiplex kit, Applera, on ABI-PRISM 310 sequencer using Genescan software) or by enzymatic restriction.

Linkage analysis was perfomed using MERLIN software and association study was performed by Transmission Disequilibrium Test (TDT) using the unphased software: http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/. A correction for multiple test of the obtained data was applied.

Statistical analysis results confirmed our previous findings of linkage of this chromosomal region to IgE in allergic asthma, but did not show significant association of the PHF11 gene SNPs with any of the studied phenotype. In our population, association might be due to other polymorphisms of the PHF11 gene or in other genes located in this chromosomal region.

P0909. Asthma and atopy in Italian families: a genome-wide linkage analysis

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To identify genetic susceptibility regions for asthma, atopy and intermediate phenotypes, 123 Italian families with at least two affected siblings were previously screened with 396 DNA microsatellite markers (Malerba G et al., Clin Exp Allergy 1999; 29 Suppl 4:27-30). Affected sib pairs (numbers in parentheses) for the following phenotypes have been studied: atopy (170), bronchial hyper responsiveness to methacholine (BHR, 95), clinical asthma (56), elevated total serum IgE (77), rhinitis (79), skin prick test reactivity to common aeroallergens (144) and dust mite (69). Nonparametric linkage analysis has indicated suggestive linkage (nominal p-value <0.001) with at least one phenotype on 4q, 5p, 9p, 13q, and 22p chromosomal regions.

With explorative purposes we investigated the presence of linkage and gene-gene interaction by Maximized LOD score (MOD score) and conditional analysis. Parametric linkage analysis, based on optimized models, accounted also for imprinting effects. The analysis indicated possible paternal imprinting on chromosome 9p for mite sensitivity (increased MOD from 3.16 to 5.32 inferring imprinting effect).

Gene-gene interaction analysis was performed selecting the families having LOD ≥ 0 for the regions showing a LOD peak ≥ 1 in the main nonparametric linkage analysis: increased allele sharing in 16p and 7p chromosomal regions for the high level IgE phenotype has been found (LOD = 2.19).

The results suggested that complementary linkage methods might help in disclosing possible epigenetic or interactive genetic effects, undetectable when standard nonparametric linkage analysis are applied.

P0910. ALOX5 promoter polymorphism and response to leukotriene inhibitors in asthmatic patients

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Leukotrienes are a family of polyinsaturated eicosatetraeoic acids derived from the arachidonic acid. They show multiple farmacologic and physiological effects and seem to play a critical role in the pathogenesis of asthma.

Drugs that modify the action of leukotrienes have demontrated to be useful in order to control mild and moderate asthma.

In leukocytes, 5-lipoxygenase (5-LO) catalyses the conversion of arachidonic acid into leukotriene which afterwards is transformed to leu-

kotrienes B4→E4.
5-LO is coded by gene ALOX5 which has a VNTR polymorphism within the transcription factor binding region at position -147 to -176 in its promoter. In human cells in vitro, the cell lines containing other than the 5 tandem repeats allele in the promoter region of ALOX5 show diminished reporter gene transcripition
We have studied 61 patients with mild and moderate persistent asthma and previously untreated with leukotriene receptor inhibitors, the correlation between the VNTR. This patients recieved treatment with Montelukast during 6 months. The response to therapy was measured by means of the variation in FEV1, number of exacerbations during the last month and number of beta2 agonists puffs per day during the last month.
There were not differences between groups defined by the genotype before therapy; Nevertheless, after it, we found statistical differences for every parameter at the end of the study. Z score value of FEV1 of patients homozygous for the allele 4 obtained a p=0.0002 when compared to 5 allele homozygous, p=0.0098 for the demand of beta2 agonists and 0.0086 for the nr. Of exacerbations within the last month. Heterozygous patients showed values similar to those of the 5 homozygous, with the exeption the FEV1 that showed intermediate values.

P0911. Evidence for association between HTR1B and males with Autism Spectrum Disorders.

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Autism is a complex neurodevelopmental disorder characterized by qualitative impairments in communication, social interaction, and restricted and repetitive patterns of interests or behaviors. Onset is generally before 3 years of age and results in life-long disabilities for affected children. From early 1960s, when hyperserotonemia was first reported in autistic children, to the modeling of serotonergic deficits seen in ASD using animal models, several different alterations in processes involving serotonergic innervation was found in ASD patients. However, the role of serotonin receptors in its pathophisiology still remains poorly understood. In this study, we present results from a case-control association study within 14 polymorphisms in five serotonin receptors genes *(HTR1A, HTR1B, HTR1D, HTR2A, HTR2C).* Positive results were found for *HTR1B* haplotypes (p=0,0448) in a sample of 242 ASD patients. Transmission disequilibrium test (TDT) was then performed for *HTR1B*, which shown evidence for transmission disequilibrium (p=0,0415) in a sample of 163 trios. A sex-specific analysis showed an increased effect in our male sample (p=0,0015, table 1), and no effect in the female sample (p=0,3). Our results confirm previous publications describing a sex-specific genetic contribution in the etiology of ASD. FAPESP
Table1- Transmition disequilibrium test for HTR1B gene in a male sample.

Haplotypes*	Transmited	Non transmited	TDT
C-A-C-G	14	20	
G-T-C-G	42	29	
T-A-C-C	35	33	
T-A-C-G	39	33	
Others	2	17	
Total	132	132	GI obal p=0,0015

*NCBI id-rs11568817-rs130058-rs130057-rs6296

P0912. DNA analysis in families with autosomal dominant polycystic kidney disease (ADPKD) in Czech Republic

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Autosomal dominant polycystic kidney disease is the most common hereditary renal disease. ADPKD is a systemic disorder, with cysts and connective tissue abnormalities involving many organs. The disease is caused by mutations of PKD1 (MIM 601313; affecting roughly 85 % of ADPKD patients) and PKD2 (MIM 173910; 14 % of ADPKD patients) genes. In the Human Mutation Database (HGMD) have been reported 270 different germline mutations of the PKD1 gene and 73 different germline mutations of the PKD2 gene.
Presymptomatic DNA diagnosis using highly polymorphic microsatellite markers for DNA linkage analysis was performed in 280 unrelated ADPKD families.
The direct detection of mutations in the non-duplicated region of the PKD1 gene was performed in 90 nonrelated families. An affected member from each family was analyzed using denaturing gradient gel electrophoresis and by sequencing. We detected 19 different mutations in 21 families. 16 mutations are unique. We identified 8 nonsense mutations, 6 missense mutations, 2 frameshifting mutations and 3 mutations in splice site.
The direct detection of PKD2 mutation was performed in 147 nonrelated families. An affected member from each family was analyzed by heteroduplex analysis and by sequencing. We detected 14 different mutations in 30 families. 11 different mutations are unique. We identified frameshifting mutation c.203dupC (p.Pro68fsX23) in 10 nonrelated families and the nonsense mutation c.478C>T (p.Gln160X) in 5 nonrelated families.
The identification of new mutations in both PKD genes could help to find the functional important areas of both polycystins.
Supported by the grant projects IGA NR/9427-3 and VZ0021620806

P0913. High frequency of AZFc microrearrangements in infertile and normozoospermic French population

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The *Azoospermia factor c* (AZFc) region on the Y chromosome long arm consists essentially of multiple palindromes prone to rearrangements. The impact of the various types of deletions (gr-gr, b1-b3, b2-b3), duplications or deletions followed by duplications on male fertility is discussed. The most studied genes in AZFc are *DAZ* and *CDY1*, present in 4 and 2 copies respectively. We screened 344 infertile and 136 control men for AZFc deletions and duplications. PCR analyses of specific AZFc STS (sY1192, sY1291, sY1197), DAZ SNV sY587 and quantitative dosage of sY255 and sY586 for DAZ genes were used to detect AZFc microrearrangements. CDY1 gene dosage used RT-PCR with sY639 and PCR-digestion assay of CDY7750. All patients were haplotyped for YAP, 12f2, and 92R7 polymorphisms, and individuals with AZFc rearrangements also for the Tat, M9, and SRY-4064 SNPs, defining altogether five haplogroups (Yhg): E, J, K(xN3,P), N3 and P, and one paragroup Y*(xD,E,J,K).
Forty-two (8.7%) rearrangements were identified representing a high frequency of chromosomal recombinations, with similar frequencies of deletions and duplications in infertile and control populations. The analysis of DAZ and CDY1 SNVs and dosage of CDY1 copies by RT-PCR allowed to better characterise these rearrangements and their mechanisms. Complex rearrangements (deletion / duplication) were found in infertile patients only, including 2 from Yhg-N3 and 2 from Yhg-J. This study brings complementary data about AZFc rearrangements and their potential impact on male infertility.

P0914. A case of Y chromosome microdeletion transmission from a father to his azoospermic sons

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Y chromosome microdeletions especially in the AZF regions which include DAZ genes are usually *de novo* deletions associated with primary oligo- and azoospermia. It has been evaluated that 5-15% of men with spermatogenic failure harbour microdeletions of Yq. The present study refers to the investigation of azoospermia in two brothers and

their father.
We received DNA samples from blood of all 3 individuals and sample from testicular biopsy from the younger brother. Cytogenetic analysis (GTG-high resolution) was performed in both brothers. Y microdeletion analysis was carried out including SRY gene (Yp) and 23 STS (Yq) in AZFa, AZFb, AZFc regions. We investigated also DAZ-like gene (3p24) polymorphisms in exons 2 and 3. CFTR gene mutation screening and IVS8-polyT polymorphism was also performed.
Cytogenetic analysis was normal in all 3 members of the family and also in testicular tissue sample of the younger brother. A Y microdeletion was detected, encompassing 10 STS and DAZ gene copies, identical in the fertile father and the two azoospermic sons. No polymorphism in exons 2 (nt 260) and 3 (nt 386) of DAZL gene was detected. CFTR screening was negative. This study provides additional evidence that the clinical phenotype of azoospermia can neither be attributed to the Y microdeletion itself, nor to probable expansion of the deletion during the vertical transmission from the father to his two sons as it has been reported previously. It is likely that azoospermia in these two cases is probably due to other environmental and genetic factors involved in spermatogenesis.

P0915. Balkan endemic nephropathy (BEN) - impact of Cytochrome P450, GSTs and NAT2 gene polymorphisms in its etiology

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BEN is a chronic, noninflamatory kidney disease spread among the rural population of alluvial valley regions along tributaries of the Danube River in Bulgaria, Bosnia, Croatia, Romania and Serbia. Although the etiology of BEN is still unclear, it was supposed that the disease may develop in genetically susceptible individuals exposed for a long time to multiple environmental toxins. To evaluate this hypothesis we launched case-control study, focused on the identification of common variants in genes, coding for proteins participating in the metabolism and transport of xenobiotics and their association with BEN.
Genomic DNA from 58 BEN patients and 104 healthy individuals from Vratza`s district of Bulgaria was used. PCR-RFLP (*CYP1A1*, *CYP1A2*, *CYP2E1*, *CYP3A4*, *GSTP1, NAT2* variants*)*, tetra-primer PCR (CYP2D6*4 allele 1934G>A), triplex PCR (simultaneously detection of the null deletions in *GSTM1* and *GSTT1*) and TaqMan assay (*CYP2C19*, *CYP2D6*, *CYP3A5*, *MDR1* polymorphisms) were performed.
Fourteen of the studied variants did not show statistically significant genotype distribution between the explored groups. The polymorphic variant 4889A>G in *CYP1A1* and the deletion allele CYP2D6*5 were prevalent in BEN patients (p=0.036 and 0.020). Conversely, carriers of at least one GSTM1 wild type allele were more common among BEN patients compared to controls (p=0.005). Combined genotype distributions demonstrated statistically significance for the combinations: CYP1A1/CYP2D6*5 (p=0.021), CYP1A1/CYP3A4*1B (p=0.017), CYP1A1/CYP3A5*3 (p=0.051), GSTM1/CYP1A1 (p=0.007), GSTM1/CYP2D6*3 (p=0.046), GSTM1/CYP2D6*5 (p=0.008), GSTM1/CYP2E1 (p=0.001), GSTM1/CYP3A4*1B (p=0.023), GSTM1/CYP3A5*3 (p=0.025).
Conclusion: The genetic heterogeneity in xenobiotic metabolizing enzymes CYP1A1, CYP2D5 and GSTM1 alone or in combination with other Phase I enzymes associates with BEN.

P0917. The molecular basis of bitter taste genetics: a study in genetically isolated populations

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Some individuals are taste blind to bitter compounds having the thiourea moiety (-N-C=S), such as phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP). Taste sensitivity to PTC/PROP is bimodally distributed: nontasters make up approximately 30% of the adult Caucasian population and tasters make up the remaining 70%. Tasters can be further divided into 2 sub-groups, medium- and super-tasters. These percentages can vary, depending on geographic location and ethnic origin.
Evidence suggests that the expression of phenotype reflects the interaction between the TAS2R38 gene and enviromental factors.
In this study, we determined the PROP phenotype distribution and the correlation between taster status and TAS2R38 genotype in two different genetically and geographically isolated populations: Carlantino (Southern Italy) and Stoccareddo (Northern Italy).
A sample of 696 adults, from 15 to 89 years old of age, was recruited from the two villages.
Our data showed that the percentage of PROP non-tasters was comparable to other Caucasian populations, although the percentage of super-tasters in Stoccareddo was very low.
The contribution of TAS2R38 alleles to the phenotype was lower in Carlantino (64%), compared to Stoccareddo (76%). For this reason we analyzed, only in Carlantino, three additional bitter taste receptor genes (TAS2R16, TAS2R4, TAS2R14) and BDNF gene. Linear regression and stepwise algorithms were used to determine the influence of the selected genes on the PROP/PTC phenotype.
Preliminary results showed no correlation between the PROP/PTC phenotype and these genes. A whole linkage analysis will be performed to identify other genes or genetic determinants that might contribute to this phenotype.

P0918. Quantitative trait genetic linkage analysis of Body-Mass Index in familial coronary artery disease using a variance component approach and a regression-based procedure.

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Body mass index (BMI) is one of the most reproducible and commonly used proxy for obesity and is known to be influenced by many environmental causes as well as genetic factors.
No susceptibility genes for BMI regulation have been unequivocally identified. Reasons for these controversial results are both methodological and related to obesity aetiology.
A genome-wide linkage analysis was performed to localise QTLs influencing BMI levels in a large cohort collected in the PROCARDIS coronary heart disease study consisted of 1812 informative families. Multipoint linkage analysis for BMI was conducted using both a variance component approach and a model-free regression method implemented by MERLIN and the resulting LOD-scores were compared. Maximum LOD-score was detected on chromosomes 13 (LOD 1.6). Other regions showing a LOD-score greater than 1 were observed on chromosomes 3, 5, 11, 12 and 15. Results were mainly confirmed by the three different approaches used in the analysis.
Our study did not find any locus strongly supporting evidence for linkage to BMI even in such a large sample which could provide a power equal to 80% in detecting a putative QTL. Nevertheless our results seem to confirm the substantial genetic heterogeneity influencing BMI regulation emerged from the majority of genome scans so far published.

P0919. Retrospective analysis of genetic polymorphisms and prospective genetic testing in atopic bronchial asthma (ABA) patients

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100 pregnant women affected with ABA and their 100 newborns were tested for genetic polymorphisms of 7 genes such as GSTT1 (del), GSTM1(del) CCR16 (G38A), TNFA (-238G/A;-308 G/A), IL4 (C590T), IL4R (Q576R) and NOS1 (AAT repeats in intron 20 GSTM1 0/0 genotype was recorded in 57% ABA women compared to 39% in the control. Respective figures for GSTT10/0 genotype were even more impressive -40% in ABA and 21% in the control. Homozygotes for GSTM1 or GSTT1 deletions were registered in 70% of ABA patients while the number of double homozygotes (GSTM10/0;GSTT1 0/0) in the ABA

women group more than 5 times exceeded these ones in the control (30% and 6,5% respectively). Alleles and genotypes frequences for polymorphisms of CC16, NOS1, IL4 & IL4R in ABA group were found within control limits. 22% of newborns from ABA women had no adverse genotypes or alleles for GSTM1,GSTT1,IL-4 or IL4R(Q576), (C590), while 43% of newborns possessed at least 3-4 unfavorable (ABA predictive) genotypes or alleles and they were enrolled in ABA-prone group. So far chronic atopic dermatites and other manifestations of allergy were registered in 83% and 50% respectively of newborns from the ABA high-risk group and these complications were twice more often in this group when compared to the ABA-mother born childs without obvious genetic predisposition. Genetic testing of childs or could be now efficiently implemented for more sophisticated presymptomatic selection of individuals with elevated inborn risk of ABA.

P0920. Impact of ACE (Ins/Del), ApoB (R3500Q) and ApoE (E2/E3/E4) polymorphisms on myocardial perfusion: correlation with myocardial single photon emission computed tomographic imaging

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Coronary artery disease (CAD) is associated with multiple genetic and environmental risk factors, whose cumulative effects have been the focus of intensive research. Several studies have shown an association between CAD and polymorphisms in genes implicated in the control of blood pressure and lipid metabolism. We studied 182 CAD patients with exercise-rest Tc-99m tetrofosmin myocardial perfusion single photon emission computed tomography (SPECT) and calculated the summed stress score (SSS), summed rest score (SRS) and summed difference score (SDS) indexes. Genetic analysis for ACE (Ins/Del), ApoB (R3500Q) and ApoE (E2/E3/E4) polymorphisms were determined by polymerase chain reaction (PCR) and RFLP analysis on agarose gel electrophoresis. A significant correlation ($p<0.001$) was found between ACE, Apo E, ACE +ApoE (SUM) and SSS, SDS SPECT indexes, while no association was found between ApoB (R3500Q) and either SSS or SDS. Patients with a low SUM score (≤ 2) had significantly better myocardial SPECT studies ($p<0.001$) and a generally better performance during exercise testing compared to patients with a SUM score ≥ 5. Only one of sixty patients with a normal SPECT study had a genetic SUM score ≥ 5, while 39 patients had a SUM score ≤ 2. Furthermore, only two patients with abnormal myocardial SPECT had a SUM value ≤ 2. These data provide the first evidence for an association of ACE and ApoE polymorphisms with SPECT studies and suggest that the proposed effect of ACE and ApoE polymorphisms in the process of CAD may be modified by the ACE and ApoE genotype.

P0921. A Polymorphic Purine-Rich Sequence at the 1.5 kb Downstream Region of the Human Caveolin-1 Gene

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Caveolins are a family of proteins that coat the cytoplasmic face of caveolae, vesicular invaginations of the plasma membrane. Aberrant expression of the CAV1 gene is implicated in the pathogenesis of a wide variety of disease phenotypes. Mechanisms of gene expression and regulation in this gene may shed light on the pathophysiology of the related disorders. We report a novel polymorphic purine stretch containing GGAA tetranucleotide repeats in the 1.5kb downstream region of the human CAV1 gene with likely effect on the expression of the respective gene. The allele length of this polymorphic locus ranges from 40 to 134 bp, and contains motifs for the Ets transcription factor family members. The effect of this polymorphic purine stretch on the expression of CAV1 and possible association of the alleles of this region with disease phenotypes remain to be clarified.

P0922. Polymorphic Purine-Rich Complex Located at the Enhancer Regions of the Human Caveolin-1 Gene

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Caveolin-1 (CAV1) is the principal structural protein of caveolae membranes that are found in most cell types. Aberrant expression and mutation of this gene are associated with a wide range of disorders including neurodegenerative disorders, various cancers and autoimmunity. We report a novel polymorphic purine complex located at between -1698bp to -1549bp of the enhancer region of the human CAV1 gene. This region contains GGAA and GAAA motifs, the consensus binding sites for the Ets and IRF family transcription factors, respectively, and is highly conserved in distantly-related non-human primates in respect with location and motif sequence. The effect of this complex sequence on the expression of CAV1 and possible association of the haplotypes of these regions with disease phenotypes remain to be clarified.

P0923. A whole-genome scan reveals significant linkage of celiac disease to 6q21-22 and 22q13 in extended pedigrees from Hungary and Finland

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Celiac disease is a complex genetic disorder caused by inflammatory responses to gluten. Apart from the known susceptibility genes at HLA-DQ locus, the search for additional risk genes continues. We performed a whole-genome linkage scan in two extended four-generation pedigrees consisting of multiple individuals with celiac disease. Our aim was to identify genomic regions shared by the affected individuals within the pedigrees, regions harbouring genetic factors influencing susceptibility to celiac disease.

Approximately fifty thousand single-nucleotide polymorphisms were genotyped with the Affymetrix 50K microarray system and analysed by affecteds-only non-parametric linkage analysis. We selected individuals separated by as many meiosis as possible in order to minimise random sharing of genomic segments and to narrow down the disease-linked genetic regions. Our material consisted of one pedigree from central Hungary and one from Finland. Six patients from the Finnish family were genotyped and seven from the Hungarian family.

In addition to the well known HLA-DQ risk genes, we identified linkage in both families to a locus on chromosome 6q21-22 (LOD= 2,01 p=0.0012). The Finnish family also showed linkage to a locus on chromosome 22q13 (LOD= 1,29, p=0.007).

These regions have previously been suggested to be involved in celiac disease in European populations, but the primary risk genes at these loci remain unknown. Further finemapping in our larger independent family materials will be performed to narrow down the linked region and to identify the disease-associated gene(s). Characterization of novel genetic factors in celiac disease will help us understand the pathogenesis of this complex disorder.

P0924. Linkage and association study of the *CELIAC2* locus (5q31-33) in Hungarian and Finnish families with celiac disease

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Celiac disease is an autoimmune inflammation of small intestinal mucosa. It is triggered by dietary gluten in genetically predisposed individuals and it affects approximately 1% of the Caucasian population. The only known genetic risk factors, *HLA-DQ2* and *-DQ8* on chromosome 6p21.3 (*CELIAC1*), are necessary but not sufficient alone for the disease onset. The search for other risk genes in several candidate regions continues. The most promising risk locus for celiac disease after the HLA locus is on chromosome 5q31-33 (*CELIAC2*). However, no risk genes have been identified from this region yet. This region har-

bours several genes that have an immunological function, and which could therefore have a putative role in celiac disease.
41 single-nucleotide polymorphisms that tag 17 immunologically relevant genes in a region spanning nearly 20 Mb (130.7-150.0 Mb) at the *CELIAC2* locus were selected for this study. They were genotyped in a total of 541 Hungarian and Finnish affected sib-pair and trio pedigrees.
The analysed region showed linkage to celiac disease in the combined Finnish and Hungarian material (p=0.0015) and in the Finnish population alone (p=0.004). Borderline significant linkage values were observed in the Hungarian population (p=0.03). Several genes and haplotype blocks showed suggestive association with celiac disease.
More finemapping will be needed to identify the gene or genes possibly associated with celiac disease in the region. Functional studies of the associated genes will also be required to confirm their role in the pathogenesis of celiac disease.

P0925. Gene expression variability of immunologicaly important genes in blood and intestine of celiac patients: a comparative study

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Is it possible to estimate the extent of inflammation in the intestine of a celiac patient by monitoring changes in gene expression in the peripheral blood? We are trying to find an answer to that question by comparing the expression of 94 relevant immune genes between inflamed tissue and blood in two celiac cases and two controls. Among genes we are testing there are cytokines, chemokines, growth factors, immune regulators, apoptosis markers, ischemia markers, tissue-specific markers, and other genes taking part in immunological responses. For this purpose we are using pre-designed Low Density Arrays based on TaqMan® technology. Blood samples and biopsies from the duodenum and small intestine are taken at the same occasion in all individuals.Our preliminary results are showing that a number of genes expressed in the intestine are not measurable in blood. On the other hand, the altered expression of several genes in the intestine corresponds to an altered expression of the same genes in the peripheral blood, which makes them potential markers for inflammation. Our aim is to investigate if some of those genes may co-act in a distinct inflammatory pathways and whether measuring expression of one gene can predict the expression level of other genes. Moreover, we are trying to establish a method to correlate changes in gene expression in peripheral blood to a level of intestinal damage in celiac cases.

P0926. Demyelinating Autosomal Recessive Charcot-Marie-Tooth disease (ARCMT1): Estimation of loci frequencies in 34 consanguineous families originated from the Mediterranean basin and middle Est

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CMT is a pathological and genetic heterogeneous group of hereditary motor and sensory neuropathies. Two major types have been distinguished on neuropathological and electrophysiological grounds: demyelinating and axonal CMT. Nine loci have been reported and 7 genes identified in demyelinating ARCMT. We selected 34 consanguineous families in whom 62 subjects presented ARCMT1 in a total of 156 individuals. These families are originated from the Mediterranean basin and middle Est. All the individuals were screened for 54 microsatellites markers covering the 9 known loci. PCR products were resolved in an ABI-Prism 3100 automated sequencer and analyzed with Genescan and Genotyper software (ABI™). Assignment of the families to each locus was established by homozygocity mapping and linkage analysis using Allegro1.2. When a linkage was suspected the coding sequences of the corresponding gene were sequenced. The fragments were analyzed with sequencing analysis and SeqScape software (ABI). We identified 14 families (41%) with linkage to one of the known loci. CMT4B1 (*MTMR2*) and CMT4C (*SH3TC2*) are the most frequent (14.9% each). They represent about 30% of the known loci, in our series and are found in families originated from Algeria, Bosnia, France, Morocco, and Saudi Arabia. CMT4D (*NDRG1*) is less frequent (5.9%). CMT4A (*GDAP1*) represents 2.8% of the known loci. CMT4A and 4D were found only in Italian families. CMT4B2 (*MTMR13*) and CMT4F (*PRX*) were suspected in one Tunisian family (2.8%). Sequencing of the corresponding genes is in progress. 59% of families were not associated with the known loci, demonstrating further genetic heterogeneity.

P0927. A molecular genetic study of Charcot-Marie-Tooth Type 1 in Turkey

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Charcot-Marie-Tooth Type 1 (CMT1) is a genetically heterogeneous neuropathy that is associated with PNS demyelination. It is characterized with reduced NCV values (<38m/s) and segmental demyelination on nerve biopsies.
The PMP22, MPZ, and LITAF genes have been associated with autosomal dominant CMT1 and *Cx32* is responsible for the X-linked form of the disease. About 60-70% of CMT1 patients have duplications on chromosome 17p11-11.2 that contains the PMP22 gene.
In this study, the molecular basis of demyelinating dominant CMT in the Turkish population was investigated in a total of 140 patients.
The CMT1A duplication was identified in 46 CMT1 patients (32.8%) screened by STR analysis. Consanguineous marriages were observed among parents of 45 patients raising the possibility of recessive inheritance. Thus, the duplication frequency was recalculated and found to be 48.4% (46/95) that was still a low value compared to that of other populations. Twenty-five of the duplications were familial mutations and eleven were sporadic. Pedigrees were not available for ten cases.
Patients negative for the duplication were further investigated for mutations in the *PMP22, MPZ, Cx32*, and *LITAF* using SSCP and subsequent sequencing analyses. Six Cx32, five MPZ, four PMP22, and two LITAF mutations were identified in heterozygous condition. The phenotypes of the mutated patients were in accordance with the identified genotypes with respect to the site and type of the mutations.
Autosomal dominant inheritance and low NCV values among 32 cases (33.6%) for which mutations could not be identified suggests further genetic heterogeneity of CMT1 disease.

P0928. Relationship between MTHFR C677T and A1298C and maternal risk for having a child with chromosomal aneuploidy

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Maternal impairments in folate metabolism due to polymorphisms in 5, 10- methylenetetrahydrofolate reductase (MTHFR) gene have been suggested as a risk factor for having an offspring with chromosomal aneuploidy. Two common polymorphisms in the MTHFR gene, C677T and A1298C, are known to reduce the activity of this enzyme, which can lead to DNA hypomethylation and abnormal chromosomal segregation during meiosis (non-disjunction). Contradictory results are published in recent studies concerning the relationship between chromosomal nondisjunction and MTHFR polymorphisms. In this study we examined the C677T and A1298C polymorphisms in the MTHFR gene by single base extension reaction using SNaPshot multiplex kit and subsequent analysis on ABI 310 genetic analyzer. We analyzed 47 women; 24 with a child with Down syndrome and 23 with a prenatally diagnosed fetus with aneuploidy (10 with trisomy 21, seven with trisomy 18, three

with trisomy 13, two with Turner syndrome and one with Klinefelter syndrome). The MTHFR C677T allele (C-57,4% and T- 42,6%) and genotype frequencies (C/C-36,2%, C/T-42,5%, T/T- 21,3%), as well as the MTHFR A1298C allele (A-68,5% and C-31,5%) and genotype frequencies (A/A-52,2%, A/C-32,6%, C/C-15,2%) among women with a child with chromosomal aneuploidy did not differ from the frequencies observed in the general population in the Republic of Macedonia. Our results failed to support the relationship between MTHFR C677T and A1298C polymorphisms and the risk of having a child with aneuploidy.

P0929. Genetic factors determining predisposition to chronic course of virus hepatitis and fibrosis in liver

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Polymorphic variants in *IL4* (C-590T), *IL4RA* (I50V), *TNFα* (G-308A) genes were studied for association with chronic viral hepatitis (HCV or HBV) and extent of the disease chronization which is marked by hepatic fibrosis stage. Association study was performed for levels of interleukins 4, 10, 12, tumor necrosis factor - α, fibronectin, collagenase, protease inhibitors, macroglobulines, elastases, free and protein-bound hydroxiproline.

It has been found that Val/Val genotype of *IL4RA* (I50V) polymorphism is associated with HBV: in the patients, this genotype was registered with frequency of 3.08% whereas in control group and in patients with HCV frequency of Val/Val genotype was 15.6% and 15.3%, correspondingly. In the group of patients with weak fibrosis, a higher frequency of "A" allele of *TNFα* (G-308A) was found (24,5%), comparing to the population sample (11.2%). Decreasing of "A" allele frequency was observed from patients with weak fibrosis to patients with cirrhosis (8.7%). "CT" genotype of *IL4* (C-590T) was more frequent in patients with cirrhosis (68,2%) than in patients with moderate fibrosis (39,1%).

It has been shown that "A" allele of *TNF*α (G-308A) polymorphism is associated with decreased TNF-α, increased IL-4 and IL-12, as well as with low level of protein-bound hydroxiproline. In addition, association of "CT" genotype of *IL4* (C-590T) polymorphism and high level of protein-bound hydroxiproline has been identified.

The results suggest that "A" allele of *TNF*α (G-308A) polymorphism is associated with favourable course of chronic viral hepatitis, and "CT" genotype of *IL4* (C-590T) polymorphism is associated with severe course of the disease.

P0930. BCL3 gene association analysis with nonsyndromic cleft lip and/or cleft palate in Latvia

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Background: Nonsyndromic cleft lip and/or cleft palate (CL+/-CP) is one of the most common congenital malformations with multifactorial trait. The incidence of CL+/-CP is in the range of 1/700-1/1000, the disease frequency in Latvia is 1 in 700 newborns,

The BCL3 (*B-cell leukemia/lymphoma-3*) gene has been considered a susceptibility locus for nonsyndromic cleft lip with or without cleft palate, based on association and linkage studies in some populations.

Matherials and methods: Four SNPs (rs10401176, rs7257231, rs8103315 and rs2927456) in the BCL3 gene were analysed with MALDI-TOFF technique for allelic association with the nonsyndromic CL+/-CP in 75 families (proband with both parents) from Latvia. Observed data analysed with transmission disequilibrium test (TDT).

Results: Significant association of BCL3 gene in patients with CL+/-CP had been found with rs10401176 (P = 0.0001, df 1) and rs7257231 (P = 0.0046, df 1). Borderline association of BCL3 gene in patients with CL+/-CP had been found with rs8103315 (P = 0.045, df 1). Association was not found with rs2927456 (P = 0.317, df 1).

Conclusion: BCL3 gene plays a role in development of CL+/-CP.

P0931. Founder effect in CMT-families with mutations in *GJB1* gene from different regions of Russia

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Charcot-Marie-Tooth disease (CMT), as known as hereditary motor and sensory neuropathies (HMSN), that is characterized by symmetrical distal mussel weakness and atrophy, sensory and tendon reflexes depressed, fridrich's foot deformation, typical change of gait, ataxia, tremor. The X-linked dominant form of CMT (CMTX1) is associated with mutations in gap junction B1 (GJB1) gene. We analyzed four families with Arg142Trp mutation, five families with Arg164Gln mutation and three families with Val181Met mutation. We performed haplotype analysis of mutant chromosomes by PCR-AFLP method using eight microsatellite markers from Xq13 region: DXS1275, DXS8040, DXS1216, DXS8111, DXS983, DXS8107, DXS8052, DXS8060. We find out that all families with Val181Met mutation have common haplotype for all markers. In two families with Arg142Trp mutation we revealed common haplotype in all markers too. In all families with mutation Arg164Gln common haplotype was not detected. Existence of a common haplotypes of chromosomes with Val181Met mutation allows us to speculate on the existence of founder effect. Common haplotype chromosomes with mutation Arg142Trp also indicate the founder effect. In families with mutation Arg164Gln the founder effect doesn't exist, and probably codon 164 is a "hot spot" in GJB1 gene.

P0932. Copynumber alterations of *TEKT3* in Charcot-Marie-Tooth disease

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The most frequent form of demyelinating CMT (CMT1a) is linked to chromosome 17p11.2. Intrachromosomal duplications, mainly of a 1.5Mb region harboring the *PMP22* gene, or mutations in this gene are found in the majority of CMT1a patients.

Here we describe an aberrant chromosome 17 duplication proximal of the *PMP22* gene in several CMT1 patients. In these patients mutations in the coding region of *PMP22* or the non-coding first exons of the two alternative transcripts were absent. This rare copy number polymorphism (CNP), starting approximately 33 kb centromeric of *PMP22,* encompasses the entire *TEKT3* gene, in addition to the uncharacterized transcript *CDRT4*/*LOC653497* and co-segregates with the disease in two affected families that were studied. The duplication is 186 kb in size and identical in nine different individuals from five different families. Sequence analysis and microarray CGH both show that this duplication fully maps within the commonly seen 1.5 Mb duplication and deviates from it at both ends. This CNP was not detected in 2124 normal chromosomes nor in 40 patients with CIDP. We therefore propose that this rare variant is the cause of CMT in these patients and most probably results in the disease through effects on expression of *PMP22*. Of note, most diagnostic tests for CMT will not detect this CNP.

P0933. Quantitative fluorescent PCR in the detection of duplication/deletion of the PMP22 gene in Belarussian patients with CMT1A and NHPP.

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Charcot-Marie-Tooth disease type 1A (CMT1A) accounts for 70-80% of CMT1 cases and is most frequently caused by the duplication of a 1.4-Mb genomic fragment on chromosome 17p12 containing PMP22 gene. For molecular diagnosis of CMT1A, based on quantitative PCR analysis of short tandem repeats (STRs), we used 5 markers located within the duplicated region. D17S2218, D17S2220, D17S2223, D17S2226 and D17S2229 were analysed in two multiplex PCR reactions followed by automated capillary electrophoresis on the ABI Prism 310. Heterozygosity values of the selected STRs in Belarus population are 81.2%, 94.5%, 72%, 77,6% and 84,3% respectively. Using this panel we genotyped 130 unrelated individuals with presumable clinical diagnoses of CMT neuropathy and 130 their family members. 36 duplications of the PMP22 gene in affected individuals from 17 families, and 3 gene deletions responsible for hereditary neuropathy with liability to

pressure palsy (HNPP) in one family were identified. The duplication detection rate for D17S2218 is 15/36, D17S2220 - 28/36, D17S2223 - 17/36, D17S2226 - 11/36 and D17S2229 - 15/36. The high detection rate of D17S2220 can be explained by the complex structure of the polymorphic marker, which have not only tetra but also dinucleotide repeat inside. No individual marker was informative in every patient. However, in all positive cases we were able to detect three different alleles of at least one STR. The described analysis is fast simple and reliable for pre- and postnatal diagnosis of CMT1A and NHPP in Belarussian families.

P0934. A coeliac disease genome-wide association study identifies a novel susceptibility locus

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Coeliac disease is a common (1% prevalence) chronic inflammatory disease of the small intestine, caused by an immune response to dietary wheat, rye and barley. HLA-DQ2 (present in 95% of coeliacs) is necessary to present wheat epitopes to CD4 T cells, but not sufficient for disease (present in 30% of the population). Previously, linkage studies were the only cost-effective way to map and isolate non-HLA genes contributing to coeliac disease. We have performed a genome-wide association study and genotyped 310,605 SNPs with minor allele frequency >1% in 778 coeliac cases and 1422 population controls from the UK using the Illumina HumanHap300 BeadChip. Overall SNP call rate was 99.87%. An extended region of highly significant association was seen around the HLA locus (Ï‡2=769.1, P<10-19, OR 7.04 [95% CI 6.08 - 8.15]). Excluding the HLA region, we observed one other SNP that remained significant after permutation testing (P=2.0 x 10-7, empirical genome-wide significance P=0.045). This finding was independently confirmed in Dutch and Irish collections (meta-analysis P=3.8 x 10-11, OR 0.66 [95% CI 0.58 - 0.74]). This SNP maps around a biologically plausible candidate gene and genetic variation in this gene may predispose coeliac patients towards unwanted immune responses to cereal antigens. In addition to the replicated SNP, a greater number of significant SNPs were observed than expected by chance: P<10-6 (2 vs 0.3 expected) and P<10-5 (12 vs 3 expected). All SNPs with P<0.005 are currently followed in multiple independent cohorts.

P0935. A genome-wide linkage scan in an extended Maltese family with a high incidence of coeliac disease

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Gluten-sensitive enteropathy, or coeliac disease, is an autoimmune disorder characterized by inflammation, villous atrophy and hyperplasia of the small intestinal mucosa. Coeliac disease is caused by both environmental and inherited factors. A small number of family based linkage studies were performed so far, where the HLA locus and other chromosomal regions were linked with the disease. In this study, linkage analysis was performed in a Maltese family with a high incidence of coeliac.

A whole genome linkage scan using 400 microsatellite markers was performed in seventeen family members, four of whom where diagnosed as coeliac by biopsy and another two were symptomatic but were following a gluten free diet. Multipoint parametric and non-parametric linkage analyses were performed by EasyLinkage v4.01 using GENEHUNTER v2.1, assuming dominant and recessive modes of inheritance with variable penetrance. Disease allele frequency was assumed to be 0.001.

Highest NPL (5.27; p=0.0039) and LOD (1.46) scores were observed to marker D10S1731. NPL and LOD scores of 4.12 (p=0.0313) and 1.46, respectively, were observed to another marker on chromosome 11p12. These results were confirmed after fine mapping at these regions. No evidence of linkage was observed to the HLA region on chromosome 6, where sequencing of HLA-DQA1, DQB1 genes confirmed that neither DQ2 nor DQ8 HLA heterodimers were found in this family.

These results suggest that non-HLA genes might be responsible for the onset of coeliac disease in this Maltese family. Further investigations of the indicated loci are going to be performed by sequencing of candidate genes.

P0936. COL4A3/COL4A4 mutations in Cypriot families explain the recurrent hematuria-thin basement membrane nephropathy that progresses to focal segmental glomerulosclerosis and occasionally to end stage renal failure. Extended founder effect phenomena and mutation dating

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Focal Segmental Glomerular Sclerosis is a frequent histological finding of heterogeneous aetiology caused by primary and secondary clinical conditions. In 11 families with autosomal dominant inheritance of FSGS and microscopic hematuria, 372 patients and healthy relatives were investigated. Linkage analysis was performed with markers around four chromosome regions and screening for mutations was accomplished by the SURVEYOR plant endonuclease, followed by automated DNA sequencing. For locus 2q36 the total LOD score was 7.75, clearly implicating mutations in either the *COL4A3* or *COL4A4* gene. DNA sequencing revealed two *COL4A3* and one *COL4A4* mutations in 90 patients. In one family there was compound heterozygosity and cosegregation of two mutations in *COL4A3* while two individuals had inherited both mutations and developed the Alport syndrome. Mutation *COL4A3* - G1334E was found in eight families that share a common *COL4A3* haplotype, from three geographic locations of Cyprus. Five are from Kaimakli, a city near Nicosia, thereby documenting a founder effect. This finding may prove to explain a high frequency of patient referrals with chronic renal failure from Kaimakli. Mutation dating suggested that this mutation is about 300 years old. This is the first detailed investigation of Cypriot families with COL4 pathology with Thin Basement Membrane and FSGS as a prominent feature. Our data enhance the observation that isolated microscopic hematuria is not always a benign condition. Further epidemiological studies are needed in the Cypriot population for investigating additional founder phenomena and providing proper genetic counselling in concerned families and practicing physicians.

P0937. The association of a polymorphism within the COL5A1 gene an Achilles tendon injury in a second Caucasian population

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As many as 30-50% of sports injuries are tendon injuries of which a large proportion are Achilles tendon injuries (ATI). The exact mechanisms underlying ATI are poorly understood, however it is widely accepted that ATI is a multifactorial condition caused by the interaction of both intrinsic, which includes a genetic component, and extrinsic risk factors. Our group has reported an association between polymorphisms within the COL5A1 gene with ATI in a physically active Caucasian population. COL5A1 codes for a polypeptide component of type V collagen which is involved in the regulation of fibrillogenesis. The aim of this study was therefore to repeat this previous association study within a second Caucasian population.

The study comprised 152 Caucasian individuals from Australia, of whom 91 were diagnosed with ATI and 61 asymptomatic control (CON) individuals. The BstUI restriction fragment length polymorphism was used to genotype all the individuals for the C>T transition at nucleotide 414 of exon 66 of COL5A1 (rs12722).

There was a significant difference in the allele frequencies of the COL5A1 BstUI RFLP between the ATI and CON subjects (p<0.001). The frequency of the TC genotype was significantly higher in the ATI group

(69.2%) than in the CON group (36.1%) (odds ratio of 4.0; 95% CI 2.0-7.9; p<0.001).
These findings provide additional evidence that the COL5A1 BstUI RFLP is associated with Achilles tendon injuries.

P0938. COL5A1 genotype effects on range of motion measurements

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A reduced joint range of motion (ROM) has traditionally been postulated to increase the risk of musculo-tendinous, including Achilles tendon, injuries during exercise. There is, however, conflicting evidence with regard to the role of musculo-tendinous flexibility in Achilles tendon injuries (ATI). We have recently shown that the BstUI restriction fragment length polymorphism (RFLP) within the COL5A1 gene is associated with ATI. Mutations within this gene have been implicated in Ehlers Danlos syndrome which is characterised by joint hypermobility. The aim of this study therefore was to investigate the possible association of the BstUI RFLP within the COL5A1 gene and musculo-tendinous flexibility in subjects with Achilles tendon injuries and asymptomatic control subjects. Lower limb ROM measurements, including the sit and reach (SR) test and the passive straight leg raise (SLR), were conducted on 118 physically active Caucasian subjects, which comprised 50 subjects with chronic Achilles tendinopathies and 34 with acute Achilles tendon ruptures, and 34 asymptomatic control subjects. The BstUI RFLP was used to genotype all these individuals for the C>T transition at nucleotide 414 of exon 66 of COL5A1 (rs12722). Gender, age and the COL5A1 BstUI RFLP genotype contributed significantly to the optimal SLR model. The factors contributing significantly to SR were weight, age and the COL5A1 BstUI RFLP genotype. This data suggests that the COL5A1 gene may be associated with lower limb flexibility.

P0939. Analysis of a functional catechol-O-methyltransferase gene polymorphism in healthy females: association with aggressive behavior

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Several lines of research have reported that functional polymorphism in the catechol-O-methyltransferase (COMT) gene that is responsible for substantial variability in COMT enzymatic activity is associated with aggression and impulsivity. The most of recent researches have been conducted on males therefore we suggest that it is important to investigate an association of aggression with COMT genotypes in females. A common low-activity variant of the enzyme contains a Met residue at amino acid 158 of COMT whereas the common high activity variant has a Val at this site. Considering the role of COMT in dopamine metabolism and the involvement of dopaminergic pathways in the formation of aggression, we screened athletes (78 females) and non-trained control group (83 females) to determine whether a behavioral association with the COMT polymorphism exists or not. Case-control analyses suggested a strong association between the ValVal genotype in both groups and high aggressive and dangerous behavior (P < 0.04). Also we have found that allele distribution is different in the groups and it could be the evidence of genetic propensity in sports.

P0940. Congenital cataract - a national wide study in Denmark

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Inherited congenital cataract leads in most cases to loss of vision if it not treated. Congenital cataract is present at or develops shortly after birth. Mutations in more than 20 human genes have now been associated with congenital cataract and mainly in combination with autosomal dominant inheritance.
A study of 30 Danish families with inherited congenital cataract has been initiated in order to identify the disease causing mutations. Mutations were found in 16 families and analyses of the remaining families are in progress. Mutations have been identified in seven different genes revealing both known and novel mutations.
The methods include genome wide linkage analyses, locus specific STS marker analyses of cataract loci or direct DNA sequencing of cataract genes. The analysis comprises all known congenital cataract-associated genes and the analyses may be applied for medical genetic counseling in the future.
The results from the first 16 families demonstrated mutations in the alpha-, beta- and gamma- crystallin gene families, the alpha gap junction gene family, the heat shock factor genes and in regulatory genes.
Several of the families represent distinct cataract phenotypes as the ant-egg-cataract (1) and the combination of microcornea and cataract. The results, the geno-/phenotypic relations and the structural and functional consequences of the mutations will be presented at the meeting.
1) Hansen L, Yao W, Eiberg H, Funding M, Riise R, Kjaer KW, Hejtmancik JF, Rosenberg T. The congenital “ant-egg” cataract phenotype is caused by a missense mutation in connexin46. Mol Vis. 2006; 12:1033-1039.

P0941. Linkage screening in a large Iranian family with congenital motor nystagmus (CMN)

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Idiopathic congenital motor nystagmus (CMN) is a genetic disorder characterized by bilateral ocular movements that start within early months of life. To date different typs of inheritance patterns have been reported for CMN, but no causative gene has been determined for CMN so far. This Iranian pedigree shows a typical X-linked dominant pattern with reduced penetrance (about30%) among obligate carrier females. There is no male to male transmission. Investigations have been done so far indicated five out of six families with X-linked CMN to be linked to a nearly distinct area in long arm of chromosome X . We designed linkage screening for some microsatellite markers previously confirmed for linkage on chromosome X. Linkage study was performed on Xp by DXS993 ruled out linkage to this locus. The second marker on Xq,DXS1047 , was not informative in our Iranian population. Our study on other markers on Xq is under way.

P0942. Insertion/deletion polymorphism of Angiotensin Converting Enzyme (ACE) gene in children with connective tissue dysplasia.

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Connective tissue dysplasia (CTD) is a group of geterogenous diseases with hereditary and inborn collagen synthesis disturbance. These patients have different neurological complaints, such as, headache, weakness, fatigue, dizziness, syncope, lypothymia, associated with cerebral hemodynamic disturbance. Previous studies have suggested an influence of ACE gene alleles on cerebrovascular disorders (CVD) in adults and children.
The aim of our study was to investigate the relationship between ACE gene polymorphism and cerebrovascular disorders in these children.
One hundred and three CTD children (36 girls, 68 boys) were included in our study. The mean age of patients was 12.97 ±3.93 years, 61,0% had CVD. Total genomic DNA was extracted by a standard method and ACE genotypes were determined using polymerase chain reaction (PCR) followed by analysis of PCR products by agarose gel electrophoresis in 41 children.
We have revealed the next genotypes and alleles distribution in these children: II-31,7%, ID-39,0%, DD-29,3%; I-51,2% and D-48,8%. We have no significant differences in ACE genotypes and alleles distribution between boys and girls. The incidence of homozygous deletion (DD) genotype in children with CVD was 37,5% compared to 25,0% in children without CVD. Children with II genotype had CVD only in 16,7% cases, but children with ID genotype had CVD in 42,9% cases and children, carriers of DD genotype had CVD in 50,0% cases.
Conclusion: the role of ACE gene insertion/deletion polymorphism in cerebrovascular disorders in children with connective tissue dysplasia still needs to be determined.

P0943. Ignoring intermarker linkage disequilibrium induces false-positive evidence of linkage for consanguineous pedigrees when genotype data is missing for any pedigree member

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Missing genotype data can increase false-positive evidence for linkage when either parametric or nonparametric analysis is carried out ignoring intermarker linkage disequilibrium (LD). Previously it was demonstrated by Huang et al (2005) that no bias occurs in this situation for affected sib-pairs with unrelated parents when either both parents are genotyped or genotype data is available for two additional unaffected siblings when parental genotypes are missing. However, this is not the case for consanguineous pedigrees, where missing genotype data for any pedigree member within a consanguinity loop can increase false-positive evidence of linkage. The false-positive evidence for linkage is further increased when cryptic consanguinity is present. The amount of false-positive evidence for linkage is highly dependent on which family members are genotyped. When parental genotype data is available, the false-positive evidence for linkage is usually not as strong as when parental genotype data is unavailable. Which family members will aid in the reduction of false-positive evidence of linkage is highly dependent on which other family members are genotyped. For a pedigree with an affected proband whose first-cousin parents have been genotyped, further reduction in the false-positive evidence of linkage can be obtained by including genotype data from additional affected siblings of the proband or genotype data from the proband's sibling-grandparents. When parental genotypes are not available, false-positive evidence for linkage can be reduced by including in the analysis genotype data from either unaffected siblings of the proband or the proband's married-in-grandparents.

P0944. Polymorphism of cytokines genes in COPD patients from Russia

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The purpose of this study was to investigate the possible roles of the cytokines genes in the development of chronic obstructive pulmonary disease (COPD). Polymorphisms in the genes encoding *IL1b*, *IL-1RN, TNFA*, *LTA*, *IL6, IL8* & *IL10* were investigated in COPD patients (N=319) and healthy individuals (N=403) living in Ufa, the Republic of Bashkortostan.

We observed that *IL1RN**2/*IL1RN**2 genotype of *IL1RN* gene was associated with susceptibility for COPD (9.8% vs 4.67%; χ^2=5.45, *df*=1, *p*=0.02; OR=2.21). Analysis of the *LTA* gene polymorphic locus 252A/G showed that in patients with COPD, the frequency of the GG genotype was significantly higher than that in the control group (7.84% vs 3.72%; χ^2=5.00, *df*=1, *p*=0.025). The increase of this genotype was significant in case of IV stage of COPD (11.18% vs 4.79%; χ^2=3.075, *df*=1, *p*=0.07). Frequency of genotype combination *TNFA*-308 G/G and *LTA*252 A/A significantly decreased in COPD group (38.55% vs 46.93% in control group; χ^2=8.82, *df*=1, *p*=0.0039). The frequency of GG genotype of the *IL6* gene was higher in the patients with IV stage of COPD (43.75% vs 31.54%, χ^2=4.14, *P*=0.041).

Our results indicate that the genotype frequency of the -511 T/C, 3953 T/C of *IL1B*, -308G/A of *TNFA* , -174G/C of *IL6,* -251A/T of *IL8* and - 627C/A of *IL10* genes polymorphisms was similar in COPD and healthy control groups.

P0945. Alleles of the Toll-Like-Receptor signaling pathway in Coronary Heart Disease patients

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Atherosclerosis is characterized by signs of chronic inflammation and systemic immune responses. Bacterial infection and inflammation have been implicated in the pathogenesis of coronary heart disease (CHD). Toll Like Receptors (TLRs) are the initial sensors of invading microbes, and interact with endogenous ligands such as oxLDL. They activate multiple pathogen-specific immune responses, e.g. cytokine expression.

To elucidate CHD predisposing genotypes of the TLR pathway, we analysed the complete haplotype information of 16 genes within this pathway, encoding for receptors, ligands, and signal transducers (TLR1, TLR2, TLR4, TLR5, CD14, CD36, MyD88, TOLIP, Traf6, TAB1, TBK1, TAK1, TRAM, TIRAP, IRAK1 and IRAK4). In a large scale case-control association study (1103 early-onset CHD patients and 736 controls), 136 tagging single nucleotide polymorphisms (tSNPs) were genotyped using the high-through-put SNPlexTM technology. To adjust for all conventional CHD environmental risk factors, multiple logistic regression analysis was performed with stepwise selection (Wald). Before and after adjustment, polymorphisms of TLR4 and TLR5 showed significant p-values. The tSNPs of TLR4 and TLR5, showing significant p-values after stratification for environmental factors (p=0.029 and 0.018, respectively) were replicated with a second population of further 2206 CHD patients and controls. Genotyping was performed using the TaqManTM technology, and associations were adjusted by logistic regression. Before and after adjustment, no evidence for associations with CHD was detected for the replicated tSNPs at both, the allele and genotype level.

Thus, the study does not indicate any association of a TLR-pathway specific allele with an increased risk of CHD susceptibility in the Caucasian population.

P0946. Functional gene variants in the regulatory regions of COX-2 gene and non-melanoma skin cancer after organ transplantation.

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Overexpression of COX-2, which results in excessive prostaglandine production, has been observed in human epidermal keratinocytes after UVB injury, in squamous cell skin cancer, in actinic keratoses and in early stages of carcinogenesis. The dysregulation of COX-2 expression observed in carcinogenesis can in part be due to functional changes affecting regulatory elements in the 5' or 3' UTR regions of the gene. Two polymorphisms (-765G>C, and -1195A>G) in the promoter and one polymorphism in 3'UTR (8473T>C) regions have been described. To elucidate if these COX-2 variants can be associated to non-melanoma skin cancer (NMSC) susceptibility after transplantation, we genotyped 240 North Italian transplant patients (107 cases and 133 controls). The distrubution of COX-2 univariant genotypes, and of estimated haplotypes was not significantly different between the overall NMSC group and controls. However, stratification by kind of tumors (SCC or BCC), and age at transplant, showed that allele -765C was never present in BCC cases who underwent transplantation before 50 years (CC+CG vs GG Fisher exact test P=0.00042) suggesting a protective effect of allele -765C in this subclass of patients. To determine if other polymorphisms in these regions can contribute to NMSC susceptibility, heteroduplex screening of the proximal 5' and 3' regulatory regions of the gene was performed. One rare polymorphism (-62C>G), which was present in both study groups was identified. No other variant was found suggesting that sequence variations in these regulatory regions of the COX-2 gene do not likely contribute to NMSC risk in this population.

P0947. Maternal mosaicism of a RYR2 mutation.

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Mutations in the cardiac ryanodine receptor (RYR2) gene have been reported to cause autosomal dominant catecholaminergic polymorphic ventricular tachycardia (CPVT). Exposure to stress or exercise in these patients can lead to ventricular arrhythmias and sudden cardiac

death. More than 60 mutations in RYR2 have been reported. They cluster in 3 regions: the amino terminus, a central domain and the carboxyl terminus.
The diagnosis CPVT was made in a 10year old boy. He presented with syncope at the age of 9 years. Family history was remarkable: one sister died at the age of 3 weeks while crying and another sister died suddenly at the age of 13 years while being angry. Both parents had not experienced symptoms of ventricular tachycardias.
In the index-patient, a mutation in the RYR2 gene was identified. The mutation c.12446A>C, resulted in a p.Tyr4149Ser amino acid change. Tyr4149 is located in the I-domain, a hydrophobic RYR2 region that is postulated to transduce cytoplasmic events by regulating the Ca2+ pore-forming domain. This domain is a hot-spot for arrhythmia-linked RYR2 mutations.
DNA sequencing of the RYR2 gene in the parents suggested that they were both homozygous for the wild-type allele. A de novo mutation was not expected, because the parents already lost two daughters with symptoms compatible with CPVT. Further investigation (dHPLC and digestion with Bse1) indicated that the mother was somatic mosaic for the mutation.
This finding confirms the occurrence of somatic mosaicism and has implications for the genetic counselling of apparently de novo cases of CPVT.

P0948. Association of variants of the *IL23R* and *ATG16L1* genes with susceptibility to pediatric Crohn's disease

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Two recent genome wide association studies reported association of Crohn's disease (CD) in adults with single nucleotide polymorphisms (SNPs) in the interleukin-23 receptor (*IL23R*) and autophagy-related 16-like 1 (*ATG16L1*) genes. The aim of this study was to examine the impact of these SNPs on risk of CD in children. Utilizing data from our ongoing genome-wide association study, we investigated the association of the previously reported SNPs at the *IL23R* (rs11209026) and *ATG16L1* (rs2241880) genes in a preliminary cohort of 142 pediatric CD cases with the childhood form of this disease and 281 matched controls. The minor allele frequency (MAF) of SNP rs11209026 in the cases was 1.75% while it was 6.61% in controls, yielding a protective odds ratio (OR) of 0.25 (95% CI 0.10 - 0.65; one-sided P = $9.2x10^{-4}$). A transmission disequilibrium test with 65 trios derived from our initial patient cohort confirmed the significant association with rs11209026 (one-sided P = 0.0017). Similarly, the frequency of allele G of rs2241880 in the cases was 63.73% while it was 51.96% in the controls, yielding an allelic OR of 1.62 (95% CI 1.21 - 2.18; one-sided P = $6.93x10^{-4}$). The ORs in our pediatric study are comparable with those reported previously in adult IBD case-control cohorts. As such, these variants confer a similar magnitude of risk of CD to children as for their adult counterparts. Our findings provide an independent confirmation of the effect of variants of these two genes on risk of CD.

P0949. Crohn's disease and polymorphisms of the NOD2/CARD15 and TNFA genes

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Crohn's disease (CD) is a complex multifactorial disorder, which is thought to result from the influence of environmental factors on genetically predisposed host. We studied the mutation frequencies (Gly908Arg, Arg702Trp,1007insC) in NOD2/CARD15 gene in the group of patients with Crohn's disease and in the control group. The frequencies of these mutations were following: Gly908Arg - 3,2% Arg702Trp-3,8%, 1007insC - 4,4%. 20,5% of the patients had at least one mutation in the NOD2/CARD15 gene. We analyzed also polymorphic variants of TNFA gene within a promoter region. Recently it was reported that this polymorphism is associated with a level of TNFA proinflammatory cytokine production and therefore is implicated in an inflammatory process. The frequency of *-308G/-308A* genotype was significantly higher in the group of patients (p=0.003, OR=3.89; 95% CI:1.57-9.65). According to the odds ratio it was found that the carriers of the *A*-allele of *TNFA* gene have 3-fold increase of Crohn's disease risk (OR=3.20, 95% CI:1.43-7.15). Interestingly that combined heterozygous genotype *-238A/238G* + *-308A/-308G* of *TNFA* gene resulted in considerable increase of CD risk (OR=18.8; 95% CI:1.02-345.97). TNFA is a key component of inflammatory process development and a target for anti-TNFA therapies which are used as a second or third-line treatment. Therefore further research may help to develop of new therapeutic strategies for the treatment of chronic inflammatory diseases by anti-TNFA medicines.

P0950. Variations in C-reactive protein gene and COPD: tagging SNPs analysis in case-control study

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BACKGROUND Chronic low-grade systemic inflammation (i.e. increased C-reactive protein, CRP) has been recognized to be present in COPD and, in turn, may have a systemic impact. However, remarkably little is known about the underlying mechanisms of systemic inflammation in COPD. Systemic inflammatory response might be modulated by genetic background. The aim of this study was to examine whether variations in CRP gene coding for acute phase protein are associated with susceptibility for COPD.
METHODS A total of 265 clinically stable patients with moderate-to-severe COPD (mean age 64 years, FEV1 39% pred, FEV1/VC, 44.6%) entering pulmonary rehabilitation and 90 healthy smokers (mean age 56 years, FEV1 109% pred, FEV1/VC, 77%) were enrolled. All subjects were Dutch Caucasians.
Six tagging single nucleotide polymorphisms (SNPs) (rs3091244, rs1800947, rs113084, rs1205, rs2808630, rs3093077) within CRP gene have been selected for genotyping from Seattle SNPs database (r2 0.8, MAF 5%).
RESULTS All tested SNPs were in Hardy-Weinberg equilibrium both in cases and controls. We found that carriers of 5237TT genotype (rs2808630) had 50% lower risk of having COPD (p=0.006, recessive model). In addition, two trends have been shown: homosigosity of 1444A (rs1130864), previously associated with elevated CRP plasma levels, has increased the disease risk by 2.1-fold (p=0.06, recessive and additive models), and carriage of 1059C allele (rs1800947) also was found to be associated with increased risk of COPD (p=0.07, OR=1.93, additive model). Analysis of CRP haplotypes confirmed these findings.
CONCLUSIONS Results indicate that SNPs in CRP gene might be associated with susceptibility for COPD.

P0951. Family based association analysis of TGFB1 as modifier gene in Cystic Fibrosis

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Cystic fibrosis (CF) is a lethal, multi-system autosomal recessive genetic disorder primarily affecting Caucasian populations, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Severity of clinical presentation in CF, particularly the pulmonary manifestation, are highly variable, even among CF patients presenting the same genotype. This variability is only partially explained by allelic heterogeneity at the CFTR gene. Literature data suggest that the severity in CF may be correlated with other genetic factors. Two polymorphisms (-509C/T and Leu10Pro) of the TGFB1 gene, which encodes for a cytokine involved in inflammation and tissue repair and expressed by several cells, have recently been associated to a more severe CF pulmonary manifestation in the American population (Drumm et al, NEJM 353:1443; 2005). We here report a TDT analysis of three TGFB1 functional polymorphisms (-509C/T, Leu10Pro e Arg25Pro) in Italian CF patients. Eigthy-three family trios were collected through a CF patient attending the Veneto Regional CF Centre of Verona. All the 83 patients were severe/severe CFTR mutation carriers and were clinically evaluated for respiratory parameters, gastrointestinal and nutritional status parameters, and other clinical variables related to the common CF complicances (diabetes, DIOS, etc). We found evidence of association between Arg25 homozygotes

and FEV1 (p=0.018). No association was found among other polymorphisms and studied clinical parameters. In order to confirm these preliminary data, we have enlarged our population, and we are now genotyping a second group of 60 unrelated Italian CF patients.

P0952. Syntaxin 1A: a possible modifier of lung disease in cysic fibrosis (CF)

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Phenotypic presentation, primarily progression of lung disease, varies significantly even among patients with the same CFTR genotype. There is growing evidence that polymorphic variants in genes besides CFTR play an important role in phenotype determination. Mainly genes that modulate the chloride-transport or the cycle of inflammation may lead to progressive lung disease. Syntaxin 1A (STX1A), a SNARE protein essential for the docking and fusion of exocytotic vesicles, binds to the N-terminal tail of CFTR and down-regulates its function by direct protein-protein interaction making it a promising candidate as a CF modifier.

DNA from 63 F508del homozygous and clinically well characterized patients was screened for mutations in the STX1A gene using our very sensitive (98%) SSCP/HD method followed by direct sequencing of the variants. Eight different sequence variants (c.31-21T>C, 3.17%; c.150C>T, p.N50N, 6.35%; c.204T>C, p.D68D, 37.30%, c.284-66G>A, 2.38%; c.467-38A>G, 42.06%; c.540+52C>T, 42.86%; c.790-15C>T, 3.97%, c.959+92C>T, 3.17%) were identified and tested for associations with lung function parameters such as functional residual capacity determined by whole-body plethysmography (FRCpleth), lung clearance index (LCI), trapped gas (VTG) and indexes of flow limitation (FEV1, FEF50). Most significant associations were found between c.204T>C and c.467-38A>G and the lung parameters LCI and FEF50.

Homozygosity for F508del combined with both c.204T/T and c.467-38G/G predicts a progressive and severe lung disease (n=11), combination with c.204C/C and c.467-38A/A, however, is associated with a much milder course (n=18; p = 0.001 for LCI, p=0.002 for FEF50). Thus, the STX1A gene seems to act as a modifier of lung disease in CF patients.

P0953. Cystic Fibrosis modifier genes and outcome of the disease. Correlation with the age of the patients.

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In Cystic Fibrosis (CF) patients, the CFTR genotype is not a good predictor of pulmonary disease, which is the direct cause of death in over 90% of CF patients. Genotype-phenotype studies showed a high variation in severity of pulmonary disease, even between patients sharing the same CFTR genotype, suggesting that outcome is under the influence of other genetic or/and environmental factors.

The aim of this study was to study the role of several potential modifier genes and if this role changes as the disease progress.

Sixty one cystic fibrosis patients over 23 years were initially included in the study. CFTR mutations were identified and genotype was carried out for the following genes: PI (alpha-1-antitrypsin), TNFA, TGFB1 (codons 10 and 25), IL10, IL6 and IFNG.

In order to minimise the role of the CFTR genotype, patients with mild mutations were excluded of the study. Data corresponding to the basal forced expiratory volume in 1s (FEV1), Crispin-Norman score, and forced vital capacity (FVC) were retrospectively recorded.

Results showed a double distribution of modifying effect: PI and IFNG showed good correlation to FEV1 and FVC between 10 and 15 years, whereas for TGFB and IL6 we found good correlation in patients over 18 years. Crispin-Norman score showed good correlation only with TGFB1 codon 10 genotype. We did not find correlation neither for IL10 nor for codon 25 of TGFB1.

These findings suggest that the role played by the modifier genes deppends on the stage of the disease, and changes as the disease progress

P0954. *Polymorphisms of the IL1B and IL1RN genes are associated with essential hypertension in Tatars from Russia*

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Essential hypertension (EH) is a common disease and a major risk factor for many cardiovascular events. The hereditary nature of hypertension is well established. Inflammation plays a pivotal role in the pathogenesis of cardiovascular disease. Inflammatory damage of endothelium leads to impairment of the vascular tone. Interleukin-1 (IL-1) regulates the expression of genes involved in inflammatory response. IL-1 receptor antagonist (IL-1Ra) is an endogenous inhibitor of IL-1 signaling. The rare allele (*IL1RN*2*) of a variable number tandem repeat polymorphism in the *IL1RN* gene was demonstrated to significantly reduce IL-1Ra level.

The aim of the present study was to investigate the association between *IL1B* and *IL1RN* polymorphisms with EH in Tatar ethnic group (Bashkortostan, Russia).

Our study comprised 360 hypertensive patients. The control group consisted of 242 healthy individuals. Genomic DNA was isolated from peripheral blood by phenol-chloroform extraction. Genotyping was performed using polymerase chain reaction followed by *Ava*I digestion (for *IL1B-511T/C* polymorphism). Genotypes and allele frequencies distribution was estimated using Fisher's exact test. Odds ratios with 95% confidential interval were calculated.

*IL1RN*2* allele was found to be associated with risk of early onset (before 45 years) of EH (OR=3.27, CI: 1.65-6.49). We have shown that *IL1B-511*C/*C* genotype was less frequent among hypertensive patients who had stroke (37.5% versus 57.52% in group of patients with non-complicated EH, P=0.03). We may conclude that *IL1B-511*C/*C* is associated with decreased risk of stroke in patients with EH (OR=0.44, CI: 0.22-09).

Our results suggest a significant role for *IL1* family genes in the development of cardiovascular disease.

P0955. Screening for SLC26A4 gene mutation in unilateral hearing impairment and same-side EVA

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Mutations of the SLC26A4 (PDS) gene are involved in either syndromic deafness, characterized by congenital sensorineural hearing loss and goitre (Pendred syndrome), or congenital isolated deafness (DFNB4). In our previous studies, we identified mutations in cases with bilateral hearing impairment and unilateral enlarged vestibular aqueduct (EVA) in two cohorts (Pendred and DFNB4). In the present study, we screened SLC26A4 by DHPLC in 25 patients presenting with unilateral hearing impairment associated with same-side EVA. None of these patients had two deleterious genetic changes in trans. Seven of them had a single heterozygous SLC26A4 variation. Among these variations, we found three established mutations and three potentially deleterious genetic changes.

We conclude that causative SLC26A4 mutations in patients with unilateral deafness and same-side EVA are at best infrequent.

P0956. No evidence for an association of the methylenetetrahydrofolate reductase gene *C677T* polymorphism with major depressive disorder

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Major Depressive Disorder (MDD) is a complex disorder thought to result from multiple genes interacting with environmental and developmental components. The 5,10-methylentetrahydrofolate reductase

gene (*MTHFR*) is considered to be an important candidate gene of MDD. A single base mutation *C677T* (rs1801133) results in the production of a mildly dysfunctional thermolabile enzyme. The *MTHFR 677T/T* genotype and, to a lesser extent the *677C/T* genotype, is associated with a significant elevation in the circulating concentrations of the homocystein and a decrease in serum folate concentrations, which may parallel a similar reduction in 5-methyltetrahydrofolate in CNS and may lead to a reduction in monoamine neurotransmitter function and elevated risk of MDD. To test the hypothesis that *MTHFR C677T* polymorphism can be involved in predisposition to MDD we conducted the association study in sample of 1222 patients with recurrent MDD and 833 control subjects. The statistical power of our sample to detect an odds ratio of 1.5 for *677T/T* genotype was 88%. There are not significant differences in genotype/allele frequencies between depressive patients and controls, neither in total samples, nor in females and males separately. We also failed to confirm the association of this gene with MDD using meta-analysis of 4 case-control studies. Our results exclude a major role for the *MTHFR C677T* polymorphism in conferring susceptibility to recurrent unipolar depression in British population. However, there are many rare mutations in the *MTHFR* gene, also associated with minor changes in the enzyme activity of MTHFR, which could play a role in susceptibility to depression.

P0957. Association of INSR and IRS-1 gene polymorphisms with type 2 diabetes in the Northern Greek population

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Type 2 diabetes is a heterogeneous disorder. Several genes have been proposed to contribute to its pathogenesis. Associations found in a particular population were often not confirmed in others, suggesting regional differences. In the present study we tested the possible association of variants in the genes for insulin receptor (INSR) and insulin receptor substrate-1 (IRS-1) with type 2 diabetes in the Northern Greek population. The distribution of three polymorphisms of the INSR gene (3559C>T or Y984Y, 3560G>A or V985M and 3781C>T or H1058H) and one polymorphism of the IRS-1 gene (3932G>A or G972R) were determined in 100 unrelated diabetic patients and 100 age-matched unrelated control subjects. Genotyping was performed by PCR amplification followed by restriction digestion with appropriate enzymes. Samples with certain patterns were further sequenced in order not to conceal the coupling between the Y984Y and V985M polymorphisms.

Genotypic and allelic frequencies were compared between the two groups. No association was found for the four polymorphisms with diabetes. The analysis of composite genotypes revealed that individuals heterozygous for the Y984Y polymorphism and homozygous normal for the other three, were found more frequently among the controls (p=0.033). This genotype might be protective against type 2 diabetes in Greek individuals.

P0958. Using high-density SNP genotyping to fine-map the diabetic nephropathy locus on 3q in patients from Finland

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Diabetic nephropathy (DN) is the leading cause of end-stage renal disease and affects about 30 % of all diabetic patients. Our previous genome scan using discordant sib pairs from Finland identified the 3q locus with a suggestive linkage (LOD 2.6, P=0.0004) to DN (Kidney International 2007;71:140). To capture genetic variants in LD with the causal SNP, we carried out a high-density SNP genotyping strategy for fine-mapping the 3q locus. Based on the HapMap database, 3072 tagged SNPs (r2>0.7, MAF>2%) covering 28 Mb, from 124 to 152 Mb on chromosome 3 (build 35), were selected by the LD-based tagging method and were genotyped in 643 samples (456 Finnish and 187 Icelandic) using the Illumina SNP genotyping platform. In results, 635 samples (98.7%) and 2820 SNPs (92%) reached the threshold of data quality controls. In association analysis, 154 SNPs in the Finnish samples and 149 SNPs in the Icelandic samples showed allelic associations (P<0.05) between cases and controls. Eight SNPs with the same direction in both two sets showed significant associations with DN. In combined sample analysis using the Mantel-Haenszel model, 43 SNPs (1.5%) reached the significant level of association (P<0.01). The P values for two most significant SNPs among these 43 SNPs were 0.0000038 and 0.00006. respectively. For the follow-up studies, a large DN cohort containing more than 1000 cases and 1000 controls from the FinnDiane project in Finland is used. Genotyping of these 43 SNPs in the FinnDiane cohort for replication studies is ongoing.

P0959. Association of a dopamine transporter gene (DAT1) polymorphism with alcohol dependence in Russian population from West Siberia

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The dopamine transporter (*DAT1* or *SLC6A3*) plays the important role in dopamine metabolism, serves as a critical regulator of dopaminergic neurotransmission. The association of *DAT1 VNTR* with alcoholism was found in many populations. Two polymorphisms in *DAT1* gene, *VNTR* in 3'-untranslated region and 1342 *A/G* polymorphism in exon 9 were investigated in three groups: alcoholics (n=92), non-alcoholic controls (n=81) and population samples from three Russian populations of Tomsk area (n=583). For both loci the genotype frequencies obeyed the Hardy-Weinberg equilibrium in all groups. No association of exon 9 *A/G* polymorphism with alcoholism was found. As to *VNTR* polymorphism the protective effect of 10/10 genotype was revealed: the odds ratio (*OR*) for this genotype for alcohol dependence were 0.49 (95 % *CI* 0.25 - 0.96; *P* = 0.025) in patient/control and 0.64 (95 % *CI* 0.40 - 1.02; *P* = 0,048) in patient/population samples comparisons. Thus, obtained data testify for importance of *DAT1* variability in predisposition to alcoholism. This work was supported by the Russian Foundation for Basic Research (project no. 05-04-98007-p_ob_a).

P0960. Contribution of the dopamine transporter (*SLC6A3*) and the dopamine receptor D2 (*DRD2*) genes to cocaine addiction: a case-control association study in the Spanish population

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Drug addiction is a complex neuropsychiatric disorder in which environmental, social and genetic factors are involved. Several evidences suggest that the dopaminergic system may play an important role in cocaine abuse and dependence and that decreased dopaminergic function underlies cocaine addiction. The dopamine transporter (DAT1) and the dopamine receptor D2 (DRD2) mediate cocaine effects in the central nervous system. In this regard, cocaine binding to DAT1 causes an overstimulation of the dopamine reward system, and decreased DRD2 availability has been reported in cocaine addicts. In addition, consistent associations have been reported between cocaine addiction and several polymorphisms in the dopamine transporter (*SLC6A3*) and the dopamine receptor D2 (*DRD2)* genes. We aimed to replicate these results by genotyping the Int8 VNTR of the *SLC6A3* gene and the *Taq*IA and *Taq*IB RFLP polymorphisms within the *DRD2* gene in a Spanish sample of 85 cocaine addicts and 85 sex-matched healthy controls. The case-control study showed an overrepresentation of the 5R/5R genotype of the *SLC6A3* gene in the group of cocaine abusers when compared to controls (p=0.009). However, no significant association was detected when allele frequencies of the *SLC6A3* Int8 VNTR or genotype, allele or estimated haplotype frequencies of the *DRD2 Taq*IA and *Taq*IB SNPs were considered. We also evaluated potential epistatic effects between the *SLC6A3* and *DRD2* genes but found no evidence supporting the existence of interactions. Although additional research in other datasets with an increased sample size is required, our preliminar results suggest that the *SLC6A3* gene may predispose to cocaine addiction.

P0961. Mutational screening in Dysferlin gene

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Mutations in dysferlin gene (*DYSF*) cause different muscular dystrophy phenotypes with autosomal recessive inheritance including Limb Girdle Muscular Dystrophy 2B (LGMD2B), Miyoshi Myopathy (MM) and Distal Anterior Compartment Myopathy (DAT). *DYSF* gene, that maps to chromosome 2p13, has 55 exons and codifies a protein of about 237 kDa.

Dysferlin protein is expressed in skeletal muscle and peripheral blood monocytes. The genomic analysis of the *DYSF* gene has proved to be time consuming because its long size. We designed a mutational screening strategy based on cDNA from monocytes to find out whether the mutational analysis could be performed in mRNA from a source less invasive than the muscle biopsy

We studied 50 patients from 31 families clinically diagnosed as MM, LGMD2B and DAT through: 1) the analysis of dysferlin expression by immunohystochemistry in muscle biopsies and Western blot in peripheral blood monocytes and, 2) the screening of mutations in *DYSF* gene by sequencing monocytes cDNA, amplifying 14 fragments that covers the 55 exons. Finally, the results were confirmed in genomic DNA.

We identified mutations in *DYSF* gene in the thirty one families studied: 17 of them in a homozygous state and 14 in a heterozygous state. We found the two mutated alleles in twenty eight families and only one mutated allele in 3 families. Seventeen mutations were missense, 10 nonsense, 13 frameshit and 2 splice site mutations.

This report furnish evidence of reliable mutational analysis using monocytes cDNA and constitutes a good alternative to genomic DNA analysis.

P0962. Haplotypic classification of DEB in Tunisia: evidence for genetic variability among the different geographic regions

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The dystrophic epidermolysis bullosa (DEB), a group of heritable blistering skin diseases, is characterized by abnormalities in the anchoring fibrils at the dermal-epidermal basement membrane zone. Mutations within COL7A1 gene that encodes type VII collagen, the major component of the anchoring fibrils, is responsible for DEB.

In order to study the mutational spectrum of DEB in Tunisia, we classify the DEB Tunisian patients on the basis of their haplotype. Thirty recessive DEB patients, belonging to 24 families, have been genotyped with five microsatellite markers overlapping the COL7A1 region.

The genetic investigation showed that there are two common haplotypes, 3-1-6-3-3 and 1-11-14-6-6, which are shared by 6 and 2 families respectively. The most frequent haplotype, 3-1-6-3-3, shared by 25% of the families, is mainly found among families originating from Central Tunisia, and the second one, 1-11-14-6-6, shared by 8% is found among families originating from North Eastern region. The other studied DEB patients have different haplotype and scattered on different cities of the country. All together, seven different haplotypes have been identified. This study shows haplotypic heterogeneity among Tunisian DEB families, thus suggesting a mutational heterogeneity.

P0963. The *ACE* insertion/deletion genotype is associated with elite athletic performance

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Background: Growing evidence suggests a significant genetic contribution to human physical performance. The deletion (D) allele of angiotensin-I converting (*ACE*) gene has been associated with higher ACE activity. In the current study we evaluated the association between *ACE* ID polymorphism and elite endurance athletic ability.

Methods: Ninety-two elite athletes, classified as either endurance type performers (51 long distance runners and triathletes) or power athletes (41 sprinters and long jumpers), were genotyped for the *ACE* ID polymorphism using polymerase chain reaction on leukocytes DNA. Their *ACE* genotypes were compared to those of 405 healthy individuals.

Results: Allele and genotype frequencies differed significantly between the groups. The frequency of the D allele was 0.78 in the elite long distance runners and triathletes, 0.66 in the control group, and 0.57 in the sprinters and long jumpers (X^2 = 10.04, P = 0.006). Moreover, the *ACE* DD genotype was significantly more prevalent among the long distance runners (0.63) compared to the controls (0.43), and compared to the sprinters and long jumpers (0.34) (X^2 = 21.99, P = 0.0002). Importantly, in the group of elite athletes the odds ratio of *ACE* DD genotype for endurance performance was 3.24 (95% confidence interval 1.38-7.61), and of *ACE* II genotype was 0.25 (95% confidence interval 0.06-0.97).

Conclusions: The present study clearly indicates a positive association of the *ACE* D allele with sustained high-level endurance performance. These data support the notion that increased ACE activity may be of benefit to endurance-type elite athletes.

P0964. Association of TNF-alpha G-308A promoter polymorphism with human embryo viability

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Tumor necrosis factor alpha (TNF-alpha), a multifunctional cytokine, is expressed in embryonic tissues practically at all stages of human development. It is believed that TNF-alpha boosts death signaling to kill the embryo if damages triggered by detrimental stimuli may culminate in structural anomalies, and stimulate protective mechanisms if the repair of these damages may prevent maldevelopment. Nonetheless, no data about the influence of *TNF-alpha* G-308A promoter polymorphism for embryo viability itself are found. Samples of trophoblast tissue from delayed miscarriages (n=118) and dried blood spots of newborns (n=300) were analyzed for *TNF-alpha* G-308A polymorphism. The genotypes -308GA and -308AA *TNF-alpha* were more prevalent among newborns (24%) than in the group of delayed miscarriages (14.4%; P=0.03). Carriers of allele -308A predominated in the neonatal group to compare with delayed miscarriages (12,3% vs. 7,2%; P=0.04). In conclusion, high frequency of mutant *TNF-alpha* allele among newborns reflect the possible advantage for embryo survival, that in turn, may lead to its stable frequency in population.

P0965. Polymorphism of IL4RA gene is associated with endometriosis

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Endometriosis is a common multifactorial disease. Typical endometrioid cells are characterized by increased cytokine activity. Cytokine genes are highly polymorphic that results in synthesis of proteins with various functional activities. This study is devoted to definition of role of allelic variants of IL4 and IL4RA genes in pathogenesis of endometriosis.

DNA samples from the patients with endometriosis (n=36) and control group of women without any gynecologic complications (n=69) were included in the study. Polymorphisms of IL4 (-590T>C) and IL4RA (1902A>G or Gln551Arg) were defined by PCR-RFLP assay.

The distribution of IL4 and IL4RA genotypes was in agreement with the HWE law (p>0.05). The frequencies of alleles and genotypes of IL4 gene did not differ between studied groups (p>0.05). However the frequency of Arg/Arg genotype of IL4RA gene was significantly higher in patients with endometriosis (16.7%) than in control group (1.4%, p<0.01).

The results of this pilot study demonstrate a role of IL4RA gene polymorphism in development of endometriosis. According to odds ratio the presence of Arg551/Arg551 genotype more than 13-fold increases the risk of endometriosis (OR=13.60; CI: 2.43-76.26). Obtained data allow defining Gln551Arg polymorphism as perspective prognostic marker of endometriosis.

P0966. Novel gene loci for the Enlarged Vestibular Aqueduct Syndrome

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Background: Enlarged Vestibular Aqueduct Syndrome (EVA-Syndrome) (MIM 603545) is the most common form of congenital inner ear abnormality seen in radiological assessment, associated with sensorineural hearing impairment or syndromic forms of deafness such as Pendred syndrome (MIM 274600). Up to now only mutations in the SLC26A4 gene, located on Chromosome 7q31, have been made responsible for Pendred- and EVA-Syndrome. This gene product, a transporter of iodide and chloride, is called pendrin. In this study we analyzed 64 patients with EVA and hearing loss to distinguish between the Pendred- and EVA-syndrome.
Methods: Individual exon and intron transitions of the SLC26A4 gene of patients were PCR amplified. Direct automatic sequencing of variant fragments was performed with the same primers. A genome-wide linkage analysis was accomplished using the Affymetrix 10K/50K XbaI SNP GeneChip® mapping array.
Results: In the analysed patient collective with Pendred syndrome and/or enlargement of the vestibular aqueduct, a total of eighteen SCL26A4 mutations were detected. A mutation could not be detected in 36 % of the cases. With a genomewide linkage analysis, of these families, using SNP technology, it was possible to identify potential new gene loci for the EVA-Syndrome.
Conclusions: The novel gene loci will be analyzed for additional genes involved in the development of the EVA-Syndrome. Our results indicate evidences of a accessory gene for this Syndrome.

P0967. Clinical and genetic familial study of 61 children showing different epileptic phenotypes

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During the last 10 years many advances in the genetics of epilepsies have been done. In particular, several mutations have been detected in genes encoding ion-channels, suggesting that epilepsies are “channelopathies”. Nevertheless, the genetic basis of idiopathic epilepsies remain unknown for a large number of cases and the genetic transmission of these diseases appear to be “complex” because of the great genetic heterogeneity and the coexistence of different epileptic types in each familial cluster. Moreover, in different epilepsies, mutations in the same gene have been reported.
We performed a clinical and genetic study on 60 Italian families (61 probands) showing idiopathic epilepsies by sequencing DNA regions previously associated to epilepsies in order to collect data on the type and frequency of ion channel mutations. Partial epilepsies represented 28% of the sample, whereas the remaining 72% was constituted by generalised epilepsies, subdivided in myoclonic (JME, BMEI, MAE, SMEI) and non-myoclonic (GEFS+, GTCS, CAE, FS) epilepsies.
We observed a genetic complexity in all phenotype groups: any epileptic type may be transmitted in either an autosomal dominant or a recessive manner, and furthermore even within a single family, epilepsy can be transmitted in either manner.
No significant phenotype identity among generations was observed. Moreover, we found an excess of transmitting mothers but no differences in the sex of children, suggesting a possible role of mitochondria in the disease pathogenesis. The frequency of known mutations in the analyzed regions resulted very low, while a new missense mutation in SCN1A was identified in one subject.

P0968. The analysis of association of six polymorphisms with development of ESRD in Romanian population - a preliminary report

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Diabetic nephropathy is the leading cause of ESRD. The literature suggests that genetic predisposition to ESRD is very complex.
In this study we assessed the association of six polymorphisms with ESRD in Caucasian subjects from South part of Romania.
We analyzed blood samples from unrelated dialyzed patients (etiology: T1DM: 83, T2DM: 87, chronic glomerulonephritis: 84) and healthy controls (n=494). Six genes polymorphisms associated with extracellular matrix proliferation (HSPG BamH1, TGF-beta-509C/T) or regulation of blood flow and vascular function (ACE ID, AGTR1, eNOS, MTHFR) were genotyped by PCR or PCR - RFLP. Genotype distribution for both cases and controls is in respect with Hardy-Weinberg equilibrium for all studied polymorphisms.
Our results showed that eNOS ID polymorphism increases the risk for ESRD in all groups. The highest value was observed in T1DM patients (OR_{DD}:3.9844, CI95%:1.9198<OR<8.2694). The risk for renal failure was increased by AGTR1 A1166C in T1DM patients (OR:1.401, CI95%: 0.6753<OR<2.9066) and by TGF-beta -509 CC (OR = 1.9) in T2DM patients. A little differences in distribution of MTHFR C677T (OR_{TT}:1.48, CI95%:0.8197<OR<2.6722) and ACE ID (OR_{DD}:1.2374, CI95%:0.8134<O.R.<1.8824) was observed only in dialyzed nondiabetic patients. HSPG BamH1 polymorphism has not a significant contribution to ESRD. We observed no difference regarding the contribution of these genotypes or alleles between men and women whatever renal disease is. In conclusion, our preliminary results suggest that the genetic predisposition to renal failure depends on the etiology of renal disease. Further clarification is anticipated as we increase our lots size. (This study was supported by Romanian CEEX53/2005 grant).

P0969. Variations in ERα gene predict the outcome of ovarian stimulation in in vitro fertilization

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Introduction - The outcome of *in vitro* fertilization (IVF) depends substantially on the effectiveness of controlled ovarian hyperstimulation (COH) by administration of follicle stimulating hormone (FSH). Endogenously produced estrogens extend the action of exogenous FSH in promoting folliculogenesis in COH. In the current study we determined the association between genetic variations in *ER*α and *ER*β genes and different causes of infertility, and analyzed the influence of these variations on the COH outcome in regards to the age and clinical parameters of IVF patients. Material & methods - *Pvu*II T/C (rs2234693) and *Xba*I A/G (rs9340799) SNPs, and $(TA)_n$ microsatellite polymorphism in *ER*α gene as well as *Rsa*I G/A (rs1256049) SNP and $(CA)_n$ microsatellite polymorphism in *ER*β gene were genotyped in 159 IVF patients. Results - Women‘s age was associated with poorer COH outcome in linear manner. In addition, patients with endometriosis represented diminished ovarian response to FSH compared to tubal factor infertility. *ER*α *Pvu*II T/C, *Xba*I A/G and *ER*β *Rsa*I G/A were associated with the length of the microsatellites of the respective genes. Shorter *ER*α $(TA)_n$ gave a higher risk for unexplained infertility. Longer *ER*α $(TA)_n$ repeat in association with *ER*α *Pvu*II C allele were associated with better COH outcome in an age-independent manner. Conclusions - Variations in *ER*α gene, in addition to the woman’s age, predict the outcome of COH in IVF.

P0970. A Genomewide scan in Spanish BRCAX Families: Three new candidate loci for susceptibility in Familial Breast Cancer

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Introduction: The two major breast cancer susceptibility genes BRCA1 and BRCA2 have been shown to be involved in a significant proportion (25%) of families affected with breast and ovarian cancer. On the other hand, it is also clear that a large proportion of families segregating

breast cancer alone are not caused by mutations in BRCA1 or BRCA2 (BRCAX families). Unfortunately, the discovery of additional breast cancer predisposition genes has so far been unsuccessful, presumably because of genetic heterogeneity, low penetrance, and/or recessive/polygenic mechanisms.
Material and Methods: With the aim of finding breast cancer predisposition genes, we genotyped 6000 SNPs across the genome using a high-throughput technology (Illumina Linkage panel IV), in 41 BRCAX families from Spanish population. SNP data analysis has been performed using the new version of Merlin (MERLIN v.0.10.2) to model marker-marker linkage disequilibrium in order to avoid artefacts due to LD among SNPs.
Results: These data analysis have allowed us to identify two regions with suggestive linkage (Dominant Parametric LOD score ~ 2.3, alpha ~0.3) in chromosomes 3q and 6q, and one region with complete linkage (Dominant Parametric LOD score > 3.2, alpha ~0.5) in chromosome 21q. In order to confirm these regions, and narrow them if possible, we are genotyping 2 cM density microsatellite map within them.

P0971. Cardiovascular symptoms associated with mutations in the genes encoding fibrillin-1 (*FBN1*) and transforming growth factor beta receptor type II (*TGFBR2*)

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Objectives: Mutations in the genes encoding fibrillin-1 (*FBN1*) and transforming growth factor beta receptor type II (*TGFBR2*) are known causes of Marfan syndrome (MFS) and related disorders. The objective of the present study was to assess whether the type of mutation is linked to a particular clinical subtype of the cardiovascular condition.
Methods: The genetic and clinical records of 36 patients referred to us for molecular genetic diagnosis were reviewed. The frequency of a number of clinical signs was determined for the following three groups of patients: 1. carriers of a *FBN1* mutation that affects a Ca2+-binding epidermal growth factor-like domain (cbEGF); 2. carriers of a mutation associated with a premature termination codon, PTC, in *FBN1*; 3. individuals with no mutation detected in either *FBN1* or *TGFBR2*.
Results: Throughout the study cohort, the incidence of aortic dissections per se did not depend on the type of mutation. However, we found that mutations affecting the calcium-binding epidermal growth factor-like domain were more frequently associated with a dissection of distal parts of the aorta than mutations that lead to a premature termination codon (χ^2_1: p=0.013), suggesting that the spatio-temporal pattern of vascular deterioration may vary with the type of mutation.
Conclusion: Routine genetic testing of patients with suspected MFS or thoracic aortic aneurysms/dissections could provide further insight into genotype/phenotype correllations related to aortic dissection.

P0972. Fragile X syndrome screening in families with consanguineous and nonconsanguineous marriages in the Iranian population

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Fragile X syndrome is the most common form of inherited mental retardation (MR). It is caused by the expansion of CGG triplet repeats in the fragile X mental retardation 1 (FMR1) gene. Normal individuals have <50 CGG repeats and permutation carriers have 50-200 repeats, but affected individuals with full mutation have over 200 repeats, which are generally hypermethylated. The objective of this study was to screen for and compare the frequency of fragile X syndrome in families afflicted with MR having consanguineous and nonconsanguineous parents. We examined a total of 1000 families afflicted with MR that had been referred to the Genetics Research Center (GRC) and Kariminejad-Najmabadi Pathology and Genetics Center. Of these, 558 families had consanguineous parents and the rest of the families had a putative or established X-linked inheritance pattern. In order to detect fragile X syndrome, we performed molecular and cytogenetic detection methods simultaneously.
From the total number of families (1000), we identified 127 (12.7%) families with fragile X syndrome, 21 of which also had other chromosomal abnormalities. Among the consanguineous families, 8.2% were positive for fragile X syndrome.
Fragile X syndrome accounts for ¼ of X-linked mental retardation cases, therefore one could assume that up to 30% of the families in our population with the autosomal recessive mental retardation (ARMR) pattern of inheritance could contribute to X-linked mental retardation. Our findings clearly demonstrate that the genetic counselor should rule out the X-linked gene in ARMR inheritance as part of the routine diagnostic protocol.

P0973. A PCR screening test for recurrent mutations associated to X linked mental retardation FRAXA / FRAXE / ARX / PQBP1

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This study presents molecular investigation of recurrent mutations associated to 4 syndromes presenting with mental retardation : CGG expansion in exon 1 of FMR1 gene for Fragile X syndrome (FRAXA), CCG expansion in FMR2 gene for FRAXE mental retardation syndrome, c.428_451dup24 and c.333_334ins(GCG)7 in exon 2 of Aristaless related X gene (ARX) and insertions/deletions frameshift mutations in exon 4 of PQBP1 gene. The aims of the underlying study were to validate the FRAXA PCR screening on different genotypes of FMR1 gene including rare patients who are mosaic for a full mutation and an allele in the normal range, to estimate the prevalence of FRAXE, ARX and PQBP1 syndromes among individuals referred for FRAXA testing and eventually to enlarge the clinical phenotype of ARX and PQBP1 syndromes.
We have first validated our protocol on well defined samples with the recurrent genes mutations and have used it for the diagnostic evaluation of 624 male and 283 female probands, including 15 affected sib-pairs, combined with a retrospective study of 172 males known to be normal by southern blotting at FRAXA locus. In the male group we have identified one PQBP1 mutation in two brothers and no FRAXE expansion or ARX duplication. FRAXA full mutation was identified in 10 male and 4 female patients. In frame deleting variations were detected in both ARX exon 2 and PQBP1 exon 4, as well as duplication in ARX gene that are likely to be non pathogenic.
The test can be used for performing diagnoses, postnatal and prenatal.

P0974. Sub-Components of Event Related Potentials (ERP) Associated with Polymorphisms in Glutamate, GABA and Dopamine Neurotransmitter Receptors

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Event related potentials (ERPs) reflect perceptual and cognitive processes and therefore provide an electrophysiological window on to brain function during cognition. P300 component as well as spectral components of ERPs is highly heritable. Potential candidates for the genetic determinants of ERPs are genes encoding several most important neurotransmitter receptors. In this study, we aimed to identify associations of functional polymorphisms of genes encoding glutamate receptor 2A subunit, (NMDAR2A), GABA receptor gamma-2 subunit (GABRG2) and dopamine receptor D2 subunit (DRD2) with auditory ERPs. EEG recordings and genetic analysis of 72 Turkish male healthy volunteers were performed in this study. Groups were formed according to their polymorphism types for each of the three neurotransmitter receptors. Three cognitive paradigms were designed to generate audi-

tory ERPs. ERP recordings of each polymorphic group were analyzed in the time domain by measuring P300 amplitude and latency, and furthermore, in the time-frequency domain by decomposition of ERP signals by using wavelet transform with analysis of variance (ANOVA). Results provide evidence of strong effect of GABRG2 polymorphism with ERP characteristics both in time domain and in time-frequency domain. The effects of NMDAR2A and DRD2 polymorphisms are less significant on P300 wave. However, time-frequency decomposition of ERP data showed other effects could be observed in specific frequency bands of all three polymorphisms that were not reflected in the time-domain representation of the data. The results of this study show that extended analyses on the correlations of genetic differences among normal population on electrophysiological parameters may extend our view on the genetic basis of cognitive activities.

P0975. GDAP1 gene mutation study among four Iranian families; Axonal recessive Charcot-Marie-Tooth type 4 disease

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Autosomal recessive Charcot-Marie-Tooth (CMT) disease (CMT4) is a complex group of severe childhood motor and sensory neuropathies, characterized by an early age of onset with rapidly progressive distal limb weakness and atrophy. CMT disease caused by mutations in the ganglioside induced differentiation-associated protein 1 (GDAP1) gene is a a severe autosomal recessive neuropathy originally reported in families with either demyelinating CMT4A neuropathy or axonal neuropathy with vocal cord paresis, which maps to the CMT4A locus on chromosome 8q21.1

we studied four families with 5 affected patients. Significant evidence for linkage was found for several markers from chromosome 8q (*D8S279*, *D8S551*, *D8S1474*, *D8S1289*,

and *D8S84*).

Following an initial indication for linkage of the family to the CMT4A locus on chromosome 8, we sequenced the Ganglioside-induced differentiation-associated protein 1 (GDAP1) gene whose genomic structure contains six exons, and identified mutation. We conclude that a novel GDAP1 mutation is associated with AR-CMT.

P0976. Genome wide linkage of a large serbian family with GEFS$^+$

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Generalized epilepsy with febrile seizures plus (GEFS$^+$) is a familial epilepsy syndrome characterized by heterogeneous phenotypes including febrile seizures (FS), FS$^+$ in which children had seizures with fever in early childhood that continued beyond age 6 years, or were associated with afebrile tonic-clonic seizures, and FS$^+$ with other types of generalized (absence, myoclonic, or atonic) or partial seizures. GEFS$^+$ is an autosomal dominant disorder with incomplete penetrance. Mutations in the SCN1B gene on 19q13 cause GEFS$^+$ type 1. Mutations in the SCN1A gene on 2q24 cause GEFS$^+$ type 2. Mutations in the GABRG2 gene on 5q31.1-q33.1 cause GEFS$^+$ type 3. GEFS$^+$ type 4 has been mapped to chromosome 2p24. Mutations in the GABRD gene can cause GEFS$^+$ type 5. Mutations in the SCN2A gene causes febrile seizures associated with afebrile seizures. Recently, a novel FS locus was reported on chromosome 21q22 in a family with 13 individuals affected by FS and afebrile seizures. We identified a large multigenerational GEFS$^+$ Serbian family with 20 affected individuals and we performed genetic linkage analysis for exclusion of known candidate genes and loci. Subsequently, we conducted a genome wide scan by the Linkage Mapping set version 2.5 (Applied Biosystems) genotyping 382 microsatellite markers located at on average of 10cM distance thoughout the genome. We used a dominant genetic model with 0.90 penetrance and a disease allele frequency of 0.001. Genome scan revealed linkage at some chromosomal loci and analysis of implicated regions is in progress.

P0977. Genetic polymorphisms in metabolizing genes and susceptibility to childhood leukemia in the Russian population.

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Genetic polymorphisms in metabolizing genes have been reported to be individually associated with increased susceptibility to childhood leukemia. A case-control study including 332 patients with Acute Lymphoblastic Leukemia (ALL), 71 patients with Acute myelogeneous leukemia (AML) and 490 healthy individuals was conducted. All patients were 0-18 years old, all controls were 18-34 years old and so failed the possibility to develop childhood leukemia. The polymorphic variants of genes *CYP1A1* (4887C>A, 4889A>G, 6235T>C), *CYP2D6* (1934G>A, 2637delA), *GSTT1* (deletion), *GSTM1* (deletion), *MTHFR* (677C>T, 1298A>C), *MTRR* (66A>G), *NQO1* (609C>T), *CYP2C9* (430C>T, 1075C>T), *CYP2C19* (681G>A) and *NAT2* (341T>C, 481C>T, 590G>A, 857G>A) have been studied using allele-specific hybridization with Pharmagen-biochip (EIMB). We found that "rapid acetylator" *NAT2* genotype 341T/T,481C/C,590G/G, as well as combination of a "rapid acetylator" *NAT2* genotype with "null" *GSTT1* genotype, "null" *GSTM1* genotype and double "null" *GSTT1*/*GSTM1* genotype more frequently occurred in patients than in population control and this difference is statistically significant. Also polymorphic variant *CYP1A1* *1/*2A was found to be more frequent among children with relapse comparing with primary patients (OR = 2.11, p = 0.0291), while *GSTT1* "null" genotype was less frequent among relapsed patients (OR = 0.55, p = 0.0265). Our findings suggest that polymorphic variants of *GSTT1, GSTM1, NAT2* genes may consider as risk factors modulated the susceptibility to leukemia among children in Russia, while polymorphic variants of *CYP1A1, NAT2, GSTT1, GSTM1* genes may have an impact on clinical outcome.

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P0978. Effect of 5HTT genetic polymorphism on agression in atheletes

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Genetic variations of serotonin transporter gene (5HTT) are closely related with human adaptive ability to control emotion and very attractive in investigation of athletes whose life is accompanied by high emotional pressure. Present study investigated the effect of genetic polymorphism of 5HTT on aggression of athletes, 86 synchronized swimmers and 83 non-trained female controls were genotyped. 73 (age 8-18) of synchronized swimmers are actively engaged in competition, 64 of them participated in psychological testing of aggression (Buss-Durkee Hostility Inventory). Analysis of primary of this test reveals, that Indirect Hostility is increased with both short alleles SS compared with other groups (SL and LL).

Furthermore, it was shown that the frequency of 5HTT alleles in groups of elite athletes of different kind of specialization are differ between kind of sport and between athletes and control group. Interrelation of 5HTT genotype, aggressiveness and athletic successfulness are vigorously discussed.

P0979. Genome-wide association mapping of the QT-interval in a 500k scan: confirmation of the NOS1AP locus and identification of a spectrum of additional QTLs

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Background: The electrocardiographic QT-interval is a normally distributed quantitative trait in the general population with over 30% heritability, which is associated with sudden cardiac death. In a previous100k scan we have identified a QTL for QT-interval at the NOS1AP gene which explained approximately 1,5% of trait variance.

Aim: To comprehensively map the spectrum of QTLs we now under-

took a 500k genome-wide scan in a larger sample of individuals.
Method: From the population-based KORA S3 survey 1,644 randomly selected individuals were genotyped on Affymetrix 500k arrays. Association was calculated using single and multi-marker tests under additive, dominant and recessive models adjusting for covariates known to explain parts of QT variance.
Results: In the 500k dataset the QTL at the NOS1AP gene gave a clear unmistakably association signal. 60 SNPs throughout a 500kb genomic region were associated with significance levels down to 1e-7.5. In addition we identified 55 additional QTLs between 1 and 5 SNPs in size with significance levels smaller 1e-5. Five of these QTLs were confirmed in a n=1.200 replication sample.
Conclusions: The QTL at the NOS1AP gene was confirmed as the single most significant signal from a 500k-genomewide scan. Its strong signal is due to its high allele frequency (MAF=0.35), 500kb long LD relationship and relatively strong effect size. Newly identified QTLs display a spectrum of different effect sizes, allele frequencies, surrounding LD patterns and coverage by genotyping arrays, which in combination decreased their detection probability and explains why they went undetected in the previous association scan.

P0980. Gestational hypertensive disorders, newborn birth weight and maternal IGF2/ApaI and H19/RsaI polymorphisms

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Gestational hypertensive disorders are among the leading causes of maternal death. Genomic imprinting has an important role in fetoplacental development. Polymorphism of the imprinted *IGF2* gene has been associated with obesity and cardiovascular risk predisposition. The maternally expressed *H19* gene does not code for a protein, but the RNA has growth suppressing functions. In the present study, the association among gestational hypertensive disorders, newborn birth weight (NBW) and maternal *IGF2/Apa*I and *H19/Rsa*I genotypes were investigated. Blood samples of 235 pregnant women [56 with pre-eclampsia or eclampsia (PE/E); 40 with gestational hypertension (GH); 34 with chronic arterial hypertension (CH); and 105 healthy controls] were obtained for DNA extraction, PCR and genotyping. Statistical analyses were performed by Qui-square, G, Kolmogorov-Smirnov and Kruskal-Wallis Nonparametric Tests. There was no influence of „skin color". Around 80% of the patients presented at least one copy of the alleles B (*H19*) and G (*IGF2*), concomitantly. Although the *IGF2* and *H19* genotypes were not significantly associated with gestational hypertensive disorders and/or NBW, a tendency for higher birth weight in newborns of mothers with AA (*IGF2*) genotype [mean = 3176g versus 2939g (AG) and 2772g (GG)] was observed. To our knowledge, this is the first study regarding the influence of *H19/Rsa*I polymorphism in hypertensive disorders of pregnancy.

P0981. GJB2 mutations in Croatian patients with non-syndromic hearing loss

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Mutations in GJB2 (connexin 26) gene represent a major cause of prelingual non-syndromic hearing loss (NSHL) worldwide. Among them, 35delG mutation accounts for approximately 70% of all GJB2 mutant alleles in most European populations.
The aim of the study was to evaluate the frequency and type of mutations in the exon 2 of GJB2 gene and frequency of (GJB6-D13S1830)del in GJB6 gene in 48 patients with recessive NSHL from Croatia.
The coding region of the GJB2 gene was sequenced and the GJB6 deletion was analyzed by two specific PCR reactions.
A half (26/48 or 54%) of our patients presented with one or two mutations in the GJB2 gene. Among 50 mutated chromosomes found in patient with NSHL, 41 (82%) carried 35delG mutation. Other common mutations - L90P, 313del14, V37I and W24X, accounted for 3.1% - 1.0% of analyzed chromosomes. We have also found a novel variant, -24A>C, reported here for the first time. GJB6 deletion was not found in our tested subjects. The high mutation rate (50/96 or 52%) in the coding region of the GJB2 gene indicates the importance of the GJB2 gene testing in Croatian patients with recessive NSHL.

P0982. No modifier effect of the Glu298Asp polymorphism of ENOS gene in autosomal dominant polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic hereditary diseases. It is characterized by a substantial variability in the severity of renal phenotype, primary assessed by the age at end - stage renal disease (ESRD). The role of modifier genes has been shown in different hereditary diseases, including ADPKD. ENOS, the gene coding for the endothelial nitric oxide synthase is considered to have modifier effect in ADPKD. In this study we investigated the influence of one of the most studied polymorphisms of ENOS gene, the Glu298Asp polymorphism, on the age at ESRD. We analysed a total of 100 ADPKD unrelated patients and 104 healthy cohorts from Greece. The frequencies of the three genotypes GG, GT, TT of the Glu298Asp polymorphism were 0.48, 0.38, 0.14, respectively in the group of patients and 0.47, 0.46, 0.075, respectively, in the control group. Analysis of the dada regarding progression to ESRD within 5 years or after more than 5 years following clinical diagnosis of ADPKD provided no evidence of statistical difference. In contrast to some earlier studies our results do not support that the frequent of Glu298Asp polymorphism of ENOS is associated with a 5 year lower mean age at ESRD in this subset of ADPKD patients.

P0983. Glucocerebrosidase gene mutations are associated to Parkinson's disease in a population from Southern Italy

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Recent studies have reported clinical, neuropathological and genetic associations between Parkinson's disease PD) and Gaucher's disease (GD), an autosomal recessive disorder caused by mutations in the *GBA* gene, coding for the lysosomal enzyme glucocerebrosidase. Screenings for *GBA* mutations in PD subjects belonging to different populations have suggested that heterozygosity may be a susceptibility factor predisposing to PD.
In order to elucidate the role of the GBA gene in PD in our population we screened 401 PD patients coming from Calabria (Southern Italy) for the two most frequent mutations, N370S and L444P. Forty-nine patients (12,2%) were familial. A control group consisting of 495 subjects originating from the same geographical area of the patients was used to determine the mutation frequency in the general population. Genotyping was performed by using PCR amplification followed by restriction enzyme digestion with XhoI for the N370S substitution and NciI for the L444P substitution. Mutation frequencies in cases and controls were compared using Fisher's exact test.
We found 11 patients (2.7%) carried a heterozygous mutant *GBA* allele: three of them had the N370S mutation and eight had the L444P mutation. In the control group 1 subject (0.5%) carried a heterozygous L444P mutation. These distributions are significantly different (p=0.0018).
This study was performed on the most consistent PD group so far investigated for *GBA* mutations, revealing a significant association with the disease. Our results thus contribute to identify genetic factors influencing PD susceptibility in our population.

P0984. Growth hormone receptor codon 440 G/T polymorphism is associated with first-year growth response in patients with GHD

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Context: GH replacement is an effective therapy for GH deficient (GHD) children. However, growth velocity post-GH replacement therapy varies between individuals.
Objective: To investigate possible influences of gene polymorphisms on the growth response to GH in GHD children.

Setting: The study was conducted at the China Medical University Hospital, Taichung, Taiwan.
Design: A retrospective observational study.
Patients: A total of 100 GHD children who underwent GH therapy for one year were recruited.
Interventions: PCR-RFLP (restriction fragment length polymorphism) and PCR experiments were carried out to detect single nucleotide polymorphisms (SNPs) in the following genes: growth hormone receptor (GHR), Janus-activated kinase 2 (JAK2), signal transducers and activators of transcription-5a (STAT-5a), STAT-5b, suppressor of cytokine signaling-2 (SOCS-2), Insulin Growth Factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and acid-labile subunit (ALS). We then evaluated the correlations among these gene polymorphisms with various parameters (gender, age, bone age, parents' heights, serum GH concentration, birth weight) on first year growth velocity.
Main Outcome Measure: Growth velocity was measured every two months for a total of 12 months.
Results: GHR codon 440 G/T was the only polymorphism of the GHR gene which correlated with increased growth velocity. The G homozygote was associated with low growth velocity, the G/T heterozygote corresponded with moderate growth velocity and the T homozygote correlated with high growth velocity. The GHR codon 440 T allele showed higher transcriptional activity and stronger Stat5 Tyr694 phosphorylation.
Conclusion: The GHR codon 440 T allele is associated with the therapeutic efficacy of GH replacement therapy.

P0985. Exceptional mosaicism in grandpaternal haemophilia patient in a family with isolated haemophilia A

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About one third of cases of haemophilia have no family history of the disorder and 20% are thought to be due to a new mutation. A proportion of such mutations occurs in a single germ cell but some, occurring during early embryogenesis produce a germline or somatic mosaic. In haemophilia somatic mosaicism is generally observed in women. Only 3 cases have been reported in grandfathers.
We report here a case of somatic mosaicism in the asymptomatic grandfather of a male patient with a severe haemophilia A. The mutation identified in the proband was found in the mother and her sister, suggesting the grandmother was a carrier. This mutation was not found in her leucocytes, buccal and uroepithelial cells, eliminating somatic mosaicism. Haplotypes analysis using intragenic and extragenic markers allowed to identify the origin of the deleterious allele in the grandfather. Analysis of his leucocytes, buccal and uroepithelial cells by PCR-sequencing revealed the presence of the allele with a proportion estimated between 15-20%. Somatic mosaicism, varies from 0.2 to 25%, is not always detected with conventional methods. This requires mutation-enrichment procedures that are not used during routine tests analysis. Analysis with denaturing-high-liquid-pressure-chromatography, which is now widely used, increases the proportion of allele in our patient.
This report suggests that somatic mosaicism is probably underestimated and points to the need of using methods with higher sensitivity in such sporadic cases and if necessary, to perform linkage analysis to identify the origin of the deleterious allele.

P0986. Association study of ALOX5AP gene variants with the risk of coronary artery disease.

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Leukotrienes are a group of proinflammatory lipid mediators that are implicated in the pathogenesis and progression of atherosclerosis. Arachidonate 5-Lipoxygenase-Activating Protein gene (ALOX5AP), which encodes an essential regulator of the biosynthesis of the leukotriene A4, has been recently associated to the risk of thrombotic disease.
The aim of this study was to explore the role of variants of the ALOX5AP as possible susceptibility factors for coronary artery disease (CAD) and myocardial infarction (MI) in patients with or without angiographically proven CAD. A total of 1,431 patients with or without angiographically documented CAD were examined simultaneously for seven ALOX5AP SNPs, allowing reconstruction of the at-risk haplotypes (HapA and HapB) previously identified in the Icelandic and British populations (Nat Genet 2004;36:233-239; Am J Hum Genet 2005;76:505-509). Using a haplotype-based approach, HapA was not associated with either CAD or MI. On the other hand, HapB and another haplotype within the same region (that we named HapC) were significantly more represented in CAD versus CAD-free patients, and these associations remained significant after adjustment for traditional cardiovascular risk factors by logistic regression (HapB: OR 1.67, 95% CI 1.04 to 2.67; P=0.032; HapC: OR 2.41, 95% CI 1.09 to 5.32; P=0.030). No difference in haplotype distributions was observed between CAD subjects with or without a previously documented MI. This study points out a possible role of ALOX5AP in the development of the atheroma rather than in its late thrombotic complications such as MI.

P0987. The genetic background of β⁺IVSI-6: a primary step, in determining the origin and spread of β-globin mutations in Romania.

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Romania belongs to a low-prevalence area of beta-thalassemia. In the last few years we have identified the most frequent causative mutations of beta-thalassemia in the Romanian population. To elucidate the origin and the flow of these mutated alleles, we have started our work, by studying one of the most frequent mutations: the β⁺ IVSI-6 which has the highest frequency at homozygous state among the other mutations found in Romania. Seven restriction fragment polymorphisms (RFLP) haplotype have been determined in a sample including homozygous IVSI-6 patients and carriers. Our study revealed a strong linkage of IVSI-6 to haplotype VI (-++---+) 70%**,** relative to VII (+-----+) 18% and V (+----+-) 12%. This result confirms what was found in the Mediterranean countries regarding the association of β⁺-IVSI-6 mutation to haplotype VI, the presence of haplotype V seems to demonstrate the gene flow between Romania and Bulgaria.
The diversity of the β⁺-IVSI-6 genetic backgrounds found in Romania reinforces the picture of the β-globin gene cluster as highly dynamic. On the other hand this diversity highlighted the old age characterizing the β-thalassemia alleles. Furthermore this study should be continued to examine normal individuals and all the thalassemia alleles to determine the relationships between Romanian and neighboring populations.
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P0988. Mapping Genes Influencing Human Stature - Where are We?

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Human stature is a highly heritable complex trait which is oligo- or polygenic in nature. Despite many genome-wide efforts attempting to localize genes influencing stature findings have been difficult to replicate and convincing evidence for quantitative trait loci (QTL) has been sparse.
In order to facilitate the mapping of stature genes and prioritizing positional candidate loci we have collected the results of the published genome-wide linkage studies on a publicly available web site www.genomeutwin.org/stature_gene_map.htm which is updated accordingly. From these published results it seems likely there are multiple minor loci underlying the genetic background of human stature although converging evidence from multiple independent studies also suggests that some loci such as 3p, 6q, 7q and 9q probably harbour stature genes of

moderate to major effect.
We are coordinating two large multi-national collaborative stature gene mapping efforts: 1) the GenomEUtwin and the 2) Marshfield Mammalian Genotyping Service stature projects. Both studies include family data from multiple populations with genome-wide genotype data - the former containing 8.450 individuals from 3.817 families and the latter 11.149 individuals from 3.272 families. Using genome-wide linkage analyses we have localized multiple putative QTLs for stature on 2p, 2q, 3p, 3q, 8q, 9q, 10p, 11q, 20q and Xq25, some shared across multiple populations. Data from further fine mapping analyses of these major loci will be presented.
In line with analyses of other complex traits it has become obvious that these types of massive data sets are needed to provide solid evidence for QTLs contributing to human stature.

P0989. Modulation of HFE-hemochromatosis penetrance by the BMP2, BMP4 and HJV genes of the hepcidin regulation pathway

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Most cases of genetic hemochromatosis (GH) are associated to the HFE C282Y/C282Y genotype in Caucasian populations. The biochemical or clinical symptoms expressed by C282Y homozygotes are extremely variable and only a few suffer from an overt disease. Several studies have suggested that, in addition to environmental factors, a genetic component could explain a substantial part of this phenotypic variation, though very few genetic factors have been identified so far.
The aim of the present study was to search for genes modifying hemochromatosis penetrance in a large sample of C282Y homozygotes, using pre-therapeutic serum ferritin level as marker of hemochromatosis penetrance. We used a candidate gene approach and focused on 2 biologically relevant gene categories: genes involved in non HFE-GH (TfR2, Hamp, SLC40A1) and genes from the BMP hepcidin regulation pathway (BMP 2, BMP4, HJV, Smad 1, Smad4 and Smad5). We also considered the IL6 gene involved in the inflammation hepcidin regulation pathway.
A significant association between serum ferritin level and a SNP in the BMP2 genic region (rs235756, P=4.42 x 10-5) was detected. Mean ferritin level adjusted for age and sex was 651.97 ng/ml among TT genotypes, 518.01 ng/ml in TC genotypes and 350.72 ng/ml in CC genotypes. Testing the biologically relevant gene interactions along the BMP regulation pathway, we detected a significant interactive effect of BMP2 and HJV with a small additive effect of BMP4 on ferritin level.
All together, our results suggest that the HJV-BMP complex genes are involved in the modulation of iron burden in C282Y homozygotes.

P0990. Association of Interferon-gamma (IFN-g) Polymorphisms with Susceptibility of Hepatitis B Virus (HBV) Infection in the Hong Kong Chinese

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Background: Hepatitis B virus (HBV) infection is the most common cause of acute hepatitis and may progress to chronic liver disease, including cirrhosis or hepatocellular carcinoma (HCC). Host genetic factors are important for this progression. Interferon-gamma (IFN-γ) is a pro-inflammatory T-helper 1 (Th1) cytokine. It plays crucial roles in downregulation of HBV replication and its clearance.[1] Serum IFN-γ levels were lower in the chronic hepatitis B patients than the normal controls, supporting that IFN-γ is involved in the progression of HBV infection.[2]
Objective: We aimed to determine whether a functional single nucleotide polymorphism (SNP) of IFN-γ may affect the susceptibility of HBV infection in Hong Kong Chinese population.
Methods: We recruited 460 chronic HBV carriers and 87 individuals who had spontaneously recovered from HBV infection as evidenced by the presence of anti-HBs and anti-HBc antibodies. The SNP of IFN-γ at +874 A/T at intron 1 at the 5' end of a CA repeat microsatellite sequence was detected using Genescan analysis.
Results: For the spontaneous recovered individuals, the genotype frequencies were 54.0% for A/A, 39.1% for A/T and 6.9% for T/T. For the chronic HBV carriers, the genotype frequencies were 72.8% for A/A, 24.6% for A/T and 2.6% for T/T. The A/A genotype was predominant in the chronic carriers (odd ratio=4.09, CI=1.35-12.4), and its frequency was significantly higher than those with spontaneous recovery after adjusting for age and gender (p<0.0001).
Conclusion: The polymorphism of IFN-γ gene at position +874 may confer susceptibility to persistent HBV infection.
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P0991. Detection of mutations in the C1 inhibitor gene: the confirmation by using different methods of molecular biology

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Hereditary angioedema (HAE) is an autosomal dominant disease with incomplete penetrance that affects one in 10 000 -50 000 persons. It is characterised by quantitative or qualitative deficiency of C1 inhibitor (C1 inh) - the main control element of enzymatic activity of the first component of complement cascade. Type I HAE (85% of all patients with HAE) is characterised by low antigenic and functional levels of C1 inh, type II HAE (15%) is defined by normal or elevated levels of C1 inh with low functional activity. The C1 inhibitor (C1INH) gene maps to chromosome 11q12-q13.1. The large number of different mutations may have differential effects on HAE phenotype. However, most of molecular defects causing low serum C1 inh level in type I HAE are poorly characterized. In this study, we focused on mutations causing splicing defects which typically result in type I HAE.
Basically, we screened the C1INH gene for mutations by denaturing gradient gel electrophoresis (DGGE) in 33 unrelated patients. Maximal sensitivity was reached if different methods were used for detection and confirmation of mutations, including sequencing and restriction analysis. Using this approach, we detected four novel splice site mutations (g.14255g/a, g.4351-2a>g,g.4486delG, g.4487_4498del12) in flanking regions of exons 3, 4 and 7, respectively, in four families (12 %). Mutations that affect splice sites are supposed to reduce or abolish normal splicing by either exon skipping or activation of cryptic splite sites. Inhibition of splicing was proved in one case.
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P0992. Whole Genome Scan in a Maltese Family with a rare case of Hereditary Persistence of Fetal Hemoglobin

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Hereditary Persistence of Fetal Hemoglobin (HPFH) is an inherited condition resulting in high fetal hemoglobin (HbF) levels in adults. Although this condition is caused by large deletions, removing large segments from the human β-globin cluster or point mutation within the human γ-globin gene promoters, there have been few cases where the HbF-increasing genetic determinant is located outside the human β-globin locus, e.g. in chromosomes 6q22.3-q23.1, 8q and Xp22.2-22.3.

We here describe a Maltese family with high HbF levels and heterocellular HbF distribution. The proband had 20% HbF [HbF/F-cells=9.9 g/dL] and was homozygous for haplotype IX. Her two daughters had 5.6% [HbF/F-cells=5.53 g/dL] and 8.8% [HbF/F-cells=6.13 g/dL] HbF respectively, and both bearing haplotypes III/IX, while all other family members carry normal HbF levels. Extensive molecular haplotyping and DNA sequencing across the β-globin locus excluded both point mutations and deletions associated with HPFH. The γ-globin chain ratios of the family members with high HbF levels have been found to be within the fetal ratio, *i.e.*, 66/33. Whole genomic DNA of this family has been done using the 250K Affymetrix GeneChip SNP Array and linkage analysis was performed using ALLEGRO software. Genomic regions with increased LOD scores will be subsequently analyzed by a genomic scanning approach to narrow down on significant areas of the genome that are likely candidates for the HPFH phenotype.

P0993. HPLC a new technology for trinucleotide diseases analysis

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High performance liquid chromatography (HPLC) has proved to be time saving and effective screening method for identifying point mutations and sequence variations. Trinucleotide expansions are an important mutational form specifically in neurogenerative disorders. The diagnostic is impaired by the dynamic character of mutation. Thus larger expansions require specific and mostly time consuming methods for identification, such fragments length analysis by electrophoresis or Southern blot. We tested the effectives and reliability of HPLC for diagnosis in trinucleotide repeats disorder. Huntington Disease is an autosomal dominantly inherited neuropsychiatric disorder caused by an unstable expansion of CAG repeats. The number of CAG in normal individuals varies up to 35. In affected patients the expanded allele contains 40 or more repeats. The exact determination of both alleles on the molecular level is very important for clinical diagnosis and prognosis. We have analyzed 22 patients - clinically diagnosed with HD - from the DNA bank of the Institute of Human Genetics, University of Leipzig. Following a novel PCR protocol, fragments enclosing the CAG repeat region were sized using HPLC in comparison to a 20 bp DNA standard. In order to evaluate the HPLC against one of the classical methods, the lengths of fragments were determined using ABI Prism®. Our results indicated highest accuracy and consistency of the data obtained with the HPLC method between 60-280 bp (± 2 CAG). We conclude that HPLC can be used as a sensitive and efficient alternative diagnostic method to conventional techniques for fragment length measuring in Huntington Disease.

P0994. Huntington disease-like phenotype due to trinucleotide expansions in the /TBP/ (SCA17) and /JPH3/ (HDL2) genes in Caucasian patients

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Huntington disease (HD) (OMIM 143100) is an autosomal dominant disorder of the central nervous system. It is clinically characterized by involuntary choreic movements, progressive motor impairment, cognitive decline and behavioural anomalies. HD is caused by the expansion of a $(CAG)_n$ tract within the first exon of the *HD* gene (4p16.3). This mutation accounts for 54,6% (124/227) of the cases received for confirmation or exclusion of HD at our lab, in the last 3 years.

We selected 93 patients without *HD* mutation but presenting clinical features resembling HD (Huntington-like patients), and analysed them for mutations on previously described HD-like genes: an extra octapeptide repeat (192 bp) in the *PRNP* gene (HDL1); a CAG/CTG repeat in the *Junctophilin-3* gene (HDL2); and CAG expansion in two dominant SCAs which may also have some overlapping symptoms with HD (DRPLA and TBP/SCA17). Age at onset was 45,6 ±19,1 years, ranging from onset in childhood up to age 83 years.

Expansion of CAG repeat in *ATN1* gene (DRPLA), as well as, the octapeptide insertion on *PRNP* gene (HDL1) were excluded in all our patients.

We found a CAG/CTG expansion in the HDL2 locus in a Brazilian family. The patient carried 47 repeats and onset was at age 44 years. Clinical manifestations included bradipsychism, mutism, dysarthrya, cognitive deterioration and coreic movements, as well as ataxic gait. He showed cortical atrophy. The likelihood of the patient's ancestrallity was estimated 97,4% European, 1,8 % Amerindian and 0,7% African. We found also a CAG expansion (44 repeats) in the TBP gene in a Portuguese patient with behavioural disturbances, epilepsy, aphasia, unbalance, and gait ataxia.

This work stresses the importance of performing the diagnosis of TBP and HDL2 in patients with HD-like disease of various ethnical origins.

P0995. Polymorphic variation at P2X receptor genes is associated with differences in blood pressure

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Objectives: Large family based association study was performed to examine the association of genetic variation in P2X4, P2X6 and P2X7 genes and hypertension.

Background: Hypertension is one of the major risk factors for cardiovascular diseases. Blood pressure is a quantitative trait clustered in families with heritability estimated between 30-60%. However, identification of the genes responsible for this condition has proved difficult. Purinergic receptors are ligand-gated ion channels activated by ATP. They were analysed as candidate genes for hypertension due to their broad range of expression and their implication in ion exchange, renal function and neural impulse transmission.

Methods: 1428 individuals from 248 families ascertained via a proband with hypertension were studied. Blood pressure was measured using 24h ambulatory monitoring. 28 TagSNPs were selected for analysis in the region of the gene and 15kb upstream and downstream using the HapMap data for population of European decent (r^2>0.8 and MAF≥0.5). SNPs were genotyped on a Sequenom MassArray platform and PCR-RFLP. Genotypes were checked for errors and quantitative analysis was performed using QTDT software.

Results: Common haplotype frequencies obtained were similar to the HapMap data. A highly significant association was observed between genotypes at the rs591874 polymorphism and blood pressure. Other significant results are listed in the table.

Gene	SNP	Office			Day			Night			Day	Night
		Systolic	Diastolic	Pulse	Systolic	Diastolic	Pulse	Systolic	Diastolic	Pulse	HR	HR
P2X7	rs591874	0.019	0.006		0.022	0.004		0.035	0.002		0.008	
	rs656612								0.007			
P2X4	rs2303998	0.016			0.022		0.040					
P2X6	rs9625334	0.025										
	rs8141816	0.016	0.032									
	rs2255371	0.032	0.034									
	rs2277838										0.04	0.02
	rs2541953				0.049							

Conclusions: The present results suggest that genetic variation at purinergic receptor genes may contribute to variations in blood pressure.

P0996. Association studies of obesity and high blood pressure to variants of the genes *LEP, LEPR, ADRB2, PPARG, PLIN, RETN, INSIG2, ACE, eNOS, GNB3* and *AGT* in afro-derived Brazilian populations

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Obesity and hypertension are common diseases determined by genetic as well as environmental factors. Many genes have been investigated as possible candidates to these conditions in different populations, but studies in African-derived populations are rare. Quilombos are rural Brazilian populations founded by runaway or abandoned African slaves. They have remained partially isolated until recently, thus representing an interesting model for the study of complex diseases. We report the results we obtained in a sample of 528 adult individuals, living in remnants of Quilombos, in which we investigated the pos-

sible association of obesity and hypertension with polymorphic markers. Polymorphisms in the genes *LEP*, *LEPR*, *ADRB2*, *PPARG*, *PLIN*, *RETN*, *INSIG2*, *ACE*, *eNOS*, *GNB3* and *AGT* were investigated in all subjects in order to assess their effect on BMI (Body Mass Index) and blood pressure. Blood pressure and obesity related phenotypes were analyzed both as dichotomous and continuous variables, adjusting for other covariates such as age and gender. No significant associations were detected between these genetic polymorphisms and BMI. A significant association was found between homozygosis CC at the polymorphism C825T in the *GNB3* gene and hypertension in women ($p < 0,001$). Regression analyses showed that systolic blood pressure is significantly associated with the polymorphism in *GNB3* among women (p=0,030), and that diastolic blood pressure is significantly associated with the polymorphism rs7566605 in the *INSIG2* gene (p=0,027).

P0997. Autosomal recessive congenital ichthyosis: Mutations in *ichthyin* associated with specific ultrastructural changes in the epidermis

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of skin disorders. Several mutant genes are identified in ARCI but the association between genotype and phenotype is poorly understood. In search for genotype-phenotype correlations in ARCI we selected 27 patients from 18 families with specific ultrastructural features of the epidermis. The electron microscopy (EM) picture was characterized by abnormal lamellar bodies and elongated membranes in stratum granulosum and was classified as ARCI EM-type III. DNA samples from a subset of the affected individuals were screened for homozygous genomic regions and a candidate gene region was identified on chromosome 5q33. The region coincides with the *ichthyin* gene, previously reported as mutant in ARCI. Mutation screening of *ichthyin* revealed missense or splice site mutations in 25 out of the 27 individuals (93%) with specific EM characteristics of type III. In a control group of 18 ARCI patients without EM findings consistent with type III we found only one individual with a mutation in *ichthyin*. Our findings indicate a strong association between ARCI caused by *ichthyin* mutations and characteristic ultrastructural findings in epidermis. These results suggest that EM provides a specific diagnostic tool for a subgroup of ARCI patients.

P0998. Endothelial nitric oxide synthase (*NOS3*) haplotype is associated with diabetes type 1 and its microvascular complications.

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Diabetes is a common, chronic disease that profoundly impacts health and quality of life. Type 1 diabetes (IDDM) is an autoimmune disease with a complex polygenic inheritance. Polymorphism of NO-synthases genes could be one of the earliest marker of vascular damages in diabetes.

To study contribution of NOS3 gene into IDDM and its microvascular complications, a group of patients has been investigated (N=154 mean age 13,56±2,67; 72 females, 82 males). Control group consisted of 241 healthy donors.

C-691T, VNTR, C774T and G894T gene *NOS3* polymorphisms were analyzed by PCR-RFLP. Frequencies of haplotypes were estimated using the E-M approach and a more computationally-intensive Bayesian approach as implemented in PHASE.

The „C-B-C-G" haplotype was more common in IDDM patients (51%) and group of patients with microvascular complications (51%) than in healthy controls (40%; p<0.05).

In conclusion, the „C-B-C-G" haplotype may have a negative influence in the pathogenesis IDDM and its microvascular complications. Further studies should be conducted to address the molecular basis for such an effect.

P0999. IL-23 receptor 3'UTR C2370A variant in inflammatory bowel disease: differential profile in Crohn's disease and ulcerative colitis

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Crohn's disease (CD) and ulcerative colitis (UC) are two main clinical presentations of inflammatory bowel disease (IBD). The pathogenesis of IBD is complex and both environmental and genetic factors contribute to its etiology. The IL-23 receptor (IL23R) gene on chromosome 1q31 encodes a subunit of the receptor for the proinflammatory cytokine IL-23. In a genome-wide association study Duerr et al. (Science, 2006; 314:1461-1463) verified IL23R as an inflammatory bowel disease associated gene. Amongst the identified SNPs, the C2370A variant (rs 10889677) in the 3' UTR was found to confer risk for IBD in non-Jewish population. Our aim was to obtain quantitative data on prevalence rates of IL23R 3' UTR C2370A separately on CD or UC affected group of patients using another approach, a case-control study. A total of 330 patients with IBD (124 CD, 206 UC) and 235 controls were genotyped for the SNP using PCR/RFLP method. The A allele frequency was 38.3% in the CD group, 32.0% in the UC group, and 30.0% in the controls ($p<0.05$ vs CD). We found increased AA genotype frequency in the CD group (15.3%), compared to the controls (5.5% $p<0.05$). A statistically not significant trend to increase was found in the prevalence of AA genotype in the UC group (9.2%), compared to the controls (5.5%). Logistic regression analysis adjusted for age and gender confirmed, that the AA genotype represent independent risk factor for CD (OR=3.090, 95% CI: 1.470-6.494, p=0.003), but not for UC.

P1000. Methods to detect imprinting using linkage data: parent-of-origin-based penetrance vs. independent male-female recombination fractions

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Genes whose expression depends on the sex of the parent contributing the gene, i.e., imprinting, are being recognized. We tested two methods for detecting imprinting using linkage data.

Maximizing the lod score:

1) over parent-of-origin penetrances (PP)

2) over independent male-female recombination fraction (RF).

We simulated family data with and without imprinting, defined as penetrance in offspring dependent on the parent contributing the allele. We compared the max lod score assuming equal parameter values from mothers and fathers to the global max over all combinations. We calculated the chi-squared for the null hypothesis of no imprinting and the power to detect imprinting over a range of data set sizes. We tested the confounding effects of reduced penetrance, heterogeneity, differential male-female penetrance and biased ascertainment, and studied the distribution of the nested likelihood ratio test statistic under the null hypothesis.

Without heterogeneity and reduced penetrance, the PP method has more power. Imprinting detection power actually increases for PP if reduced penetrance exists. If one sex had higher penetrance than the other, PP could falsely indicate imprinting, while RF was not fooled. Neither method gave biased results because of parent-specific ascertainment bias.

When there is no imprinting, without heterogeneity or reduced penetrance in the generating model, the likelihood ratio test was conservative for both PP and RF, however the type I error rate is strongly affected by locus heterogeneity and the ascertainment criteria. Families selected for having a high density of affected members also resulted in an increased Type I error rate.

P1001. A North African founding mutation causes male infertility by disrupting meiotic cytokinesis

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An estimated 80 million people face reproductive difficulties worldwide. An important proportion of these cases is believed to be caused by genetic defects, yet few genes have formally been associated with infertility in the human. We performed a genome-wide microsatellite scan on 14 unrelated infertile men, all originating from North Africa and born from first degree cousin. All presented very characteristic sperm parameters with close to 100% abnormal spermatozoa with anomalies of the head, several flagellae and polyploidy. A common homozygous region harboring the Aurora Kinase C gene (AURKC) known to be highly expressed in the testis and involved in cytokinesis and mitosis/meiosis was detected in 10/14 patients. Sequence analysis of AURKC coding sequence showed the presence of a homozygous single nucleotide deletion in all 14 patients. This mutation results in premature termination of translation thus yielding a truncated protein which lacks its kinase domain. We conclude that the absence of AURKC leads to male infertility due to the production of «large-headed multiflagella spermatozoa».

P1002. The study of Insulin, Igf2 and NAIP mutations in romanian population

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The balance between proliferation and apoptotic processes is involved in some pathologic phenotype, including type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and obesity. The genetic markers from IDDM2 region were associated with these phenotypes. Neuronal apoptosis inhibitory protein (NAIP, 5q) was found also to have anti-apoptotic effects in different cells and to be up-regulated during adipocytes differentiation.

The aims of our study were to test the contribution of Insulin, IGF2 and NAIP polymorphisms to diabetes onset and obesity.

Clinical information and blood samples were collected from unrelated obese T2DM subjects (n=70, BMI between 32,5-39 kg/m2), nonobese T1DM (n=70, BMI between 20-25kg/m2) and 150 controls (non obese, non-diabetic subjects). All samples were genotyped for Insulin -23Hph, Insulin +1127Pst1, IGF2Apa and NAIP exon 5 polymorphisms.

We observed a higher frequency (4%) of NAIP exon 5 homozygous deletion in control group compared with obese T2DM (2,85%) and T1DM (1,42%) patients. This value is also higher than in other populations. Interesting, all subjects with homozygous absence of NAIP exon 5 have low BMI .The IGF2 AA genotype was more frequent in T2DM comparing with T1DM and control subjects (OR=1,9) and polymorphisms from insulin region (OR-23Hph AA= 2,7, OR +1127Pst CC = 2,0983) were more frequent in T1DM subjects.

Taken together, we found a high frequency of -23Hph AA and +1127Pst CC in T1DM patients, of IGF2 AA in T2DM and of NAIP exon 5 homozygous deletion in control group.

P1003. Analysis of *IGF1* gene polymorphism in newborn and elderly people from North-West region of Russia

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The serum insulin growth factor IGF-1 level differs in various human ages. The individual variations in the level of this enzyme depend on the number of CA repeats in the promoter region of *IGF-1* gene. In this study *IGF-1* (CA repeats) gene polymorphism was analyzed by PCR method in 102 newborns and also in 136 elderly people from North-West Region of Russia. Increasing of the frequency of 20/- genotype in elderly people when compared to newborn group (26.7%, 44.1%, respectively, p=0.0034) with concomitant age-related decrease of 19/19 genotype frequency (51%, 27.9%, respectively, p=0.0001) were found. According our data 19/20 genotype in male newborns was twice more frequent than in female ones (23.2%, 11.3%). The same ration remained constant in elderly people as well but the proportion of 19/20 genotype in this group increased almost twice (44.4%, 21.1%, respectively, p=0.025). The association of 20CA allele with lower body weight (Z=2.2, p=0.028) and reduced height in female newborns has been registered. We suggest that increased number of CA repeats (more than 20 CA) being responsible for low production of IGF-1 enzyme is associated with longevity in man as well as with reduced body weight and the height in female newborns. Allele 19 responsible for the high level of serum IGF-1 might be associated with longevity for women. Our final finding was the correlation between 20 CA allele of IGF-1 and cataract (Z=2.4, p=0.018). There was no correlation between *IGF-1* gene polymorphism and stroke, essential hypertension, coronary heart disease and Type 2 diabetes.

P1004. Interleukin-23 receptor (IL23R) gene C2370A polymorphism in scleroderma patients

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Scleroderma or systemic sclerosis is a chronic connective tissue disease generally classified as one of the autoimmune rheumatic diseases. The cause of scleroderma is still unknown but there are several factors presumed: abnormal immune or inflammatory activity stimulating the fibroblasts to produce too much collagen, as well as not yet discovered genetic and enviromental factors. Interleukin-23 plays an important role in the Th17 mediated immune response. The IL23R gene located on chromosome 1p31 encodes a subunit of the IL23-receptor. Recent investigations (Duerr et al, Science, 2006; 314:1461-1463) identified several IL23R gene polymorphisms as risk factors for inflammatory bowel diseases. Theoretical considerations also suggested possible association of these SNPs with other autoimmune diseases. Our aim was to test whether the 3'-UTR C2370A SNP of the IL23R gene (rs10889677), one of the verified IBD susceptibility genes also confers risk for scleroderma. We performed genotyping using DNA samples collected from 244 patients with scleroderma and 135 unrelated, healthy controls. The genotypes were analysed using PCR/RFLP-methods. We found no significant difference between the allele frequencies of the two groups (73% and 71% for the C allele and 27% and 29% for the A allele in patients with scleroderma and in the control group, respectively). Our results show that eventhough the C2370A polymorphism of IL23R can associate with selected autoimmune diseases like the Crohn's disease, but not with others, including the scleroderma, thereby it is not an universal autoimmune disease associated susceptibility factor.

P1005. Interleukin-23 receptor 3'-UTR C2370A SNP confers risk for rheumatoid arthritis

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INTRODUCTION: Interleukin-23 (IL23) is a proinflammatory cytokine that plays a crucial role in the development of chronic inflammation, being a master regulator of the IL17/23 molecular pathway. IL23-receptor gene (IL23R) on chromosome 1p31 encodes one subunit of the IL23-receptor. Duerr et al. (Science, 2006; 314:1461-1463) using genome-wide association study found association between Crohn's disease and the IL23R gene; amongst the reported SNPs the 3'-UTR C2370A (rs10889677) conferred risk for Crohn's disease in a non-Jewish population. Since the IL17/23 pathway is known to associate with experimental allergic encephalomyelitis, inflammatory bowel disease, multiple sclerosis, collagen-induced arthritis, rheumatoid arthritis (RA), and perhaps other autoimmune diseases, we theoretized that this SNP described originally for Crohn's disease might also have significance in the development of RA. Therefore, the aim of the present study was to test this hypothesis. METHODS: Genotyping was performed on 226 well-characterized RA-patients and 135 age-and sex-matched controls using a PCR-RFLP method. All RA-patients were characterized for RF- and anti-CCP seropositivity. RESULTS: We observed a highly increased prevalence of the homozygous AA genotype compared to the controls (15.0% vs. 4.44%; p<0.05). DISCUSSION: Our results

show that the AA genotype means a 4-fold risk for the development of RA (χ^2=8.55, p=0.003, OR=3.81, 95%CI: 1.55-9.33). The data of the study reported here provide direct evidence first in the literature, that besides Crohn's disease the IL23R 3'-UTR single nucleotide polymorphism C2370A is an independent risk factor also for RA.

P1006. Genetic Association of TAPBP, IKBL, and MIF Polymorphisms with Juvenile Rheumatoid Arthritis in Mexican Population.

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Juvenile rheumatoid arthritis (JRA) comprises the most common chronic autoimmune arthropathies of childhood. Strong evidence is emerging to suggest that the disease likely involves multiple susceptibility genes, a feature common to many autoimmune disorders. Single nucleotide polymorphisms (SNPs) in some genes of system immune like TAPBP 260 C/G, IKBL - 62 T/A and MIF - 173 G/C, have been found associated with ARJ in different populations,. Our aim was to identify whether TAPBP, IKBL, and MIF polymorphisms are associated with JRA in a sample of Mexican patients. We performed a case-control association study in 133 pediatric patients with ARJ, and 350 unrelated, healthy Mexican controls. Allelic discrimination was carried out by TaqMan assay. The genotypes and allele frequencies were compared between cases and controls by x2 test. Genotype frequencies were in Hardy - Weinberg equilibrium. When genotype and allelic frequencies were compared between cases and controls we observed that the 260G TAPBP and - 173C MIF alleles ((p=0.01, OR 1.42, 95% CI 1.06- 1.89 and p=0.03, OR 1.4, 95% CI, 1.02 - 1.84, respectively) were associated with susceptibility to JRA in Mexican population, while- 62 T/A IKBL (p= 0.01, OR 0.7, CI 95%, 0.52 - 0.93) was found associated with protection.

P1007. Bone mass and genetic study of a 113 year-old man

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Osteoporosis is a common disease that affects postmenopausal women and elderly people. Aging induces loss of bone density and quality resulting in a progressive incidence of fractures with significant morbidity and mortality. The *KLOTHO* gene has been related to the ageing process. *Klotho* knock-out mice display multiple disorders that resemble human aging, including osteopenia. Furthermore, polymorphisms in *KLOTHO* have been associated with life span and bone mineral density (BMD). On the other hand, the *LRP5* gene has been related to variations in bone mass. In particular, the Gly171Val mutation has been associated with a high bone mass phenotype (HBM).

Here we describe the bone mass and a limited exploratory genetic study of a 113 year-old man and several first-degree relatives. No fractures have been suffered by any of them and their BMD values are listed in Table 1.

Regarding the *KLOTHO* gene, no mutation was detected in either the index case or any of his relatives, with the exception of a previously described polymorphic variant: one of the proband's daughters presented one copy of the KL-VS allele, which has been associated with longevity and increased bone mass. Finally, mutation Gly171Val of the *LRP5* was not present in any individual. These data rule out these potential genetic contributions. The identity of other longevity and/or high bone mass genes or environmental factors remains to be defined.

Table 1 Values of bone mass in the index case and four relatives

Individual	Age	BMD				T-Score		Z-Score	
		L2-L4	Femoral neck	Total hip	UD	L2-L4	Femoral neck	L2-L4	Femoral neck
Index case	113	0.880	0.533	0.742	0.294	-3.00	-4.10	-1.45	-1.01
Daughter	77	1.033	0.768	0.914	0.335	-1.40	-1.80	-1.4	0.9
Daughter	81	0.939	0.731	0.853	0.251	-2.20	-2.10	0.28	0.0
Nephew	85	1.227	0.839	1.004	0.565	-0.10	-1.80	2.08	1.2
Brother	101	1.163	0.922	1.043	0.440	-0.60	-1.10	0.60	1.75

L2-L4: lumbar spine; UD: ultradistal radius

P1008. Focal Idiopathic Torsion Dystonia: A new locus identified in a Large French family.

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Focal Idiopathic Torsion Dystonia (FITD) is a group of movement disorders, which is usually autosomal dominant with reduced penetrance. Commonly described forms of FITD include cervical dystonia, blepharospasm, oromandibular dystonia, laryngeal dystonia and limb dystonia. We studied a large French family presenting with varied symptoms of adult-onset FITD. The family is composed of 30 subjects (six definitely affected and one asymptomatic obligate carrier). The average of onset is 43 +/- 20 years. The three loci known to be implicated in FITD: *DYT6*, *DYT7* and *DYT13* have been studied and excluded. Genome-wide linkage analyses have been performed with a parametric model and incomplete age-dependant penetrance. This study identified a new highly probable locus, *DYTL*, with several lod scores > +2 for contiguous markers and a maximum of 2.37 (maximum lod score estimated in the family: 2.62) defining a 40 cM candidate region. Concurrently, another project has been established in collaboration with practitioners implicated in the follow up of dystonic patients in France and associations of patients to recruit other families presenting with FITD. Forty-three families presenting with intrafamilial heterogeneous phenotypes and corresponding to 170 affected patients have been identified and are in recruitment. The preliminary results of the *DYTL* analysis in 14 families show possible segregation of this locus with the disease in ten families and exclusion in 4 families. This last result further illustrates the great genetic heterogeneity of FITD and is in favor of the existence of more than one unassigned genes for this pathology.

P1009. Association of VEGF gene variant with left ventricular hypertrophy in athletes

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Left ventricular hypertrophy (LVH) in endurance-oriented athletes is generally understood to be a limiting factor for improving maximal oxygen uptake (VO2max). Studies in related and unrelated individuals clearly demonstrate that a high proportion of interindividual variability in left ventricular mass and risk of LVH is attributable to genetic factors. Vascular endothelial growth factor (VEGF) has been identified as one of the key regulators of angiogenesis and, therefore, VO2max. Several studies have shown that human VEGF gene polymorphisms are associated with VEGF gene expression and VO2max before and after aerobic exercise training. However, the influence of VEGF gene variants on cardiac growth of athletes has not been examined. The purpose of the study was to investigate the VEGF promoter G-634C polymorphism for association with LVH in athletes. Seventy one Russian athletes (all-round speed skaters and rowers) of national competitive standard (sub-elite and elite) were studied. VEGF gene G-634C polymorphism was determined by PCR-RLFP. Echocardiography was performed for the measurement of left ventricular mass and function. We found that left ventricular mass (LVM) and LVM index (LVMI) in male sub-elite speed skaters was significantly greater in GG genotype carriers than in heterozygotes (GC) (LVM: 333 (21) g vs. 254 (21) g, p=0.002; LVMI: 169 (10) g/m2 vs. 130 (18) g/m2; p=0.015). It has been shown previously that -634C allele is associated with increased VEGF expression, and, therefore, can be considered to be protective against LVH. Thus, VEGF G-634C polymorphism is associated with development of LVH in athletes.

P1010. Are common variants in leptin and leptin receptor genes associated with specific eating patterns in the Czech population?

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INTRODUCTION: Mutations in the leptin and the leptin receptor genes were previously reported to cause rare obese syndromes, however, both leptin and leptin receptor play also an important role in common multifactorial obesity. Therefore, increasing attention is being paid to specific eating patterns as obesity determining traits that undoubtedly display a heritable component. In this study, we determinated whether genetic variations within the leptin and leptin receptor genes underlie specific eating patterns.

METHODS: The case-control study comprised a total of 45 obese individuals ((BMI>=30) and 38 healthy controls aged 18.6-68.2 y whose uptake of nutrients was determined by using 7-d food records; with special attention paid to the use of excessive portion sizes or irregularity in eating. They were genotyped for the LEP -2548G/A (5'UTR) and LEPR Gln223Arg (exon 4) variants by means of PCR-based methodology.

RESULTS: No statistically significant associations of both examined polymorphisms with age, BMI, systolic and diastolic blood pressure, history of sterility or infertility or waist-to-hip ratio were observed. Obese carriers of examined allelic variations in leptin or the leptin receptor gene did not express an increased risk to display extreme snacking behavior or to eat excessive portion sizes; however, the AG carriers of LEPR Gln223Arg tended significantly to prefer the low-fibre diet (p=0.03).

DISCUSSION: These results do not provide evidence for a preferential transmission of some alleles of the LEP of LEPR polymorphism in obesity, but support the hypothesis that LEPR Gln223Arg confers susceptibility to specific eating patterns, such as low fibre diet.

P1011. Molecular analysis of the CAPN3 gene by both dHPLC, direct sequencing and cDNA analysis identify new mutations in LGMD2A patients

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Limb-Girdle muscular dystrophies (LGMD's) are a group of neuromuscular disorders presenting great clinical heterogeneity. Among these, LGMD-2A is the most prevalent and assumed to account for at least 30% of LGMD's, LGMD-2A is inherited recessively and caused by mutations in the CAPN3 gene which encodes a skeletal-muscle-specific member of the calpain superfamily.

We report here on the molecular analysis of the CAPN3 gene in 41 patients suspected for LGMD2A.

The CAPN3 gene of 18 patients were screened for mutations by dHPLC and/or direct sequencing of the entire coding and intron flanking sequence, whereas the remaining patients were investigated by RT-PCR and CAPN3 cDNA sequencing. Seven of the patients in whom no mutations were found by dHPLC/exon sequencing were also sequenced for their CAPN3 cDNA. In total we identified 14 different mutations of which 6 were previously unknown. In eight patients we identified both mutant alleles. In additional two of the patients only one mutation could be identified on the genomic level, however CAPN3 cDNA analysis demonstrated that both patients only expressed the mutant allele, indicating that both harbor an unidentified allel that somehow compromise CAPN3 RNA maturation. In three of the remaining patients only one mutation could be identified. Interestingly, all three patients had a highly abnormal western blot for calpain-3 and clinical characteristics of LGMD-2A, however the mutations were all clearly heterozygous on full-lengthCAPN3 cDNA.

P1012. Lipoedema associated with familial growth hormone deficiency

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Lipoedema is a poorly understood condition that does not appear in the medical textbooks. It is frequently mistaken for lymphoedema but instead of fluid accumulating in the tissues there is heavy deposition of fat. The cause is unknown but it leads to increased swelling, marked floppiness and looseness of the tissues, tenderness and sometimes pain and bruising. It is distinct from morbid obesity and apparently affects females only after puberty. No endocrine or hormonal abnormalities have been identified. A family history is common, and it appears to be a sex limited condition even within families.

We report a family of 4 generations presenting with short stature and lipoedema. The proband, a 60 year old Caucasian lady, is 132cm and has suffered with gross lipoedema of her arms and legs since puberty. Her mother and grandmother also exhibited short stature and lipoedema. Her son is short but has no lipoedema. Studies show that both the proband and her son have growth hormone, TSH and prolactin deficiency.

This pattern of combined pituitary deficiency is suggestive of mutations in the *PIT1* (*POU1F1*) gene. We have found a mutation in *PIT1* (P24L in exon1), which acts in an autosomal dominant manner. The proline is in the transactivation domain of the protein, and is highly conserved. It is previously described in one sporadic case with no clinical details.

Growth hormone is associated with insulin regulation, which in turn is important in adipose formation and cardiovascular disease. This is the first gene associated with lipoedema.

P1013. Sudden cardiac death in a Swiss family is potentially caused by two distinct genetic elements

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Introduction: Congenital long QT syndrome (LQTS) is characterized by a prolonged QT interval on the electrocardiogram, leading to syncope or sudden death. Ten specific LQTS loci (LQT1-LQT10) have been found. We identified a four-generation Swiss family with several cases of sudden death and displaying two heart phenotypes: LQTS and conduction defects of the cardiac electrical impulse (CD). These two anomalies were present as independent entities, since some individuals exhibited only one of them, while others suffered from both.

Methods: Microsatellites linkage analysis was performed for three of the most frequent LQT loci (LQT1, LQT2, LQT3), accounting for ~ 80% of all LQTS cases. The *KCNQ1* gene (LQT1) was screened by direct DNA sequencing.

Results: We found perfect co-segregation between LQTS and a specific LQT1 haplotype. Sequencing of *KCNQ1* revealed the previously described mutation p.A344A (GCG>GCA) in all individuals with LQTS. However, this mutation was absent in patients (one of whom died suddenly) with CD and no LQTS. We then tested LQT2- and LQT3-associated markers for co-segregation with CD and could clearly exclude these two loci as being responsible for this phenotype.Conclusion: The mutation identified in *KCNQ1* is responsible for LQTS in this family, but cannot explain all cases of sudden death, nor the cardiac conduction defects also present in some individuals. It is therefore likely that another genetic cardiac anomaly, potentially lethal, segregates independently in this family. We plan to fully genotype the members of this family, in hopes of identifying this second gene.

P1014. Haplotype analysis of G72/G30 genes polymorphisms in major depressive disorder

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Major depression disorder (MDD) is a common, severe, chronic, and often life-threatening illness. There is evidence that overlapping genes G72/G30 (13q32-33) are transcribed in brain and involved in the etiol-

ogy of affective disorders. Interestingly, G72 protein interacts with the gene for D-amino-acid oxidase on 12q24 to regulate glutaminergic signaling through the N-methyl-D-aspartate receptor pathway, whereas the function of the G30 gene remained unclear. Recently, an association between the G72/G30 genes and bipolar disorder and schizophrenia has been reported. The aim of the present study is to determine whether the G72/G30 genes are a susceptibility factor for major depressive disorder. Three SNPs - rs2391191, rs3918342 of the G72 gene and rs1341402 of the G30 gene - were investigated in a case-control study included 108 individuals who had MDD and 244 healthy controls from Russia. There were no statistical differences between MDD patients and healthy controls in the genotypic and allelic distribution of the G72/G30 genes investigated polymorphisms. Maximum likelihood analysis of haplotype distribution demonstrated the presence of linkage disequilibrium between the two polymorphisms (rs2391191, rs1341402) both in control subjects (D'=0.999), and in cases (D'=0.35). Analysis of distribution of the estimated haplotype frequencies revealed significant difference between depressive subjects and controls ($\chi2$=14.54, df=3, p=0.0023). Further analysis showed overrepresentation of the haplotype CA ($\chi2$=20.13, p<0.001) in depressive group compared to control one. Our findings indicate the contribution of the G72/G30 genes to susceptibility for MDD, but further research of these genes on case-control phenotypic groups is of high importance.

P1015. Common variation in the ABO glycosyltransferase is associated with susceptibility to severe *Plasmodium falciparum* malaria.

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A link between host ABO blood group and susceptibility to severe *Plasmodium falciparum* malaria has long been suspected. Previous serological studies have suggested that blood group O individuals are relatively protected from severe disease; however the association has often proved difficult to confirm. Using a combination of family- and population- based studies from three African populations (almost 10 thousand individuals) we tested the molecular genetic variation underlying the ABO system. Our results confirm that the common frame-shift mutation underlying blood group O is associated with protection from severe disease (Odds Ratio 0.8, p-value $2x10^{-7}$). The high frequency of the common ABO alleles means that even modest differences in susceptibility could significantly affect the health of millions of people in malaria endemic regions. Using our genetic data, and additional resources from the International HapMap project, we went on to investigate the evidence for a possible parent-of-origin effect related to ABO alleles, and the signals of balanced evolutionary selection at the ABO locus.

P1016. Analysis of Y chromosome markers in patients with spermatogenesis abnormality.

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AZF region microdeletions of Y chromosome are one the most frequent causes of spermatogenesis abnormality. We investigated 131 unrelated males with azoospermia and oligozoospermia. Microdeletions were revealed in 11.45%. The spectrum of microdeletions was the following: AZFc - 5.34 %, AZFa+b+c - 1.5 %, AZFa+c, AZFb+c, AZFb - 0.76 %. Haplogroups determination was carried out using 19 diallelic markers of Y chromosome in 117 patients of Russian, Tatar and Bashkir ethnic origin with spermatogenesis abnormalities. The control group consists of 300 fertile men of Tatar (100), Bashkir (100) and Russian (100) ethnic origin. The comparison of group of Bashkir and Russian ethnic origin there were statistically significant differences in R1a, R1b3, I, N3 haplogroup frequency distributions (p=0.013,chi-square=6.271; p=0.005, chi-square=37.955; p=0.005,chi-square=8.482; p=0.003,chi-square=9.76, respectively). Haplogroup R1b3 frequency distribution were found between control groups of Bashkir ind Tatar ethnic origins (p=0.0005,chi-square=9.770). Haplogroup R1b3 found to be protective in spermatogenesis abnormality in males of Bashkir ethnic origin (p=0.0024; chi-square=12.4419; OR=0.29).The analysis of gr/gr deletions of AZFc locus was spent in 132 males with spermatogenesis abnormalities and in 300 control individuals. There were no statistically significant differences in deletions frequency distribution, but we determined association of gr/gr deletions with N haplogroups of Y chromosome (p=0.0005, chi-square=63.115) both in patients and controls irrespective of ethnic origin of individuals.Thus, characteristic features of Y chromosome haplogroups and gr/gr deletions of AZFc locus distribution were revealed in 3 ethnic groups from the Volga-Ural region and in patients with spermatogenesis abnormality.

P1017. Distribution of the MAOA-VNTR polymorphism in groups of elite athletes

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Monoamine oxydase (MAO), as a catabolic enzyme, regulates monoamine transmitter levels in the central nervous system. The activity of this enzyme is genetically regulated. The gene encoding the A form of human monoamine oxidase enzyme (MAOA) is located on the short arm of X chromosome. One of the common polymorphisms is a 30 bp functional VNTR polymorphism (MAOA-uVNTR).

In this study, we examined distribution of the MAOA-uVNTR polymorphism in groups of elite athletes of different specialization. 100 athletes and 105 non-trained controls were genotyped. Analysis of this test reveals that the frequency of MAOA alleles differ between groups of complex-coordination, playing and endurance kind of sport. Frequency of high active allele increases in groups of complex-coordination and endurance sportsmen. Frequency of low active allele decreases in groups of complex-coordination and playing sportsmen.

Interrelation of MAOA genotype and athletic successfulness are discussed.

P1018. Homozygosity mapping of primary microcephaly in Iranian families revealed novel mutations and novel phenotype

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Primary microcephaly (MCPH) is a genetically heterogeneous disorder showing an autosomal recessive mode of inheritance in the majority of cases. Affected individuals present with head circumferences more than 3 standard deviations below the age- and sex-matched population mean, accompanied by mental retardation without further associated malformations and with no manifest etiology.

Six genomic loci have been identified so far (MCPH1-6) and for four of these, the underlying genes are known.

For this study we have ascertained patients with primary microcephaly and additional family members from 30 consanguineous Iranian families. In addition to a thorough clinical characterisation, karyotype analyses were performed for all patients. For linkage analyses, several Microsatellite loci were selected for each known MCPH locus and used for genotyping in all available family members.

Our investigation enabled us to detect linkage to the *ASPM* (MCPH5) region in three families. Two families showed linkage to MCPH2 and one to *MCPH1*. For the remaining 24 families linkage to one of the six known loci could not be established. Subsequent sequencing revealed one novel mutation in *MCPH1* and two in *ASPM*. It is interesting that all carriers of *ASPM* mutations in this study also show short stature. This has not been observed before in and thus widens the spectrum of clinical manifestations of sequence changes in *ASPM*. In addition to that our results indicate that, in keeping with previous findings for other populations, *ASPM* mutations are the most common cause for primary microcephaly in the Iranian population.

P1019. Polymorphisms of the MDR1 gene in Hungarian Roma population samples

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The human multidrug resistance gene (MDR1) product P-glycoprotein (P-gp), a member of the ATP-binding cassette superfamily of transporters, plays crucial role in the bioavailability, absorption, distribution and excretion of various natural substrates and xenobiotics. Therefore its activity has significant pharmacokinetic and pharmacodinamic consequences. Among many SNPs of the MDR1 gene, C3435T in exon 26 and G2677T/A in exon 21(Ala893Ser/Thr) are the most extensively studied in relation to interindividual variability of P-gp expression and activity, and show linkage disequilibrium with C1236T in exon 12. The aim of this study was to investigate the frequency of these three major functional SNPs in the Hungarian Roma population: 251 unrelated Hungarian Roma subjects and 139 controls of Caucasian origin were examined. The genotypes of polymorphic positions C1236T, G2677T/A and C3435T were determined by PCR-RFLP. No significant differences were found in genotype distribution in exon 21 and 26 between the two groups. The observed allele frequencies were 48.6%, 1.4% and 48.2% in the Roma population, and 43.8%, 0.40% and 51.8% in controls for the alleles 2677T, 2677A and 3435T, respectively. These results were similar to that of other Caucasian populations in Europe. In exon 12 the Roma population had significantly higher frequency for the 1236T allele (57.8%) and showed increased frequency of TT genotype (34.3%) compared to controls (42.8% and 20.1%, respectively, $p<0.05$). The differential profile can have consequences for choice of treatment with drugs metabolising via this system.

P1020. Elucidating the molecular causes of autosomal recessive mental retardation in a systematic fashion: a progress report

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Mental retardation (MR) is a common disorder with severe socio-economic consequences. Most moderate and severe forms have genetic causes. These include cytogenetically visible chromosomal rearrangements, submicroscopic deletions and duplications as well as X-linked gene defects, which have recently received much attention.

There is reason to believe that autosomal recessive MR (ARMR) is far more common than XLMR, but to date, no more than 3 ARMR genes have been identified. In part, this is due to small family sizes and low consanguinity rates in industrialized societies, which has greatly hampered gene mapping and identification. These constraints do not exist in most Arabic populations, nor in Iran or Pakistan.

In 2003, we have set out to perform systematic clinical and molecular studies in large consanguineous Iranian families with several mentally retarded children. Here we report on the results of SNP array-based homozygosity in more than 120 families. So far we have identified 12 novel loci for non-syndromic ARMR, 8 of which with a LOD score above 3. None of these intervals show overlap with the 3 previously known ARMR loci. Mutation screening has so far enabled us to identify 2 novel genes.

The recent introduction of novel high-throughput sequencing technologies and our access to hundreds of consanguineous families with ARMR should enable us to greatly expand our ongoing search for novel ARMR genes. This collaborative effort will shed more light on brain function and will improve the prospects for molecular diagnosis, genetic counselling and eventually, therapy of mental retardation.

P1021. Association of HLA allele and level of streptococcus mutans in saliva of normal and mentally retarded Egyptian children

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Streptococcus Mutans (SM) is thought to play a major role in the etiology of dental caries. The aim of this study was to evaluate the relationship between HLA DRB1*0701-DRB1*0702 and DRB1*1101-DRB1*1104 loci and levels of SM in normal and some mentally retarded (MR) Egyptian children. Subjects and methods: subjects were 68 normal and 58 MR children, their ages ranged between 6-14 years. Subjects were classified according to caries index into free (0 caries index), low (1-6 caries index) and high (>6 caries index). Results: in MR children high levels of SM (mean: 84±127) were positively associated with absence of HLA DRB1*1101-DRB1*1104 alleles compared to normal children were high level of SM (mean 107±129) were positively associated with absence of DRB1*0701-DRB1*0702 allele, but not associated with DRB1*1101-DRB1*1104 alleles. A significant association was observed between caries level and HLA DRB1*0701-DRB1*0702 in MR children. No significant association was observed between DRB1*1101-DRB1*1104 allele and caries level in both groups. In conclusion, these results support the hypothesis of an association between HLA class II genetic profile and colonization of SM as pathogens for dental caries. However, further studies are needed to evaluate other HLA alleles as genetics variants in dental caries.

P1022. SNP association analysis of adipokines and metabolic traits in obesity and hypertension

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Essential hypertension and obesity are multifactorial traits determined by a complex interplay of genetic and environmental factors. Obesity is frequently associated with elevated blood pressure (BP), insulin resistance, dyslipidemia and abnormal glucose tolerance. However, many individuals with comparable degrees of obesity and similar environmental exposure have normal levels of BP, lipids and glucose tolerance. This suggests the existence of underlying regulatory functions which might confer protection from expression of inflammatory mediators and cardiovascular injury in the obesity/normal BP phenotype. Adipokines are proteins produced by fat cells with pro or anti-inflammatory function. The purpose of this study was to determine if genes involved in adipokines regulation are associated with metabolic phenotypes in obesity and hypertension. A cohort of 275 African American unrelated subjects was studied. The European admixture in this cohort was estimated between 12.7 and 13.6%. Metabolic traits quantified included insulin sensitivity, lipids and urinary albumin. DNA was examined by DNA sequence analysis and TaqMan. SNPs of candidate genes including pro-inflammatory *PAI1* and *IL6*, and anti-inflammatory adiponectin (*APMI*), as well as Calpain-10 were studied. Statistical analyses were performed by regression of the trait phenotypes on the groups defined by the SNP genotypes, adjusting for age, sex and BMI as covariates when appropriate. SNP 712 of the *APMI* gene showed significant association with insulin resistance ($p=0.005$). Despite the relatively small sample, our results indicate that genes that regulate adipocyte function may have a regulatory role in the expression of metabolic traits in obesity associated hypertension.

P1023. Prevalence and heritability of the metabolic syndrome and individual components in a Dutch isolate: the Erasmus Rucphen Family study

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Objective: In western countries, the metabolic syndrome (MetS) is a growing cause of morbidity and mortality. The MetS includes both an environmental and genetic component. We aim to determine heritabil-

ity and find novel loci for the MetS (IDF, 2003) and correlate inflammation and visceral obesity with MetS, in a Dutch isolate.
Methods: The Erasmus Rucphen Family study (ERF) consists of some 3000 individuals that descend form a limited set of founders. Waist circumference (WC), blood pressure, HDL-C, triglycerides and glucose levels were obtained. Variance component analysis was applied to extended family data to test for evidence of heritability (SOLAR).
Results: The prevalence of MetS, according to the IDF definition, in the ERF cohort is 36.8% in males and 31.0% in females. Our results indicate that HDL-C and WC are the main contributors to the MetS. Heritability of the MetS (as binary trait) was 14.3% ($P < 0.0001$). The heritability for the MetS corrected for household, decreased to 10.6% ($P = 0.012$). Also the heritability of individual components of MetS were analyzed (as quantative traits). The highest heritability was obtained for HDL-C (42.9%, $P < 0.0001$).
Discussion: The heritabilities of MetS in the ERF population are found to be highly significant. Moreover, our results indicate that the HDL-C component is a main contributor to the MetS and was found to have the highest individual heritability. Our data on prevalence and heritability of the MetS, indicate that determining loci for MetS by linkage analysis, with emphasis on the individual traits, is feasible.

P1024. Homozygous silencing of the T-box transcription factor *TBR2/EOMES* locus. Results in a microcephaly syndrome with polymicrogyria and corpus callosum agenesis

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Mechanisms regulating brain size during neurogenesis include the regulation of neural progenitor proliferation and migration. We report an autosomal recessive microcephaly syndrome co-segregating with a homozygous balanced translocation between chromosomes 3p and 10q in a large inbred family. The translocation was found at the homozygous status in all affected individuals (46,XY,t(3;10)(p24;q23)2x), while unaffected parents were heterozygous. We established a physical and characterized the BACs that encompassed the breakpoints on 3p24 and 10q23 (BAC RP11-9a14 and RP11-104H24). Interestingly, neither of the two translocation breakpoints disrupted a known or predicted gene coding sequence.
However, we showed that a position effect at the breakpoint on chromosome 3 silences the Tbox-brain2/Eomesodermin (*TBR2/EOMES)* transcript. Together with its expression pattern in the developing human brain, our data suggest an involvement of *TBR2/EOMES* in neuronal division and/or migration. Thus, mutations in not only mitotic and apoptotic proteins but also transcription factors may be responsible for malformative microcephaly syndromes.

P1025. Identification of a gene involved in hereditary microcytic anemia due to defective iron absorption in a Sardinian family

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We report the study of a large sardinian family where five patients show iron deficiency resulting in microcytic hypochromic anemia, not responsive to oral treatment but partially responsive to parenteral treatment. Known causes of hereditary microcytic anemia such as thalassemia, hemorrages, gastrointestinal disorders have been excluded by specific tests. By the pedigree analysis it seems an autosomal recessive disorder. Since clinical data suggest that it could be a defect of iron metabolism, especially a defect of iron absorption and/or mobilization of intracellular iron storage, initially we studied all the known gene involved in iron metabolism (Tf, TfR, ZIRTL, HJV and DMT1). Linkage analysis for these genes resulted negative, therefore we supposed that a different gene could be involved. Wide genome screening using 300 microsatellite markers was performed and a chromosome 22q13 region showing 5.6 multipoint LOD SCORE near marker D22S1177 and 5.4 multipoint LOD SCORE near marker D22S423. This region spans 7.2 cM and the affected subjects share two homozygous tracts. No recombination has been observed.
A further linkage analysis based on SNPs study was performed to better define the locus limits. This study confirmed the homozygous tract but did not allow to narrow the interval of interest. These results reinforce the hypotesis that the locus for hereditary microcytic anemia is on chromosome 22q13.
The 7.2 cM critical region of chromosome has been completely sequenced and is available from published databases. A large number of genes have already been characterized in the region.

P1026. Exclusion of ADAMTS10 gene for the isolated microspherophakia in a consanguineous multiplex Tunisian family

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Microspherophakia (OMIM 251750) is an eye disease characterized by a small and spherical crystalline lens with increased anteroposterior thickness. Autosomal recessive Weill Marchesani Syndrome is due to mutations within ADAMTS10 gene. The locus responsible for the isolated form of microspherophakia is still unknown because the reported cases are rare and sporadic. A consanguineous family with two patients affected with isolated microspherophakia has been identified in Central Tunisia. In order to check if ADAMTS10 gene is involved in microspherophakia in this family, a linkage analysis was performed using microsatellite markers flanking this gene. Genetic investigation showed an exclusion of linkage between ADAMTS10 gene and the disease locus in this family. This result suggests that isolated microspherophakia is not an allelic disorder to Weill Marchesani Syndrome.

P1027. Mitochondrial mutations in maternally-inherited non-syndromic deafness: whole-mitochondrial-genome screening using a microarray resequencing mitochondrial DNA chip

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Mitochondrial DNA (mtDNA) mutations have been implicated in non-syndromic hearing loss, with various degrees of penetrance. As only part of the mitochondrial genome is usually explored in deaf patients, their prevalence is probably under-estimated. Among 1350 families with sensorineural deafness collected through a French collaborative network, we selected 29 large families with clear maternal inheritance and screened them for the known mtDNA mutations in 12S rRNA, tRNAser(UCN), and tRNAleu(UUN) genes. When no mutation could be identified, a whole-mitochondrial genome screen was performed using the MitoChip v2.0 microarray resequencing chip (Affymetrix, Inc). Known mtDNA mutations were found in 9 of the 29 families: A1555G in five, T7511C in two, 7472insC and A3243G in one each. In the remaining 20 families, the resequencing Mitochip detected 258 mitochondrial homoplasmic variants and 107 potentially heteroplasmic variants. The more likely pathogenic variants are displayed based on allelic frequency and conservation between species. The whole-genome analysis elicited 5 additional families with a possibly pathogenic mitochondrial DNA variant: T669C, C1537T, G8078A, G12236A, and G15077A. These results indicate that the new MitoChip platform is a rapid and reliable tool for (the) identification of new mtDNA mutations, whether in deafness or other mtDNA-related diseases.

P1028. Multiple Ligation Probe Amplification analysis in the *DMD* gene: hidden results

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Duchenne and Becker muscular dystrophies (DMD/BMD) are the most common form of dystrophinopathies, with a reported incidence of 1:3500 and 1:18000 birth males repectively.
About 65% of DMD/BMD cases are attributable to large deletions of the DMD gene, whereas the remaining cases are caused by duplica-

tions or point mutations of the gene. More than 98% of deletions of the *DMD* gene are readily detectable in affected males by using a PCR reaction. We routenly use three multiplex PCR reactions to analyse 25 exons and the muscle and brain promoters. Recently, we started to use the Multiplex Ligation-dependent Probe Amplification (MLPA) technique, with the two commercial dystrophin probe mixes (P034/P035 MRC Holland). This system allows the simultaneous hybridisation and ligation of several probes, followed by PCR amplification and analysis by capillary electrophoresis. We analysed with this technique 200 affected males and 60 female relatives for deletions and duplications detection.
An sporadic DMD patient, presenting absence of dystrophin in the immunohystochemistry, was analyzed firstable with by multiplex PCR and no deletion was observed. Subsequently a deletion of exon 12 was observed with the P035 MLPA probe mix. The sequencing of exon 12 showed the c.1438G>T, p.Gly480X mutation. This mutation changes the penultimate nucleotide of the ligation site sequence and consequently originates an apparent absence of exon 12.
In case of a single exon deletion pattern in MLPA reaction, it is mandatory to perform the multiplex PCR and the sequencing of the exon in order to check a point mutation.

P1029. High prevalence of GCK mutations and low prevalence of HNF1A mutations in Italian MODY patients

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Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous group of disorders characterised by early onset non-insulin-dependent diabetes mellitus, autosomal dominant inheritance and primary defect in pancreatic beta cells function. Six genes have been associated with different subtypes of the disease, but 15-20% of MODY families do not exhibit any mutations in these genes. MODY2, caused by glucokinase (GCK) mutations and MODY3, caused by hepatocytes nuclear factor (HNF1A) mutations, are the most common forms; while MODY1, 4, 5 and 6 are rare disorders. Aim of our study is to assess the relative prevalence of MODY2 and MODY3 in Italian patients.
96 unrelated probands fitting MODY criteria were screened for GCK mutations and, when negative, for HNF1A mutations. The analysis was performed by DHPLC and direct sequencing. Mutations in the GCK gene were detected in 33 of 96 (34.3%) families. 7 mutations were previously undescribed: 3 missense (E372D, C382X, H424Y), 2 deletions (Q106_M107 delinsL and G295fsdel CA), 1 splicing mutation (IVS7+2T>C) and a stop codon suppression (X465insQ465+1_X+145). Mutations in HNF1A gene were detected in 3 (3,1%) probands, consisting in 2 new missense mutations (R159P and E508K) and the known G31D. All mutations co-segregated with affected family members, except for the *de novo* R159P mutation.
Our study indicates that defects in GCK/MODY2 gene are a very common cause of MODY in Italian population, whereas HNF1A/MODY3 has a lower prevalence. Our data broadens our knowledge of the naturally occurring GCK and HNF1A mutations repertoire.

P1030. Resolving a genetic paradox for Kostmann disease through PGD

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Introduction: Kostmann disease is a congenital immunodeficiency syndrome amenable to bone marrow transplantation. The family described, has a son affected with this disease. Genetic analysis identified a known dominant mutation in the ELA2 gene in the affected child, but also in the asymptomatic father. The parents requested preimplantation genetic diagnosis (PGD), coupled with HLA matching, to obtain a healthy suitable donor for the affected child.
Method: A PGD protocol was developed for the known mutation in the ELA2 gene. The protocol was based on multiplex nested PCR for direct analysis of the mutation, flanking polymorphic markers in the ELA2 gene locus and HLA typing. The protocol was calibrated and applied to single leukocytes isolated from the father, the mother and the affected child.
Results: The amplification efficiency of the mutation was >90% in single leukocytes from the affected child but only 67% in the father, suggesting somatic mosaicism for the mutation. Analysis of single haploid sperm cells from the father, using the same protocol, demonstrated 3 different sperm-cell populations: 1) sperm cells harboring the ELA2 mutation with the suspected allele, shared with the affected child, 2) Sperm cells without the ELA2 mutation and the normal haplotype, and 3) sperm cells without the ELA2 mutation but with suspected allele, shared with the affected child.
Conclusion: This was taken as evidence of somatic mosaicism for the ELA2 mutation in the father, explaining why he is asymptomatic. These data were also taken into consideration when deciding which embryos to transfer.

P1031. A novel locus for Autosomal Dominant Distal Motor Neuronopathy maps to chromosome 4q-ter

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Distal hereditary motor neuronopathy (dHMN), also known as distal spinal muscular atrophy, is a rare genetically and clinically heterogeneous early-onset disorder, characterized by weakness and wasting of distal limb muscles with possible pyramidal dysfunction, in absence of overt sensory abnormalities. To date nine and three loci for autosomal dominant dHMN and autosomal recessive dHMN have been described respectively. We have previously reported a four generation kindred characterized by atrophy and weakness of distal leg muscles associated with pyramidal features without sensory abnormalities. Linkage analysis excluded association to all the known loci for autosomal dominant dHMN suggesting further locus heterogeneity for dHMN. A genome wide search was performed by using 206 microsatellite markers from the ABI PRISM Linkage Mapping Set LD 20. All genotyped markers generated negative or nonsignificant LOD scores at all recombination fractions tested, except for markers on chromosome 4. A maximum LOD score of 3.19 at marker D4S408 was obtained, providing evidence of linkage between the disease and this region. All affected individuals in the family shared a commom haplotype between D4S1552 and D4S426 ,which allowed the identification of a 20 cM interval. The locus region contains many genes, including SNX25, a member of the sortin nexin family. This gene was screened by using DHPLC followed by sequencing of the variants. A nucleotide variation was identified in the IVS13, but it was also present in the unaffected members of the family suggesting that it is a polymorphism.

P1032. Association of mtDNA polymorphism with hypertension and its complications

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Hypertension is among most frequent cardiovascular diseases. It is known that failing of energetic processes in cardiomyocyte mitochondira plays substantial role in pathogenesis of cardiological diseases. Mitochondrial DNA contains genes which encode subunits of electron-transfer chain. Polymorphisms in these genes may influence cardiovascular system function. To evaluate associations of mtDNA polymorphisms with development of arterial hypertension, we have studied sample of 147 patients (51 females, 96 males) with hypertension and 137 healthy Russian individuals (81 females, 50 males). Mean age in the samples was 48,3±5,5 and 47,6±10,2 years, respectively. In the groups, ultrasound examination and 24-hours monitoring of blood pressure was performed. Statistical analysis has shown that in the hypertensive patients, prevalence of polymorphisms in positions 16292-16298 was revealed, so this locus might be designated as risk factor for hypertension (OR=1,86; p=0,046). Frequencies of mitochondrial polymorphisms were compared in the groups of patients with or without left ventricular hypertrophy. It has been found that haplogroup H was more frequent in hypertensive patients without hypertrophy (OR=0,36; p=0,043) whereas haplogroup T was more frequent in the patients with hypertrophy (OR=9,33; p=0,018). The findings suggest

that carrying of particular mtDNA haplogroup may have some impact on energetic processes and may be a predisposition factor for arterial hypertension and its complications. The work was supported by partial support of Russian Foundation for Basic research (RFBR) grants 04-04-48792, 04-04-48732, 06-04-08326.

P1033. Mitochondrial DNA depletion syndrome

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The mitochondrial genome (mt) is a small (16.5kb) DNA molecule that is normally present in multiple copies in individual mitochondria. MtDNA depletion syndrome (MDDS) is a recently recognized disorder involving a quantitative defect of mtDNA, that is inherited in an autosomal-recessive mode. The patients are born after an uneventful pregnancy and often normal at birth, but deteriorate in the neonatal period or early childhood. There are two main clinical presentations : myopathic and hepatocerebral. In the first group children usually present with devasting myopathy and neurological abnormalities. In the second condition patients suffer from early progressive hepatic failure with hypotonia, hypoglycaemia, lactic acidosis and progressive neurodegeneration as described in Alpers syndrome. We used real-time PCR techniques to quantify the level of mtDNA in fibroblast, blood, and muscle or liver tissue of patients suspected clinically and on the basis of the biochemical data. Of all patients clinical presentation of MDDS, mtDNA depletion was documented in ten. Molecular analysis of known nuclear mutant genes was undertaken and the exons of the TK2, DGUOK and polG genes were sequenced. In two patients DGUOK gene mutations were revealed, while in four other patients recessive polG mutations were seen. It is known that mutations in these genes count only for a fraction of MDDS cases. Recently mutations in two other nuclear genes, SUCLA and MPV17, segregating with mtDNA depletion were identified in patients with MDDS.

P1034. Myosin IXB: Initial evidence for association to multiple sclerosis in Finnish MS study sample

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Multiple sclerosis (MS) is a chronic inflammatory disease with a distinct autoimmune component affecting the myelin of CNS. Our group has previously identified MS-associated allelic haplotypes of the gene encoding protein kinase C alpha (PRKCA) in Finnish and Canadian populations. In vitro evidence suggests that PRKCA is a downstream target of RhoA in a pathway regulating blood brain barrier permeability. In this pathway, RhoA is regulated by myosin IX class proteins, of which MYO9B was recently associated with two autoimmune diseases, celiac disease and inflammatory bowel disease.
We genotyped 21 single nucleotide polymorphisms (SNPs), covering the MYO9B gene, in the nationwide collection of 970 Finnish MS families. Two SNPs (rs17533945 and rs12986130), located 22 kb apart in introns 2 and 10, provided evidence for linkage (Pseudomarker, Linkage p-values 0,0004 and 0,001, respectively). rs12986130 also showed evidence for association assuming linkage in a set of families stratified based on the PRKCA haplotype (Pseudomarker LD assuming Linkage p-value 0,001; Gamete competition p-value 0,002). The results lend support to our hypotheses that PRKCA and MYO9B may operate in the same biological pathway, potentially relevant for the disease pathogenesis of MS. The replication effort is ongoing in 217 MS trios and 3000 case-control samples from Denmark, Sweden and Norway.

P1035. Sequencing: a time consuming but robust highway to multifactorial diseases associated polymorphisms

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The HapMap project has characterized sets of Single Nucleotide polymorphisms (SNPs) to be picked for disease association studies. However, divergence of opinion still exists on how to optimise the selection of markers to capture the genetic variation underlying complex traits.
Cytokine gene polymorphisms are known to influence susceptibility and disease course of many autoimmune diseases. Interleukin 4 (IL4), Interleukin 13 (IL13) and their receptor (IL4R) have been partially investigated as candidate genes for MS by linkage and association studies with contradictory results.
In order to perform an exhaustive investigation of Il4-IL13 cluster and IL4R gene polymorphisms in susceptibility to MS we sequenced the complete region of each gene (promoter, exons and introns), in collaboration with the National Center of Sequencing (Evry, France) in 128 MS trio families.
Among the 145 SNPs within the IL13-IL4 cluster, 63 were newly identified by our study. Note that 54 previously reported SNPs were monomorphic in our sample. Using pairwise linkage disequilibrium (LD) we determined that 93 SNPs would need to be genotyped to represent the 145 SNPs with r2=0.8. Concerning IL4R gene, 71 sites were newly identified, 44 previously reported are not polymorphic in our sample, leading to a total of 157 SNPs which can be tagged by 74 SNPs.
Comparison with the tags proposed by HapMap is in progress. Preliminary results show that at least 10 of the 43 common SNPs of the IL13-IL4 cluster would not have been tagged if markers were selected based on the HapMap CEU data.

P1036. Investigation of the IL4-IL13/IL4R pathway in French Multiple Sclerosis patients

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Multiple Sclerosis (MS) is a multifactorial disease, in which genetic and environmental factors intervene. Apart from HLA, little is known on the other genetic factors involved. Two genome scans [Broadley et al, 2001; Babron et al, 2004] suggested the presence of a risk factor in the chromosomal region 5q31. The location of the IL4 and IL13 genes, coding for cytokines involved in the immune response, in this region makes them good candidates for susceptibility to MS. Previous studies on type I diabetes, another autoimmune disease have suggested interactions between IL4/IL13 and their receptor IL4R [Bugawan et al, 2003].
We investigated the role of this pathway in a sample of 124 French trios (an affected child and his two parents). Sequencing of all the individuals allowed identification of a total of 249 SNPS. Among them, 14, 23 and 86 for IL13, IL4 and IL4R, respectively, had a minor allele frequency greater than 1%. None of these, taken individually, showed significant association with MS.
However, in multifactorial diseases such as MS, combinations of two or more variants in the same pathway may be involved. Thus, as proposed by Jannot et al [2003], we investigated combinations of two variants, one in IL4 or IL13 and one in IL4R. Among the 3219 combinations tested, 12 were significant at the nominal 1% level, and were more significant than each SNP analysed separately. These combinations will need to be tested in independent samples for replication.
We thank REFGENSEP for data sharing and ARSEP for funding.

P1037. A Multiple Imputation approach to test the role of the CD28-CTLA4-ICOS gene cluster in Multiple Sclerosis susceptibility

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Genes involved in Multiple Sclerosis (MS) are difficult to identify because of their small effect and their possible interaction with other

factors. This is well illustrated by the numerous studies of the role of CTLA4 in MS, which showed an association with the CTLA4 gene in three cohorts (France, Italy and Portugal) in the subset of patients carrying the HLA-DRB1*1501 allele (Alizadeh et al, 2003) whereas this association was not replicated in a British sample (Roxburgh et al, 2006). However, since the CTLA4 gene is located close to two other genes, CD28 and ICOS, also involved in the immune response, it is possible that the former association detected reflects an association with other polymorphism(s) in any of these three genes. To study this hypothesis, 19 markers spanning this cluster were tested in a sample of 450 French MS cases-parent trios. Since these markers were not consistently genotyped among the different families, power to detect the association was not the same at the different markers and comparison was therefore very difficult. To help solve this problem, a multiple imputation approach (Croiseau et al, 2006) has been used. After a complete association study including a stepwise approach and tests of interaction, no marker other than the one previously reported in CTLA4 showed significant association with MS. However, since only a few markers in this cluster have been investigated, it is difficult to rule out the possibility of their involvement in MS.
We thank the REFGENSEP, coordinated by Bertrand Fontaine for data sharing.

P1038. Searching for factors modifying disease expression: Application to Multiple Sclerosis.

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A modifying locus is a polymorphic locus, distinct from the disease locus, which leads to differences in the disease phenotype, either by modifying the penetrance of the disease allele, or by modifying the expression of the disease. The effect of such a locus is a clinical heterogeneity that can be reflected by the values of an appropriate covariate, such as the age of onset, the severity of the disease.
We designe the Ordered Transmission Disequilibrium Test (OTDT) to test for a relation between the clinical heterogeneity, expressed by the covariate, and marker genotypes of a candidate gene. The method applies to trio families with one affected child and his parents. Each family member is genotyped at a bi-allelic marker M of a candidate gene. To each family is associated a covariate value; the families are ordered on the values of this covariate. As the TDT (Spielman et al, 1993), the OTDT is based on the observation of the transmission rate T of a given allele at M. The OTDT aims to detect whether T depends on the value of the covariate.
We illustrate this method on Multiple Sclerosis trio families ascertained by REFGENSEP (Réseau Français d'Études Génétique sur la Sclérose En Plaques) which are genotyped for several candidate genes. The EDSS (Expanded Disability Status Scale) of 268 patients is used to compute the MSSS (Multiple Sclerosis Severity Score)(Roxburgh et al, 2005), which we use as a covariate.
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P1039. A simple model for a complex disease: PLOSL and multiple sclerosis - are two demyelinating diseases related?

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PLOSL is a recessively inherited disease characterised by demyelination in the CNS and bone cysts. The causative mutation in Finnish patients is a 5.3 kb deletion in TYROBP on 19q13.12, while mutations in TREM2 on 6p21.1, encoding another molecule in a receptor complex with TYROBP, have been identified in PLOSL patients from other populations. Demyelination in the CNS and the role of TYROBP and TREM2 in inflammatory cell activation makes PLOSL-genes potential candidates behind demyelination in MS.
Since all the Hapmap SNPs in TYROBP or TREM2 were not polymorphic or had very low minor allele frequency among Finns, we re-sequenced parts of the TYROBP gene in 12 patients and identified four novel SNPs. These SNPs and additional 24 polymorphic SNPs flanking the genes were genotyped in 970 Finnish MS families. To increase the allelic information content, two flanking STS markers (D19S876 and D6S1575) were also genotyped. Pseudomarker program was used to monitor for linkage and association. Eight SNPs mapping to 6p21.1 provided some evidence for linkage, the highest LOD (2.51) observed with the SNP rs6923053 located 24 kb from TREM2 and 8.5Mb from the MS associated HLA region. Polymorphic variations in TYROBP region showed no evidence for linkage or association in MS families. We conclude that although the exceptionally low number of SNPs in both TYROBP and TREM2 prevent final conclusions, no obvious relation with the increased risk of MS for the carriers of specific alleles of these demyelination-associated genes was observed in this set of Finnish MS families.

P1040. The association of single nucleotide E-selectin gene polymorphism with Multiple Sclerosis

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E-selectin (endothelial leukocyte adhesion molecule; ELAM-1), a cell surface glycoprotein expressed on endothelial cells, is an important adhesion molecule involved in lymphocyte recruitment into the brain, which represents a crucial step in the pathogenesis of multiple sclerosis (MS). Several polymorphisms have been described within the ELAM-1 gene. A single nucleotide polymorphism in the coding region of the gene (A561C) causes a conservative change of a serine with an arginine at codon 128 (S128R). Previous study showed that the presence of this variation confers not only an increased capability of binding lymphocytes, but also a different specificity in their recruitment. Thus, the S128R E-selectin molecules may be an important variable with regard to susceptibility to MS. Two hundred ninety-two patients were genotyped for the presence of this polymorphism in the ELAM-1 gene, through a case-control study involving Southern Italian patients (Calabria region) with definite MS, and 245 age and sex-matched healthy controls from the same geographical area. Our results showed no significant differences in the allele and genotype distribution of the ELAM-1 polymorphism between MS patients and controls. In addition, in patients no difference was observed in the age of MS onset, according to ELAM-1 genotypes; furthermore, dividing patients according to disease type, EDSS, or age and gender did not yield significant differences for the examined polymorphism. The current findings demonstrate that ELAM-1 gene is not a major susceptibility or modifying gene for MS in Southern Italian population.

P1041. Chromosome 19q13 and multiple sclerosis

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Previous studies have suggested a role of allelic variation on chromosome 19q13 in multiple sclerosis (MS) susceptibility. The object of the study was to test three subregions of 19q13 for association with MS in 440 families using the transmission disequilibrium test. A marker in the 19q13.1 area showed nominally significant association with MS. This marker resides at a suggestive linkage peak, and associated with MS in a previous Italian study. Another association was found with an APOE haplotype on 19q13.2, supported also by a separate case-control analysis. Previous suggestions on ILT6 deficiency or association with D19S585 on 19q13.3-q13.4 were not replicated in this study.
These results show that two subregions of chromosome 19q13 are associated with MS warrating further studies on their implication with the disease .

P1042. NFATC4 gene variation is associated with muscle fiber composition of athletes

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There is strong relationship between muscle fiber type distribution and human physical performance. Studies reveal that skeletal muscle can exhibit a high or a low percentage of type I fibers. It has been shown recently that the genetic variation component for the proportion of type I fibers in human muscles is of the order of 40 to 50%. Calcium/calcineurin/NFAT pathway has been suggested to play a role in the regula-

tion of several slow fiber-specific genes. To investigate the question of the influence of NFATC4 gene polymorphism on the proportion of fibers types of m. vastus lateralis, we have analyzed the muscle biopsies obtained from 27 elite Russian athletes (all-round speed skaters). M. vastus lateralis was chosen for muscle biopsy because of great individual variability of muscle fiber type composition (i.e. 5-90% for type I fiber). The immunoperoxidase technique was employed for immunohistochemical identification of myosin isoforms. Fiber distribution was expressed as a ratio of the number of fibers of each type in a section to the total number of fibers. DNA was extracted from mouthwash samples. NFATC4 gene Gly160Ala polymorphism was determined by PCR-RLFP. NFATC4 Ala allele frequency in athletes was 48.1%. Mean percentages of type I fiber in Ala/Ala homozygotes were significantly higher than in NFATC4 Gly allele carriers (Ala/Ala - 74.2±2.8%, Gly/Ala+Gly/Gly - 63.1±2.3%; p=0.019). We speculate that Gly160Ala substitution in NFATC4 is associated with increased transcript activity. Thus, NFATC4 Gly160Ala polymorphism is associated with muscle fiber composition in athletes.

P1043. The -1159 A/C polymorphism of interleukin-12B gene in myocardial infarction in Russians and Tatars from Bashkortostan

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Objective: Inflammation plays an important role in the pathogenesis of atherosclerosis. The aim of this study was to test whether the -1159 A/C polymorphism of the interleukin-12B gene (IL12B) is associated with myocardial infarction (MI) in Russians and Tatars from Bashkortostan region of Russia.

Methods: The -1159 A/C polymorphism of IL12B gene was studied by polymerase chain reaction - restriction fragment length polymorphism method in 306 male patients with MI and 245 healthy controls from Bashkortostan region of Russia. For comparison of genotype and allele frequencies between the patients and the controls we used two-tailed Fisher test and odds ratio (OR).

Results: Genotype frequencies for IL12B gene polymorphism in Russian controls: 55 A homozygotes (53.4%), 39 A/C heterozygotes (37.86%), 9 C homozygotes (8.74%), and in Tatar controls 64 (60.95%), 34 (32.38%) and 7 (6.67%), respectively, and were similar to those reported for Italian Caucasian healthy controls. In the MI patients, the genotype frequencies in Russians were 125 A/A (65.45%), 50 A/C (26.18%), 16 C/C (8.38%), and in Tatars 61 (53.04%), 44 (38.26%) and 10 (8.70%), respectively.

No significant differences in the genotype and allele frequencies were found between the Tatar patients and the control group. In Russian patients the frequencies of the A/A genotype were significantly higher than in the control group (P=0.046, OR=1.65).

Conclusion: Our results suggest that IL12B gene polymorphism may be a genetic risk factor for the development of MI in Russian patients.

P1044. A family-based test of association between alleles at the 5‘ UTR polymorphism of the myocilin (MYOC) gene and high myopia in subjects from the UK

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Purpose: Mutations in the myocilin gene (MYOC) on chromosome 1 can cause juvenile-onset open-angle glaucoma and are associated with 2-4% of cases of primary open angle glaucoma in adults. Three previous studies (Wu *et al.* (1999), Leung *et al.* (2000), Tang *et al.* (2004)) have tested for association between MYOC polymorphisms and high myopia. For a dinucleotide repeat polymorphism in the 5’UTR (untranslated region), 2 of these studies reported a significant association.

Methods: DNA was collected from 96 high myopes (≤-6.00 D) and both their parents. The polymorphism in the 5’UTR and another in the 3’UTR of MYOC were genotyped using standard techniques. Association was assessed using FBAT.

Results: For the 5’UTR polymorphism, 3 common (13, 14 and 15-repeats) and 2 rare (12 and 16-repeats) alleles were found. FBAT showed weak evidence of decreased transmission of the 15-repeat allele (Z = -2.26, *P* = 0.02) when high myopia was considered as a dichotomous trait. However, after accounting for multiple testing, this result did not reach statistical significance. For the 3’UTR, no association was detected.

Conclusion: Our findings do not support the hypothesis that MYOC is a high myopia susceptibility gene in Caucasian subjects. However, the pattern of allele transmissions was consistent with previous studies, and therefore it may be that our sample size was insufficient to disclose a real, yet small, relative risk at this locus. We are currently examining a larger sample to resolve whether MYOC polymorphisms impart an increased risk of high myopia in Caucasian subjects.

P1045. Intergenerational contraction of the CTG repeat in French-Canadian myotonic dystrophy (DM1)

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The aim of this study is to determine the occurence of intergenerational contraction of the CTG repeats and its recurrence among siblings in French-Canadian DM1 families. We examined 122 French-Canadian DM1 families. The contraction of the CTG repeats occurred in 4 families (3,3 %) of paternal transmission while no contraction occurred in cases of maternal transmission. In family 1, the number of the CTG repeats was 650 for the father and 250 for all 4 affected children. In family 2, two brothers with 650 and 500 repeats had all DM1 offspring with CTG repeat contractions (210 and 210 CTG repeats for the first and, 160 and 350 CTG repeats for the second father). In family 3 and 4, the fathers with 700 and 550 CTG repeats have DM1 offspring with 230, 300 and 170 CTG repeats, respectively. In all cases, DM1 offspring had a less severe phenotype than the father. We did not observe a case with regression of CTG repeats and another with expansion within the same sibship. These observations indicate that:1) the frequency of intergenerational contraction of the repeats in DM1 French-Canadian population is lower than that previously reported (6,4%) in other populations; 2) the paternal transmission of DM1 and the presence of CTG repeats contraction in a child greatly increase the probability of CTG repeat contraction in the other affected sibs; 3) large contractions are associated with a less severe phenotype than the transmitting parent’s phenotype. It may exist a familial paternal factor that restricts the expansion.

P1046. Simple genetic protocol for detection of normal and expanded alleles in myotonic dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is an autosomal dominant multisystemic disease which is caused by expansion of CCTG repeats in intron 1 of ZNF9 gene on chromosome 3q21. The range of expansion varies widely, starting from 75 up to 11000 repeats. Such expansions can usually be detected by Southern blot after restriction enzyme digestion of genomic DNA. However, this method is time consuming, requires large amounts of genomic DNA and fails to detect approximately 20% of affected individuals. Therefore, the aim was to introduce a simple and non-radioactive method for molecular diagnosis of MD2. Our specially designed PCR-based protocol allows amplification of normal and very long repeat tracts that are visualized after oligonucleotide hybridization. Very low amounts of genomic DNA (as little as 30 ng) are needed for this method which is simple, relatively fast and thus reduces the cost of diagnostic laboratory processing of DM2 patients. We tested 78 patients showing different symptoms related to myotonic dystrophy but with negative test results for myotonic dystrophy type 1 (DM1) as part of molecular diagnosis of DM2 in Croatia. DNA analysis confirmed mutation of ZNF9 gene in 8 patients. This result indicates that laboratories should establish a protocol for molecular genetic testing of DM1 negative patients for a mutation causing DM2 due to their overlapping clinical symptoms.

P1047. N-acetyltransferase 2 (*NAT2*) gene polymorphisms in psoriasis and colon cancer patients from the Moscow population

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N-acetyltransferase 2 (NAT2) is one of the key enzymes of a phase II detoxification of xenobiotics, including carcinogens. The decrease in activity of NAT2 enzyme may correlate with accumulation of harmful intermediate metabolites and influence on the risk to develop different multifactorial diseases. *NAT2*-biochip has been developed including 17 SNP in the *NAT2* gene, most significant for European populations (282C/T, 341T/C, 481C/T, 590G/A, 803A/G and 857G/A). The biochip has been tested on more than 450 clinical DNA samples of 166 patients with psoriasis, 104 patients with rectal cancer, 84 patients with various dermatological diseases and 99 healthy individuals. We found that patients with psoriasis and rectal cancer had the increased frequency of mutation 282T comparing with population control (OR=1.3, p = 0.134 and OR = 1.58, p = 0.045, respectively). On the contrary, the mutation 481T had a reduced frequency in patients with psoriasis comparing with healthy controls (OR=0.89, p = 0.567). No differences have been found in distribution of the same mutations in male and female. Also patients with psoriasis and rectal cancer revealed the increase in frequency of 590A mutation in comparison with control group (OR = 1.48, p = 0.055 and OR = 1.501, p = 0.0656, respectively). Thus, the data presented suggest association of separate polymorphic variants of gene *NAT2* with such multifactorial diseases as psoriasis and rectal cancer.

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P1048. A new substitution in *Aqp2* gene in a family with history of three affected children with Nephrogenic Diabetes Insipidus

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Nephrogenic diabetes insipidus (NDI) is characterized by inability to concentrate the urine, which results in polyuria (excessive urine production) and polydipsia (excessive thirst). NDI is most commonly inherited in an X-linked manner (~90% of individuals), but can also be inherited in an autosomal recessive manner (~9% of individuals) or in an autosomal dominant manner (~1% of individuals). Autosomal NDI is caused by mutation in the gene encoding the aquaporin-2 water channel (*Aqp2*), which maps to chromosome 12q13.We report on a family with history of two daughters and a son affected with Nephrogenic diabetes insipidus, who developed the clinical and biochemical features of metabolic acidosis and hypernatremic dehydration with generalized hypotonia and moderate mental retardation in the first year of life. They suffered from unconsiousness spell, polyuria and polydypsia without any ophthalmologic cystinosis. Laboratory tests indicated severe anemia, asotemia, thrombocytopenia and they died from the disease in the first 5 years of life.We determined by sequencing analysis that the two parents were both heterozygote for the G175R mutation in *Aqp2* gene. The mutation is already known but the base pair change is new. It is thus likely that the deceased infants were homozygotes for this mutation and suffered from repeated episodes of dehydration due to non-linked Nephrogenic diabetes insipidus. The prenatal diagnosis for this family revealed that the fetus was heterozygote for the same mutation.

P1049. MGP gene T-138C and G-7A polymorphisms in patients with nephrolithiasis.

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Kidney stone formation is the result of cascade of events to mineralize calcium salts in the urinary space causing pathological nephrolithiasis condition. The mechanism of nephrolithiasis is unknown and usually calcification and stone formation is associated with the bone specific proteins. One of them is human matrix Gla protein (MGP). It is also shown that calcium containing stone growth concurs with the increase of MGP expression along with the other proteins. MGP gene is located on chromosome 12p13.1 - p12.3, consists of 4 exons. Its promoter region contains dense binding sites for variety of transcription factors. In addition to T-138C and G-7A polymorphisms, primer design for the analysis of promoter sites has been achieved using Workbench Biology 3.2. Promoter screening and polimorphic analysis were done by SSCP and PCR-RFLP methods using NcoI and BsrSI restriction enzymes. For this, 89 patients with familial and 111 patients with sporadic nephrolithiasis, totalling 200 neprolithiasis patients and 94 normal subject DNAs from their peripheral blood samples were used. The results showed that there is no significant difference between patient and control groups for T-138C and G-7A polymorphisms. Therefore, no association can be attributed to T-138C and G-7A polymorphisms in patients with nephrolithiasis.

P1050. No evidence of Neuregulin 1 plays a role in the continuum model of psychosis

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Although family and twin studies support independent transmission of schizophrenia and bipolar disorder there are some evidence suggesting that schizophrenia may be genetically related to mood disorders with regard to psychotic symptoms (continuum model of psychosis). In consensus with this model, we have previously reported an inbred multigeneration family overloaded with distinct psychotic disorders (schizophrenia, schizoaffective, bipolar and unipolar disorders with psychotic features) in 18 affected members of the pedigree (Prog. Neuro-Psychopharmacology and Biological Psychiatry, 2004, 28; 255-266). Chromosome 8p12 region containing neurogulin 1 (NRG1) gene was excluded by both linkage and haplotype analysis for this particular family. A total of 134 parent-offspring trios with psychotic disorders (53 schizophrenia; 5 schizoaffective; 70 bipolar affective disorders with psychosis and 6 unspecified psychosis) were further genotyped with the polymorphic DNA marker, D8S1810 located in the core at-risk haplotype of the NRG1 gene. There was no evidence for overtransmission of a spesific allele of the DNA marker D8S1810 to affected offspring using broad model including distict types of psychotic disorders . We have observed an overtransmission for 206 bp allele (allele 6) of D8S1810 to affected offspring when we used a narrower (schizophrenia and schizoaffective disorder only) model in the TDT analysis (p= 0.0081 after correction p= 0.07). These preliminary results do not support the hypothesis that NRG1 also plays a role in influencing distict types of psychotic disorders. (Supported by Hacettepe University Research Foundation: 06 01 101 020; NARSAD: 2003II)

P1051. The MLPA-dHPLC approach of the NF1 gene analysis: a french experience

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The identification of mutations in the NF1 gene causing type 1 neurofibromatosis (NF1) is still presenting a considerable amount of work mainly because of the large size of the gene (350 kb - 60 exons) and the restricted number of recurent mutations. The high frequency of NF1 which is affecting 1 in 3500 individuals made us choose two complementary methods to perform NF1 gene analysis:
- the multiplex ligation-dependant probe amplification (MLPA) for the large deletion and duplication detection (P081-P082)

- and the automated denaturing high performance liquid (dHPLC) screening method.
The MLPA method was validated by the detection of 18 known large NF1 gene deletions. The dHPLC was optimised for a rapid screening of the 60 exons and the splice junctions of the NF1 gene. The dHPLC conditions were validated by the detection of 260 known variants located in two third of the NF1 exons.
The sensitivity was evaluated in a MLPA/dHPLC analysis of a panel of 100 unrelated french NF1 patients with at least two consensus diagnostic criterias.
Four large deletions were detected by MLPA and mutations were identified in 91 patients with a global mutation detection rate of 96%. The mutations included 18 deletions, 10 insertions, 33 non sens mutations, 11 missense mutations, 16 splice mutations and 3 complex rearrangements.
Our results confirm that the association of the MLPA and dHPLC techniques provides an accurate and fast method for the identification of NF1 mutations.

P1052. NOD2/CARD15 mutation analysis and genotype-phenotype correlation in Russian patients with Crohn's disease

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Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract with variations in localization and behavior. Mutations in the *NOD2/CARD15* gene on chromosome 16q have been implicated in the pathogenesis of the disease and three main sequence variants, all single nucleotide polymorphisms (SNPs). We collected a cohort of 78 CD patients and 54 population controls to determine the prevalence of *NOD2/CARD15* SNPs and their association with phenotypic expression of the disease. All patients and controls were genotyped for Arg702Trp, Gly908Arg and Leu1007fsinsC.
At least one mutation was present in 21,7% of patients compared to 10.0% in controls (p=0,02), in patients with Crohn disease, the allelic frequency of R702W, G908R, and L1007fs was 22%, 8.3%, and 1,3%, respectively. Compound heterozygotes and homozygotes occurred in 26,9% of patients and in none of the controls.
The correlation of genotype-fenotype showed a significant association with early onset in patients with R702W or G908R, but not in patients with L1007fs. There was not a significant association between this SNPs carriership and inflammatory localization (p=0,08). Association between carriage of this allels and colonic complication (p=0,004) such as stenosis, penetration and perianal exhibitings were revealed in our study.
In a Russian population SNPs of the *NOD2/CARD15* gene were a marker of susceptibility to Crohn disease and were associated with colonic complication. Carriers of the R702W and G908R alleles showed early onset of Crohn disease.
This work was pertly supported by Russian President's grant NSh5736.2006.7

P1053. Opitz-Kaveggia (FG) and Lujan syndromes are allelic having mutations in the MED12 gene

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FG syndrome is an X-linked mental retardation (XLMR) syndrome first described by Opitz and Kaveggia in 1974. It is characterized by MR, relative macrocephaly, hypotonia and constipation. Five different loci have been reported for FG syndrome on the X chromosome. Analysis of a candidate gene, MED12, in 24 XLMR families linked to Xq13, identified a specific nucleotide substitution, c.2881C>T, in 2 FG families. This change results in a missense mutation, p.R961W. Subsequent analysis of an additional 119 patients with FG identified another 7 patients with the same mutation, meaning 7.4% of the FG patients tested had this mutation. The mutation was not observed in more than 1400 normal males.
Independently, a systematic screen of 737 annotated Vega genes in 250 XLMR probands, identified another base change, c.3020A>G in MED12 in the proband from the original Lujan family. This change segregated in the family and was not observed in greater than 1400 normal males. The c.3020A>G results in a missense mutation, p.N1007S. Subsequent studies of 111 FG and 40 Lujan patients found another family with the same mutation. The phenotype of the family, K9359, consisted of height in 80th - 90th centile range, weight at the 40th centile, normal head circumference, tall narrow face, high nasal root and behavior disturbances. This closely overlaps with Lujan syndrome: tall, asthenic habitus, tall narrow face, high narrow palate, behavior abnormalities.
These findings in the original families with Opitz-Kaveggia (FG) and Lujan syndromes, indicate these two XLMR syndromes are allelic, with mutations in MED12.

P1054. A novel type of canine osteodysplasia resembling human *Osteogenesis imperfecta*

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Recently completed genetic analysis indicates that the dog genome holds a wealth of information that will benefit human health. Most of the canine inherited diseases are shared with humans. Complex genetic problems can be simplified in dogs since each pure breed represents a group of genetically similar animals that have descended from only a few ancestors. We have characterized a novel congenital disorder involving multiple skeletal defects among Brazilian Terriers. Affected dogs have typical craniofacial features, growth retardation, joint hyperlaxity and osteopenia based on radiographical examination. The clinical symptoms are severe and dogs have failure to thrive within a few weeks of life. The affected puppies have to be euthanized at an early age. Histological analyses reveal abnormalities in bone formation including the thinning of cortical bones and reduced amount of cancellous bone. These pathological features of the canine osteodysplasia resemble that of human osteogenesis imperfecta (OI). In humans, most of the OI cases are caused either by dominant mutations in COL1A1 and COL1A2 or recessive mutations in the cartilage associated protein gene (CRTAP). We have excluded these three genes as candidates using microsatellite-based association analyses. Pedigree analysis indicates a recessive mode of inheritance. A whole genome wide study with canine SNP chip arrays has been initiated to map the causative gene for this novel type of canine osteodysplasia.

P1055. Predictive value of cytokine gene polymorphisms for the development of osteonecrosis

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Osteonecrosis of the femoral head is a disease of unknown etiology that usually progresses to hip joint destruction necessitating total hip arthroplasty. Cytokines, other signaling molecules and angiogenic factors may lead to more rapid healing and filling of defects with biomechanically competent and viable bone. Cytokine production is determined by polymorphisms in the relevant genes. In this context, we explored the hypothesis that these polymorphisms of the cytokine genes may be potential genetic susceptibility factors for the progression of osteonecrosis. The IL-1α(-899), IL-1β(-511), IL-4Ra(+1902), TGF-β (cd10), TNF-α(-308/-238), IL-4(-1098/-590) and IL-10(-1082/-592) gene single nucleotide polymorphisms were studied in 30 healthy donors and 50 patients with osteonecrosis. Genotyping of the polymorphisms was detected by PCR followed by restriction fragment length analysis. A significant association in the genotype distribution

of IL-1α(T/C), IL-1β(-511)(T/T) and TGF-β(cd10/25) (CG/TG) was observed when comparing the osteonecrosis group with the control group ($p<0.05$). A high percentage of the patients (30%) carried simultaneously either the genotypes T/C(IL-1α) and T/T(IL-1β) or T/C(IL-1α) and CG/TG(TGF-β) while only one patient carried all three polymorphisms; in contrast in the controls 3 out of 30 (10%) were associated with the polymorphisms IL-1α(T/C) and TGF-β(CG/TG). There was no significant difference in IL-4Ra, TGF-β(cd10), TNF-α, IL-4 and IL-10 genotype and allele distributions between patients with osteonecrosis and controls. These results imply that the genotypes (T/C), (T/T) and (CG/TG) of IL-1α, IL-1β and TGFβ, respectively, are associated with increased risk for osteonecrosis and that the simultaneous carriage of more than one may further increase the risk for osteonecrosis.

P1056. Fine mapping by SNP linkage disequilibrium analysis of a candidate region in 1p36 for bone mineral density

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Low bone mineral density (BMD) is one of the major risk factors for osteoporosis. Epidemiological studies support the hypothesis that a large part of the variation in BMD is caused by genetic susceptibility factors. We performed a whole genome scan for linkage in a total of 40 Caucasian families recruited through probands with low BMD. A region on 1p36 near marker D1S214 received support as a candidate locus for femoral neck BMD from both linkage (max LOD = 3.53) and linkage disequilibrium analysis ($p < 0.01$) with microsatellite markers. In an attempt to better characterize the genetic risk factors for low BMD located in this genomic region, we have now genotyped the same group of families for 1095 SNPs across 11 Mb on chromosome 1p36 at an average spacing of about 10 Kb in length. Linkage disequilibrium and association analysis have been carried out using the quantitative trait linkage disequilibrium (QTLD) test and transmission disequilibrium test (QTDT) implemented in SOLAR. We have identified several SNPs potentially associated with BMD in our samples, with minimum $p=0.00004$ for femoral neck BMD, $p=0.00001$ for lumbar spine BMD, and $p=0.00010$ for trochanter BMD. Among the candidate genes in this region, it is suggested that association may exist for *RPL22*, *SHREW1* and *CAMTA1* with at least one of the BMD traits.

P1057. The MTHFR c.677C>T polymorphism and osteoporotic phenotypes in Spanish postmenopausal women

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Osteoporosis is a multifactorial disease, with a genetic component. Several candidate genes have been proposed, among them *MTHFR* (encoding methylenetetrahydrofolate reductase). This gene, associated with several diseases, has been hypothesised to play a role in osteoporosis through interference of collagen maturation caused by elevated homocysteine. Additionally, a QTL for bone mineral density (BMD) was mapped to Chr1, where *MTHFR* is located. In particular, the c.677C>T polymorphism of this gene, is responsible for increased homocysteine plasma levels. Recently, an association of this polymorphism with lower BMD and increased risk of fractures has been described in several populations. Our aim was to replicate these findings in a Spanish postmenopausal cohort, performing association analyses with femoral neck (FN), lumbar spine (LS) BMD and osteoporotic fracture. Genotyping was performed by PCR-RFLP in 950 postmenopausal women (mean age 55.6 ± 8.7). The association analysis was performed by lineal regression for BMD and by logistic regression for fracture. The minor allele frequency in our population was 39.7% and the distribution of genotypes was in Hardy-Weinberg equilibrium ($p=0.521$). No association was observed between the polymorphism and either LS BMD ($n=944$, $p=0.26$ recessive model), or FN BMD ($n=564$, $p= 0.49$ recessive model). For the presence of osteoporotic fracture, the results were also negative ($n=835$, $p=0.20$). In conclusion, this polymorphism is not associated with these osteoporotic phenotypes in our cohort.

P1058. Vitamin D receptor and osteocalcine *HindIII* gene polymorphism and bone mineral parameters in people, who lived in Blockaded Leningrad during 1941-1944.

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Osteoporosis is well-known phenomena in old population. Starvation of Leningrad children during blockade 1941-1944 resulted in total dystrophy. Hungry children weren't accumulate peak bone mass and they have high risk of osteoporosis realisation.

The mean age of 48 patients, included in our study was 8,64±5,12 years in 1941 was. Bone mineral parameters were detected by DEXA. *ApaI, TagI* vitamin D receptor (*VDR*) and *Hind III* osteocalcine genes polymorphism were detected by PCR.

Femur neck osteopenia people were elder in the end of blockade, when people without OP ($p=0,006$). We have revealed differences in *TagI* genotypes distribution between people with and without osteopenia (OP) Wards zone ($p=0,04$), trochanter ($p=0,05$), L_1-L_4 ($p=0,01$), and differences in *ApaI* genotypes distribution between males ($p=0,05$), all patients ($p=0,03$) with and without OP L_1-L_4. Males with t allele had significant higher BMC femur neck, BMC, BMD, T_{score} Wards zone, BMD trochanter, females had higher T_{score} Wards zone, BMC, T_{score} trochanter, BMC, BMD L_1-L_4. Females with A allele had higher BMC, BMD L_1-L_4.

Females with and without OP L_1-L_4 had differences in *HindIII* genotype ($p=0,05$) and alleles distribution ($p=0,049$). People with and without OP proximal part of femur had differences in genotype ($p=0,03$) distribution. Females with H allele had significant higher BMC, BMD, Tscore of femur neck, Wards zone, proximal part of femur (total), L_1-L_4.

Conclusion: TT genotype and T allele of *TagI*, aa genotype *ApaI VDR* and h allele of *HindIII* osteocalcine gene polymorphism associated with osteoporosis in people, who lived in Blockaded Leningrad during 1941-1944.

P1059. Research of MspI(a) and PvuII(a) polymorphism of PAH gene in Kazakhstan

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We investigated the frequencies distribution of polymorphic *MspI(a)* locus in 8 intron and *Pvu*II*(a)* locus in 2 intron of *PAH* gene. For research we have taken DNA from blood of 100 not related representatives of the Kazakh nation. In the Kazakh population the most often genotypes were a *Msp*I*(à)*À/*à* (0,58) and *Pvu*II*(a)*À1/*À2* (0,57). There were no deviation in distribution of frequencies of a *Msp*I*(à)* and *Pvu*II*(a)*-genotypes from Hardy-Weinberg equilibrium. In the Kazakh populations allele *Msp*I*(à)*À* (frequency 0,57) and *Pvu*II*(a)*À2* (0,59) were most often.

We have done the comparative analysis of distribution of frequencies *MspI(a)* and *Pvu*II*(a)* alleles of a gene *PAH.* We have found out distinctions in the *MspI(a)* alleles between sample of the Kazakhs and Russian ($\chi^2=5,122$, $\delta=0,034$), French ($\chi^2=5,785$, $\delta=0,024$), Udmurts, Mordvins, Maris($\chi^2=5,122$, $\delta=0,032$) and Chinese ($\chi^2 = 27,955$, $\delta< 0,001$) [Daiger et al., 1989; Akhmetova V., 2001]. Observed heterozygosity was 0,57. This meaning was higher than in populations of Volga-Ural region of Russia (0,50). In the *Pvu*II*(a)* alleles we have found out distinctions between sample of the Kazakhs and populations of Germany ($\chi^2=7,445$, $\delta=0,01$), Bulgaria($\chi^2=25,293$, $\delta<0,001$), Czechoslovakia($\chi^2=5,789$, $\delta=0,024$), China ($\chi^2=11,524$, $\delta=0,001$) and Japan ($\chi^2=10,402$, $\delta=0,001$)[Daiger et al., 1989]. Observed heterozygosity was 0,57.

P1060. Germline mutation analysis in patients with nonsyndromic Pheochromocytoma or Paraganglioma

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Paragangliomas and phaeochromocytomas are neuroendocrine tumors derived from parasympathetic and sympathetic nervous systems. Three genes have been associated with non syndromic paragangliomas: *SDHB*, *SDHC* and *SDHD*.
We studied the coding sequence of these genes by dHPLC (denaturing High Performance Liquid Chromatography) followed by sequencing of the shifted bands. We developed MP/LC (Multiplex PCR Liquid Chromatography) for the detection of large genomic rearrangements. We analyzed 21 positive control cases and detected 100% of variants: 20 variants by dHPLC and one deletion of the exon 1 of *SDHB* by MP/LC.
We studied 123 unrelated index cases presenting paraganglioma and/or phaeochromocytoma for which syndromic possibilities were excluded (negative sequencing of the genes *RET* and *VHL*). The sequencing of abnormal profiles in dHPLC allowed identification of 38 different variants (including 11 unpublished in literature): 17 polymorphisms, 3 variants with unknown pathogenic caracteristics and 18 pathogenic mutations: 8 in *SDHB*, 4 in *SDHC* and 6 in *SDHD* (7 missense, 3 nonsense, 7 frameshift and 1 splice site mutation). For 14 remaining negative patients we looked for large genomic rearrangements by MP/LC but none variant of this type was highlighted.
Genetic analysis with dHPLC and MP/LC allows us to systematically analyze *SDHB*, *SDHC* and *SDHD* for small mutations or large rearrangement in all our patients. We discuss genotype-phenotype correlations about our patients.
We use complementary genetic technics to complete clinical studies of a patient and his family. This approach can be extended to whole genetic pathologies tested in diagnosis.

P1061. *Parkin* analysis in early onset Parkinson Disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder with a prevalence of 2% over 65 years of age. A strong genetic component is present when the disorder begins before 40 years of age. The *parkin* gene (PARK2 - 6q25.2-27; OMIM: 602544) is the most common cause associate with autosomal recessive-juvenile parkinsonism.
146 PD patients (26.7% familial - 73.3% sporadic) with onset ≤40yy have been analyzed in order to asses the frequency, nature and associated phenotypes of PARK2 mutations. The 12 exons of the gene were screened for point mutations by DHPLC and/or sequencing analysis and, when necessary, a gene-dosage assay has been done using RealTime PCR.
A total of 17 patients were found to carry *parkin* mutations: 13 (9%) had two *parkin* mutations, meanwhile single gene mutations were found in 4 probands (2%). Eleven point mutations were detected: 6 have been previously reported (pR42P, pA82E, pM192L, pT240M, pR275W, pE409X) and 5 are novel variants (pC212_CfsX224, pA230T, pC238W, p.C253Y). Gene-dosage analysis identified deletions of exons 3, 3-4, 3-5, 5-6, 6, 6-7, and duplications of exons 2 or 3 in homozygous or heterozygous state. The parkin phenotype is similar to typical idiopathic PD: patients with 2 mutations were younger at onset and more likely with symmetric symptoms. According to previous European studies we found that the more frequent amino acid change is the pR275W.

P1062. Molecular Analysis of the LRRK2 Gene in Familial and Isolated Turkish Parkinson's Disease Patients

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Parkinson's disease (PD) is the second most common neurodegenerative disorder in the Western world after Alzheimer's disease. Every year 16/ 100,000 people are diagnosed with PD. James Parkinson was the first person in 1817 to describe six individuals with symptoms of "shaking palsy" but it was some 60 years later that Jean Martin Charcot named the condition "Parkinson's disease". Typically, this progressive movement disorder produces bradykinesia, tremor, rigidity, and impairment of postural reflexes. PD is characterized by a depletion of dopamine in the striatum. Recently, several genes have been shown to result in PD phenotype. Mutations in these genes may lead to autosomal dominant (α-synuclein, LRRK2), autosomal recessive (parkin, PINK1, DJ1) or sporadic PD. In a recent paper, the frequency of the LRRK2 G2019S mutation has been shown to reach 41% in the North-African population compared to 3% in the European population. The aim of this study is to identify the frequency of the LRRK2 G2019S mutation in Turkish patients with PD. The presence of the G2019S mutation was analyzed in 72 Turkish PD patients and their 43 relatives by direct sequencing of the LRRK2 gene (47 familial and 25 isolated cases). Direct sequencing of exon 41 in 115 Turkish PD patients and their relatives revealed a heterozygous G2019S mutation in a 69-years-old female patient, with disease onset at 62 years. The results obtained so far in 72 Turkish patients indicate that the frequency of the G2019S mutation is in accordance with the European populations.

P1063. Mitochondrial DNA haplogroups and risk of Parkinson's disease in patients of Tatar ethnic origin from the Volga-Ural region of Russia

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There is the increasing evidence that genetic variants of mitochondrial DNA may be involved in Parkinson's disease (PD) development. We analyzed mtDNA variation in 153 unrelated patients of Tatar ethnic origin, aged 46-89 from the Volga-Ural region and 183 age- and ethnically-matched controls. Patients were clinically examined in Department of Neurology of Bashkir State Medical University and diagnosed PD.
In PD patients and control subjects all the examined Western Eurasian and Eastern Eurasian mtDNA haplogroups were detected. There was a highly significant difference in the overall haplogroup distribution between PD patients and control subjects. Haplogroup H was significantly overrepresented in PD patients and underrepresented in controls (χ^2=15.56, p=0.0007, OR=2.69, CI=1.61-4.49) whereas UK haplogroup cluster was significantly overrepresented in controls and underrepresented in patients (χ^2=18.26, p=0.0005, OR=0.31, CI=0.17-0.55). The frequency of haplogroups J, T, A, C, D, I, W, F didn't differ significantly in the compared groups. The frequency of U and K haplogroups distribution between patients and controls, analyzed separately, showed highly significant results. The frequency of haplogroup U in PD patients was 14.38%, whereas its frequency in control subjects was twice higher - 28.96% (χ^2=9.39, P=0.003, OR=0.41, CI=0.23-0.74). Haplogroup K was revealed in 1.31% of PD patients and 8.19% of control individuals (χ^2=6.86, P=0.009, OR=0.15, CI=0.02-0.69). So, individuals classified as haplogroup U and K demonstrated a significant decreased risk of PD versus individuals, belonging to haplogroup H, confirming that the "uncoupled" European mtDNA haplogroups are strongly protective of PD while the "coupled" European haplogroups are strongly predisposing to PD.

P1064. A Common Haplotype in the Annexin A5 (ANXA5) Gene Promoter Is Associated with Recurrent Pregnancy Loss

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We sought to verify whether variation in the promoter of the placental anticoagulant protein annexin A5 (ANXA5) gene represents a risk factor for recurrent pregnancy loss (RPL). Sequence analysis of 70 German RPL patients, all known to carry neither factor V Leiden nor a prothrombin mutation, revealed four consecutive nucleotide substitutions in the ANXA5 promoter that were transmitted as a joint haplotype (M2). Reporter gene assays revealed that M2 reduces the in vitro activity of the ANXA5 promoter to 37-42% of the normal level. The possible relationship between M2 and RPL was evaluated by comparing RPL patients with two independent control groups recruited from the regis-

try of the Institute of Human Genetics in Münster and the PopGen biobank in Kiel, respectively. Carriers of M2 were found to exhibit a more than two-fold higher RPL risk than non-carriers (odds ratio = 2.42, 95% confidence interval: 1.27 - 4.58) when using unselected controls (PopGen), and an almost four-fold higher risk when using the Münster 'super-controls', i.e. women with successful pregnancies and no previous history of pregnancy losses (odds ratio = 3.88, 95% confidence interval: 1.98 - 7.54). This statistically significant association should facilitate the development of improved prognostic algorithms for RPL, involving a more precise assessment of individual disease risks, and provide a guide to offering adequate therapies where relevant.

P1065. Application of genetic markers for prognosis of physical performance of athletes

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The aim of the study was to investigate genotype and allele distribution of *ACE* (I/D polymorphism), *ACTN3* (R577X), *NOS3* (5/4), *UCP2* (Ala55Val) and *UCP3* (-55C/T) genes in rowers (*n*=230) and controls (*n*=855), and to find an association of genotypes with physiological parameters. Genotyping was performed by restriction fragment length polymorphism analysis. Physiological parameters were evaluated by PM 3 Rower Ergometer and MetaMax 3B Gas Analyzer. The frequency of *ACE* II genotype (34.6% vs. 23.6%, *p*=0.017) was significantly higher in elite rowers (*n*=107) than in controls. Amongst all athletes the frequency of unfavorable for speed and power qualities *ACTN3* XX genotype was two times less than in controls (6.2% vs. 12.8%, *p*=0.01). Moreover, the frequencies of *ACE* I, *ACTN3* R, *UCP2* Val, *UCP3* T alleles increased in athletes with the growth of skills. Furthermore, *ACE* I (when maximal power production capacity was measured), *NOS3* 5 (power at the anaerobic threshold data), *UCP2* Val (VO_2 data) and *UCP3* T (power at the anaerobic and aerobic thresholds and VO_2 data) alleles were associated with high values of aerobic performance. Thus, *ACE* I, *ACTN3* R, *NOS3* 5, *UCP2* Val and *UCP3* T alleles can be considered as genetic markers associated with enhanced physical performance, and can be included in the diagnostic complex for the prognosis of human physical performance.

P1066. The $(TAAAA)_n$ microsatellite polymorphism in the *SHBG* gene influences serum SHBG levels in women with polycystic ovary syndrome

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Hyperandrogenaemia and hyperandrogenism are common features of polycystic ovary syndrome (PCOS). Since the sex hormone-binding globulin (SHBG), a specific plasma transport glycoprotein for sex steroid hormones, regulates the access of the hormones to their target cells, the *SHBG* gene was proposed as being a PCOS candidate gene. We investigated a possible influence of the microsatellite polymorphism $(TAAAA)_n$ in the *SHBG* gene on serum SHBG levels in 123 PCOS patients (study group) and 110 age-matched healthy volunteers (control group). Peripheral blood samples were obtained in the early follicular phase of the menstrual cycle or randomly in amenorrhoeic patients. Genotyping of the polymorphism was performed and serum SHBG and total testosterone (TT) levels were determined. The *SHBG* alleles with 6-11 TAAAA repeats were found; however, none of the alleles or genotypes was characteristic for PCOS patients. Serum TT levels were significantly elevated ($P < 0.001$), while serum SHBG levels were significantly lower ($P < 0.001$) in PCOS patients compared with controls. Moreover, serum SHBG levels were found to be strongly influenced by the $(TAAAA)_n$ *SHBG* polymorphism, in both the PCOS (55.3%) and the control (33.1%) group of patients. According to our results, the $(TAAAA)_n$ *SHBG* polymorphism might be an important predictor for serum SHBG levels and, consequently, for hyperandrogenaemic clinical presentation of PCOS.

P1067. Polymorphic genetic markers and birth weight

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This study aimed to evaluate a possible correlation between birth weight and four polymorphic genetic markers: ESD, GSTT1, GSTM1 and the microsatelite promoter region of IGF1 gene. We studied blood samples of mothers and their neonates from two maternities at Ribeirão Preto, São Paulo state, Brazil. The first group was composed of 509 mothers and their neonates, born at University Clinical Hospital maternity (HC). The second group was composed of 339 mothers and their neonates, born at Airport Complex maternity (Mater). In order to exclude factors that may or may not interfere in birth weight, all mother's medical records were analyzed for: age, number of pregnancies, miscarriages, previous deliveries, number of medical consultations, smoking, alcohol consumption and morbidity during actual pregnancy. Babies were analyzed for: sex, birth weight and length, gestational age, APGAR score and congenital anomalies detected at birth. Birth weight was associated four molecular genetic markers using the Generalized Linear Model. An association has not yet been shown between ESD, GSTT1 and GSTM1 (maternal and fetal) phenotypes and neonates´ birth weight. Some alleles in the IGF1 locus showed correlation with birth weight and, in the group from Mater, a strong association between *IGF1*22/*22* homozygote neonates and lower birth weight was noticed (P=0,0008). The *IGF1*20/*20* genotype was associated with higher birth weight (P=0,0445). This data strongly suggest that such markers may play a direct or indirect role in the modulation of birth weight.

P1068. The influence of endothelin-A receptor polymorphism on the progression of renal diseases

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease. IgA nephropathy (IGAN) is a mesangial proliferative glomerulonephritis characterized by diffuse mesangial deposition of immunoglobulin A. Endothelin-1 (ET-1) has been suggested to be a major disease promoting factor in renal diseases. The vasoconstrictor effect of ET-1 is mediated by ET-A receptor. We have investigated influence of C/T polymorphism in exon 8 of ET-A receptor gene.

A total number of 193 patients (pts) (87 males, 106 females) with ADPKD entered into this study. Patients were divided into three groups: 1. 47 pts with end stage renal disease (ESRD) later than in 63 years (slow progressors), 2. 49 pts with ESRD before 45 (rapid progressors) and 3. 97 pts with ESRD between 45-63 years. Moreover, we examined a group of 153 pts with histologically proven IGAN (116 males, 37 females). Pts were divided into two groups: 1. 79 pts with ERSD during 5 years of the (rapid progressors) and 2. 74 patients with normal renal function (slow progressors). As a control group we used 200 genetically unrelated healthy.

The distribution of C/T polymorphism did not significantly differ between rapid and slow progressors of ADPKD and IGAN. The comparison of ESRD ages showed that CC females with ADPKD failed significantly later than CT heterozygotes: CC (57.4±8.1), CT (53.0±9.1) and TT (54.5±6.4) (t-test, p=0.018).

To conclude, CC genotype could be protective in ADPKD females. This genotype was described to be associated with lower pulse pressure.

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P1069. Combined effect of hemostatic gene polymorphisms and the risk of myocardial infarction in patients with advanced coronary atherosclerosis.

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Relative little attention has devoted until now to the combined effects of gene polymorphisms of the hemostatic pathway as risk factors for

Myocardial Infarction (MI), the main thrombotic complication of Coronary Artery Disease (CAD). We studied a total of 804 subjects, 490 of whom with angiographically proven severe CAD, with or without MI (n=306; n=184; respectively). An additive model considering ten common polymorphisms [Prothrombin 20210G>A, PAI-1 4G/5G, Fibrinogen β -455G>A, FV Leiden and "R2", FVII -402G>A and -323 del/ins, Platelet ADP Receptor P2Y12 -744T>C, Platelet Glycoproteins Ia (873G>A), and IIIa (1565T>C)] was tested. The prevalence of MI increased linearly with an increasing number of unfavorable alleles ($\chi 2$ for trend = 10.68; P = 0.001). In a multiple logistic regression model, the number of unfavorable alleles remained significantly associated with MI after adjustment for classical risk factors. As compared to subjects with 3-7 alleles, those with few (£2) alleles had a decreased MI risk (OR 0.34, 95%CIs 0.13-0.93), while those with more (³8) alleles had an increased MI risk (OR 2.49, 95%CIs 1.03-6.01). The number of procoagulant alleles correlated directly (r=0.49, P=0.006) with endogenous thrombin potential. The combination of prothrombotic polymorphisms may help to predict MI in patients with advanced CAD.

P1070. Frequency of C-1055T gene polymorphism in IL-13 in Russian population and its association with asthma, IgE and IL-13.

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Bronchial asthma (BA) is a chronic disease associated with elevated IgE levels. It was demonstrated that IL-13, as well as IL-4, plays a crucial role in IgE production regulation. IgE - regulating effect of IL-13 is confined to activation of IgE's ε-chain gene transcription.

The aim of our study was to reveal the association between C-1055T, IL-13 gene polymorphic modifications and level of total IgE and IL-13 in BA patients.

The C-1055T gene polymorphism in Il-13 was analyzed by the PCR-RFLP, using restriction enzyme BstFNI in 136 asthma patients and 64 healthy control subjects. Total IgE and IL-13 levels were measured by ELISA.

The total serum IgE level was found to be elevated in BA patients group: 190(47,5;495)kE/l, and within normal range in the control group: 25,5(19;100)kE/l. The levels of total IgE in BA patients and controls differ significantly, p<0,001.

The serum IL-13 level was elevated in BA patients group: 165(22;320)kE/l, and normal in the control group: 70(15;130)kE/l. The levels of IL-13in BA patients and controls differ significantly, p<0,001.

The frequency of allele T was 40% in the BA patients group and 61% in control group, this parameter differed significantly, (p=0,005). In spite of the high frequency of T allele, the number of homozygotes was less than 2% within the whole group. Therefore we failed to determine the association of C-1055T polymorphism with total IgE level and serum IL-13 level. Thus in our study we didn't revealed the association between C-1055T and IL-13, total IgE levels and atopy.

P1071. TNF-a and Lt-a gene polymorphisms and suceptibility to COPD in a spanish population

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Several cytoquines that contribute to airway inflammation, including tumor necrosis factor a (TNF-a), have implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). Some polymorphisms of the TNF-a and Lt-a gene may be associated with COPD in different ethnic populations. We investigated the importance of certain variants/haplotypes of seven single nucleotide polymorphisms (SNPs) of these genes in the course of the disease or whether they predispose to a more severe form of COPD in a Spanish population.

Case-control study. We analyzed the SNPs: -857(C/T), -376(G/A), -308(G/A), -238(G/A) and +489(G/A) of the TNF-a gene and +249(G/A) and +720(C/A) of the Lt-a gene in 323 Caucasian individuals. The study included 143 COPD patients (62 ± 10 years; 96 men and 47 women) with airflow obstruction (55%±20 media predicted (FEV_1)) recruited from a outpatient pulmonary clinic and 180 control subjects without any respiratory disease included in a cohort of 15000 subjects of the Canary Islands.

Each subject was genotyped by a multiple polymerase chain reaction followed by a SNaPshot® reaction. A random number of PCR amplified products were sequenced as a confirmational method. We determined TNF-a and Lt-a haplotypes by PHASE v2.1 software.

Alleles at the individual loci studied, both in the patient and control group, were in Hardy-Weinberg equilibrium. The polymorphisms analyzed revealed no differences in the allele or haplotype distribution in patients compared to control subjects.

We conclude that the TNF-a and Lt-a gene polymorphisms analyzed are not major genetic risk factors for COPD at least for the studied population.

P1072. Association of the FADS gene cluster with polyunsaturated fatty acids in serum and erythrocytes

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Polymorphisms of the human delta-5 (FADS1) and delta-6 (FADS2) desaturase genes have been recently described to be associated with the levels of several long-chain n-3 and n-6 polyunsaturated fatty acids (PUFAs) in serum phospholipids in an East German population. We report a study in an Italian population that replicates the association, extends it to erythrocyte cell membrane PUFAs, and to the FADS3 gene as well.

Thirteen polymorphisms located in FADS1-FADS2-FADS3 gene cluster (chromosome 11q12-13.1) were genotyped in 658 adults.

The markers showed the presence of linkage disequilibrium (LD) and the LD-block structure of the gene cluster was determined.

Polymorphisms and statistically inferred haplotypes showed strongest associations with the acid arachidonic acid (C20:4n-6) in serum phospholipids and in erythrocyte cell membranes (rs174545 adjusted-p-value=6.48x10-22 and adjusted-p=1.4x10-10, respectively). Other significant associations were observed for eicosadienoic (C20:2n-6), linoleic (C18:2n-6), alpha-linolenic (C18:3n-3), stearidonic (C18:4n-3) and eicosapentaenoic (C20:5n-3) acids. No significant association was observed for docosahexaenoic acid (C22:6n-3).

P1073. Positive allelic and haplotype association of two 5q markers with schizophrenia

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The study is based on literature data of genomewide linkage studies in numerous families with schizophrenia during the period of 1997-2001 that identified putative schizophrenia locus at 5q region. Those findings were supported with positive associations of chr 5 trisomy and schizophrenia occurence. However, limited number of population based association studies replicated positive result pinpointing to Epsin4, a plausible functional candidate gene in the 5q region.

In our study, total of 102 age and sex matched individuals donated their blood specimens after their informed consent to participate. Patients fulfiling DSM-IV and ICD-10 criteria for schizophrenia diagnosis were recruited. Healthy individuals were evaluated in the interview with psychiatric specialist for control grouping. DNA extraction, PCR for D5S818 i CSF1PO markers and detection on automated DNA sequencer were performed using standard protocols. Data were analyzed for allelic, genotype and haplotype association.

Case and control groups are in HWE for both analzyed markers (P>0.05). Single locus allele case-control test based on Exact P-value (Nielsen and Weir 1999) showed statistical significance for both D5S818(P=0.01) and CSF1PO (P=0.013), but not for genotype level association (P=0.226 and 0.337, respectively). These results were also confirmed using allelic and genotypic association test based on $\chi 2$ test. Two locus haplotype trend regression (Zaykin et al. 2002) showed significant association based on $\chi 2$ test in 1000 permutations (P=0.0097).

P1074. Polymorphism of nitric oxide synthases in preeclampsia

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Preeclampsia is multifactorial disease of third trimester of pregnancy affecting up to 15% of women. The etiology of preeclampsia still remains unknown. Pathological changes lead to generalized endothelial dysfunction in mother and further complications of pregnancy. Nitric oxide plays an important role in regulating vascular processes, acting as a vasodilator and second messenger. Nitric oxide synthases (NOS) catalyze nitric oxide production. There are three types of NOS encoded by three different genes (NOS1, 2 and 3). Polymorphism of these genes correlates with NO level and thus certain genetic alleles may predispose to preeclampsia.

DNA samples were obtained from the women with pure preeclampsia (n=39) and control group of women without any gynecologic complications and background disorders (n=98). Polymorphisms of NOS1 (AAT repeats), NOS2 (CCTTT repeats) and NOS3 (894G>T and 4a/4b) were studied by PCR-RFLP assay.

The distribution of genotypes of all NOS genes was in agreement with the HWE law (p>0.05). There was no significant difference in frequency of genotypes of NOS1, NOS2 and NOS3 (894G>T) genes between studied groups (p>0.05). Although there were some tendencies for certain alleles of NOS2 (protective affect of allele 15) and NOS3 (894G) genes in patients but they were not significant. However the frequency of 4b/4b genotype of NOS3 gene was significantly higher in preeclamptic women (79.5%) than in controls (59.8%, p<0.05).

In conclusion it can be suggested that NOS1 polymorphism unlikely affects preeclampsia and polymorphisms of NOS2 and NOS3 genes probably participate in pathogenesis of this disorder what needs verification in further studies.

P1075. Association studies of candidate genes for Premature Ovarian Failure (POF)

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Premature Ovarian Failure (POF) is a disorder defined by cessation of menses before 40-45 years of age and characterized by elevated levels of gonadotropins. POF has an estimated frequency of 1-2% and a relevant genetic component. Despite familial segregation of the condition is a frequent finding, its genetic inheritance remains unclear and heterogeneous. POF genes identified so far confirmed the heterogeneity of the condition as they are responsible of an autosomal recessive form (i.e the FSHR gene) or X-linked dominant (i.e the BMP15 gene) but they can also contribute as risk-factors (i.e the FMR1 premutated allele) thus suggesting a multifactorial inheritance of POF.

To investigate the genetic basis of the condition we evaluated POF candidate genes as risk-factors by analysing their association in case-control studies. To this aim a cohort of more than 500 Italian POF patients was established by collaboration with gynaecological and endocrinological clinics. Controls were selected as women with normal age (>48) at physiological menopause collected in the same geographic areas. Among a number of candidate genes for POF, we started analysing genes involved by X/autosome balanced translocations. The analysis of the DIAPH2 gene gave us first evidence of its contribution as a susceptibility gene. Allelic frequencies at two polymorphic loci showed significant enrichment in the POF cohort and defined an associated haplotype spanning the proximal portion of the gene. Replication of the association results and functional studies are in progress to evaluate the prevalence of these variants in the etiology of POF.

P1076. Genome-wide linkage analysis for familial primary cutaneous amyloidosis: a comparison between single-nucleotide polymorphisms and microsatellites assays

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Primary cutaneous amyloidosis (PCA) is a relatively common skin disorder in South America and Southeast Asia. The pathogenesis of PCA remains unclear. Most cases of PCA are sporadic but familial aggregation has been reported from South America and Taiwan. The different susceptibility among ethnic groups suggests that genetic factor may play an important role in its pathogenesis. In this study, we performed genome-wide linkage analysis using both single-nucleotide polymorphisms (SNPs) and microsatellites (STRs) assays across nine families with familial primary cutaneous amyloidosis (FPCA) to map the disease gene(s) for FPCA.

Multipoint linkage analysis of both SNPs and STRs assays identified significant lod scores (lod >3) with a peak lod score of 3.73 (NPL score = 5.39, p-value = 0.000057) produced by the SNPs assay on chromosome 5q region. However, the critical region of FPCA was further narrowed down from 7.25 cM to 2.37 cM by SNPs assay. The linkage peaks obtained by both SNPs and STRs sets had a high degree of correspondence in general, with SNPs consistently producing higher lod scores. Additional lod > 1 regions were observed on chromosome 2, 11, 15, and 16 with the SNPs set. There are 12 known genes in the candidate region of chromosome 5 identified by SNP linkage mapping. Re-sequencing of some of these genes are underway to identify the possible disease gene of FPCA.

We conclude that genome-wide linkage analysis by using high-density SNP markers provided higher lod scores compared with the standard 10 cM microsatellite marker assays.

P1077. Proopiomelanocortin gene variability and chronic heart failure: no association so far

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Background: Chronic heart failure is characterized by persistent activation of the hypothalamic-pituitary-adrenocortical axis where proopiomelanocortin (POMC) plays a crucial role. In this study, we therefore aimed to investigate possible associations of the POMC RsaI (rs3754860) and C1032G (rs1009388) variants in the non-coding regions of proopiomelanocortin gene with chronic heart failure.

Methods: The case-control study comprised a total of 374 patients of caucasian origin with chronic heart failure (functional classes NYHA II-IV, ejection fraction (EF) < 40%) and 202 age-matched healthy controls. They were genotyped for the POMC RsaI (5´-UTR) and C1032G variants (intron 1) by means of PCR-based methodology.

Results: In univariable analyses, neither genotypes nor alleles of both examined polymorphisms were associated with BMI, older age, male sex, ejection fraction (EF), hypertension, left ventricular hypertrophy (LVH), diabetes mellitus (DM), and chronic kidney disease (CKD) (P≥0.05 for each); no case-control differerences in genotype distributions or allele frequencies were observed. We also constructed POMC RsaI/1032 C/G haplotypes and have not found a significant association with BMI, EF, LVH, DM or CKD.

Conclusions: Our results do not clearly indicate that both the genotypes of alleles of RsaI and C1032G polymorphisms within the POMC genes are associated with chronic heart failure in the Czech cauacasian population.

P1078. Novel associations of several polymorphisms and haplotypes in *5-alpha-reductase gene* (*SRD5A2*) in Czech prostate cancer patients

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Introduction & Objectives: SRD5A2 is a key member of androgen methabolic pathway converting testosterone to dihydrotestosterone and has been suggested to play a role in the transformation of prostate cells. Several SRD5A2 SNPs have previously been desribed as potential risk markers. The aim of this study was to evaluate potential SNP and haplotype association with prostate cancer.

Material & Methods: The case-control study included 339 unrelated individuals with clinically verified prostate cancer and 231 unrelated

controls with clinically verified benign prostatic hyperplasia. DNA was extracted from venous blood and genotyping of a set of 17 SNPs at the *SDR5A2* locus was carried out by PCR and cycling-gradient capillary electrophoresis (CGCE), a technique based on heteroduplex analysis in temperature gradient. Case-control association analyses were performed using PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/data.shtml).
Results: We have identified 5 SNPs showing significant allele frequency and genotype distribution differencies between cases and controls. The SNP markers with their respective p values are: rs4952219 (p=0.0186), rs413836 (p=0.0261), rs2300697 (p=0.0124), rs2208532 (p=0.0142) and a novel marker assigned as SRD5A2_SNP4 (p=0,0358). In addition we have identified 5 haplotypes showing strong association with p values between 0,001 and 0,005.
Discussion: Many of the significant SNP markers are from noncoding regions, therefore the haplotype association may mostly be related to alternate gene regulation. Haplotype analyses on a larger patient cohorts is desirable in order to evaluate potential of the identified SNPs and haplotypes for identification of risk groups.
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P1079. Linkage to 13q in a novel autosomal dominant pseudoarthrogryposis-like syndrome

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Distal Arthrogryposis (DA) Syndromes are characterized by nonprogressive, congenital contractures of two or more different body areas without primary neurological and/or muscle disease that affects limb function. Features common to all DA syndromes include a consistent pattern of distal joint involvement, limited proximal joint involvement, an autosomal dominant inheritance pattern, and widely variable expressivity. Mutations in genes encoding the fast skeletal muscle regulatory proteins troponin T, troponin I, and beta-tropomyosin have been shown to cause DA syndromes. Pseudoarthrogryposis-like syndrome (PAG-L), a novel disorder, has been observed in a large Turkish kinship. Manifesting multiple contractures in patients, and an autosomal dominant mode of inheritance, the syndrome may be classified as a new form of DA. However, PAG-L is progressive with a preadolescence age of onset, which makes it distinct from DA syndromes. A genome-wide scan has identified a locus on chromosome 13q suggestive for linkage. Further genotyping in the candidate locus with additional family members is in progress.

P1080. Genome-wide association studies for complex diseases using samples from the Quebec founder population: examples with psoriasis and ADHD

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Genizon is involved in the discovery of disease susceptibility genes in more than 20 diseases using samples from the Quebec founder population (QFP). We have successfully completed nine genome-wide association studies for complex diseases. To illustrate our process and the evolution of our strategy, examples from the psoriasis and ADHD studies are shown. The psoriasis study was performed using 500 trios and a custom marker map of 80,654 SNPs spaced according to the variation in the extent of local LD across the genome in the QFP. The ADHD study was performed using 459 trios with an upgraded custom marker map of 374,187 SNPs, consisting of the HAP300 chip from Illumina supplemented by 56,683 SNPs, optimizing the SNP selection to the QFP samples. For both studies, single-marker and haplotype association analyses were performed and genome-wide significance of the obtained P values was evaluated based on permutation tests. For both studies, regions with P-values that met the criteria of genome-wide significance were identified. We describe the identified regions and the encoded genes. Many regions contained a single, druggable gene representing immediate opportunities for drug development and genes with biologically relevant function. Genes in novel pathways were identified in both studies. Additional disease genes were also found from conditional and sub-phenotype analyses. The disease genes were then used to infer a GeneMap that consists of a network of interacting disease genes and their biological pathways. The shown GeneMap reveals the genetic etiology of the disease and represents a comprehensive tool toward personalized medicine.

P1081. Genetic refinement of PSORS4 and ATOD2 susceptibility loci in Italian samples

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Psoriasis (PS) and atopic dermatitis (ATOD) are chronic inflammatory skin disorders triggered by both genetic and environmental factors. A susceptibility locus mapped on chromosome 1q21 have been identified in both the diseases (PSORS4-ATOD2). Recently we refined the PSORS4 and ATOD2 susceptibility loci by using a LD approach in two cohorts of 128 PS and 120 ATOD Italian trios. We showed that PS and ATOD shared a risk-haplotype defined by STRs markers MIDDLE and ENDAL16. We failed to reveal evidence of association for *LOR* gene located within the risk-haplotype although a differential gene expression has been observed in PS and ATOD.
In order to reveal the identity of the susceptibility factor of PSORS4 and ATOD2 we newly refined the risk haplotype and its surrounding chromosomal regions (650 Kb) by typing a selection of 31 SNPs in our familial cohorts of trios.
Preliminary statistical analysis identified three distinct associated haplotypes within the selected region: the first, Hap1 (9.7 kb) generated significant association in both the diseases (PS p-value 0.0229; ATOD p-value 0.0077), the second, Hap2 (8.8 kb) is associated in the only ATOD cohort (p-value 0.0257); the third, Hap3 (38.5 kb) generated significant p-value in the only PS cohort (p-value= 0.0270).
The weakness of association data reported could reflect the low penetrance of PSORS4 and ATOD2 but need to be confirmed in additional samples. Threfore, an independent set of sporadic psoriatic patients (n=300) and further 60 ATOD trios are being typed at the moment.
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P1082. Detection of large deletion of 4.5 Mb in PTCH region in a Croatian Gorlin syndrome case by semi-quantitative fluorescent multiplex PCR

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Gorlin syndrome or Nevoid Basal Cell Carcinoma Syndrome (NBCCS) is a rare autosomal dominant disorder characterized by developmental abnormalities, cysts of the skin, jaws, and mesentery and cancer predisposition to basal cell carcinomas (BCC), medulloblastomas, meningiomas, fibromas of the ovaries and heart.
The syndrome is caused by mutations in the human homolog of the Drosophila patched gene, PTCH. PTCH is a tumor supressor gene, located at 9q22.3, and encodes a 12 transmembrane glycoprotein that acts as an antagonist in the Hedgehog signaling pathway.
We present a Gorlin syndrome patient with typical phenothypical features of widespread basal cell carcinomas, jaw malformations, strabismus and mental retardation, with family history that beside basal cell carcinomas includes lung cancer and gastrointestinal carcinomas. Since we found no mutations in exons of PTCH gene with conventional methods of SSCP, dHPLC screening and direct sequencing, we developed a new method of semi-quantitative fluorescent multiplex PCR with polymorphic markers surrounding PTCH gene. With this method we defined a deletion of 4.5 Mb in size between markers SHGC-110746 and SHGC-132418 (9q22.3-9q31.1).

Those results confirm previously reported findings that large deletions in PTCH region may also cause Gorlin syndrome through haploinsufficiency of PTCH gene.

P1083. Mutation analysis of the *PVR and PVRL2* genes in patients with non-syndromic cleft lip/palate

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Non-syndromic cleft lip with or without cleft palate (nsCL/P, MIM 119530) is one of the most common major birth defects. Genetic linkage and association studies have implicated loci in the 19q13 in nsCL/P. Several candidate genes in the 19q13 region have been studied and allelic associations with nsCL/P reported. We carried out mutation analysis of the *PVR* and *PVRL2* genes in this chromosomal region due to their close homology to *PVRL1*, a gene involved both in an autosomal recessive CL/P syndrome, CLPED1, and in nsCL/P in patients from northern Venezuela. We screened a total of 73 nsCL/P patients and 105 healthy controls from North America, and 94 patients and 94 controls from Venezuela for sequence variants in *PVR* and *PVRL2* as candidate genes for nsCL/P. We detected a total of 10 variants in the *PVR* gene and 2 variants in the *PVRL2* gene; however, none of these showed individual significant association with nsCLP in cases versus controls. Indeed, only one non-synonymous *PVR* variant, A67T, was more frequent in the nsCLP patients than in controls, though this difference was not significant. Altogether, no variants were significantly associated with risk of nsCL/P in both populations. Together, these data suggest that variants of *PVR* and *PVRL2* genes are not major genetic risk factors for nsCL/P, at least in the North American and Venezuelan populations studied.

P1084. Gene dosage quantification by using multiplex polymerase chain reaction and capillary electrophoresis

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Many genetic diseases are known caused by the presence of point mutations, small insertions and deletions in respective genes; however, the number of diseases caused by deletions and duplications involving large DNA genomes are significantly increasing. It would lead to under-express or over-express according to changes in gene dosage. In contrast, the methods for the detection of point mutations, small insertions or deletions are well established, but the detections of larger genomic deletions or duplications are more difficult. Due to the lack of efficient and in-house protocol for gene dosage quantification, we hereby describe a diagnostic protocol employing a combination of available methods. The efficient and accurate gene dosage quantification platform is combined the multiplex PCR with capillary electrophoresis (CE), and applied on several entities of genes, including *SMN*, *PMP22* and alpha-globin genes. The reliability of the novel methodology demonstrated it is relative speed and low-cost procedure as a significant tool in genetic diagnosis. Its sensitivity and specificity for identify the deletions and duplications genotypes are 100%. Moreover, once we have established this powerful system, we will further apply this technique on rapid detection of trisomy syndromes and microdeletion syndromes, including trisomy 13, trisomy 21, Down syndrome, DiGeorge syndrome and others.

P1085. New approaches for the estimation of renin-angiotensin-bradykinin system genes polymorphism

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The rennin-angiotensin-bradykinin system is regulated by the number of genes and possesses crucial role in the development of cardiovascular diseases. However, interpretation of multi-gene associations usually encounters substantial problems in evaluation of particular gene polymorphism contribution in pathogenesis of the disease. So, there is a clear cut necessity for the development of new approaches for more objective evaluation of gene testing studies.

Present work focuses on the different approaches based on stand-art χ-test, the "score" analysis with using Mann-Whitney U test and Bayesian statistical approaches coupled to Markov chain Monte Carlo (MCMC) techniques. Analysis of REN (I9-83G>A), AGT (M235T), ACE (I/D), AGTR1 (1166A>C), AGTR2 (3123C>A), BKR2 (-58T>C and I/D) genes in children with arterial hypertension was shown that application MCMC techniques with/or the "score" analysis can be used for studing multi genes associations.

P1086. Association study of IRF5 gene with Rheumatoid Arthritis in the TUNISIAN population

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Rheumatoid Arthritis (RA) is an autoimmune, systemic disease which is characterized by inflammation of synovial tissues, leading to cartilage and bone destruction. It affects ~1% of the general population. The susceptibility to RA involves genetic and environmental factors. In the present study we have analysed the Interferon Regulatory Factor 5 (IRF5) which is involved in the production of cytokines implicated in RA pathophysiology, such as tumor necrosis factor alpha, interleukine 6 and type I Interferon. In order to search an association of IRF5 rs 2004640 T allele in Tunisian population, we have analysed 101 unrelated patients affected with RA with a mean age of 54 years and a sex ratio (F/M) of 5.6/1 and 100 healthy controls. DNAs genotyping was carried out with a TaqMan 5' allelic discrimination assay on an ABI 7500 real time PCR machine (assay:C___9491614_10). Data were analyzed by χ2-test, and Odds Ratio (OR) with 95% confidence interval (95% IC) was calculated. Our results showed that T/T genotype was more frequent in RA patients compared to controls (44% vs 24.7%; p= 0.007) (OR = 0.68; IC = [0.46-1.01]). While in the RF positive subgroup the frequency of the T allele and T/T genotype were significantly increased compared with the controls (p=0.011; p= 0.003, respectively) (OR = 0.57; IC = [0.36-0.88]). In conclusion, our results support the involvement of IRF5 gene in the genetic susceptibility to RA in the Tunisian population.

P1087. Evidence for Further Genetic Heterogeneity in Restless Legs Syndrome

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Introduction: Restless legs syndrome (RLS) is a common neurological disorder characterised by a distressing need or urge to move the legs, usually accompanied by an uncomfortable sensation in the legs described as a crawling, muscle ache or tension. The symptoms follow a circadian pattern with a significant increase during the evening or night which leads to nocturnal sleep disruption and daytime somnolence. Molecular genetic approaches have identified five loci on chromosomes 12q, 14q, 9p, 20p, and 2q, in RLS families from different populations. No disease-causing gene has yet been identified.

Aim: The goal of this research was to localise and identify the gene responsible for the syndrome in a newly identified Irish autosomal dominant RLS family.

Method: Fourteen members of the new RLS3002 family participated in the study; ten members are affected and four are unaffected. The five current RLS loci were examined for linkage.

Results: The results indicated exclusion of linkage to the five identified RLS loci.

Conclusion: The newly recruited Irish RLS pedigree is not linked to the currently described genetic loci. This provides evidence of further genetic heterogeneity for RLS. A new unidentified RLS locus therefore awaits identification. Future work includes a genome wide scan to identify the novel locus in this Irish family.

P1088. Association study in the 5q31-32 linkage region for schizophrenia using pooled DNA genotyping and family-based controls

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BACKGROUND: Schizophrenia is a common disorder with high heritability and increased risk for relatives of the affected probands. We have chosen to study a strong region of linkage to schizophrenia: 5q31-32 in order to establish polymorphic markers associated with the disorder. METHODS: We have undertaken association analyses using 90 microsatellites markers. We saturated the 14 Mbp area in 5q31-32 region at 150 kb intervals. We genotyped the microsatellites markers in three sets of DNA pools: a proband pool comprising 300 SZ patients, a parents pool (N=600) and a control pool comprising 615 health individuals, all of Bulgarian origin. RESULTS: Nine markers were selected for individual genotyping (cut-off p<0.15). Individual genotyping in the parent-proband trio sample confirmed the pooling results for two of the markers: D5S2017 (p=0.004) and IL9 (p=0.014) located neighbor to SPRY4 and IL9 genes. We are now planning detailed investigation of these regions and fine mapping with a dense map of additional SNPs markers.

P1089. Association analysis of 22 SNPs in the DISC1 gene in schizophrenia and bipolar affective disorder in the Polish population

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Linkage between schizophrenia and chromosome 1q42.2 markers has been reported independently in different populations worldwide. This region contains the gene Disrupted In Schizophrenia 1 (DISC1) which had been found to be disrupted by a balanced translocation and to co-segregate with schizophrenia in a large Scottish pedigree. Association studies investigating DISC1 single nucleotide polymorphisms (SNPs) in a number of samples were promising but not yet compelling. In the present study, we aimed at investigating the possible contribution of DISC1 variants on the pathogenesis of schizophrenia and bipolar affective disorder in the Polish population.

We investigated 22 DISC1 SNPs, chosen on the basis of previous association findings, using MALDI-TOF mass spectrometry-based SNP genotyping (Sequenom's iPLEX technology). The study sample comprised 501 DSM-IV diagnosed patients with schizophrenia, 418 DSM-IV diagnosed patients with bipolar affective disorder and 530 controls. All individuals participating in the study originated from the Polish population.

When single marker analysis was performed, two DISC1 SNPs (rs1000730 and rs1411776) showed association with bipolar affective disorder at the genotypic level (p=0.036, and p=0.018 respectively), and one DISC1 SNP (rs1000730) was associated with schizophrenia (p=0.012). Allelic analysis gave significant result for rs1411776 in bipolar affective disorder only (p=0.009).

Our results provide modest evidence for an involvement of rs1000730 in the predisposition to both schizophrenia and bipolar affective disorder, and for the contribution of rs1411776 to schizophrenia susceptibility. However, these results do not withstand correction for multiple testing. More detailed analyses, including haplotype and phenotypic subgroup analysis are currently underway and will be presented.

P1090. Copy Number Variations in 97 Schizophrenia Patients

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Genetic aberrations at the DNA copy number level are common causes of human genetic disorders. We have undertaken a genome-wide analysis of copy number changes by Tiling Path BAC Array Comparative Genome Hybridisation in a cohort of 97 patients with schizophrenia diagnosed according to DSM-IV criteria that were recruited in Bulgaria. Patients' DNA was hybridised against reference DNA consisting of sex-matched pools. DNA was available on all parents to test fpr *de novo* formation of chromosomal imbalances. Data were analysed with the CGHPRO software that has been described previously (Chen et.al, 2005). All data were normalised by subgrid lowess. The log2ratio of test to reference intensity was calculated and copy number gains and losses were resolved by using a conservative threshold of 0.3 and -0.3. Deviant signal intensity ratios involving three or more neighbouring BAC clones were considered as genomic aberrations. To eliminate polymorphic copy number variants, conspicuous findings were compared to relevant databases and other cohorts, including several hundred healthy controls and patients with unrelated disorders. Aberrations were validated with Affymetrix 250K SNP genotyping arrays. We identified 18 DNA copy number changes that satisfied the above criteria. Their size ranged from 0.1 to 1.4Mb. Two of them are likely to be pathogenic: one segregates with the illness in the family, and one because it is has arisen *de novo* and partially overlaps the Prader-Willi critical region.

P1091. Association study of 5' end of NRG1 gene polymorphisms with schizophrenia in Ahwaz province of Iran

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Schizophrenia is a severe neuropsychiatry disorder with symptoms such as hallucination, delusion and thought disorder. It is a complex disorder, and genetic components may play a crucial role in its pathogenesis. The heritability of schizophrenia has been estimated to be around 80%. Among candidate genes for schizophrenia, NRG1 is one of the most significant which has been confirmed by several studies. NRG1 is located on 8p a locus which shows a strong linkage to schizophrenia. Also NRG1 plays an important role in central nervous system especially in signaling and neurotransmission which makes it a plausible candidate gene for schizophrenia. Several polymorphisms of NRG1 have been shown to be associated with schizophrenia in different population. However there isn't any information from Iranian population. We are investigating the association of NRG1polymorphisms with schizophrenic patients from Ahwaz province of Iran. To high extent this is an isolated population from south-western part of Iran which makes it appropriate for association studies. Our study includes new homogenous Asian population and might bring supportive evidence for the association of NRG1 with schizophrenia.

P1092. DNA pooling in myelin-related genes in schizophrenia using whole-genome association scan with Affymetrix 500K arrays

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Abnormal myelination and oligodendrocyte abnormalities have been implicated as pathogenic processes in schizophrenia (SZ) by a diverse range of experimental approaches including gene expression analysis, neuropathology, and neuroimaging. With the aim of establishing whether such abnormalities are of primary aetiological relevance to schizophrenia pathogenesis, we performed a whole-genome association study using DNA pooling on Affymetrix GeneChip Mapping 500K SNP arrays (250K Sty / 250K Nsp). We constructed pools of ~648 SZ cases and ~712 age- and gender-matched controls. Depending on the quality of the hybridization we replicated each pool on between 8 and 11 arrays, which provided from 6 to 8 good replicates for analysis.

We restricted the present analysis to 9985 SNPs within/around genes

relevant to myelination and oligodendrocyte function. In order to validate the pooling predictions, we performed individual genotyping of 54 SNPs from the top 100. This largely confirmed the predicted allele frequency differences. Twenty-two of the 54 SNPs showed nominally significant levels for association with SZ ($p<0.05$). The correlation between the allele frequency differences predicted by pooling and those produced by individual genotyping was 0.88. This work demonstrated the accuracy of our pooling method. Our results emphasize the feasibility of using genomic DNA pooling in whole genome association studies for the detection of association with complex diseases.

P1093. Sequencing and detection of novel single-nucleotide polymorphisms (SNPs) in the steroid 21-hydroxylase gene and association with the C4A/C4B copy number polymorphism in 96 individuals

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The steroid-21-hydroxylase gene plays a crucial role in the synthesis of steroid hormones. There are two 21-hydroxylase genes in man, A (inactive) and B (active), and these have been localized at the MHC III class region on chromosome 6, in a strongly linked haplotype block. This block contains 4 genes: a kinase (RP), complement C4A or C4B, 21-hydroxylase and tenascin-X. We found that the short term mortality of acute myocardial infarction (AMI) patients was influenced by the copy number of the C4B genes. Carriers of low C4B gene number (0 or 1) (C4B*Q0) were found to have significantly higher risk for short-term mortality of AMI. The aim of the present study was to find a molecular explanation for this observation. We assumed that C4B*Q0 carrier state may result in impaired function of the neighboring CYP21B gene, associated with inadequate mobilization of steroid hormones during stress in critical situations. We have determined the number of C4A and C4B genes, and sequenced the 21-OH active gene from 96 genomic samples. Sequences were compared to all other published 21-OH sequences. We found that both the +1106 (A) and +1113 (C) variant alleles of 21-OH gene were strongly linked to C4B*Q0 ($p=0.015$, $p=0.006$), respectively. This linkage was independent of the other MHC class III gene variants. In addition, five novel polymorphisms were found in the 3'-untranslated region for the 21-OH gene.
This observation indicates that the association between AMI mortality and C4B*Q0 may be due to the strongly associated SNPs of the 21-OH gene.

P1094. Integrating the BigDye® XTerminator™ Kit into Various Laboratory Workflows

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The BigDye® XTerminator™ Kit is a new single-tube method for purifying DNA sequencing reactions prior to electrophoretic analysis. The kit strives to not only ensure effective dye terminator removal, but also provides flexible protocols to fit into all types of workflows and accommodates low to high throughput levels. Advantages of this novel method include a simple and rapid protocol, less hands-on time allowing researchers more time to focus on their research, quick turn around time, improved sample stability, and efficient desalting. BigDye XTerminator kit also has unique workflow advantages over conventional purification schemes such as ethanol precipitation or spin column purifications. Automation issues will also be described.

P1095. Association analysis of the A1438G polymorphism of the serotonin receptor gene *HTR2A* with level of intellectual development (IQ) of the person

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Serotonin receptor gene *HTR2A* (13 q14-q21) is one of the basic genes, which determine efficacy of serotoninergic neuromediators system. We analyzed the associations between polymorphism *A1438G* located in promotor area of gene *HTR2A* and a level of intellectual development (IQ) of at 246 unrelated individuals (18-35 years) by non-verbal Kettel test.
According to the IQ scores examinees were divided into three groups: with a normal level of intellectual development (IQ within the limits of 90-110 points), high (above 110 points) and low (below 90 points). The group with normal level IQ has served as control group for two others. In order to characterize **A1438G** polymorphism, we performed PCR followed by digestion with restriction enzymes (MspI).
Genotypes **A/*A, *A/*G, *G/*G* met frequency 1) 10 %, 2) 64.67 %, 3) 25.33 % in group of comparison, 1) 3.33 %, 2) 85 %, 3) 11.67 % in group with high parameters IQ and 1) 0 %, 2) 77.78 %, 3) 22.22 % in group with low level IQ. The analysis of associations has shown statistically significant distinctions in distribution of frequencies genotypes of gene *HTR2A* between control group and group with high IQ scores ($c2=11.925$, $P=0.003$) via enhancement of frequency of genotype *HTR2A*A/*G* (85 % against 64.67 % in group of comparison;$P=0.021$; $OR=1.315$; 95%CI 1.087-1.) and diminution of frequency of genotype *HTR2A*G/*G* (11.67 % against 25.33 % in group of comparison; $P=0.046$; $OR=2.172$; 95%CI 1.012-5.166) in group of persons with high parameters IQ.

P1096. Serotonin Transporter gene (5-HTT): association analysis with Temporal Lobe Epilepsy.

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Two functional polymorphisms, a 44-base-pair insertion/deletion polymorphism in the 5' regulatory region and a variable number of tandem repeat (VNTR) polymorphism in the second intron of the serotonin transporter gene (5-HTT), were previously identified and suggested to modulate transcription. The current study was designed to determine the contribution of these polymorphisms within the 5-HTT gene to susceptibility to temporal lobe epilepsy (TLE). Two hundred and seventy six patients with TLE and 309 age- and sex- matched healthy controls from Calabria (Southern Italy) were studied. Patients and controls were genotyped using the WAVE TM DNA Fragment Analysis System for the insertion/deletion polymorphism in the promoter region (5-HTTLPR) and GENESCAN TM System for the (VNTR) polymorphism in the second intron of the 5-HTT gene (5-HTTVNTR). The program UNPHASED was used to compare genotype, allele and haplotype frequencies between cases and controls, including age and gender as covariates in the model. No significant differences were observed for 5-HTTLPR, but significant association was obtained for the 5-HTTVNTR polymorphism, both modelling genotypes (P-value=0.0145) or alleles (P-value=0.0086). Compared with controls, patients with TLE showed lower frequencies of the 10 repeat at 5-HTTVNTR (26.2% in patients vs. 40.8% in controls). A lower frequency of homozygous individuals for the 10 allele was observed among patients compared to controls (5.2% of patients were 10/10 vs. 18.8% of controls). Haplotype analysis did not increase the evidence for association. These results suggest, for the first time, that the serotonin transporter gene may play a role in the aetiology of TLE.

P1097. The most common mutation of *SERPINA1* gene PIZ molecular analysis in patients with cystic fibrosis.

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Cystic fibrosis (CF) and Alpha-1 antitripsin deficiency, are the most common genetic diseases, and reason of mortality and decreased life quality in Caucasians.
The aim of the study was to analyse PIZ mutation in *SERPINA1* gene and common polymorphism variants frequency in patients with Cystic Fibrosis.
Methods: Analysis was done on 28 CF patients and 111 persons as control group. PIZ mutation analysis performed with artificial restriction site creation PCR method. Common polymorphism variants were detected by Maldi-Tof technique.

Results: From 28 CF patients *SERPINA1* gene mutation Z was detected in homozygote state in 1 person and heterozygote state in 2 persons.
SNP g. 135575 in gene *SERPINA1* exon III in control group was met SNP T/T variant; 66,66%, C/T; 30,63% and C/C; 2,70%.
From CF patients T/T; 60,71%, C/T; 35,71% and C/C; 3,57%. Obviously, the distribution by SNP groups in both cases is rather similar and SNP T/T is most common variant in both groups.
Small sample size does not allow to make any definitive conclusions, that presence PIZ mutations in CF patients, does not affect severity of disease.

P1098. Early influence of UDP-glucuronosyltransferase 1A1 gene promoter polymorphism on the clinical course of sickle cell disease

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The accelerated erythrocyte destruction in sickle cell disease (SCD) often leads to chronic hyperbilirubinaemia. The hepatic enzyme UDP-glucuronosyltransferase 1A1 (UGT1A1) mediates the conjugation of bilirubin into a water-soluble form. A di-nucleotide repeat polymorphism $(TA)_{5-8}$ in the TATA box of *UGT1A1* is associated with differential gene expression and enzyme level.
The aim of this study was to determine whether this UGT1A1 activity polymorphism could modify bilirubin metabolism in SCD, thereby influencing the development of cholelithiasis subsequently leading to cholecystectomy.
We studied 161 SCD patients with an average of 10 years of age. *UGT1A1* promoter TA repeat number was assessed by PCR and GENE SCAN. Steady-state haemoglobin and total bilirubin levels, cholelithiasis and cholecystectomy were investigated.
Several *UGT1A1* genotypes were found, the most frequent being 5/6 (n=9), 6/6 (n=37), 6/7 (n=61) and 7/7 (n=29). These groups did not significantly differ in age, sex and haemoglobin level. Total bilirubin levels were significantly different between groups (p=0.000) with an increased TA repeat number being associated with higher hyperbilirubinaemia. Cholelithiasis episodes (p=0.071) and cholecystectomy (p=0.034) were both augmented with TA repetition, although the former difference was not statistically significant. The odds ratio of having cholelithiasis for the genotypes 6/7 (OR=1.455; CI=0.578-3.666) and 7/7 (OR=2.057; CI=0.708-5.980) were increased when compared to 6/6. The same comparison for cholecystectomy revealed an increased risk for genotype 7/7 only (OR=4.714; CI=0.869-25.581).
In conclusion, the *UGT1A1* promoter polymorphism may represent an important genetic modifier of SCD even at a young age.
(Work partially supported by FCT grant IME/MGI/49853/2003).

P1099. Familial SIDS caused by LQT Syndrome

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On his 17th day of life the first born son of apparently healthy parents suddenly experienced syncope, apnea and pulselessness. Resuscitation was unsuccessful. Upon autopsy SIDS was diagnosed, no genetic investigation was initiated.
A second son born three years later suddenly lost tone, turned pale and stopped breathing on the 27th day of his life. A physician noted bradycardic sinus rhythm (10/min), initiated CPR and epinephrin infusion and restored sinus rhythm (160/min). In hospital the child showed repolarization disturbances characterized by QTc up to 500ms, multiple T-waves with low amplitudes and severly reduced cardiac function, attributed to traumatization during CPR. The boy survived but suffers from severe ischemic brain damage.
A marginal prolongation of QTc was noted in the mother (450 ms) while the father and the sister showed no abnormalities. Molecular diagnostic testing for LQT-Syndrome revealed a novel nonsynonymous D259N change in Exon 4 of the KCNH2 (LQT2) gene in our patient and his father in heterozygous state. In the light of the repolarization abnormalities in the mother and the uneventful history of the father we speculate that the mother may carry another, yet unknown molecular variant.
This case exemplifies three lessons for the diagnostic and therapeutic management of LQT and SIDS: (a) A proportion of SIDS cases (estimated 5-10%) can be attributed to monogenic LQT-Syndrome. (b) Sequencing of LQT-disease genes is mandatory in a diagnostic SIDS workup. (c) In severe forms of LQT compound mutations (Westenkow et al., Circulation 2004) may be more common than generally recognized.

P1100. SimStudy: A simulation program to generate data for case-control and quantitative trait studies

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The SimStudy program can generate population based data which is extremely beneficial in evaluating tagSNP selection, haplotype reconstruction and population admixture/substructure. Additionally the program can be used to assess type I and type II errors of statistical methods used to detect gene main effects as well as gene x gene and gene x environmental interactions. The SimStudy program can generate di-and multiallelic marker loci data which are in linkage equilibrium or disequilibrium for qualitative (case-control) or quantitative trait data. Cases-control status is determined by penetrance models or genotypic relative risks under a specified genetic model (i.e. multiplicative, additive, etc), or based upon threshold values for a quantitative trait. Quantitative trait loci are generated based upon genotypic means, variances and specified distributions (e.g. normal, log normal, exponential, etc). For both qualitative and quantitative traits it is possible to model gene x gene (epsitatis) and gene x environmental interactions as well as locus and allelic heterogeneity. Additionally class specific data can be generated based upon for example age, sex, or environmental exposures. Missing data and genotyping error may also be incorporated in the generation of genotype data. The program can quickly generate a large number of replicates for data sets with an almost unlimited number of marker and trait susceptibility loci. For the simulated data, SimStudy can generate reports with pair-wise r^2 and D' values between all marker and susceptibility loci. The SimStudy program is written in C and is available free of charge for Unix, Linux and Windows platforms.

P1101. Carrier Frequency of Spinal Muscular Atrophy in the Iranian Population

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INTRODUCTION: Spinal muscular atrophy (SMA) is the second most frequent autosomal recessive disorder. The incidence of SMA is one per 10,000 live births with a carrier frequency of 1/50. The SMA determining gene, called the survival motor neuron (SMN) gene, is present on 5q13 in two copies SMN1 (telomeric) and SMN2 (centromeric). Homozygous deletions of exons 7 and 8 of SMN1 occur in >95% of patients with SMA.
MATERIAL & METHODS: DNA extraction was performed according to the standard protocol from 400 normal individuals from different ethnic groups of Iran as listed in the WHO report (Persian 51%, Azari 24%, Gilak & Mazandaran 8%, Kurd 7%, Arab 2%, Baluch 2%, and Turkmen 2%). Exon 7 of the SMN gene was analyzed using a PCR-based protocol followed by digestion using DraI restriction enzyme. A total of 40 obligate carriers were studied to determine the telomeric centromeric ratio for carrier detection.
RESULTS & CONCLUSION: According to the T/C ratio, 52 (13%) individuals are carriers, while the remaining are normal for the SMN gene copy number. In conclusion, our results demonstrated a high frequency of SMA carriers in the Iranian population, about five times higher than that of the European population.

P1102. A possible association between Bam H1 perlecan gene polymorphism and the risk of spinal muscular atrophy in Romanian patients

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Perlecan is a heparan sulfate proteoglycan with various biological functions, including the neuromuscular one. Recent data have revealed functional mutations of perlecan gene in disorders characterized by severe myotonia. No correlation study between perlecan gene polymorphisms and spinal muscular atrophy (SMA) was performed before.

The aim of our study was to investigate the correlation between the BamH1 perlecan gene polymorphism and the risk for spinal muscular atrophy in Romanian patients.

We investigated 63 spinal muscular atrophy patients and 100 normal control subjects. The SMA patients were diagnosed according to the SMA International Consortium and the informed consent was obtained for all the subjects. The Bam H1 perlecan gene polymorphism was detected using PCR-RFLP method.

The values obtained for the perlecan genotypes are shown in the table.

We performed the χ^2 test for both lots, the results confirming that the populations are in a Hardy - Weinberg equilibrium (χ^2 = 2.73, DF =1, p = 0.05 for the SMA patients lot; χ^2 = 3.62, DF=1, p = 0.05 for the control lot). For the risk genotype G/G we obtained an OR = 1.164.

These are the preliminary results only. Therefore, in a future prospective study, by increasing the subject number, we hope to establish a more accurate correlation between BamH1 gene polymorphism and the risk for SMA.

Genotypes	SMA patients	Control subjects
T/T	31 (49.2%)	53 (53%)
T/G	30 (47.6%)	34 (34%)
G/G	2 (3.2%)	13 (13%)

P1103. Carrier Frequency of Spinal Muscular Atrophy in the Largest Island of Persian Gulf (Qeshm)

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INTRODUCTION: Spinal muscular atrophy is a neuromuscular disorder caused by the degeneration of α-motor neurons of the spinal cord anterior horns. It is an autosomal recessive disorder with an incidence of 1/6000 to 1/10000 and a carrier frequency of 1/40 to 1/50. Qeshm the largest island of Persian Gulf has population of more than 100,000 people. The main ethnic group is Persians with minorities of Arabs, Balouch, Indians, Portugese and African Blacks. The large size of Qeshm, its ethnic variety and tribal life style and relatively large population, make this island a proper site for genetic study. MATERIAL & METHODS: One hundred seventy eight healthy individuals participated in this study in order to find carrier frequency of SMA in this in this Island. Exon 7 of the SMN gene was amplified, followed by PCR products digestion using DraI restriction enzyme. In our study, analysis of the ratio of the telomeric to centromeric portion (T/C ratio) of the SMN gene after enzyme digestion was performed using LabWork Software in order to differentiate carriers, normal and affected individuals.

RESULT & CONCLUSION: Our result shows 22 out of 178(16%) were carriers while the remaining was normal. In conclusion our findings indicate SMA carrier frequency in the Island Qeshm is six times higher than European and American population.

P1104. Alpha-synuclein promoter haplotypes and dementia in Parkinson's disease

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Dementia is a common complication of Parkinson's disease (PD). It correlates significantly with the presence of cortical, limbic or nigral Lewy bodies, mainly constituted of alpha-synuclein. Mutations of the alpha-synuclein gene have been linked to rare familial forms of PD, while association studies on the promoter polymorphisms have given conflicting results in sporadic patients. In a previous study we did not find association between the Rep 1 polymorphism of the *SNCA* promoter and PD in our population. Since haplotype analysis has proven to be a more reliable method in association studies, in this work we analyzed a more extended region of the the *SNCA* promoter . We performed a case control study to investigate whether genetic variability in the promoter of the alpha-synuclein gene could predispose to dementia in PD. A total of 114 demented patients and 114 non-demented patients with sporadic PD were included in the study. Six polymorphic loci (including the Rep1 microsatellite) in the promoter of the *SNCA* gene were examined. Each marker, taken individually, did not show association to dementia and no significant differences were observed in the inferred haplotype frequencies of demented and non-demented patients (p=0.73). Our data suggest the lack of involvement of the *SNCA* promoter in the pathogenesis of dementia in PD. Further studies in other populations are needed to confirm these results.

P1105. Further evidence for additional loci for split hand/foot malformation in chromosome regions 4q32-q35 and 6q16-q22

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On the basis of the Human Cytogenetic Database (HCDB) we collected from the literature 102 cases with chromosomal aberrations and split hand/foot malformation or absent fingers/toes. Statistical analysis revealed highly significant association (p<0.001) between the malformation and the chromosomal bands 4q32-q35, 5q15, 6q16-q22 and 7q21-q22 (SHFM1). Considering these findings we suppose additional SHFM loci on chromosome 4q, 6q and probably 5q. The regions 4q and 6q have already been discussed in the literature as additional SHFM loci. We now show further evidence. In the proposed regions there are interesting candidate genes such as SNX3, GJA1, HEY1, HEY2, Tbx18, HAND2, FGF2, LEF1, BMPR1B, MSX2, FLT4, PTX1 and PDLIM7.

P1106. Unravelling the genetic aetiology of stroke in Pakistan

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The incidence of stroke is on the rise in developing countries. In search of quantitative trait loci at candidate genes underlying the aetiology of ischaemic stroke (IS) in Pakistan, we designed an association study with 210 IS cases and 350 disease-free controls. Associations of genetic dimorphisms with IS were tested by single-point and haplotype analyses. The three following loci were directly associated with clinical IS diagnosis:

Phosphodiesterase 4D (*PDE4D*): amongst three *PDE4D* genetic markers (SNP32, 83 and 87, see *Gretarsdottir* et al*., Nat Genet. 2003;35:131-138*), TT genotypes of SNP83C>T conferred a significant risk for IS on both univariate and multivariate analyses (P<0.005).

Paraoxonase genes (*PON1*, *2* and *3*): amongst eight markers, *PON1* 192Q>R, *PON2* 148G>A and *PON2* 311C>S were associated with IS, and QAS haplotypes indicated a 1.87-fold increased risk of developing IS (P=0.01).

ATP-binding cassette transporter A1 gene (*ABCA1*): we studied five intragenic markers, and first established that the RL haplotype (combination of R219K and V825L marker mutations) was strongly associated with decreased HDL-c levels (OR=8.33, P=0.001). We then found that allele 774P of mutation T774P showed the strongest direct association with IS (OR=4.06, $P<10^{-7}$). We further demonstrated that a three-point VPL haplotype (combination of V771M, T774P and V825L markers) was indicative of a very strong increased risk for IS (OR=10.34, $P<10^{-7}$).

Upon applying an overall, single multivariate model, we established that two major gene effects (*ABCA1* and *PON*) and one minor gene effect (*PDE4D*) account for 65.3% of IS in the studied Pakistani population.

P1107. Association analysis of the triallelic polymorphism of the serotonin transporter gene with suicide

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Serotonergic dysfunction has been implicated in the pathophysiology of suicidality. The serotonin transporter (5-HTT) strongly modulates serotonin function and is a major therapeutic target in several psychiatric diseases, including anxiety, depression, and suicide. 5-HTTLPR, a functional polymorphism of the 5-flanking region of the 5-HTT gene has been intensively studied for association with suicidal behaviour. Some, but not all, studies using biallelic S/L genotyping have demonstrated a higher frequency of the low activity S allele and with suicidal behavior. A>G polymorphism (rs25531) has been detected within 5-HTTLPR: L_G haplotype is equivalent to the lower expressing S allele. The aim of our study was to examine the relationship of a triallelic 5-HTTLPR polymorphism to suicidal behavior in a gender-specific manner. 231 suicide attempters (79 men, 152 women) and 264 healthy volunteers (160 men, 104 women) without history of suicidal behaviour from the general population of Russia were genotyped for the triallelic 5-HTTLPR polymorphism using PCR-RFLP technique. All individuals were considered as LL carriers (L_AL_A), LS carriers (L_AS, L_AL_G) or SS carriers (SS, L_GS, L_GL_G). The distribution of allelic and genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. We revealed no significant differences in allele and genotype distribution between suicide and control groups. In women there was a tendency of a lower frequency of the LS carriers in suicide group compared to that in control group (chi2=3.5; p=0.06; OR=0.62, 95%CI 0.38-1.02). Our findings indicate a possible contribution of the 5-HTT gene to susceptibility for suicidal behaviour in females only.

P1108. Analysis of type I collagen α1 and α2 chains genes in patients with syringomyelia from Bashkortostan

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Syringomyelia is a rare disease of the spinal cord that causes neurological deficit. A mendelian transmission of syringomyelia (autosomal dominant or recessive) has been proposed in approximately 2% of reported cases. The association of syringomyelia with hereditary diseases (Noonan's syndrome, phacomatoses) has been mentioned frequently in the literature. However the possible involvement of a genetic component in some cases of syringomyelia is debated. In Bashkortostan republic syringomyelia rate is one of the highest around the world (3.2 - 130 cases per 100 000 inhabitants).

The aim of the present study was to test two polymorphisms in genes encoding proteins of α1 and α2 chains of collagen (*A/Msp*I of the *Col1A1* gene, *B/Msp*I of the *Col1A2* gene) for association with syringomyelia in patients from Bashkortostan. 132 patients presented with magnetic resonance imaging (MRI)-proven syringomyelia and 196 controls were typed for the above-mentioned gene variants using polymerase chain reaction technique.

Our data indicate the contribution of *Col1A1*A* (p=0.013, OR=1.28, 95%CI=1.07-1.99) and *Col1A2*N* (p=0.011, OR=1.37, 95%CI=1.12-2.03) alleles to susceptibility for syringomyelia in samples from Bashkortostan. Further analysis showed overrepresentation of the *Col1A1*A/*A* (p=0.023, OR=1.6, 95%CI=1.05-1.59) and *Col1A2*N/*N* (p=0.007, OR=1.96, 95%CI=0.75-2.4) genotypes among patients compared to controls and these genotypes can be possible markers of syringomyelia.

Advances in knowledge about the genetic basis of syringomyelia offer the prospect of developing new approaches to treatments of this disorder.The work was supported by RSCI grant #05-06-06168a.

P1109. Association of Interferon Regulatory Factor 5 (*IRF5*) polymorphisms with systemic lupus erythematosus (SLE)

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Background:

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by the dysfunction of immune cells, leading to hyperactivity of B cells and over-production of autoantibodies and the formation of immune complexes.

The level of IFN-α, a type I interferon, is correlated with both SLE disease activity and severity, and is therefore suggested to be involved in the pathogenesis of SLE. Activation of transcription factors, including Interferon Regulatory Factors (IRFs) 3, 5 and 7 can modulate the expression of type I IFN genes. IRFs control inflammation, immunity and apoptosis. Irf5 knockout mouse also shows reduction of pro-inflammatory cytokines, including IL-6, IL-12 and TNF-a production. Recently several association studies in different populations have reported that IRF5 gene is a susceptibility gene of SLE.

Methods:

We hypothesized that polymorphisms of *IRF5* may affect the susceptibility and severity of SLE in the Hong Kong Chinese population. SNP rs2004640 creates a 5' donor splice site for alternate isoform of transcript in exon 1, whereas rs10954213 creates a functional polyadenylation site in 3' UTR and affects the expression of transcript variants. The 2 SNPs were genotyped in 444 SLE patients and 410 healthy controls, using sequencing.

Results:

No association of IRF5 gene polymorphisms with SLE was found. However, an overall difference in the distribution of the haplotype frequencies between SLE patients and controls was detected. The haplotype TA was identified as a probable risk haplotypes associated with SLE.

P1110. Association study of the $GABA_A$ cluster on 15q11-13 with Tourette Syndrome in the French Canadian population.

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Tourette Syndrome (TS) is a complex neuropsychiatric disorder manifested by motor and vocal tics and often associated with behavioral abnormalities. Various neurotransmitters involved in the cortico-striatal-thalamo-cortical circuits have been implicated in TS, including the dopaminergic, glutamatergic, GABAergic, serotonergic, and noradrenergic systems. However, most of the genetic studies have focused on candidate genes implicated in dopamine neurotransmission. The goal of this study was to investigate the genetic association between single nucleotide polymorphisms (SNPs) in the cluster of $GABA_A$ receptor subunit genes on 15q11-13 and TS in the French Canadian (FC) population. This region has been consistently implicated in several neurodevelopmental disorders and behavioral abnormalities.

With an average spacing of 33 Kb, 26 SNPs spanning the $GABA_A$ receptor subunit genes (GABRB3, GABRA5, GABRG3) were selected based on minor allele frequency >0.1, spacing and haplotype blocks. We performed a family-based association study by typing these SNPs in 212 FC trios with TS. Transmission/disequilibrium test (TDT) and haplotype analyses of various SNP-windows were performed using the TDTPHASE program. Because these genes may be subject to genomic imprinting, we also performed analyses of transmission by parental sex.

TDT of individual markers and haplotype analyses did not provide positive association results. When analyzed separately, paternal and maternal transmissions provided various p values < 0.05, but these results did not remain significant after correction for multiple testing. These data suggest that the $GABA_A$ cluster on 15q11-13 is not a susceptibility locus for TS.

P1111. Use of SNP array analyses for diagnosis of autosomal recessive heterogeneous diseases : identification of the second TRIM32 mutation in limb-girdle muscular dystrophy type 2H

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Molecular diagnosis of rare autosomal recessive diseases with genetic heterogeneity represents a real challenge because clinical data do not always suggest a particular defective gene. Consanguinity is frequent in such families. Genome-wide SNP array is a recent tool that allows, by searching for homozygous regions in such patients, to select few candidate genes in which to search for mutations. We report the case of a 51 years old woman who presents a moderate limb-girdle muscular dystrophy, diagnosed at the age of 37 years. We identified six homozygous regions by SNP array analysis (Affymetrix). One of the regions, on chromosome 9, contained the TRIM32 gene. This gene was previously found mutated in families with limb-girdle muscular dystrophy type 2H (LGMD2H), a mild autosomal recessive myopathy described in the Manitoba Hutterite population and in two non-Hutterite brothers from Germany with sarcotubular myopathy. A single mutation was found in these patients, D487N, located in a conserved domain of the C-terminal part of the protein. Haplotype analysis showed that Hutterite and German patients shared common ancestry. TRIM32 was also implicated in Bardet-Biedl syndrome (BBS11), again based on a single missense mutation (P130S, in the N-terminal part) found in a consanguineous Bedouin family, rising the possibility that either the LGMD or the BBS nucleotide change could be associated with disease by linkage disequilibrium rather than being disease causing. Sequencing of TRIM32 in our patient revealed a frameshift mutation, c.1753_1766dup14 (p.L589fs) in the C-terminal part. This second mutation firmly establishes the role of TRIM32 in LGMD.

P1112. Tumor Necrosis Factor polymorphisms and asthma in two international population-based cohorts (ECRHS and SAPALDIA studies)

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Genetic association studies have associated the Tumor Necrosis Factor (*TNF*) *308G/A* polymorphism with an increased asthma risk but, overall, results are inconsistent. We assessed the prevalence of atopy and asthma in adults with two single nucleotide polymorphisms (SNPs) of the *TNF* and lymphotoxin α (*LTA*) genes.

The European Community Respiratory Health Survey (ECRHS) and the Swiss Cohort Study on Air Pollution and Lung and Heart Disease in Adults (SAPALDIA) are two population based cohorts that have used comparable protocols including the questionnaires for respiratory symptoms and exposures as well as lung function and atopy tests. DNA samples from 10,736 participants from both cohorts were genotyped for *TNF-308* and *LTA+252*.

The prevalence of asthma symptoms was 6%. The *TNF-308* polymorphism was associated with an increased asthma prevalence. The adjusted odds ratio (OR) for A allele was 1.30 (95%CI 1.1-1.53) for combined sample, 1.49 (95%CI 1.22-1.81) for ECRHS and 0.94 (95%CI 0.69-1.29) for SAPALDIA study. Association pattern shows slight differences between countries. Similar risks were observed in groups stratified by sex, smoking and atopy. The *LTA+252* SNP was not associated with the prevalence of asthma symptoms or atopy. Haplotype analysis of both SNPs didn't show a combined effect, with an OR for *LTA+252* and *TNF-308* rare alleles, 1.29 (95%CI 1.09-1.52).

CONCLUSIONS: Our data suggest that genetic variation in TNF may contribute to a small extent to asthma risk but that this risk may differ between countries.

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P1113. Glucose tolerance test in the Turner syndrome families

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The chromosome diseases are polymorphic because a chromosome imbalance lowers the threshold of appearance in family pathology. The decreased homeostatic buffering in developmental pathways leads to stronger phenotypical expression of multifactorially inherited traits. For our investigation were chosen the families with Turner syndrome (TS) patients since they show many extragenital pathology. Peroral glucose tolerance test (GTT) was made for them because glucose intolerance is typically multifactorial trait.

GTT was performed for 37 TS patients, 42 their siblings (21 brother and 21 sister) and 52 parents (32 mothers and 20 fathers). The average age of TS patients was 20.8 yrs (ranking from 5 to 46 yrs), siblings 20.0 yrs (ranking interval 6-56 yrs), and in parents 48.1 yrs (ranking between 27 and 76 yrs). Glucose tolerance was found being disturbed in 7 (19.0%) probands, in 4 (9.5%) siblings and in 9 (17.3%) parents while in the control Lithuanian population these disturbances were found only in 1.7% of people at the age of 24-35 yrs and in 5.0% of those who are older than 35 yrs old. Therefore, the GTT shows glucose intolerance level being much more higher for TS patients and their relatives than in general population. These results allow us to affirm that damages of homeostasis usually present in families with chromosome diseases. The frequency of GTT disturbances in TS patients are twice more often than in their siblings.

P1114. Mutations of PEO1 gene encoding Twinkle helicase causes mitochondrial DNA depletion

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Twinkle is a mitochondrial 5'-3' DNA helicase is important for mitochondrial DNA (mtDNA) maintenance. Twinkle dominant mutations have been reported in progressive external ophthalmoplegia with multiple mtDNA deletions (adPEO) whereas Twinkle recessive mutations are associated with infantile onset spinocerebellar ataxia (IOSCA). It has been previously shown that Twinkle control mtDNA copy number renders Twinkle gene (PEO1) a candidate gene for mtDNA depletion. We selected a series of 10 patients born to consanguineous parents and presenting a severe mtDNA depletion of yet unknown origin. We then studied the segregation of microsatellite markers flanking PEO1 in these patients. Homozygosity of the microsatellite markers was found for two patients of the same family. They presented neonatal lactic acidosis, trunk hypotonia, seizures, cytolysis and cholestasis. A combined defect of complexes I, III and IV of the mitochondrial respiratory chain was found in liver of both patients as well as severe mtDNA depletion (8% of the normal mtDNA content). This prompted us to sequence PEO1 and to identify a homozygous mutation at a conserved position of the protein (T457I). The similarity of Twinkle and GP4D helicase from phage T7 prompted us to model the structure of this human helicase using the X-ray coordinates of the homohexameric GP4D protein as a tertiary template. Interestingly, the point mutation is located in the interface between two monomers of the hexameric enzyme, and can probably induce a local conformational change. This work reports the first description of a PEO1 mutation responsible for mtDNA depletion in human.

P1115. HLA B39 affects the type 1 diabetes predisposing effect of DRB1*0404-DQB1*0302 haplotypes in the Finnish population

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An autoimmune attack against the insulin-producing β-cells of the pancreas precedes the manifestation of clinical type 1 diabetes (T1D). The genetic factors regulating this process are poorly characterised. The main factors are DQB1-, DRB1- and DQA1-genes in human leuko-

cyte antigen (HLA) Class II area. However, several studies support the modifying role of HLA Class I variants. We aimed to characterize Class I modifiers of the DRB1*0404-DQB1*0302 haplotypes in relation to T1D risk and to emergence of humoral beta-cell autoimmunity.
We analysed 303 ICA-positive children who were identified in a population-based genetic screening. All individuals carried the DQB1*02/*0302 or *0302/x (x≠*02, *0301, *0602) risk genotypes. Mean follow-up time was 5.46 years. Study subjects were further tested for insulin, IA2 and GAD autoantibodies and genotyped for HLA DRB1-DQA1-DQB1, HLA-B and for the insulin gene HphI promoter polymorphism.
The DRB1*0404-DQB1*0302-HLA-B*39 and DRB1*0404-DQB1*0302-non-HLA-B*39 haplotypes conferred significantly different T1D risks, the haplotype carrying HLA-B*39 being more predisposing (HR 7,08; p<0,001). These haplotypes also had a differential effect on the appearance of insulin autoantibodies (p=0,0001). This effect was independent of the insulin gene polymorphism. The presence of HLA-B*39 did not affect emergence of GADA and IA-2A.
For the first time we showed that B*39 allele on the DRB1*0404-DQB1*0302 haplotype affects appearance of diabetes-related autoantibodies and progression to clinical disease. Importantly, its primary effect appears to be on insulin-specific autoimmunity. It is further investigated whether the B*39 allele itself or a gene in the surrounding genomic region is responsible for this phenomenon.

P1116. Association of alleles at polymorphic sites in the Osteopontin encoding gene in young Type 1 diabetic patients

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In the complex interplay of genes related to immune response and autoimmune process in Type 1 diabetes (T1D), several candidates can be envisaged as susceptibility genes. We have studied polymorphisms in the Osteopontin (OPN) encoding gene by comparison of genotype, allele and haplotype frequencies between T1D cases and unaffected controls and immunological characteristics in T1D cases. We evaluated 238 T1D patients (130 male, 108 female), and 137 unaffected control individuals (68 males and 69 females). All patients and controls were genotyped for three OPN intragenic variants: -156 (G/GG) and -66 (T/G) in the promoter region and a biallelic ins/del variant (TG/TGTG) at +245 in the first intron of the gene. The results of the case/control association study indicated that the G allele at the -66 SNP had significantly higher frequency in controls than cases. Accordingly, the B haplotype combination, that includes the -66 G allele, showed significantly higher frequency in controls than cases. Interestingly, case-control comparison in male individuals showed no significant association, whereas the association was confirmed in females.
Considering patients with 1 b-cell autoantibody versus those having two or more autoantibodies, the T allele at the -66 SNP showed a relative increase of genotypic frequency in T1D cases with high number of autoantibodies. This was also the case in patients with two or more b-cell autoantibodies who were carriers of 4 HLA-DQ risk heterodimers. These results suggest that Osteopontin can play a role as susceptibility gene, possibly by a sex-specific mechanism acting in the autoimmune process.

P1117. Gender-Specific effects of cytokine genes on childhood vaccine responses

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Background: The effects of cytokine genes on vaccine responses are context dependent. Gender-specific genetic effects have been demonstrated on many phenotypes, however, their influence on vaccine response is unknown.
Objectives: To investigate gender-specific effects of cytokine gene polymorphisms on vaccine response.
Method: In 263 2 year-old subjects selected for parental history of atopy, we investigated gene-gender interaction effects on IgG responses to diphtheria (DiphTox) and tetanus toxoids (TetTox) using a likelihood-ratio test and comparing an unrestricted (with interaction) and a restricted (without interaction) model. Interactions identified were explored by stratification for gender.
Findings: For TetTox responses, an interaction p value <0.15 was found for: IL-4 C-589T (p=0.01), IL-4 G2979T (p=0.12), IL-4Rα I50V (p=0.04) and IL-10 C-592A (p=0.13). For DiphTox responses, the interaction term for IL4 C-589T was p=0.10. After stratification, we found associations with alleles associated with atopy. Boys with IL-4 -589T alleles (CT/TT) had increased DiphTox (p=0.011) and TetTox (p=0.042) responses compared with CC homozygotes and those with IL-4Rα 50V alleles (IV/VV) had higher levels of DiphTox (p=0.039) and TetTox (p=0.077) responses compared with II homozygotes. Contrastingly, girls with IL10 -592C alleles (AC/CC) had significantly lower levels of DiphTox (p=0.028) and TetTox (p=0.018) responses compared with AA homozygotes. Overall, the most consistent pattern was a reduction in TetTox responses in girls with genotypes associated with atopy.
Conclusion: Our findings support the interaction of primary genetic and modifying factors on vaccine responses, the importance of atopic genetics to vaccine responses and investigation for gender-specific effects in genetic association studies.

P1118. The Vitamin D Receptor Gene Polymorphisms are associated with diabetes mellitus in Romanian Population

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Vitamin D receptor (VDR) gene polymorphisms were associated with development of Type 1 Diabetes Mellitus (T1DM) in some populations. In addition vitamin D may influence insulin secretion and insulin sensitivity. Based on these observations the VDR gene polymorphisms are considered potential candidate for diabetes mellitus development.
The aim of the present case-control study was to evaluate the impact of VDR polymorphisms to the T1DM in Romanian Caucasian population.
Clinical information and biological samples from 800 unrelated Romanian Caucasian subjects were collected. They were distributed into T1DM (n=200, men: women, 105:95), T2DM (n=200, men: women, 105:95), HC (n=400 healthy control) groups. These groups were matched for age, gender and ethnicity. The VDR Apa, Taq, Fok polymorphisms were analyzed using PCR-RFLP method. The distribution of genotypes in all groups was in agreement with Hardy-Weinberg equilibrium. Differences in genotypes and alleles distribution between the groups were examined by chi-square test. We observed that the VDR F, t and A alleles and FF, TT and AA genotypes are more frequent in T1DM patients than in controls. The highest difference was observed for VDR Fok (Chi-square =12,8961, p≤0.01). This polymorphism is also more frequent in T2DM than in control subjects, but the difference is less significant than in T1DM patients.

P1119. Polymorphism of the vitamin D receptor gene and osteocalcin gene in insulin-dependent diabetic children with and without osteopenia.

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Insulin-dependent diabetes is often associated with osteopenia. The mechanism of this association is not yet clear. Genetic factors play an important role in the pathogenesis of osteoporosis and osteopenia. Recent studies, aimed to identify the genes, involved in this process, suggested several candidate genes, among them vitamin D receptor gene (VDR) and osteocalcin gene. In this study we investigated the allele frequency of the VDR gene polymorphic loci, determined by the presence or absence of the restriction sites to ApaI, TaqI and BsmI ensimes and allele frequency of the osteocalcin gene polymorphic loci, determined by the presence or absence of the restriction site to HindIII endonuclease. The aim of this study was to compare the frequency of these alleles in the group of children with insulin-dependent diabetes, associated and not associated with osteopenia.
In total, 224 children (116 boys and 108 girls) with insulin-dependent diabetes wereincluded in our study. All the children were living in North-West region of Russia. Osteopenia was diagnosed in 38 subjects (28 boys and 10 girls). VDR and osteocalcin gene polymorphism were de-

tected by PCR with restriction assay.
Osteopenia was detected significantly more often among boys with diabetes compared to girls (p=0,004). TaqI, BsmI polymorphism of the VDR gene and HindIII polymorphism of the osteocalcin gene did not influence the frequency of the osteopenia among children with insulin-dependent diabetes. The A allele of the ApaI polymorphic site of VDR gene tended to appear more often in the group of the osteopenic children compared with subjects without osteopenia.(p=0,045).

P1120. The age of the founder effect for C598T mutation in the *VHL* gene in the patients with autosomal recessive erythrocytosis in Chuvashia.

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Familial congenital polycythemia (OMIM 263400) - is a rare autosomal recessive disorder that is characterized by erythrocytosis, normal leukocyte and platelet counts and usually increased erythropoietin production. This disorder is rather frequent in a small region of Chuvashia (Russian Federation) with mostly peasant citizens. All patients are Chuvashs by nationality and live in the northeastern part of Chuvashia. This disorder is caused by mutation C598T in the *VHL* gene that leads to the substitution Arg200Trp. In 2004 Liu et al calculated the age of this mutation in the world using 8 SNPs. They estimated the age of the founder effect as 14000-62000 years.
We have examined data on six microsatellite markers flanking the *VHL* gene to estimate the age of the founder effect in Chuvash population. Mean value of the generations we estimated as 52,67±24,12 generations. The age of the one generation for Chuvash peasant citizens is 27.4 years. So the C598T mutation arose in Chuvash population 1445 years ago. The mean year of the births of patients with autosomal recessive erythrocytosis that we are investigated is 1972. And mutation C598T originated from single founder approximately in 527th year.
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P1121. A novel mutation in the VDR gene in an Iranian patient with Vitamin D-dependent Rickets type II

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The vitamin D receptor (VDR), is the mediator of all genomic actions of vitamin D3 and it's analogs. Mutations in VDR results in target organ resistance to 1alpha,25-dihydroxy vitamin D [1,25(OH)2D3], the active form of vitamin D, and cause hereditary 1,25-dihydroxyvitamin D resistant rickets (HVDRR). This disease also called vitamin D-dependent rickets type II and is transmitted in an autosomal recessive mode.
We report a 5 years old girl affected with type II vitamin D-dependent Rickets, who appeared normal at birth but developed the clinical and biochemical features of calciferol deficiency with hypocalcaemia and rickets in the first year of life. She suffered from total alopecia and metaphyseal dysplasia in both hip joints and in pelvic. She was hospitalized at the age of four due to Diabetes Mellitus. Sequence analysis of all coding exons of VDR gene was performed and revealed a single homozygous point mutation in exon 7 causing a premature termination codon, Tyr295X. The Tyr295X mutation causes a truncation of the VDR protein, thereby deleting a large portion of the steroid hormone-binding domain (amino acids 295-424).

P1122. Polimorphisms of CYP2C9 and VKORC1 genes associated with the warfarin metabolism in Hungarian Roma population.

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Warfarin is an oral anticoagulant drug for the prevention and treatment of arterious and venous thromboembolic disorders. There is a great interethnic and interindividual variability in the required dose. Pharmacogenetic analysis of the S-warfarin metabolic enzyme CYP2C9 and S-warfarin target protein VKORC1 can help in individualized therapy. The aim of our work was to study the allelic frequencies of two gene variants, CYP2C9*2 C3608T and VKORC1 G1639A, in a Hungarian Roma population in comparison with the average Hungarian Caucasian population. A total of 303 Gypsy cases and 181 Hungarian controls were genotyped for the CYP2C9*2 C3608T SNP; and for the VKORC1 G1639A SNP 328 Gypsys and 193 Hungarians were tested. For genotyping PCR-RFLP assay and direct sequencing were used. Our study revealed significant difference in the presence of mutant genotype in case of CYP2C9*2 C3608T (p=0.021) and VKORC1 G1639A (p=0.012) between the Hungarian Roma and Hungarian general population. VKORC1 -1639A allele frequency was 28% in the Gypsy group, 41 % in controls, whereas the CYP2C9*2 3608T allele was present in 12% of both studied groups. The allele frequencies of the Hungarian population of both studied SNPs were very similar to the European Caucasian population (CYP2C9*2 C3608T 12%, VKORC1 G1639A 41%). Genotyping of VKORC1 G1639A SNP could be clinically important for predicting anticoagulant responses to warfarin. The results of our studies would provide new insights to the genetic variability, and their response to the drug metabolisation of the Roma population in Central-Eastern Europe.

P1123. Molecular genetic analysis of PRKAG2 in Tunisians families with Wolff Parkinson White Syndrome

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The Wolff Parkinson White (WPW, MIM 194200) syndrome is an autosomal dominant heart disease characterized by the presence of accessory pathway that arises from an aberrant conduction from atria to ventricles. This second pathway causes a premature ventricular preexcitation that manifests on the electrocardiography as a short PR interval and anomalies of QRS complex. WPW affects 1 to 3 persons per 1000 population and is associated with high incidence of sudden death in some affected families.
Several mutations within PRKAG2 gene have been shown to underlie WPW. This gene encodes the gamma2 regulatory subunit of AMP-activated protein kinase, which functions as a metabolic sensor in cells, responding to cellular energy demands by regulating diverse ATP-using pathways and ATP-generating pathways.
We report here clinical and molecular investigation of 3 unrelated Tunisian families including 6 affected members. All patients were diagnosed on the basis of clinical symptoms and electrophysiological features of WPW. Families were genotyped with two polymorphic microsatellite markers overlapping the PRKAG2 gene.
Haplotype analysis showed that this cases are likely linked to PRKAG2 gene. Two different haplotypes have been identified suggesting that there is no evidence for a founder effect to WPW in the studied families and is suggestive of mutational heterogeneity of this conduction system disease. None of the previously reported mutations within PRKAG2 gene was observed in Tunisian patients.
To our knowledge, this is the first report of a molecular study of WPW in North Africa population.

P1124. The CYP1A1 and CYP1A2 genotypes are possible factors causing chemical induced abnormal liver function

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Hepthyle, ethylebenzene-styrene, benzene are hepatotoxic in humans. In vivo, these substances are metabolized by cytochromes P450 to form the electrophilic metabolites, which may either cause cell damage or be further metabolized and detoxified by glutathione S-transferases (GSTs) and other enzymes.
The aim of this study was to estimate the predisposition of influencing possible factors causing chemical induced abnormal liver function on the basis of studying the genotypes CYP1A1 and CYP1A2. For this study, 330 workers from the petrochemical plant were enrolled. The genotypes CYP1A2 (-164C→A and -2464T→delT) and CYP1A1 (A2455G and T3801C) were determined by polymerase chain reaction and restriction fragment length polymorphism on peripheral white blood cell DNA from 73 incident cases of toxic hepatitis, 163 "groups of risk" on development of a toxic hepatitis, 94 healthy workers and

335 controls. The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin was used as the parameter of liver function.
No significant association was found between all groups of petrochemical workers among themselves.
We found that the *CYP1A1*2C* were associated with a 2.09 -fold (95% CI: 1.1-4.21) increased AST and *CYP1A2*1D* were associated with a 8.5-fold (95% CI: 1.1-75.0) increased AST and a 1.6-fold bilirubin (95% CI: 1.0-2.9). Gaplotype CYP1A2*1L had an OR (95% CI) of 14.5 (1.8-31.0), 13.1 (1.6-28.7), 44.4 (5.5-97.1) on abnormal bilirubin, AST and ALT, respectively.Our results were shown that *CYP1A2*1A* is a protective variant (OR=0.17; 95% CI: 0.9-0.27).

P1125. Molecular characterization of α-thalassemia and phenotype-genotype correlation in Sicily (Italy).

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We analyzed the α-thalassemia mutations and the genotype-to-phenotype correlations in 131 Sicilian subjects with reduced MCV and MCH, normal HbA_2 and HbF, and normal serum iron. Six different genotypes were detected, the most common (44,3% of the cases) being the heterozygous state for $-\alpha^{3.7}$ Kb deletion ($-^{3.7}\alpha/\alpha$). The $-\alpha^{3.7}/-\alpha^{3.7}$ homozygotes had a frequency of 19.8%.
Ten percent of the patients was $--^{MED}/\alpha\alpha$ heterozygotes and 9.1% was $-\alpha^{20..5}/\alpha$ heterozygotes.
The α^{HphI} mutation was found at the heterozygous state ($\alpha^{HphI}\alpha/\alpha\alpha$) in 8.4% of the patients and 6.1% was $\alpha^{HphI}\alpha/\alpha^{HphI}\alpha$ homozygotes. The $-\alpha^{3.7}/\alpha^{HphI}\alpha$ genotype was found in 2.3% of the patients.
The values of Hb and RBC were statistically significant different between the males and the females ($p<0.01$). Significant differences were found for the haematological parameters among subjects with different genotype groups. Reductions of MCV and MCH were found in those subjects with the following genotypes: $-\alpha^{3.7}/\alpha\alpha$; $-\alpha^{3.7}/-\alpha^{3.7}$; $--^{MED}/\alpha\alpha$ and $-\alpha^{20.5}/\alpha\alpha$. This reduction was more evident in both heterozygotes and homozygotes for α^{HphI} mutation.The highest values of RBC were found in the carriers of $--^{MED}/\alpha$, $-\alpha^{3.7}/\alpha\alpha$ and $\alpha^{HphI}\alpha/\alpha^{HphI}\alpha$ genotypes. Hemoglobin levels were reduced only in α^{HphI} homozygous subjects.
Our data underline that in Sicily populations, the molecular screening of α-thalassemia is useful to better characterize the clinically asymptomatic subjects with a slightly reduced MCV and MCH and normal iron status.

Po07. Normal variation, population genetics, genetic epidemiology

P1126. Metabolic and physiological changes in muscles of mice lacking alpha-actinin-3 explains the association between a common null allele in the *ACTN3* gene and human athletic performance

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The protein α-actinin-3, encoded by the *ACTN3* gene, is a highly conserved component of the contractile machinery in fast skeletal muscle fibres. Intriguingly, homozygosity for a common nonsense variant in the human *ACTN3* gene (R577X) results in complete deficiency of α-actinin-3 in ~18% of the general population - more than one billion individuals worldwide. We and other groups have demonstrated that the *ACTN3* 577XX null genotype is under-represented in elite sprinters and over-represented in endurance athletes, and is associated with poorer muscle strength and sprint performance in non-athletes. The 577X allele also shows a strong genetic signature of recent positive natural selection, suggesting that α-actinin-3 deficiency conferred an adaptive benefit during the evolution of modern humans.

To explore the mechanisms underlying the effect of R577X on muscle function we have generated an *Actn3* knockout (KO) mouse model of α-actinin-3 deficiency. We have shown that α-actinin-3 deficiency is associated with increased mitochondrial density and altered activity of metabolic enzymes, consistent with a shift in muscle metabolism towards the more efficient aerobic pathway. We have also demonstrated a specific reduction in the diameter of fast muscle fibres, reduced muscle bulk and grip strength and an increase in fatigue resistance in KO mice. These phenotypic differences provide a physiological explanation for the differing *ACTN3* genotype frequencies in sprint and endurance athletes, and may help to explain the adaptive benefit of the 577X allele during the evolution of modern humans.

P1127. Epidemiological study of progressive autosomal-dominant spinocerebellar ataxias in the Bashkortostan Republic

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Progressive autosomal dominant spinocerebellar ataxias (AD SCAs) are genetically heterogeneous group of neurodegenerative diseases, clinical characteristic of which is the disturbance of motor coordination, appearing as a result of the defeat of the cerebellum. There is significant population heterogeneity for the prevalence of AD SCAs and the molecular- genetic reasons of the disease. We have analyzed the prevalence of AD SCAs in the Bashkortostan Republic (Russia, territory of South Ural). At present in the Bashkortostan Republic (population 4069784 people) 82 patients (45 males and 37 females) from 69 families with AD SCAs were revealed. Irregular distribution of AD SCAs on the territory of the Bashkortostan Republic (2:100000 on average) is noted. The disease is registered in 22 of 54 districts and in 12 of 21 towns. The prevalence of AD SCAs in different districts varies from 0, 63 to 17, 7 per 100000. The molecular- genetic study of AD SCAs in the Bashkortostan Republic has begun. We carried out the analysis of the number of (CAG)n- repeats in the gene SCA1 in 19 unrelated families since it is one of the most frequent types of AD SCAs in Russia. However, not a single of the examined families revealed the expansion of (CAG)n- repeats. Further conducting of studies is planned.

P1128. Fine mapping of susceptibility locus for ADHD on chromosome 5p

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Attention-deficit/hyperactivity disorder (ADHD) is a behavioral diagnosis based on the presence of developmentally inappropriate levels of impulsivity, overactivity, and inattentiveness. The disease prevalence for ADHD ranges from 5%-10%, and is found to be four times as prevalent in males as in females. It is a familial condition with a complex pattern of inheritance.

Thus far five genome-wide scans have been conducted worldwide and of these two were carried out in US study samples by our group. These screenings and subsequent fine-mapping yielded four significant linkage peaks on chromosomes 5p, 6q, 16p and 17p. Chromosome 5p produced strong linkage also in two of the other genome-wide scans that were carried out in the European populations. Due to the overwhelming evidence for this particular chromosome locus we have here screened 3200 SNPs in 170 ADHD trio families. The 15 Mb wide fine-mapped chromosomal region had on average one SNP every 4.8 kb. In our preliminary analyses we found three SNPs with p-values < 0.00008. An increased frequency of haplotypes resulting in p-values < 0.01 were found on chromosome 5q11.2, all subsiding in the ARL15 gene where also the most significant single SNP (p = 0.00001) was found. The function of this gene is largely unknown.

P1129. Partially isolated communities founded by Azoreans in the Santa Catarina Island, Southern Brazil

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Costa da Lagoa (CLG) and São João do Rio Vermelho (SJRV) are communities located on Santa Catarina Island (Southern Brazil), settled on the XVIII century by immigrants came from Azores Archipelago. Demographic and genetic studies have also been indicated the presence of African and Amerindian components. CLG is considered genetically isolated due to its localization and isolate breaking is occurring in SJRV due to the increase migration to the local. Eight autosomal and five Y-linked STRs and YAP were analyzed in CLG (n=119) and SJRV (n=163) to verify the hypothesis of their different degrees of isolation. Genotyping was performed by PCR/PAGE/Silver staining and statistical analysis by ARLEQUIN, DISPAN, GDA, PHYLIP and ADMIX. The Y-linked *loci* were unable to distinguish the communities (F_{ST}=-0,001 p>0,05), but were more effective on the differentiation between them and Amerindians and Africans (0,16>G_{ST}>0,215). The communities showed more similarity in relation to Y haplotypes (F_{ST}=0,007; p>0,05), what might indicate an interchange mainly male-mediated between them. The autosomal STRs were more effective on the differentiation between these communities of recent and common origin (F_{ST}=0,005, p<0,01). Autosomal *loci* detected a major Portuguese/Azorean admixture (CLG=93,5%, SJRV=80,6%), followed by an African (CLG=4,1%, SJRV=12,6%) and a low Amerindian. The Y-linked *loci* showed a preponderantly Portuguese/Azorean (CLG=95,6%, SJRV=94,1%) and no Amerindian component. These data showed that these communities were genetically related to the Azores population. Thus, random genetic drift may have had an important effect on the CLG community, while gene flow might be the predominant evolutionary factor on SJRV.

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P1130. Analysis of *CYP1A1, CYP2D6, GSTM1, GSTT1, NAT2, CYP2C9* and *CYP2C19* gene polymorphisms in newborn and elderly people from North-West region of Russia

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Using pharmacy biochip specially constructed for population studies, 13 polymorphisms of 7 genes: CYP1A1(C4887,A4889G,T6 235C), CYP2D6(G1934A,DelA2637), GSTM1(del), GSTT1(del), NAT2(C481T,G590A,G857A), CYP2C9(C430T,A1075C) and CYP2D6(G681A) were investigated in 2 groups: 109 newborns (Group1) and 148 unrelated individuals over 70 years (Group 2) from North-West Region of Russia.

Low frequency of null allele homozygotes for GSTT1 gene in Group1 (14%) and high frequency in Group2 (28%) were obtained. The frequencies of GSTM10/0 genotype were significantly different for men in Group1 and Group2 (30.4%, 53.6%, respectively, p=0.049). The frequencies of deletion homozygotes (GSTT10/0, GSTM10/0) increased with aging from 6.5% in Group1 up to 21.4% in Group2 (p=0.058). The distribution of genotypes of NAT2 gene was significantly different

between groups (p=0.011). The frequency of N allele increased with aging from 16.8% in Group1 to 28.6% in Group2 (p=0.008). The frequency of C allele (A1075C) of CYP2C9 gene significantly increased in female from Group1 compared to Group2 (13.7%, 4.8%, respectively, p=0.009).
For estimation of combined effect of GSTM1, GSTT1 and NAT2 genes we used "score method". The carriers of 0/0(GSTM1), 0/0(GSTT1) and N/N(NAT2) genotypes were given a score of 2, N/S carriers (NAT2)-a score of 1 and carriers of S/S(NAT2), n/n(GSTM1) and n/n(GSTT1)-zero score. Combined analysis revealed significant prevalence of frequency of "score sum ≥3" in Group2 compared to Group1 (60.7%, 19%, respectively, p=0.0009).
It might be speculated that people who have certain genotypes of GSTM1, GSTT1, NAT2 genes for men and GSTT1, CYP2C9, NAT2 genes for women have some metabolic advantages for their longer survival.

P1131. Allele high-specific amplification for detection of *LCT* C/T-13910 SNP associated with adult-type hypolactasia

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Adult-type hypolactasia limits the use of fresh milk due to lactase non-persistence that causes lactose intolerance. As found recently, the CC genotype of the DNA variant -13910 T/C upstream of the lactase (*LCT*) gene is associated with lactase non-persistence [Enattah et al., 2002]. Frequency of hypolactasia varies from 2-3% in North West Europe to more than 90% in East Asia.
The aim of this study was to evaluate the applicability of *LCT* C/T(-13910) SNP detection as a diagnostic test for adult-type hypolactasia in Russia and neighboring countries.
We used allele specific amplification for determination of allele and genotype frequencies in populations of Russians, Ukrainians, Belorussians, Komi, Pamirs, Udmurt, Chukchi, Kazakh, Uigur, Buryat and Iranians. Thus, DNA diagnostics for carrying the C/C genotype of the locus C/T-13910 in individuals from populations of the European part of Russia can be considered a predictive test for development of the primary adult hypolactasia long before its phenotypic detection.
Allele specific amplification was performed by real-time PCR with SybrGree and SNPase DNA polymerase ("Bionem" Russia) which can extend a perfectly matched primer only. The obtained results have showed that the used enzyme (SNPase) provides allele high-specific amplification and can be used for SNP genotyping. In future we proposed to use this high specific enzyme for large-scale high-throughput SNP genotyping.
Application of the described method and enzyme for SNP diagnostics will be an important step toward development of the individualized medicine in Russia.

P1132. Studying Alleles Frequencies of 16 Short Tandem Repeats (STRs) Zones in Iranian Population for the First Time

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Using of molecular methods such as DNA typing has a special place in studying populations and identification of anonymous people and corpses.
Tandemly repeated DNA sequences, which are widespread throughout the human genome, are polymorphic in nature, making them important genetic markers for mapping studies, disease diagnosis, populations consideration and human identity testing. Short tandem repeats (STRs) contain repeat units that are 2-6 bp in length and can be readily amplified with the polymerase chain reaction (PCR). STRs have become popular in human genetic laboratories because low amounts of DNA, even in a degraded form, can be successfully typed. Sample mixtures can be more readily resolved with STR results than with previously used DNA typing technologies.
We have used Genetic Analyzer device to determine STR profiling of 400 DNA samples from Iranian population of different ethnic origin.
For this subject, after DNA extraction from samples by selecting molecular markers and using molecular methods such as PCR, specific places in DNA must be reproduced to obtain specific personal profile by examination and sequence determination.
Following running of the above system which is based on Capillary Array and Fluorescent Labeled Primers, the frequency of different alleles over different Iranian populations, determination of paternal relationship, diagnosis as a consequence of natural catastrophes like flood, earthquake as well as unnatural events like war.
More over, studying Iranian populations will help approved researchers to develop new and better ways of preventing, diagnosing and treating different illnesses.

P1133. Ancestry Informative Markers (AIMs) in Amerindians from Brazilian Amazon

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Ancestry Informative Markers (AIMs) are genetic loci with large frequency differences between the major ethnic groups and are very useful in admixture estimation. However, their frequencies are poorly known within South American indigenous populations, making it difficult to use them in admixture studies with Latin American populations, such as the tri-hybrid Brazilian population. To minimize this problem, the frequencies of the AIMs FY-null, RB2300, LPL, AT3-I/D, Sb19.3, APO and PV92 were determined via PCR and PCR-RFLP in four tribes (n=309) from Brazilian Amazon (*Tikúna*, *Kashinawa*, *Baníwa* and *Kanamari*), in order to evaluate their potential for discriminating indigenous populations from Europeans and Africans, as well as discriminating each tribe from the others. Although capable of differentiating tribes, as evidenced by the exact test of population differentiation, a neighbor-joining tree suggests that the AIMs are useless in obtaining reliable reconstructions of the biological relationships and evolutionary history that characterize the villages and tribes studied. The mean allele frequencies from these AIMs were very similar to those observed for North American natives. They discriminated Amerindians from Africans, but not from Europeans. On the other hand, the neighbor-joining dendrogram separated Africans and Europeans from Amerindians with a high statistical support (*bootstrap* = 0.989). The relatively low diversity (G_{ST} =0.042) among North American natives and Amerindians from Brazilian Amazon agrees with the lack of intra-ethnic variation previously reported for these markers. Despite genetic drift effects, the mean allelic frequencies herein presented could be used as Amerindian parental frequencies in admixture estimates in urban Brazilian populations.

P1134. Haplotypes in SLC24A5 gene as Ancestry Informative Markers in different populations

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Although it has been hypothesized that many genes contribute to produce different color shades in skin pigmentation, almost nothing is known about the biology of these genes. Recent work shows that the SNP rs1426654 within the SLC24A5 gene encoding for a protein belonging to the family of potassium-dependent sodium/calcium exchangers varies in frequency among several population samples according to skin pigmentation. Because of these observations, rs1426654 together with two additional intragenic markers (rs2555364 and rs16960620) have been evaluated in 471 unrelated individuals originating from three different continents (Africa, Asia and Europe), as Ancestry Informative Markers (AIMs) for forensic and evolutionary purposes.
This study supports the role of human SLC24A5 gene in skin pigmentation suggesting that variations in SLC24A5 haplotypes can correlate with human migration and ancestry. The most of haplotypes as well as combined genotypes with unknown phase analyzed exhibited dissimilar frequencies between different populations. For example

samples homozygous for the allele 2 at the rs2555364, homozygous for the allele 1 at the rs1426654 and homozygous for the allele 1 at the rs16960620, on the basis of present data could unlikely have African ancestry and show 9% and 91% of possibility to have Asian and Italian ancestry respectively. This analysis also revealed 8 combined unphased genotypes selectively found in Asian populations representing the 17.2% of the total. Haplotype analysis of SLC24A5 improved previous single allele results and can provide significant information about ancestry of unknown samples.

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P1135. Y chromosome analysis in subpopulations of Bashkirs from Russia

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The Volga-Ural region which is located between Europe and Asia has been the arena of permanent genetic exchanges among Siberian, Central Asian, Eastern European populations. We have sampled seven Bashkir subpopulations from different parts of the Volga-Ural region and neighboring areas of Russia: Orenburg (N=79), Perm (N=72), Samara and Saratov (N=51), and from Bashkortostan Republic: Abzelilovskiy (N=152), Sterlibashevskiy (N=54), Baimakskiy (N=95), and Burzaynskiy area (N=82). These samples are currently being analyzed using 24 diallelic markers of Y-chromosome (M89, M9, M20, M48, M73, M130, M170, M172, M175, M201, M207, M214, M217, M231, M253, M269, M306(M173), P15, P37, P43, SRY1532, Tat, 92R7(M74), 12f2). According to our preliminary findings Turkic-speaking Bashkirs are characterized by prevalence of R1b3 and R1a lineages. Among all subpopulations, Perm and Baimakskiy area represent with hight frequency (0.748 0.769,).It indicate there closeness with West European populations. Haplogroup R1a have frequency value 0.486 in Samara and Saratov's Bashkirs and frequency value 0.370 Bashkirs from Sterlibashevskiy area. The N3 characterize for subpopulation Bashkirs from Sterlibashevskiy area (0.537), Orenburg (0.342). Bashkirs from Abzelilovskiy area have main frequency (0.474). These differences possibly indicate that different subpopulations of Bashkirs have different origin. We found that Bashkirs from Perm district were characterized by relatively low genetic diversity, which could be explained by founder effect. Bashkirs from Orenburg region which are anthropologically closer to Ugro-Finnic populations are characterized by high frequency of N3 haplogroup. We will try to compare our results with archeologycal, historycal and anthropological data in discussed about of origin of different groups Bashkir

P1136. Studying unanimous role of MCH, MCV and HbA2 blood factors on the affecting possibility to beta thalassemia in south west of Iran.

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Thalassemia disease is the most common human disorder in the world, and controlled by one gene. Thalassemias are group of Hemoglobin(Hb) synthesis disorders. It is basal deficiency is in the reduction of β and α chains synthesis. β thalassemia (βT) as an autosomal recessive disorder increases in familial marriages. Today about 220 different mutations that causes βT have been known this disorder affect the blood indexes of defected people like CBC and Hb electrophoresis factors and cause them to increase or decrease out of normal ranges. Some of the impressed factors are HbA2, MCV and MCH. In βT patients the mount of HbA2 factors are higher and MCV and MCH factors are lower than the limit. Khuzestan state in the south west of Iran close to Persian golf with about 4 million people has the fourth degree in βT prevalence. In this study blood factors of 2000 people (1000 couple) from who referred to the counseling centre and genetic laboratory of welfare organization of Ahwaz were studied. This research had taken place at years of 2003 to 2005. The age of men and women were between 21 to 31 and 17 to 27 respectively and noticeable number of them had abnormal ranges for mentioned factors using statistical and logistic regression method for diagnosis probability of normal and abnormal individuals. Achieved data from normal peoples and liable individuals to βT show the MCV range for Iranian peoples is lower than internationl standard suggestion and it is 77 .

P1137. Improved beta-thalassemia genotyping by means of population-specific reverse-hybridization teststrips

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Beta-thalassemia is among the most common inherited diseases throughout the Mediterranean area, parts of Africa, the Middle East, India and Southeast Asia. Mutations in the beta-globin gene may lead to structural abnormalities (e.g. Hb S, Hb E, Hb C) or to haemoglobin imbalance due to the reduced synthesis or complete absence of beta-globin chains. In each at-risk population beta-thalassemia results from a limited number of common mutations and a larger, more variable number of rare mutations.
We have improved an existing reverse-hybridization assay (Beta-Globin StripAssay), originally designed for Mediterranean countries, to make it more globally applicable. Three separate teststrips, specific for the most prevalent mutations in Southeast Asia, the Middle East plus India and the Mediterranean region, have been designed. Each teststrip comprises 22 variants and represents an allele coverage of >90% in the respective area. Comprehensive beta-thalassemia genotyping is achieved by a single multiplex DNA amplification reaction and subsequent hybridization to the adequate teststrip. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be automated using robotic instrumentation. Proprietary software (StripAssay Evaluator) is available to scan, interpret and electronically archive StripAssay results. The test is simple and convenient, and requires only very small amounts of samples, which is of particular importance for prenatal diagnosis. The broad range of beta-thalassemia mutations covered by the extended StripAssay should make it an attractive and globally useful diagnostic tool. (oberkanins@viennalab.co.at)

P1138. Admixture dynamics in Brazilian afro-derived populations revealed by genetic and demographic data

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Brazilian remnant quilombo populations (Afro-derived) have heterogeneous origins depending on their region and founding groups. The Northeast semi-isolated communities of Barra (BR) and São Gonçalo (SG) exhibit reduced migration rates and high frequency of consanguineous marriages. The Southern isolated community of Valongo (VAL), formed by only seven black and one white individual, exhibit a high degree of intermarriage and a very low migration rate. Four AIMs (Ancestry Informative Markers; AT3-indel, Sb19.3-Alu, APO-Alu and PV92-Alu) and the allele CYP1A1*2C were used to investigate the histories of formation of BR (n=39), SG (n=28) and VAL (n=29). We have also examined the urban population samples of Jequié (n=44) and Florianópolis (n=31), and indigenous tribes from Brazilian Amazon (n=300). Genotyping was performed by PCR, PCR-RFLP, PAGE 6% and silver staining. Statistical analysis were performed by GENEPOP, GDA and ADMIX. CYP1A1*2C was observed in Brazilian amerindians (95%), europeans (2,8-5,8%) and africans (1,3%). Associations between unlinked markers (p<0,05) indicate a recent admixture in

SG (African/European=79%/21%, R2=0.983) and BR (68%/32%, R2=0.989). These results are not in agreement to demographic history and previous studies showing tri-hybrid admixture, which may be explained by the different nature of markers analyzed. On the other hand, those associations were not observed in VAL, probably due to its isolation and no recent admixture. The coefficient of local inbreeding (Fis=-0,136, p<0,05) indicate endogamous marriages. Admixture estimates indicate an equal african/european contribution (50%, R2=0.997) and the absence of CYP1A1*2C indicates no Amerindian contribution. These results corroborate the demographic history of formation of the VAL community.

P1139. NOTCH3 mutations in Portuguese patients with Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL): implications for diagnostic strategies

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CADASIL is caused by mutations of the Notch3 gene, on chromosome 19p13. A cluster of mutations in exons 3 and 4 was originally reported and molecular analysis restricted to these exons has been suggested for diagnostic purposes. We aimed to identify the spectrum of Notch3 mutations in Portuguese patients with CADASIL and set up an algorithm for sequential exon analysis.

One hundred consecutive patients with the clinical diagnosis of CADASIL or, at least, one of its typical clinical or neuro-radiologic features, were included in this study. DNA was extracted from leukocytes and amplified by PCR according to standard methods.

Based on published mutation data, sequence analysis of exons 3, 4, 5/6, 11, 18/19 and 22/23 was done in all patients and in exons 2, 8, 14 and 20 only in negative cases with strong clinical suspicion of CADASIL.

Ten pathogenic mutations were found in 27 patients of 16 different families: p.R141C, p.R153C, p.R169C, p.R207C (exon 4); p.G420C (exon 8); p.R558C, p.C568Y, p.R578C (exon 11); p.W1028C (exon 19); p.C1099Y (exon 20). Mutations p.R153C, p.R207C and p.R558C were found in more than one family. Mutations p.C568Y, p.W1028C and p.C1099Y have not been previously described.

About 80% of Notch3 mutations in Portuguese patients with CADASIL were found in exons 4, 11, 18/19, but these comprise only ~58% of mutations reported worldwide. Limited screening of exons 3 and 4 is clearly not appropriate in CADASIL cases of Portuguese origin. The weak criteria for patient inclusion may explain the low rate of Notch3 mutations found.

P1140. CC-Chemokine receptor CCR5 and Hereditary haemochromatosis mutations associations in viral hepatitis C patients in Latvia.

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Background. CC - chemokine receptor CCR5 has a critical role in regulating T cell functions by mediating recruitment, polarization, activation and differentiation of antiviral type 1 cytokine secreting T helper and cytotoxic T cells. As this receptor is involved in T cell mediated immune response it could be involved in susceptibility of viral infections and later to modify organism response to antiviral therapy. Hereditary haemochromatosis (HH), which is characterized with abnormal body iron storage, has been suggested to be one of VHC worsening factors. The most frequent mutations are C282Y, H63D.

Objectives. Detect frequency of Δ32 CCR5, C282Y, H63D mutations between VHC patients in Latvia and to compare its frequency in healthy individuals of neighboring populations.

Subject and methods. This study included 63 individuals with chronic viral hepatitis C (VHC), which where discovered in Infectology Center of Latvia. Mutations were detected using PCR and RFLP.

Results. The frequency of Δ32 CCR5 between VHC patients is 20 to 100 alleles (in Lithuania 11.3 to 100 alleles, in Russia - 13.9 to 100 alleles). C282Y frequency is 6 to 100 alleles, H63D is 7 to 100. There was no correlation found between Δ32 CCR5 and HH mutations.

Conclusion. Among VHC patients Δ32 CCR5 is more frequent than in neighboring populations of Latvia, suggesting necessity to continue study including clinical data and possible replay to antiviral therapy. Δ32 CCR5 and HH mutations are mutually independent worsening factors of VHC.

P1141. The need for a well characterised UK Control Population

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There have been many problems replicating significant associations between gene variation and complex diseases and population structure is widely considered to be the most significant reason. To address this problem, there are statistical methods available that attempt to allow for the effect of population structure. However, these are not really satisfactory and so the only suitable alternative is to design the studies with greater care. A powerful approach may be to characterise genetically both the cases and controls. Individuals from the controls can then be chosen to match the cases so as to minimise the stochastic differences between the two populations.

We are assembling a UK control population as a resource for future studies. It will comprise 3,500 samples (1,700 collected so far), which will have been carefully selected from throughout the UK. Rural regions are targeted to avoid the admixture observed in large urban environments and volunteers are sought who were born in the same place as their parents and grandparents to ensure historical integrity. The collection will be genotyped for around 3,000 markers, with the aim of identifying about 200 ancestrally informative markers, which will then be used to match controls to cases.

An initial pilot project on about 400-500 samples, using a variety of markers, indicates that this approach is valid. MC1R data suggest structure differentiating the Celtic Fringe from Eastern England, whilst NRY data show evidence of Norse incursions into Orkney. Preliminary admixture analyses suggest that there is an east-west gradient of Anglo-Saxon ancestry across England.

P1142. Investigation of COX-2 polymorphisms among different Iranian ethnic groups

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Cyclooxygenases-2 enzyme (COX-2) converts arachidonic acid to prostaglandins. COX-2 plays an important role in inflammation and carcinogenesis. It is shown that COX-2 -765G > C promoter polymorphism have lower promoter activity and result in decreased COX-2 expression. Also, it is reported that this type of polymorphism may have important role in increased risk of several types of cancers. In addition, genetic polymorphisms of COX-2 gene could alter enzyme expression and the response to Nonsteroidal anti-inflammatory drugs (NSAIDs). In this investigation, we assessed allele frequency of COX-2 polymorphism among different Iranian populations. The genetic polymorphisms of COX-2 -765G > C promoter has not been reported among the Iranian population so far. We initiated a study to examine COX-2 -765G > C promoter genotype in different Iranian ethnic groups. The samples were collected from healthy population from three different ethnicity groups (Fars, Turk and Rashti). We assessed the genotype patterns of *COX-2* among Iranian Fars, Turk and Rashti ethnic groups in five regions. The COX-2 -765G > C promoter genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) analysis in 90 Iranian healthy individuals. Allele frequency of COX-2 -765G > C promoter for above populations were similar and the G allele was significantly higher than C allele.

P1143. Genetic polymorphisms associated with cardiovascular disease in the Azorean population

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Background: Research on cardiovascular disease (CVD) has revealed several candidate genes associated with the worlds first cause of dis-

ability and mortality. The global distribution of the genetic polymorphisms associated with cardiovascular diseases has been widely investigated and regional variations have been observed. The Azorean population is known to be of heterogeneous origin and represents the Portuguese region with the highest mortality rate caused by CVD. Based on this fact we have analysed allelic and genotypic frequencies in 10 candidate genes.
Material and Methods: A representative sample of the population was obtained by randomly recruiting 155 unrelated subjects from Terceira Island. Genomic DNA was extracted from whole peripheral blood by *salting-out.* Identification of genetic polymorphisms was performed by polymerase chain reaction (PCR) followed by reverse hybridization (CVD StripAssay, ViennaLab Diagnostics GmbH). Genotype distributions were tested for Hardy-Weinberg equilibrium. Chi-square tests were used to evaluate statistical significant differences between our population and other Caucasian populations.

Results: Allelic and genotypic frequencies are presented in table 1.

Genetic polymorphism	Alleles	%	Genotypes	%
FV G1691A (leiden)	A	0.97	GA AA	1.94 0.0
FV H1299R (R2)	R	9.68	HR RR	18.06 0.65
PTH G20210A	A	1.94	GA AA	3.87 0.0
FactorXIII V34L	V	75.48	VL VV	41.29 54.84
FGB-455G-A	A	18.06	GA AA	30.97 2.58
PAI-1 4G/5G	4G	45.48	4/5 4/4	43.23 23.87
HPA-1a/b	b	18.71	ab bb	28.39 4.52
MTHFR C677T	T	30.97	CT TT	46.45 7.74
MTHFR A1298C	C	32.58	AC CC	47.10 9.03
ACE I/D	D	61.61	ID DD	53.55 34.84
ApoB R3500Q	Q	0	RQ QQ	0 0
Apo E	E2 E3 E4	6.13 81.29 12.58	2/2 2/3 2/4 3/3 3/4 4/4	1.29 9.03 0.65 65.81 21.94 1.29

Conclusion: We report the prevalence of various genetic CVD risk factors for the first time in the Azorean population. The frequencies obtained in our population are within the range of frequencies reported on other European populations. The high frequency of cardiovascular disease may be possibly related with other genetic or environmental factors not evaluated in this report.

P1144. Carrier frequency of cystic fibrosis transmembrane conductance regulator gene mutations in the general Moroccan population

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OBJECTIVE: Epidemiology of cystic fibrosis (CF), the most common lethal genetic disease in the Caucasian population, is poorly known in North African populations. No data is available on the native Moroccan population. We aimed to identify possible CF carriers in a sample of the general Moroccan population. METHODS: Blood samples from 150 unrelated healthy Moroccans with no symptoms of CF were tested for 33 frequent CF mutations and the intron 8 polyT variant using a commercial assay. RESULTS: Two subjects were found to be carriers for the F508del mutation and eight others were heterozygous for the (T)5 variant of intron 8. CONCLUSION: These findings indicate that the Moroccan population is at risk for CF and related diseases. The CF incidence could be in the range of that found in European populations. Larger studies are necessary to identify the clinical pattern and accurately determine the incidence and the molecular basis of CF in Morocco.

P1145. The frequency of CFTR mutations at patients from Romania - a collaborative study

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Objectives: The aim of this study was to estimate the frequency of cystic fibrosis mutations detected in patients from the National Center of Cystic Fibrosis from Timisoara (NCCFT).
Method
Based on clinical findings and pathological sweat test values, 120 patients (240 alleles) were selected. DNA was isolated from biological samples (blood, amniotic fluid) and the genetic analysis was performed using the Elucigene CF29 kit, direct PCR products sequencing and RFLP. 74 patients were investigated in collaboration with Royal Manchester Children's Hospital - Genetic Unit (UK) and 46 patients by our own laboratory (Biochemistry Department - UMF Timisoara)
Results
21 CFTR mutations (ΔF508, 2183 AA>G, W1282X, 1898+1G>A, I148T, 621+1G>T, G576X, 1717-2A>G, R553X, R735K, CFTRdel2,3(21kb), G542X, G817V, 3272-26A>G, 457TAT>G, 3849+10kbC>T, R1162X, N1303K, 3849G>A, S1235R, R117H) and four polymorphic variants - 2694T/G, 2694T/C, IVS8-5T, IVS8-7T were identified (2 new mutations - R735K and 1717-2A>G).
Conclusions
The most frequent mutations in Romania are ΔF508 (51,66%), G542X (2,91%), 621+1G>T (1,65%) and N1303K (1,25%). The rest of non-ΔF508 mutations had a frequency lower than 1%. The results show a similarity with the situation from Western Europe and the rest of the world where the most frequent mutations are also ΔF508 and G542X. However, the lower frequency for ΔF508 mutation and the great number (30%) of the alleles who could not be identified (from heterozygous patients with one identified mutation) are probably due to the genetic heterogeneity of Romanian population and to incomplete genetic analysis for rare mutations.
(Study performed through Research Grants - CEEX 5855 and CNCSISA/1188/2004-2006)

P1146. Genetic variation at D3S1358, D5S818 and D13S317 loci in Russian Siberian population

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STR loci represent a rich source of highly polymorphic markers for medical, forensic and population studies. There are notable differences in allele frequencies and heterozygosities between population groups. We present here characteristics of the allelic polymorphism for three STRs loci: D3S1358, D5S818 and D13S317 in Russians (Caucasians) living in South-West Siberia.
Allele typing was performed using PCR and subsequent high-resolution PAAG electrophoresis. We have analysed DNA samples from unrelated subjects: 262 individuals were tested for D3S1358, D13S317 STR-systems and 360 individuals were tested for D5S818. Eight alleles were identified in D3S1358 (13-20 repeats, 118-146 bp) and D13S317 loci (8-15 repeats, 169-197 bp); and nine alleles were note in D5S818 (7-15 repeats, 134-166 bp) locus. Genotype frequency distributions were consistent with Hardy-Weinberg equilibrium for every STR-systems. The level of observed heterozygosity was high: 0,821 (D13S317), 0,782 (D3S1358), and 0,769 (D5S818). Polymorphism information content (PIC) and discrimination power (pD) were: PIC=0,76, pD=0,923 for D13S317; PIC=0,74, pD=0,913 for D3S1358; and PIC=0,70, pD=0,891 for D5S818; power of exclusion (W) and marching probability (pM) - 0,638 and 0,077 in D13S317; 0,567 and 0,087 in D3S1358; 0,544 and 0,109 in D5S818, consequently.
The frequency data obtained can be used for comparison to other populations. Forensic efficiency data suggest that investigated markers D3S1358, D5S818 and D13S317 are very discriminating in Russian Siberian population.

P1147. Social aspects of deafness in the Republic Altai (south Siberia, Russia)

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Hereditary hearing impairment is a heterogeneous disability with different pattern of inheritance. The *GJB2* (Cx26) mutations account for more than a half of recessive deafness in many populations. Prevalence of particular *GJB2* mutations depends on ethnic origin, specific structure and history of population. Specific social traditions, e.g. the assortative marriage rates among deaf people, have been suggested to influence frequency of Cx26 deafness. Previously, we revealed the *GJB2* mutational diversity in Altai Republic which population (~200,000) includes three major ethnicities: indigenous Altaians (~60,000), Russians (~120,000), and Kazakhs (~12,000). Mutations c.35delG and c.235delC were found to be causative factors among Russian and Altaian patients, respectively. Cx26 deafness was detected in considerable proportion of affected Russian families whereas many Altaian multiplex families were found to be Cx26-negative. High carrier rate of c.235delC was detected in Altaian hearing controls. This study firstly evaluates the marriage patterns in deaf and hard hearing population in Altai Republic. Informative data was obtained on 112 adults with early onset of hearing impairment equally represented citizens (57) and villagers (55). Rural people constitute 3/4 of total Republic Altai population whereas the others live in the capital Gorno-Altaisk, the only city in the Republic. We revealed assortative mating rate of 0.45 in rural sample, much lower as compared to 0.84 in urban sample. Ethnic diversity, traditional Altaian marriage pattern reducing consanguinity rate, and social differences between urban and rural population could influence the prevalence of deafness in Republic Altai.

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P1148. Case-Control Study of Libyan and Maltese Patients with Type II Diabetes Mellitus

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Type II Diabetes Mellitus (DMTypeII) is a common disease with onset in middle-aged individuals, caused by an imbalance between insulin production and action. Single nucleotide polymorphisms (SNPs) and mutations in different genes may be implicated in developing DMTypeII. In this study we analyzed 9 such genes that include IPF, MTHFR, mitochondrial tRNA, Resistin, PPP1R3, ADRABbeta2, MIF, PTPN1 and TLR4. SNPs were chosen from each gene according to stringent criteria based on developing DMTypeII in other populations. SNPs were genotyped in the Libyan and Maltese patients and compared with healthy Maltese citizens. DNA was extracted from whole blood, and genotyping of each gene determined by PCR-RFLP. Concurrently, pools of DNA from random Maltese newborn were carried out using fluorometry for accurate quantification. All genes were in Hardy-Weinberg Equilibrium and statistical analysis was carried out by SPSS (student package 12). Chi square analysis of all data in between populations and across populations revealed a significant association of the ADRABbeta2 gene of both Libyan and Maltese DMTypeII patients with healthy Maltese controls ($p < 0.05$ for both). There is no difference between Libyan and Maltese Diabetics ($p=0.07$) indicating that this gene has a common predisposition to both populations. On the other hand, IPF gene was only associated with the Libyan DMTypeII and not with the Maltese population ($p < 0.05$). All other genes were not statistically significant associated with DMTypeII. The results show a strong association of the ADRABbeta2 (Arg16Gly) and IPF (missense mutation Cys18Arg) genes with DMTypeII.

P1149. Cytogenetic and cancerogenic effects of low dose radiation among liquidators in the Chernobyl accident area

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The purpose of the presented study was to estimate influence of the absorbed dose values on the cytogenetic effects and risk of malignant formation (MF) in group of liquidators.

The method of "internal comparison" made it possible to study epidemiological parameters in dependence with the documented exposure doses for 17 thousand liquidators. The cytogenetic examinations of 500 liquidators were carried out. The dose dependence of MF frequency was studied with the application of piecewise-linear splines and hypothesis about the equality of two probabilities on the basis of the statistical criterion *2S*.

Tendency toward reduction in the frequency of MF with increase of the dose in the interval of 1-85 cGy for both the age groups (younger and older than 40 years) was observed. Probability of random event when the liquidators with MF were exposed to dose in the ranges from 1 to 3 cGy or from 3 to 5 cGy statistically significantly exceeds the appropriate probability for the whole cohort . Statistically significant differences in the probabilities for other classes of diseases within dose ranges were not observed. In spite of the promote terms of cytogenetic analysis and partial elimination of dicentric chromosomes from blood „dose- effect" dependence for aberrations remains in the group of liquidators with MF.

Our cytogenetic investigations revealed the tendency to increasing of MF frequency in group of liquidators exposed to low doses. The retention of „dose- effect" dependence for the dicentrics in lymphocytes of liquidators with MF within promote postradiation periods was established.

P1150. Allele frequencies for the fifteen short tandem repeat loci in Croatian population

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We have analyzed the distribution of allele frequencies at fifteen autosomal short tandem repeats loci in the representative sample of Croatians. A total of 110 unrelated individuals (Caucasians) born in Croatia have been sampled for the analysis. All of them have been voluntary donors. Buccal swab have been used as the DNA source. Specimens were air-dried, placed in 1,5 ml tubes, and immediately transported to the laboratory. The samples have been stored at -20°C until beginning of DNA analysis. The Qiagen Dnaeasy™ Tissue Kit was used for DNA extraction. The AmpFlSTR® Identifiler® (ABI, Foster City, CA) has been used to simultaneously amplify by PCR 15 STR loci. The STR loci are: D3S1358, TH01, D21S11, D18S51, D2S1338, D5S818, D13S317, D7S820, D16S539, CSF1PO, D19S433, vWA, D8S1179, TPOX, and FGA. Similar amount of DNA (approx. 1 ng) was used in all PCR reactions. Total reaction volume was 12,5 µl. The PCR amplification has been carried out in PE Gene Amp PCR System Thermal Cycler (ABI, Foster City, CA) according to the manufacturer's recommendations. Electrophoresis of the amplification products was preformed on an ABI PRISM 3130 genetic analyzer Raw data have been compiled, analyzed and numerical allele designations of the profiles were obtained by using the accessory software: ABI PRISM® Data Collection Software v3.0 and GeneMapper™ ID Software v3.1. Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination and power of exclusion were calculated. Also, we have compared our data with data obtained from geographically neighboring European populations.

P1151. Gene expression variation of HSA21 genes in normal and Down syndrome (DS) individuals: understanding phenotypic variability in DS patients.

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Down Syndrome (DS) individuals display considerable phenotypic variability. Since DS is a disease caused by alterations of gene dos-

age, gene expression variation of human chromosome 21 (HSA21) genes in DS is likely to have a substantial impact on the penetrance of DS phenotypes.
We studied gene expression variation in 14 lymphoblastoid (LCLs) and 17 fibroblast cell lines from DS individuals and an equal number of age, sex and ethnic matched controls. Gene expression was assayed using Taqman qRT-PCR on a total of 100 and 106 HSA21 and on 26 non-HSA21 genes in LCLs and fibroblasts respectively.
Around 42% and 62% of genes in LCLs and fibroblasts were significantly overexpressed in the DS population (KW, p<0.005). According to the degree of overlap in the distribution of expression values between euploid and trisomic individuals, we could broadly classify genes in three categories: (a) genes with non-overlapping distributions (b) genes with partially overlapping distributions (c) genes with largely overlapping distributions. We hypothesize that genes in category (a) are the most sensitive to the extra copy of HSA21 and thus probably involved in the constant features of trisomy 21, those in category (b) might be involved in the determination of variable DS traits, whereas those in category (c) are not dosage sensitive and are less likely to be involved in DS pathology.
This study provides the first extensive data set on HSA21 gene expression variation in DS and addresses its potential role in underlying phenotypic variability observed in this disorder.

P1152. Birth of child with numerical chromosomal aberrations as a possible consequence of irradiation of vulnerable stages of human oogenesis

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Surveillance of Down syndrome (DS) prevalence at birth has been performed in Belarus since 1981 using a population-based registry of congenital malformations. A significant cluster was observed in January of 1987, 9 months after the Chernobyl accident (31 cases versus 13.2 expected, $P<10^{-3}$). The registered increase was highest in Gomel oblast, the mostly contaminated region (8 cases versus 2.4 expected, P<0.05). The period of birth of the cases fits with an estimated time of conception within the first two weeks after Chernobyl accident, period of highest radiation dose rates (1-3 orders of magnitude over the natural background level).
The stochastic nature of the cluster cannot be completely excluded. However, experimental data provide evidence that the "diakinesis" stage of mammal oogenesis immediately presiding conception is a period a high sensitivity to ionizing radiation. Several epidemiological studies have detected enhanced prevalence rates among newborns conceived within the periods of increased radioactivity i.e. after atmospheric nuclear weapon tests and Chenrnobyl accident.
Thus, enhanced risk of giving birth to aneuploid child by woman exposed around the time of conception could be suspected. Therefore it seems reasonable for women planning a pregnancy to restrict contacts with sources of ionizing radiation at least in the middle of ovarian cycle as well as to recommend to postpone conception in case of exposure of the lower part of abdomen and organs located in small pelvis within the given time period. Highly efficient multi-parametric prenatal screening programs might be recommended to women at supposed high risk.

P1153. The G894T polymorphism of the eNOS gene and atherosclerosis precursors in Serbian child population

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As it is known, nitric oxide play an important role in physiological regulation of vascular tone and blood pressure. Endothelial nitric oxide synthase (eNOS) is the key enzyme responsible for basal vascular wall production of nitric oxide. Some data suggest eNOS role in serum lipide level also. The G894T polymorphism at exon 7 in the gene encoding for eNOS is associated with altered plasma nitric oxide levels.
The aim of this study was to determine G894T genotype in 467 healthy children 15 years old and investigate a possible influence of this polymorphism on blood pressure, BMI and serum lipid level. G894T polymorphism was genotyped by PCR amplification of specific fragment of genomic DNA followed by digestion with the Mbo I restriction enzyme. The mean values of blood pressure (systolic and diastolic), BMI and serum lipid level (LDL cholesterol, HDL cholesterol and triglycerides) between different genotypes were compared by analysis of variance (ANOVA). We detected wild type GG genotype in 186 (39, 9%), heterozygous GT in 210 (45,0%) and homozygous mutant TT in 71 (15,1%) children. Multiple comparisons showed significant difference (p < 0,05) of diastolic blood pressure between TT and GG or GT group of children (74,1±8,42 mmHg, 72,9±8,42 mmHg and 71,6±8,4 mmHg respectively) . Also, TT genotype was associated with significantly lower HDL cholesterol (F= 4,759, p<0, 009) and triglyceride (F= 3,380, p< 0,035) level. We did not find significant association between the mutant TT genotype and systolic blood pressure or BMI in our group.

P1154. Investigation of the eotaxin gene -426 C>T, -384 A>G AND 67 G>A SNPs and atopic dermatitis in Italian children using family-based association methods.

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To learn whether eotaxin gene single nucleotide polymorphisms (SNPs) are associated with the atopic dermatitis, we investigated the genotype and allelic frequencies of -426 C>T, -384 A>G, and 67 G>A SNPs in 130 Italian families.
From these families, we recruited 130 Italian children suffering from either allergic extrinsic form of atopic dermatitis (EAD) or intrinsic non-allergic form of atopic dermatitis (IAD). Genotyping was performed using the PCR-RFLP method and automatic sequencing.
A significant difference was observed in the genotype frequency of -426 C>T SNP between EAD and IAD children (p=0.01); and between EAD and controls (p= 0.01). The frequency of this SNP was no different between the IAD children and the control group (p=0.52). In the EAD children, the frequency of -426 C>T SNP was no different between the groups of mild, moderate and severe SCORAD Index (p=N.S.).
The -426 C>T polymorphism was significantly associated with the relapse of the disease (p<0.01) in the EAD children. No significant association was observed between the -384 A>G and 67 G>A SNPs and the two groups of extrinsic and intrinsic atopic dermatitis children in respect to control group. In 48 trios selected from 68 EAD families, the transmission disequilibrium test (TDT) showed a preferentially transmission of -426 T allele from the parents to affected offspring.
In our Italian children, the -426 C>T of the eotaxin gene was associated with extrinsic atopic dermatitis. Therefore, the eotaxin gene might contribute to a characteristic "signature" for the two types of AD.

P1155. The load of Mendelian pathology in the Siberian indigenous populations

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Summarized data of medical genetic study of some Siberian indigenous populations (tuvinians, altayans and khakasians) are presented. Uniformity of the methodical principles of investigation allows comparing the load of hereditary diseases between the populations. The load of diseases with autosomal dominant, autosomal recessive, and X-linked inheritance is, respectively, 0.58, 0.92 per 1000 people and 0.49 per 1000 men in tuvinian; 0.75, 1.22 per 1000 people and 0.11 per 1000 men in altayans; 1.09, 0.81 per 1000 people and 0.65 per 1000 men in khakasians. Rare genetics syndromes are observed in each population. However, familiar cases of microtia with meatal atresia and conductive deafness are accomulated predominantly in the tuvinians and altayans. Factors that play a role in the unequal distribution of hereditary diseases in Siberian indigenous populations are under way of investigation.

P1156. STR markers in ethnic admixture studies

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A small riverine community, Portuchuelo (8º37'S, 63º49'W) in the State of Rondônia, Brazil, was studied with the aim to ascertain its health conditions and the causes of the variability of some infectious diseases. Almost all individuals (181) of Portuchuelo were included in the sample. Data on 11 STRs markers along different autossomes (UNISTS: 19943; 21182; 67015; 74530; 67557; 53216; 81816; 14863; 82795; 45947; 63378) were used to determine its ethnic composition. The contributions of Amerindian, African and European genes to the ethnic composition of the studied population are shown bellow, as well as those estimates based on classic markers (Ferreira *et al.,* 2002). Although the ethnic proportion estimates are apparently different they do not show statistical significance. The face values of the standard errors of the ethnic proportion estimates are higher than those based on classical ones, as well as some goodness of fit tests of the genotype frequency distribution. Therefore, due to poorly studied ancestral gene frequencies, higher mutation rates and heterogeneity among systems, classical markers seems to provide better estimates of ethnic composition of tri-hybrid populations.

Ethnic composition of Portuchuelo, Brazil, based on STR and classical markers data.

Ancestry	*STR (P ± s.e.)*	*Classical (P ± s.e.)*	*t*	*p*
Ameridian	0.317 ± 0.087	0.444 ± 0.064	1.182	0.240
African	0.118 ± 0.059	0.210 ± 0.046	1.224	0.224
European	0.565 ± 0.106	0.346 ± 0.069	1.732	0.086

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P1157. How old is the Val30Met mutation in transthyretin familial amyloid neuropathy?

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Transthyretin familial amyloid polyneuropathy (FAP) is a severe autosomal dominant neuropathy of adulthood, linked to the pathogenic TTR-Val30Met variant. This mutation is the only one identified in Portugal where the affection was initially described and in Sweden the second most important area of FAP in Europe. The hypothesis of a common ancestor for Val30Met kindred has been debated for years. This study analyzed such hypothesis in Val30Met carriers from Portugal, Brazil and Sweden and estimated the occurrence of the most recent common ancestor (MRCA) in each population.

Individuals from 18 Portuguese and 15 Swedish families along with 28 unrelated probands (15 from Sweden, 13 from Brazil) and 66 controls were studied. Eleven microsatellites were genotyped, 13 Mb around *TTR* gene. To estimate the age of the MRCA, we performed haplotype reconstructions and applied a previously described likelihood based method. In Portuguese, the longest common haplotype was 237-143-271-291-311-202, between markers L1 and D18S47 (700 kb). The Brazilian shared the same alleles encompassing a shorter distance (215 kb) and bounded by microsatellites L1 and L9. In contrast, Swedish presented different alleles and a common haplotype between markers D18S49 and D18S47 (1500 kb). The length of the shared regions provided age estimates of 750, 350 and 650 years for Portuguese, Swedish and Brazilian Val30Met mutation, respectively.

Our work strengthens the hypothesis of two different founders in Portuguese and Swedish Val30Met carriers and suggested a Portuguese origin of the Brazilian mutation. Moreover, it allows estimate the age of the FAP-Val30Met variant in these populations.

P1158. STRs (*introns* 13 and 22) of Factor VIII gene in Brazilian populations: allele and haplotypic frequencies and forensic parameters

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Hemophilia A is a X-linked bleeding disorder caused by a defect in Factor VIII protein. Direct analysis of Factor VIII gene (FVIII) mutations is difficult due its great number and heterogeneity. Therefore, most of the tests for the detection of the carrier and prenatal diagnosis use the study of DNA polymorphisms linking with FVIII *locus*. We characterize here the distribution of allele and haplotypic frequencies of i*ntrons* 13 and 22 STRs of FVIII gene in samples of unrelated individuals of São Paulo (SP), Rio Grande do Sul (RS) and Pernambuco (PE) states. Genotyping was performed by PCR, denaturing PAGE 12% and 11,25% and silver staining. The *Intron* 13 distribution in the three samples studied here is centered in allele *Intron* 13*20, differing from Chinese and a sample of São Paulo city. *Intron* 22 distribution is centered in allele *Intron* 22*26, also differing from Asians and that São Paulo city. The more frequent haplotype based in *Introns* 13 and 22 for both populations are the 20/26. The haplotypes composed by alleles 24/25 and 21/24, respectively, do not occur in RS and SP samples, opposed to what it would be expected by the frequency of them in the other samples. The forensic parameters indicate that both the markers are significantly informative and can be used in forensic situations where the genetic identification is necessary, respecting the characteristics of being on to chromosome X and considering the use of markers genetically linked.

P1159. Detection of gene-environment interaction: a new test based on sibling recurrence risks

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Gene-environment interactions may play important roles in complex disease susceptibility but their detection is often difficult. Here we show how gene-environment interactions can be detected by investigating the degree of familial aggregation according to the exposure of the probands. In case of gene-environment interaction, the distribution of genotypes of affected individuals, and consequently the risk in relatives, depends on their exposure. We developed a test comparing the risks in sibs according to the proband exposure. To evaluate the properties of this new test, we derived the formulas for calculating the expected risks in sibs according to the exposure of probands for various values of exposure frequency, relative risk due to exposure alone, frequencies of latent susceptibility genotypes, genetic relative risks and interaction coefficients. We find that the ratio of risks when the proband is exposed and not exposed is a good indicator of the interaction effect. We evaluate the power of the test for various sample sizes of affected individuals. We conclude that this test is valuable for diseases with moderate familial aggregation, only when the role of the exposure has been clearly evidenced. Since a correlation for exposure among sibs might lead to a difference in risks among sibs in the different proband exposure strata, we also add an exposure correlation coefficient in the model. Interestingly, we find that when this correlation is correctly accounted for, the power of the test is not decreased and might even be significantly increased. An application to type 2 diabetes will be presented.

P1160. Genetic epidemiology of the *Trypamosoma cruzi* infection

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At least 100 million people are living in risk areas of *Trypanosoma cruzi* infection, around 3 million of them are living in Brazilian risk areas. It is estimated that 17 million are infected by *T. cruzi* infection. Usually, the areas of the *T. cruzi* infection are linked to poverty and poor housing conditions. The present sample comprised 4697 individuals belonging to 886 nuclear families originated mostly from the Northeastern Brazil at the ancient "Hospedaria de Imigrantes do Departamento de Imigra-

ção e Colonização do Estado de São Paulo" in the early 70´s of the last century. From the collected data, information on gender, familial relationship, housing conditions, age and serological diagnostics were used. Statistical analyses were performed using some lab made programs, as well as SPSS and POINTER programs. Multiple regression analyses showed that gender have no influence on *T. cruzi* infection, while age and housing conditions presented significant effects. Correlation of related pairs of individuals, showed consistent positive and significant values, indicating familial aggregation. Complex segregation analysis, suggested that the best model is the dominant one, but the environmental influence cannot be excluded. The current study provides evidence of a major gene for *T. cruzi* infection, suggesting that it is an appropriate trait for further genetic analysis on the causal factors acting on the variability of Chagas disease.

Model	*d*	*t*	*q*	*H*	*τ1*	*τ2*	*τ3*	*-2 ln L*	χ^2	*P*	*test*	*ep*	*AIC*
1.Mixed*	1.000	2.096	0.605	0.421	[1]	[0.5]	[0]	672.40	-	-	-	4	680.397
2.Sporadic	[0]	[0]	[0]	[0]	-	-	-	838.63	166.238	0.000	2vs1	0	838.635
3.No major effect**	[0]	[0]	[0]	0.995	[1]	[0.5]	[0]	696.37	23.974	0.000	3vs1	1	698.372
4.No multifatorial	0.097	3.250	0.296	[0]	[1]	[0.5]	[0]	712.27	39.869	0.000	4vs1	3	718.266
5.Recessive (d=0)	[0]	2.389	0.383	0.279	[1]	[0.5]	[0]	682.78	10.381	0.001	5vs1	3	688.778
6.Aditive (d=0.5)	[0.5]	2.782	0.454	0.019	[1]	[0.5]	[0]	674.09	1.688	0.194	6vs1	3	680.085
7.Dominant (d=1)	**[1]**	**2.096**	**0.605**	**0.421**	**[1]**	**[0.5]**	**[0]**	**672.40**	**0.000**	**1.000**	**7vs1**	**3**	**678.397**
* „d" value was set to upper limit. **Model estimated by lower „-2 ln L".													

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P1161. Genetic-epidemiology study of reported number of malaria episodes in a sample from Monte Negro, RO, Brazil.

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Malaria is one of the main human infectious disease that affects 300 to 500 million people per year, causing the death of 1.5 to 2.7 million people in Africa, Asia and the Americas. In Brazil around 70% of the cases are due to *Plasmodium vivax* infection. The reported number of malaria episodes was studied on a sample of 924 individuals from Monte Negro/RO, Brazil (malaria hypo-endemic region) in order to test previous reports on a riverine population from the Western Amazonian Region (Portuchuelo, RO). The effects of gender, inbreeding, age were evaluated before a correlation study of pairs of relative was conducted. All estimates were relatively high and statistically significant showing a significant familial aggregation. It could be observed that the greatest number of malaria episodes occurs in male individuals with more than 45 years of age, probably due to a longer exposition to the causal agent. Complex segregation analysis was applied to the data, and although the free τs model showed the best fit, with somewhat non-mendelian τs values, the action of a heritable multifactorial component has to be present in order to explain the familial aggregation. The results are the same whether Duffy negative individuals are excluded from the sample or not. Further linkage studies are being conducted in order to identify probable genetic mechanisms associated to this treat.

Model	*D*	*t*	*q*	*H*	τ_1	τ_2	τ_3	*-2 ln L*	χ 2	P	test	AIC
1.Mixed	1.000	1.513	0.230	0.065	[1]	[0.5]	[0]	1179.38				1187.380
3.No major effect	[0]	[0]	[0]	0.435	[1]	[0.5]	[0]	1229.74	50.360	0.000	3 vs. 1	1231.740
4.No multifactorial	0.092	1.510	0.623	[0]	[1]	[0.5]	[0]	1201.29	21.910	0.000	4 vs. 1	1207.290
5.Recessive (d=0)	[0]	3.065	0.211	0.259	[1]	[0.5]	[0]	1170.99	8.390	0.004	5 vs.1	1176.990
7. Dominant (d=1)	[1]	1.515	0.230	0.065	[1]	[0.5]	[0]	1179.48	0.100	0.752	7 vs. 1	1185.480
8.Free τs	1.000	1.332	0.301	0.447	1.000	1.000	0.000	962.30	208.690	0.000	8 vs 5	976.300

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P1162. Uncovering German genetic landscapes of Y-chromosome

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Widespread population data on Y-chromosomal short tandem repeats (STR) haplotypes are public available. World-wide as well as continent-wide studies of Y-STR haplotypes confirm its applicability to quantification of population genetic differentiation.

Europe evidences Y-chromosomal diversity in form of clines, primarily influenced by geography (Rosser et al., 2000). While genetic differentiation has been accounted between major European populations (Ploski et al., 2002), along present-day political borders (Kayser et al., 2005) or even between micro-geographic regions (Brion et al., 2004), Germany is regarded as quite homogeneous. Small but statistically significant differences were detected between Eastern and Western Germany (Kayser, 2005).

German genetic landscapes of Y-chromosome are assessed. A genetic geographical Information system (GenGIS) was built. Y-chromosomal geographical patterns were portrayed examining 7 STR (3070 samples, recruited along 12 German locations; http://www.yhrd.org). Following the stepwise mutation model (SMM), thus taking haplotype molecular distance into account, haplotype groups were defined. First, most frequent haplotypes were identified, and then nearest neighbours, in terms of mutation steps, were clustered. Genetic landscapes were built based on spatial interpolation of haplotype and haplotype-group frequencies with GRASS GIS. Regional patterns are analyzed. The relatively genetic homogeneity attributed to German population may be interpreted as a product of superimposed genetic landscapes. GenGIS assessment of genetic landscapes provides an insight into the spatial composition of extant, highly admixed German population.

References

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P1163. Genetic polymorphism of Y chromosome short tandems repeats (Y-STRs) in the Latvian population

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Biallelic loci and short tandem repeats (STRs) positioned in the non-recombining part of Y chromosome and paternal inheritance make it a powerful tool for studying population genetics and evolution.

The aim of the present study was to evaluate Y-STRs variation in two haplogroups, I and R1a1 that form 46.6% from the total Latvian sample.

A sample of 74 paternally unrelated Latvians belonging to haplogroups (hgs) I and R1a1 were analyzed at 12 Y-STR loci. One trinucleotide STR (DYS392) and 11 tetranucleotide STRs (DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS393, DYS437, DYS438, and DYS439) were determined using PowerPlex® Y System (Promega, USA). Haplotype diversity was calculated using the Arlequin 2.0 package.

Y-STR analyses of Latvian hg I lineages revealed 12 different haplotypes (haplotype diversity: 0.957±0.016), 14-13-14-12-28-22-10-11-13-16-10-11 (defined by DYS19-385a-385b-389I-389II-390-391-392-393-437-438-439) of which occurred more frequently (25%). Among hg I Y-STRs two microsatellites were less diverse and only one allele for each of them was found (DYS392 locus-11 repeats, DYS438-10 repeats). In contrast to hg I, the haplotype diversity of haplogroup R1a1 was 0.986±0.003, where 51 distinct haplotypes were detected and six of them were found in more than one individual, comprising 11.8% of hg R1a1 Y chromosomes in Latvian males. We have detected two predominant alleles at DYS392 and DYS 393 loci (11 and 12) that make up 94% of the total microsatellite variation in the Latvian population.

The analysis of associated Y-STRs revealed differences between microsatellite diversity within two distinct phylogenetic branches, haplogroups R1a1 and I, in the Latvian population.

P1164. The association of gene variants with physical performance in middle school-age children

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The aim of the study was to determine the association between *ACE* I/D, *ACTN3* R577X and *PPARA* intron 7 G/C gene variants and anthropometrical and performance-related traits in 457 middle school-age children. The assessment of physical performance and anthropometry was conducted with a number of physical and physiological tests.

Gene variants were determined by PCR. The discovered correlations concerned primarily sprint performance traits, being in agreement with the generally accepted data: the *ACE* D, *ACTN3* R and *PPARA* C alleles and their different combinations were associated with the maximal values of height, weight, BMI, standing long-jump and handgrip strength, increased resting heart rate (RHR) and systolic blood pressure (SBP), whereas the *ACE* I and *PPARA* G alleles correlated with decreased RHR and SBP. In conclusion, *ACE*, *ACTN3* and *PPARA* gene variants are strongly associated with several anthropometrical and performance-related traits in physically active middle school-age children.

P1165. Investigating human genome-wide heterozygosity and its effects on health-related quantitative traits using dense genome-wide scans

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Aim. To investigate concordance of different measures of human heterozygosity (internal relatedness, multilocus heterozygosity - MLH) using genome wide scans of 800 STR and 317.000 SNP genetic markers; to study patterns of heterozygosity in the human genome and investigate effects on health-related traits.

Materials and methods. A sample of 1026 examinees from isolated island of Vis, Croatia, with high prevalence of consanguinity was studied. Genome-wide scan using 800 STR markers was performed in all examinees, and using 317.000 SNP markers in a subset of examinees. A set of 31 health-related quantitative phenotypes was measured (e.g. blood pressure, anthropometric measurements, spirometry and biochemical parameters).

Results. The average MLH (standard deviation) in 1026 examinees was 0.754 (±0.020) using STR markers (range 0.693 - 0.810), and 0.343 (±0.006) using SNP markers (0.317 - 0.352). The correlation between genealogical and marker-based (STR, SNP) estimates of individual genome-wide heterozygosity was statistically significant in all cases, but generally weaker than expected. The coefficient of correlation between residuals of estimates based on STR and SNP markers from the linear fit was r=0.434 (p<0.0001). Of all measured quantitative traits, the strongest correlation with MLH (after correction for potential confounding factors) was observed for biceps, triceps, subscapular and suprailiac skinfolds (p-values all <0.01), and hip, waist and brachial circumference (p-values <0.05).

Conclusions. Patterns of individual genome-wide heterozygosity in the human genome differ substantially when measured using STR versus SNP marker sets, and with varying density of genome scans. Genome-wide heterozygosity is associated with levels of certain human quantitative traits.

P1166. Applicability of commercially available dense genome-wide scans for genetic association studies: a comparison of CEPH and Croatian island founders

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Aim. To investigate the performance of two commercially available dense genome-wide genotyping arrays (Illumina BeadChip ~317.000 SNP markers and Affymetrix GeneChip ~250.000 SNP markers) in a population sample from the Croatian Island of Vis.

Materials and methods. We genotyped 63 individuals who were carefully chosen to be unrelated and to represent the founders of the present island population. In addition, genotypes were obtained for 60 unrelated CEPH individuals.

Results. The proportions of loci that were polymorphic among all marker loci were 99.74% (Vis, Illu), 99.93% (CEPH, Illu),85.36% (Vis, Affy) and 85.85% (CEPH, Affy). The proportion of markers with a minor allele frequency below 5% was 5.77% (Vis, Illu), 0.38% (CEPH, Illu), 28.75% (Vis, Affy) and 24.74% (CEPH, Affy). Estimated F_{ST} values between the two population were 0.012 (Illu) and 0.015 (Affy). The proportion of marker loci showing departure from Hardy-Weinberg equilibrium at the significance level of p<0.01 was 2.66% (Vis, Illu), 0.43% (CEPH, Illu), 6.47% (Vis, Affy) and 1.13% (CEPH, Affy). The proportion of homozygous genotypes in the whole dataset was 65.79% (Vis, Illu), 64.88% (CEPH, Illu), 72.99% (Vis, Affy) and 69.31% (CEPH, Affy).

Conclusion. The genetic distance between CEPH and this isolated Croatian island population is small, given the F_{ST} estimates obtained with the two different arrays. However, the significant effects of genetic drift in isolated populations can be noted as increase in proportion of markers with minor allele frequency approaching 0%. The Illumina array provides a higher proportion of informative markers for genetic association studies.

P1167. A recurrent mutation in Italian Gitelman patients is linked to a specific haplotype, suggesting a founder effect

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Gitelman's syndrome (GS) is one of the hereditary renal tubulopathies, characterised by hypokalemic metabolic alkalosis, hypomagnesemia, hypocalciuria, hypermagnesiuria, and hyperreninemia with normal or somewhat lower blood pressure. GS is caused by inactivating mutations in the *SLC12A3* gene localised to chromosome 16. It encodes the thiazide-sensitive NaCl-cotransporter.

In our cohort of Italian patients with a clinical and molecular diagnosis of GS we found 66 mutations distributed between missense, nonsense, insertions and deletion mutations. They were spread along the gene without any hot spot. Especially one mutation, c.1202_1203ins7bp in exon 10 (p.Ala401Alafs>X1), was relatively frequent: it was found in ten apparently unrelated alleles (~15%).

The subjects that carried the c.1202_1203ins7bp mutation were characterised, except one which was not available, by extensive genotyping with seven intragenic polymorphic markers/SNPs flanking exon 10. A multiallelic polymorphism in intron 8 of the gene revealed 100% segregation of the 258 bp allele with the mutation even though this allele was found to be linked with wild type as well. Furthermore, a specific haplotype in linkage to the mutation was demonstrated in all the patients and parents carrying the insertion.

Our centre receives samples from all over Italy and also from abroad but the geographic distribution of patients carrying p.Ala401Alafs>X1 is, at the moment, circumscribed to the northern regions of Italy.

We hypothesize that this mutation might be the result of a founder effect that originated in a common ancestor in the north of Italy.

P1168. T8T and other *GJB2* unclassified or controversial sequence variants identified in Portuguese families

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Mutations in *GJB2* are the most common cause of nonsyndromic sensorineural hearing loss (NSSHL), being recessive cases by far more frequent than dominant ones. To date, more than 100 mutations, polymorphisms and unclassified variants have been reported in *GJB2*. The identification of known mutations in this gene allows definitive etiological diagnosis of *GJB2*-based hearing loss and improves the precision of genetic counselling and risk assessment for patients and families. The identification of novel or unclassified variants, or controversial variants with unclear pathogenicity, complicates the interpretation of DNA tests results and require further analysis of this variants among different populations.

The present study represents a new contribution for the characterization of hereditary hearing loss in the Portuguese population. DNA-based sequencing of *GJB2* coding region was utilized in the molecular diagnostic of 50 patients with NSSHL, from 40 different affected families being analysed for the first time.

The results obtained provided relevant information. They therefore contribute for a better characterization of the *GJB2* variation spectrum, including the novel T8T and the nt-34 C>T variants, both found in heterozigosity, in two different families. The role of these and other unclassified or controversial variants is discussed according to genotype/phenotype analysis, their frequency in the Portuguese impaired and control population, and comparison with other populations.

These new data support and extend prior studies of the association

between *GJB2* and NSSHL in Portuguese families. They also contribute for a more precise risk prediction and genetic counselling.

P1169. Glutathione S-transferase polymorphisms in Russian populations of European part of Russia

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Cytosolic or soluble glutathione S-transferases (GST) - are a superfamily of multifunctional proteins with fundamental roles in the cellular detoxification of a wide range of exogenous and endogenous compounds. Like many other genes, genes for GSTs display polymorphisms which, as have been shown can contribute to interindividual differences in responses to xenobiotics. The allele distribution for some of frequently tested in association studies GST polymorphisms (SNPs at codons 105 and 114 for GSTP1, -69 C/T polymorphism for GSTA1, three nucleotide deletion for GSTM3 and gene deletions for GSTM1 and GSTT1) were studied in seven geographically different Russian populations (from Tver, Smolensk, Kursk, Ivanovo and Archangelsk regions) and in one Yakut population. Yakuts were used as a reference population. The analysis of GSTP1 polymorphisms did not demonstrate any statistically significant differences in allele/haplotype distribution among the most of Russian populations. At the same time all Russian populations significantly differed from Yakut population. Similar results were for GSTM3, GSTA1 and GSTM1 polymorphisms, but not for GSTT1. The frequency of GSTT1 null genotypes was substantively lower in Archangelsk population (9%) compared with other Russians (16-24%). The significance of this difference was verified with the test for homogeneity of Russian samples (we had $p = 0.035$ for all Russian populations tested and $p = 0.64$ when Archangelsk samples were excluded from the total Russian pull). Based on previously published data one may suppose that the lower frequency of GSTT1 0/0 homozygotes is an attribute for the Russian people of Archangelsk region.

P1170. *Cis-Trans* Interplay at the β Globin Locus using β Globin Gene Models from the Maltese Population

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The quantification of normal and abnormal globins of HbF-Malta-I [$^{G}\gamma$117(G19)His→Arg] heterozygotes which are in tight linkage disequilibrium with Hb Valletta [β87(F3)Thr→Pro], together with haplotyping of homozygotes and heterozygotes including the *Xmn*I dimorphism in the $^{G}\gamma$ promoter and the $(AT)_XT_Y$ polymorphism 5' to the β globin genes had suggested that the *Xmn*I dimorphism was largely inactive in the normal newborn whilst the HbF levels and the proportion of $^{G}\gamma$ globin in anemic adult β-thalassemia homozygotes and compound heterozygotes differed significantly. Here, we document the occurrence of eight newborns who were heterozygous at three globin loci permitting quantification by RP-HPLC of the six globin products in the context of genotypic variation at the *Xmn*I and $(AT)_XT_Y$ sequences. Results were compared with newborn HbF-Malta-I-Hb-Valletta heterozygotes and anemic adult β-thalassemia homozygotes/compound heterozygotes. The globin quantification together with haplotype data were analysed using the general linear model by SPSS V.12. The data excluded significant effect of the *Xmn*I dimorphism alone on relative γ/β globin gene expression in the newborn. Conversely, the $(AT)_XT_Y$ with BP1 binding sites of 19 $(AT)_7T_5$, 21 $(AT)_7T_7$, 23 $(AT)_9T_5$, or 25 $(AT)_{11}T_3$ nucleotides in *trans* supersede *Xmn*I. In contrast, it is the *Xmn*I dimorphism that over-rides the (AT)xTy diversity in the anemic adult β-thalassemia homozygotes or compound heterozygotes. The $^{G}\gamma$FMalta-I/$^{G}\gamma$0 ratio of the newborn heterozygotes with HbF-Malta-I and the $^{A}\gamma^{T}/^{A}\gamma^{I}$ ratio of the newborn heterozygotes with HbF-Malta-I and HbF-Sardinia suggested that the developmental regulation of the *Xmn*I site may be subject to *cis*/*trans* interplay with the $(AT)_XT_Y$ sequences.

P1171. High Frequency of 35delG *GJB2* mutation and absence of del*(GJB6-D13S1830)* in Greek Cypriot patients with non-syndromic hearing loss

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Mutations in the *GJB2* (Connexin 26) gene are responsible for more than half of all cases of pre-lingual recessive inherited non-syndromic deafness in Europe.

This work presents a mutation analysis of the *GJB2* and *GJB6* (Connexin 30) genes in thirty six Greek Cypriot patients with sensorineural non-syndromic hearing loss compatible with recessive inheritance. Fourteen of the patients (38.9 %) had the 35delG mutation in the *GJB2* gene. Moreover, eleven of these were homozygous for the 35delG mutation, whereas two patients were in the compound heterozygous state with the L90P missense mutation and one with the E47X nonsense mutation. Another patient with severe sensorineural hearing loss was heterozygous for the V153I missense mutation. Finally, no *GJB6* mutations or the known del*(GJB6-D13S1830)* were identified in any of the investigated Greek Cypriot non-syndromic hearing loss patients. This work confirms that the *GJB2* 35delG mutation is an important pathogenic mutation for hearing loss in the Greek Cypriot population and the findings will be used towards the effective diagnosis of non-syndromic hearing loss, improve genetic counselling and used as a potential therapeutic platform in the future for the affected patients in Cyprus.

P1172. The monogenic pathology in rural districts of Buryatia Republic

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The medical genetic study of the population of four districts (Okinsky, Kyakhtinsky, Eravnensky, Kizhinginsky) of Buryatia Republic (Russia) was performed, totally there live 85.01 thousand people. Most of the natives in these districts are Buryats.

Buryatia Republic is situated in the southern part of East Siberia. Total population is 974.3 thousand people. The most numerous ethnic groups are Russians (67.8%), Buryats (27.8%), Ukrainians (0.98%), Tatars (0.83%) and Germans (0.16%).

As a result of the study the 96 patients from 66 families with monogenic hereditary diseases were revealed. Thus, the 57 patients from 37 families were found with autosomal dominant diseases; the 15 patients from 14 families had autosomal recessive pathology; the 24 patients from 15 families were revealed with X-linked forms. The 74 patients from 47 families were Buryats, the 22 patients from 19 families were Russians. The group of hereditary syndromes predominated in the structure of autosomal recessive and X-linked pathology (37.5% and 50% respectively). The group of skeletal and connective tissue disorders (35.3%) was more often among autosomal dominant pathology. The inborn metabolism defects (37.5%) were the most prevalent among autosomal recessive diseases.

The X-linked diseases predominated in Okinsky district, the 16 patients from 9 families were revealed with this pathology. The diagnosis of X-linked ichthyosis was made in 8 families. The founder effect cannot be excluded in these cases.

The studies of the structure of hereditary diseases in Buryatia Republic are continued.

P1173. The medical genetic study of inherited pathology in Khakasia Republic

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Federation.
Khakasia Republic is situated in the centre of South Siberia; the total population is 542.7 thousand people (the urban population - 336 thousand, the rural population - 206.7 thousand). The most numerous ethnic groups are Russians (79.5%) and Khakasses (11.1%). The epidemiological study of congenital malformations among newborns during 19-years period in Khakasia was performed (1986-2004). Total spectrum of congenital malformations was registered. The overall rate of all birth defects was 32.72‰ and varied from 17.22 to 57.98‰ in some years. The structure of congenital malformations was revealed, the defects of musculoskeletal, urogenital and cardiovascular system were more prevalent. The frequency of Down syndrome and multiple congenital malformations was 1.42 and 2.46‰, respectively.
The load Mendelian pathology with different types of heredity was determined for each ethnic group, taking into account the territorial distribution: town, village. In the urban population, the load of autosomal dominant, autosomal recessive, X-linked pathology were: Russians - 0.51; 0.23 per 1000 individuals respectively, and 0.13 per 1000 male; Khakasses - 1.22; 0.87; per 1000 individuals respectively. No one case of X - linked pathology was found in Khakasses. In the rural population, the load of autosomal dominant, autosomal recessive, X-linked pathology were: Russians: 0.65; 0.27 per 1000 individuals respectively, and 0.04 per 1000 male; Khakasses - 0.97, 0.76 individuals respectively, and 0.65 per 1000 male. The load and the prevalence of hereditary pathology in Khakasia Republic were described for the first time.

P1174. HLA haplotypes associated with *HFE* C282Y mutation in São Miguel Island population (Azores)

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Hereditary Hemocromatosis (HH) is an autosomal recessive disorder of the iron metabolism. In the majority of HH cases, the defect is a single missense mutation, cystein replaced by tyrosine at the 282 position, in the *HFE* gene. Generally, the C282Y mutation lies within a celtic ancestral HLA-A*03-B*07 haplotype. In addition, other haplotypes associated with HH have been found.
Here, we infer the HLA haplotypes associated with the *HFE* C282Y mutation in São Miguel Island population. All samples were genotyped for HLA-A and -B by PCR-SSP and for *HFE* mutations (C282Y, H63D and S65C) by PCR-RFLP. Allele and haplotype frequencies were estimated by Arlequin 3.1 version software.
The sample was composed of 88 individuals divided into two groups: 47 were homozygous and carriers for the *HFE* C282Y mutation, while 41 had no mutations. The data show that alleles HLA-A*03, HLA-A*29, HLA-B*37 and HLA-B*45 are in higher frequency in the C282Y patients and carriers group (p<0.01), whereas alleles HLA-A*30, HLA-A*32, HLA-B*08 and HLA-B*44 are the most frequent in the control subjects (p<0.01). Moreover, results demonstrate that haplotypes HLA-A*29-B*45 (7.5%) and HLA-A*02-B*58 (5.3%) are detected only in individuals with the C282Y mutation (p<0.001). The ancestral haplotype HLA-A*03-B*07 is present in both groups, but at a higher frequency (5.3%) in the C282Y patients and carriers group (p<0.01). Taken together, the data suggest that these three haplotypes are associated with the *HFE* C282Y mutation, indicative of a multiple founder effect responsible for HH in the São Miguel Island population.

P1175. Establishment of a Human DNA Bank: the case of the Azorean population

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In order to characterize the genetic background of the Azorean population, a human anonymized DNA bank was built. Initiated in 2002, this DNA bank consists in 1556 DNA samples from individuals that inhabit the nine islands of the Azorean archipelago.
The strategy used consisted of blood collection from healthy, not family-related, individuals in the Hematology Department of the Divino Espirito Santo's Hospital (for the São Miguel Island) or in the Health Centres of their residence (concerning the other eight islands), after presenting the project and receiving their written informed consent. To guarantee individual ancestral origin, the blood was preferably taken from individuals whose parents were born in the same island. Nevertheless, the bank includes DNA samples from 69 individuals whose parents were born in different archipelago islands, 58 individuals with only one Azorean parent and finally 59 individuals with no Azorean parents.
In regards to the genetic characterization of the Azorean population project, the bank is composed of 1370 DNA samples from individuals (whose parents were born in the same island) with an average age of 42 years old (18 - 88 y), mostly men (71%). Up to the present moment, the bank includes a populational representativity between 0.2% (Terceira Island) and 7% (Corvo Island; *Census* 2001 - INE).
This DNA bank was used for Azorean genetic diversity studies (based on Y, Alu and STRs polymorphisms) and represents an essential resource for the analysis of genes responsible for diseases, as well as for the development of forensic and farmacogenomic studies.

P1176. Ibreeding depression in the Spanish kings of the Austrian Dynasty

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Even though inbreeding in the Spanish-Austrian dynasty (That extends since 1550 till 1700, when the last Spanish-Austrian king, Charles II, died without descendents) has been considered by historians as one of the causes of the disappearance of such, no genetic analysis of the Spanish-Austrian dynasty, to calculate the inbreeding coefficients and to detect its possible effects, has been carried out.
To estimate the inbreeding coefficients of the kings of the Spanish-Austrian dynasty two approaches have been followed: on the one hand, a 16 generations, 153 individual pedigree of the descents of the Spanish-Austrian kings and queens has been reproduced. On the other, a 2300 individuals of the different European dynasties, database has been created. Data of the family relationships extracted from these two approaches was processed with the Fast Inbreeding Computation Software FSpeed 2. Consanguinity of Spanish-Austrian kings is between F = 0,024 (Philip I) and F = 0,25 (Charles II).Table 1 for some results.
The effects of the inbreeding in the dynasty of the Spanish-Austrians have been studied by analyzing the pre-reproductive survival of the descendents of the royal families, until they were 10 years old. This quantitative character has been used to detect an inbreeding depression in the Spanish Austrian dynasty. By applying different regression models a significant negative regression between the survival of the infants and its inbreeding coefficients has been found.

	Inbreeding Coefficient
Philip I (1478-1506)	0,024
Joana I (1479-1555)	0,034
Charles I (V of Germany) (1530-1558)	0,035
Isabella of Portugal (1503-1593)	0,095
Philip II (1527-1598)	0,120
Mary of Portugal (1527-1545)	0,120
Elisabeth of Valois (1545-1568)	0,001
Anna of Austria (1549-1580)	0,105
Philip III (1578-1621)	0,216
Margaret of Austria (1584-1661)	0,138
Philip IV (1605-1665)	0,115
Elisabeth of Bourbon (1603-1644)	0,005
Mariana of Austria (1634-1696)	0,154
Charles II "The Bewitched" (1661-1700)	0,025

P1177. Retrospective study of Clinical and histological manifestations of IP in Adults

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Introduction. Incontinentia pigmenti (IP) is a rare X dominant genodermatosis related to mutations of NEMO gene. It affects mostly female patients and is usually lethal for males in utero. The skin lesions may occur in 4 classically successive diagnostic stages: erythema and vesicles, (stage 1); verrucous (stage 2); linear hyperpigmentation (stage 3); and pallor and scarring (stage 4).In adults, manifestations are skin involvement (stage 4) and teeth and nail anomalies.
Patients and Methods
25 adults patients with molecular diagnosis of IP, were enrolled for clinical examination. Skin, ocular, neurological and stomatological manifestations were recorded using a standard form. Skin biopsy was performed.
Results. 25 patients fulfilled the criteria : the diagnosis was made in adulthood in 52%of the patients. Stage 4 was constant (100%) stage 3 and 2 were found in 11 and 1 patients respectively. Other manifestations were: woolly hair (44%), nails (84%), teeth (92%), ocular (48%), mammary (28%) and neurological (12%) anomalies. Histology shows apoptotic kératinocytes, absence of follicles and sweat glands (stages 3 and 4), hypopigmentation and atrophic epidermis (stage 4).
Discussion. Diagnosis of IP was delayed in 52% of the patient and based on the constant association of stage 4 lesions and teeth anomalies. Interestingly, apoptosis persists in adulthood and was associated to absence of both sweat glands and follicles. Dermatological examination of patients with several miscarriages may be helpful to detect IP as a rare cause of spontaneous abortion. Moreover, histology confirms clinical suspicion of the disease and should be include in IP criteria.

P1178. The role of Toll-like Receptor 4 polymorphisms and CARD15/NOD2 mutations in the susceptibility and phenotype in Inflammatory Bowel Disease: reports of a survey in Southern Italy population.

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Single nucleotide polymorphisms in the Toll-like Receptor 4 (TLR4) and CARD15/NOD2 genes have recently been shown to be associated with Inflammatory Bowel Disease (IBD), but whether this susceptibility extends to all ethnic groups remains unknown. The aim of our study was to evaluate the CARD15/NOD2 and TLR4 gene mutations in a Southern Italy population.
Thirty two ulcerative colitis (UC) patients (mean age, 47 yrs), 79 Crohn's disease (CD) patients (mean age, 46 yrs) and 103 healthy ethnically matched controls were genotyped for three CARD15/NOD2 variants (R702W, G908R, and L1007finsC) and for TLR4 gene polymorphisms (D299G and T399I). The allele frequency of the different genotypes was compared and genotype-phenotype correlation was performed. Comparison of the frequency between patients and controls and the association to the phenotype was performed by chi-square test or Fisher's exact test where appropriate.
No significant association of the two TLR4 gene mutations with UC or CD was observed (allele frequency: D299G- controls 3.5%, UC 3.6%, CD 3.3%; T399I- controls 2.9%, UC 2.8%, CD 2.9%). The frequency of the frameshift mutation L1007finsC of the CARD15/NOD2 gene was significantly higher in CD patients (9.3%; p=0.0001) compared with controls (2.9%) or patients with UC (2.7%). In CD patients the frequency of R702W mutation was significantly higher (8.3%; p<0.001) than in controls (3.4%) and in UC (3.7%). No significant association of the CARD15/NOD2 gene G908R mutation was found in UC.
Our preliminary results implicate the CARD15/NOD2 gene in susceptibility to Crohn's disease also in a cohort of patients of Southern Italy.

P1179. Variability in inflammatory immune response genes in human populations of North Eurasia

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Inflammatory immune responses play a crucial role in the interaction of humans with the environment, and genes which mediate immunity in humans are a possible target for natural selection during human evolution and migrations. The aim of this study was to estimate the genetic diversity of immune response-related genes in populations living under either moderate or Arctic climatic conditions in North Eurasia. Ten populations, 3 Eastern European (2 Russian, 1 Komis), 2 Central Asian (Kirghiz), 5 Siberian (2 Buryat, 2 Altay, 1 Khant) were genotyped for SNPs in genes involved in Th2 response (CD14 C-159T, SCGB1A1 A38G, CMA1 A-1903G, ADRB2 Arg16Gly, ADRB2 Glu27Gln).
Allele frequencies of the CD14 and CMA1 genes showed significant departure from selective neutrality expectations in most of study populations. A high total level of genetic diversity in inflammatory immune response genes was found in the North Eurasian populations. Irrespective of ethnic and linguistic affiliation and geographical location, 9 of the 10 populations were characterized by high average expected heterozygosity (0.45-0.49). The degree of genetic differentiation of North Eurasian populations exhibited by Th2-related genes was relatively low (Fst=2.4%) compared to 'neutral' markers (autosomal Alu Fst=6%, Y-chromosome Fst=18%). The patterns of genetic relationships between populations revealed by phylogenetic analysis seemed not to correspond with those obtained for 'neutral' genetic systems (Alus, Y-chromosome, mtDNA). In general, a significant correlation of 'proinflammatory' allele frequency and total genetic diversity with geography and climate was revealed.

P1180. Analysis of Interleukin 10 -1082 gene polymorphism in hemodialysis patients

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Introduction: Interleukin 10 (Il-10) is an important constituent of immune response and has regulatory function on inflammatory and cell mediated immunological mechanisms.
Production of Il-10 is under strong genetic influence. One of the most studied regulatory DNA sequences is single nucleotide polymorphism A/G at position -1082 from the Il-10 gene transcriptional start site. Studies of Il-10 and its genetics are of particular interest in hemodalysis (HD) patients due to the possible role of this cytokine in the pathogenesis of disorders associated with chronic HD such as atherosclerosis and depression.
Aim: The aim of our study was determination of Il-10 -1082 genotype and its correlation with Il-10 serum level in a group of hemodialysis (HD) patients.
Methods: We assessed 70 patients on chronic HD due to the end stage renal disease. IL-10 -1082 genotyping was performed by PCR method followed by digestion with MnlI restriction enzyme. Il-10 serum level was determined by immuno assay.
Results: DNA analysis showed that 31/70 (44.29%) patients were with AA genotype, 28/70 (40.0%) with AG genotype and 11/70 (15.71%) with GG genotype at position -1082 of the Il-10 gene. There is no difference between genotype frequencies in HD patients and control group of healthy subjects. HD patients with GG genotype had higher mean Il-10 level compared to both AG and AA group of patients, but without statistical significance.
Conclusion: Further investigations will show weather Il-10 -1082 genotype and Il-10 serum level could be used in prevention of disorders affecting life quality in HD patients.

P1181. Disease frequency of Inborn Errors of Metabolism in the Irish Traveller Community

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The frequency of Inherited Metabolic Disorders (IEMs) varies between ethnic groups, reflecting founder effect, genetic isolation, and the potential effects of consanguinity. These disorders are a major cause of morbidity and mortality in "Irish Travellers", an endogamous group of nomads.
We aimed to compare the birth prevalence of IEMs in Traveller with non-Traveller children attending a tertiary level metabolic centre and to examine possible genetic factors contributing to observed differences.
A retrospective review of diagnoses in Travellers was performed for 5 years (2002-2006). Mean birth prevalence was calculated and compared with overall figures for IEMs in the total population.
Travellers constitute 9% of the total patient group, but only 0.6% of the Irish population. 15 IEMs were noted, Galactosaemia, MPS 1, Mitochondrial cytopathies, Glutaric Aciduria Type 1(GA1),GSD Type 111a, Mucolipidosis Type 11, Hyperprolinemia Type 11 being the commonest. The birth prevalence of IEMs in the Traveller group for this period was estimated to be 1/80,Whereas that for the total population for 2006 approximates 1/500. Carrier frequency for the common galactosaemia mutation (Q188R) is 1/11. The W402X homozygous mutation explains all cases of MPS 1. All GA1 patients are homozygous for the E365K mutation. Hyperprolinemia Type 11 is caused by one mutation (G521fs(+1)) in an extended pedigree.
We propose that the high incidence of IEMs in Irish Travellers may reflect initial founder effects and the increased rate of consanguinity.

P1182. Mosaic imprinting aberrations at H19, SNRPN and KvDMR1 are not common after in vitro fertilisation.

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In vitro fertilisation (IVF) potentially provides a profoundly abnormal environment for an embryo. Studies with mice, sheep and cattle have indicated that the culture environment of the embryo can affect the imprinting of genes and the phenotype of the animal. Recent studies have suggested that IVF causes a small but increased risk of imprinting aberrations such as Angelman syndrome and Beckwith-Wiedemann syndrome. Given that mosaicism for the imprinting defect has been observed in Angelman syndrome and Beckwith-Wiedemann syndrome, we hypothesised that low-level, mosaic imprinting defects may be present in phenotypically normal individuals conceived using IVF.
DNA samples from peripheral blood were obtained from 70 IVF-conceived pre-pubertal children and 70 matched controls. DNA methylation of CpG sites within the H19, SNRPN and KvDMR1 loci was accurately quantified using methylation-sensitive restriction digest followed by real-time quantitative PCR (MSQ-PCR). Global DNA methylation was also examined by using MSQ-PCR on the Satellite 2 repeat region. No differences in the percentage of methylation between the IVF-conceived and control children were observed at the examined CpG sites.
We concluded that low-level imprinting errors are not a common occurrence in children conceived using IVF. Our data provides reassurance that IVF-associated imprinting errors are sporadic and rare.

P1183. A novel variant E43K found in *KCNE1* gene

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KCNE1 gene encodes a K^+ channel ß-subunit (mink or KCNE1) that modulates voltage-gated potassium channels in various organs, namely heart and cochlea. A functional K^+ channel requires coexpression of this transmembrane protein and a α-subunit, usually KCNQ1, coded by gene *KCNQ1*. In the inner ear, the KCNE1/KCNQ1 channels play a key role in ensuring K^+ homeostasis. Loss of functional channels leads to a reduction of endolymph potencial, which can originate hearing loss. Mutations in *KCNE1* are also responsible for the prolongation of the action potential in the heart, which leads to a prolonged QT interval and to a cardiac disease known as long QT syndrome (LQTS), associated or not to severe congenital bilateral hearing loss.
In the present study we report a novel heterozygous 127G>A mutation in *KCNE1* gene. This variant was detected in a healthy individual of our control population by direct sequencing in both directions. The mutation results in an acid to basic amino acid substitution in the extra cellular domain of the protein at codon 43 (E43K), a position highly conserved among MinK proteins of different vertebrates.
Recent findings suggest that common variants located in the *KCNQ1, KCNE1, KCNH2* and *SCN5A* genes might influence the QT interval in healthy individuals and might also represent risk factors for arrhythmias or cardiac sudden death. We have thus investigated whether variant E43K could influence, by itself or in association with other variants, the channel function and, as a consequence, the length of the QT interval, leading to LQTS expression.

P1184. Genetic susceptibility to legionnaires' disease

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Legionnaires' disease is a bacterial pneumonia due to *Legionella pneumophyla*. Given the reported wide exposure to this pathogen, behavioural, microbial and environmental risk factors might not fully explain the limited known number of pathological cases thus suggesting the involvement of differences in host susceptibility.
With the aim of addressing the potential role of genetic polymorphisms, a case-control study was recently realised within the nationwide research network. Candidate genes included interleukins and respective receptors.
Herein we report on the analysis of genetic polymorphisms at CCR and TLR. Specifically, patients (n=96), exposed subjects (n=106) and controls (n=320) were recruited based on stringent inclusion criteria. Genomic DNA was isolated from peripheral blood cells and mutation detection by polymerase chain reaction. We report a significant (<0.05) association for CCR264I, a Single Nucleotide polynorphism where a G_{190} is substituted by an A_{190} in the Chemokine Receptor-2 gene. Our data together with available epidemiological and clinical evidences support the potential role for this specific polymorphism in the susceptibility to legionnaires' disease. Additional in vitro studies indicate that macrophage phagocytosis, chemokine production as well as cytotoxicity may play a role . Another SNP (CCR5D32) also resulted associated to bacterial pneuomonia but it was not specific to legionnaires' disease. Genetic polymorphisms may open up new perspectives for preventive medicine approaches in high risk human subjects exposed to *Legionella pneumophyla*.

P1185. Parametric linkage analysis of large complex pedigrees

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Due to computational limitations, it is impossible to perform exact parametric linkage analysis in large pedigrees with multiple loops. Usually, a pedigree is split to multiple small fragments. This enables exact likelihood calculations using Lander-Green algorithm. Alternative method is to break all loops and apply Elston-Stewart algorithm for approximate likelihood calculation. We compared the linkage power of two approaches using a large complex human pedigree with multiple loops. Two pedigree structures were derived from the initial pedigree: 1) single pedigree constructed by breaking loops and 2) a set of the non-overlapping fragments of limited size. Quantitative trait and genotypes for the set of five-allelic markers were simulated based on initial pedigree and ascribed to the corresponding individuals in two derived pedigree structures. We performed multipoint linkage analysis for each of two structures. For the linked markers the average values of Lod score were significantly higher for the first pedigree structure. However, it is known that simplification of pedigree structure may increase type 1 error (false positives) of linkage analysis. This may lead to an overestimation of power, when tabulated threshold values are used. To test for this possibility we simulated unlinked markers. Empirical threshold level was lower for the first pedigree structure, than for the second one. This means that increase of power observed in analysis of pedigrees with broken loops compared to analysis of pedigree frag-

ments is determined by higher portion of linkage information remaining in the former structure but not by a higher increase in type I error.

P1186. Human pigmentation genes and genetic susceptibility to melanoma.

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The aetiology of malignant melanoma (MM) remains unclear but it is known that both genetic and environmental factors influence the development of sporadic disease. The main reason for the increasing incidence of MM is greater sun exposure. Epidemiologic studies confirm that ultraviolet B radiation is the main factor involved in the pathogenesis of the disease. Among phenotypic factors, fair pigmentation and low tanning ability are the most important risk factors. MM predisposition is highest in fair skinned, blond or red haired individuals who never tan and always burn.

Human pigmentation and consequently sun sensitivity are complex characteristics. In humans there is a long list of genes known to be involved in rare pigmentary disorders such as albinism. These genes contribute most of the variation in pigmentation phenotypes seen in human populations, and they do this by regulating the level of synthesis, chemical composition, packaging and distribution of melanin.

This case-control study included 120 consecutive Spanish MM patients from the Dermatology Unit of the Gregorio Marañón Hospital and 240 control subjects frequency matched for sex and age. Phenotypic information was colleted using a standardized questionnaire.

Forty-five SNPs in seven genes belonging to the pigmentation pathway (*MC1R, OCA2, ASIP, TYR, TYRP1, SLC45A2* and *SILV*) were genotyped. The selection of the candidate SNPs was carried out using a gene-based approach. We are able to identify several individual SNPs associated with MM. Possible interactions among them will be discussed.

These results confirm the contribution of pigmentation genes to genetic predisposition to MM in Spain.

P1187. Low density microarrays for forensic DNA analysis.

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We suggest to use SNP analysis on microarrays for forensic studies of DNA. Microarray for SNP-analysis of three loci (HLA-DQA1 (6p21), ABO(9q34), AMEL(Xp22)(Yp11)) has been developed. The microarray allows the detection of nine groups of alleles of HLA-DQA1 gene (group 1 -0101XX, 0104XX, 0105, 0107 alleles; group 2 - 0102XX; group 3- 0103; group 4 - 0106; group 5 - 0201; group 6 - 03XXXX, group 7 - 04XXXX; group 8 - 05XXXX; group 9 - 06XXXX), five groups of alleles ABO gene (A, B, O1, O1V and O2 groups) and 2 alleles of AMEL gene (AMELX and AMELY), thus we are able to divide all people in to 1350 groups. The procedure take about 24 hours and includes multiplex two-stage PCR with fluorescently labeled primers and hybridization with oligonucleotide microarray. The fluorescent signals are analyzed using a chip-reader equipped with CCD-camera. The microarray has been tested with DNA samples isolated from fresh blood and saliva. The sensitivity of the method is about 25-100 pg per reaction. We tested the microarray with degraded DNA isolated from spots of blood from dead body, spots of saliva from cigarette end and glass. Allele frequencies and population genetic parameters for HLA-DQA1 and ABO loci were obtained. The presented microarray could be applied in forensic study to confine the number of suspects. It could replace blood group serological test which may give mistakes when degraded samples are studied. Probability of identification could be increased by adding other loci located on the different chromosomes.

P1188. Predictive factors of migraine in a sample of Portuguese families

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Migraine is a highly prevalent disease, reducing quality of life of migraineurs and their relatives. In a previous study, we found evidence of familial aggregation in a sample of Portuguese families. Proband's age of onset has also been associated with familial aggregation in other studies.

Our aim was to evaluate if proband's age of onset, relative's age at contact and gender were predictive factors of migraine.

We analyzed a sample of 133 Portuguese families (a total of 492 first-degree relatives of probands with migraine, from which 317 were affected and 175 were healthy).

Similarly to what was described, proband's age of onset was dichotomized (<16, 16+ years). Relatives were divided in two groups according to their age at contact (<40, 40+ years) and were also separated by gender, since this is an age and gender-dependent trait.

We performed a logistic regression analysis to evaluate if any of these variables could predict relative's affection status.

After adjusting for the remaining variables, while gender was found to be a risk factor for migraine (OR=2.74; 95% CI= 1.87- 4.01), with females being more affected than males, proband's age of onset and relative's age at contact were not. No significant interactions were found between these variables.

Our findings showed that, as expected, females had a higher risk of migraine than men. Unlike previous descriptions, however, variation in age of onset was not a predictive factor of migraine.

P1189. Analyzing of mitochondrial polymorphisms in southwest ethnics of Iran

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Mitochondrial polymorphisms are useful tool for investigations involving in the genetic structure and the history of human populations. mtDNA characteristics such as maternal inheritance, high mutation rate, high copy number and lack of recombination, produce it as a good tool to this propose. The highest degree of polymorphism in mtDNA lies in two hyper variable non-coding region, HV-I and HV-II that can be amplified and sequence separately.

In this study, three ethnical subgroups of southwest of Iran have been analyzed at HV-I sequence of mtDNA. Populations of this study were 48 cases of unrelated individuals in three groups of Arab, Bakhtiyary and Persian. Based on 59 variable positions, 44 different haplotypes were founded. The genetic diversity in Persian, Arab and Bakhtiyary were 0.918, 0.930 and 0.945 respectively. Haplotype diversity in all three populations was high and probably this is because of historical role of this area as a common population immigrant place to many of tribes and ethnics. Surprisingly, in this study we found four new variations that have not been reported in this region (HV-I) at mitomap previously. These variations are insertion of one G after 16336, one (C) after 16188 and two transversion (A to C) in 16258 and 16318 positions.

P1190. Mitochondrial DNA coding and control region polymorphisms associated with a prolonged life span in the Latvian population

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There is evidence that some mitochondrial DNA (mtDNA) coding region and control region HVSI polymorphisms are associated with longevity in humans. These findings allowed to suggest that the association of mtDNA variability with longevity may be population specific, depending on both genetic and environmental background. Studies of the control region HVSII polymorphism in association with longevity so far are very limited.

The aim was to verify if there is any association between mtDNA coding and control region polymorphisms and longevity in the Latvian population.

Objects were 351 healthy unrelated Latvians 18 - 40 years old and 98 aged 74 - 89 years. MtDNA haplogroups were determined by PCR

- RFLP analysis. Control regions HVSI and HVSII were analysed by direct sequencing.
The frequency of haplogroup J was significantly higher in the older age group (13.3%) than in younger individuals (6.6%). Subhaplogroup J2b in particular was more frequent in older individuals (3.1%) than in the younger age group (0.3%). Statistically significant differences were found for mutation frequencies in HVSII at sites 150 (more frequent among the older individuals and in haplogroups H and U4, less frequent - in the haplogroup J), 152 (more frequent among the older individuals in haplogroups T and U4, less frequent - in the haplogroup U2), 195 (more frequent among the older individuals in the haplogroup T, less frequent - in haplogroups J and U4).
Our results support hypothesis that certain inherited mtDNA polymorphisms via interaction with other loci in mtDNA may promote human longevity.

P1191. Analysis of mitochondrial DNA polymorphism in four Siberian ethnic groups

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Mitochondrial DNA polymorphism was studied in 1130 individuals from 12 populations of the most numerous Siberian peoples - Altaians (4 populations), Tivinians (3 populations), Yakuts (2 populations) and Buryats (3 populations). 308 different HVS1 haplotypes were revealed in total which belong to 34 different mtDNA haplogroups, mainly of East-Eurasian origin. Portion of "West-Eurasian" mtDNA haplogroups was the highest in Altaians (up to 46%) and Buryats (up to 20%). AMOVA analysis has shown that 95,78% of HVSI variation was within populations, 2.09% could be explained by inter-population differentiation and 2.09% was variability between ethnic groups. Test on differentiation of polymorphism in population pairs has shown that in all cases except the pair of Yakut samples the differentiation was significant. AMOVA analysis for separate ethnic groups revealed the highest degree of intraethnic differentiation for Altaians (3.78%), followed by Tuvinians (2.61%) and then Buryats (0.43%). Comparison of spectrum of haplogroups and individual haplotypes in the populations under investigation also shows significant differentiation of native Siberian populations. Only two haplotypes from haplogroup C and one haplotype from D could be considered as common for all four ethnicities. One more haplotype from C was abundant in Tuvinians, Yakuts and Buryats but rare in Altaians. Substantial number of haplotypes was population-specific. Analysis of migrations and interethnic marriages revealed various effects of these factors depending both on ethnicity and particular population. The results suggest considerable ethnic differentiation in the studied Siberian peoples, as well as geographic differentiation.
The study was supported by RFBR grant 04-04-48792.

P1192. Paleomolecular genetic analyses (mitochondrial and nuclear DNA polymorphisms) on some Thracian populations from Romania, dating from the Bronze and Iron Age

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We have performed this study on the skeletal remains of some old Thracian populations from Romania, dating from the Bronze and Iron Age. Therefore, within our research we analysed mtDNA (HVR I and HVR II regions) and nuclear DNA (vWA31A Microsatellite) polymorphisms in order to show the degree of their genetic kinship with other old and modern European populations, especially with nowadays Romanian population. We also amplified the Amelogenin gene to identify the genetic sex of old individuals. We have used three methods for DNA-extraction from human fossils and adapted them on the degradation state of the biological material: the phenol-chloroform DNA extraction method, the DNA extraction method with guanidine-tiocianate and silica-particles, and the DNA-extraction method with Invisorb Forensic Kit I.
After amplifying by PCR, the mtDNA sequences were sequenced by the Sanger method. The nuclear vWA31A Microsatellite polymorphisms and the Amelogenin gene sequences were demonstrated on PAA gel, Ag-stained.
We have compared the mtDNA sequences of 50 old Thracian individuals with mtDNA sequences of the present-day Romanian population and other European, Asian and African modern and old populations. The frequencies of vWA31A Microsatellite were compared with similar genetic data of other modern populations from all over the world.
Our results suggest that the old Thracian populations might have made an important contribution to the foundation of the modern genetic Romanian pool and also reflect an evident genetic similarity between the old Thracian populations and other modern populations from South-East Europe.

P1193. Analyses of mitochondrial and Y-chromosomal lineages in modern Hungarian, Szekler and ancient Hungarian populations

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Hungarian population belongs linguistically to the Finno-Ugric branch of the Uralic language family.
High-resolution mtDNA analysis of 27 ancient samples (10^{th}-11^{th} centuries), 101 modern Hungarian, and 76 modern Hungarian-speaking Szekler samples was performed. Only two of 27 ancient Hungarian samples are unambiguously Asian: the rest belong to one of the western Eurasian haplogroups. Statistical analyses, including 57 European and Asian populations, revealed that some Asian affinities and the genetic effect of populations who came into contact with ancient Hungarians during their migrations are seen. Though strong differences appear when the ancient Hungarian samples are analyzed according to apparent social status, as judged by grave goods. mtDNA results demonstrate that significant genetic differences exist between the ancient and recent Hungarian-speaking populations.
The Y-chromosomal base substitution "Tat", proved to be a valuable marker in the Finno-Ugric context. The Tat C allele is widespread in many Uralic-speaking populations, while it is virtually absent in recent Hungarians.
To further elucidate this finding we studied this polymorphism on 100 modern Hungarian, 97 Szekler and 4 ancient Hungarian samples. Our data revealed that only one Szekler men carries the C allele among the modern individuals, whereas out of the four skeletal remains two possess the mutation.
Furthermore we examined 22 Y-chromosomal binary markers to analyze the paternal genetic diversity of the two recent populations.
Our results show that Hungarians and Szeklers share basically the same genetic components found in other European populations, genetically closely related and close to other populations from Central Europe and the Balkan.

P1194. Polymorphisms in the methylenetetrahydrofolate reductase gene and methotrexate efficacy and toxicity in rheumatoid arthritis

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Polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene can influence the methotrexate (MTX)-related metabolic pathways. The aim of this study was to determine a possible relationship between polymorphisms C677T and A1289C of the MTHFR gene and toxicity and efficacy of MTX in patients with rheumatoid arthritis. A total of 47 patients fulfilling American College of Rheumatology criteria and on MTX therapy (7,5-15 mg/week), were evaluated. Clinical efficacy was assessed using the Disease Activity Score in 28 joints (DAS 28) at day 0 and 6 months after initiation of therapy. Drug toxicity was evalu-

ated by blood count, liver and renal function tests. Genotype analysis of C677T and A1289C mutations of the MTHFR gene was investigated by PCR and restriction analysis of DNA extracted from the patients' lymphocytes. Statistical analysis was performed using SPSS version 11.5 (ANOVA, χ^2 test). 17% of patients had genotype T/T, 47% C/T and 36% C/C for the C677T polymorphism. For the polymorphism A1289C the findings were as follows: 38% AA, 48% AC and 14% CC. We did not find significant difference in relative DAS28 decrease between genotype groups 6 months after initiation of MTX therapy. MTX-related toxicity was identified in 17 patients. According our findings the A1289C polymorphism rendered patients sensitive to MTX toxicity.
The polymorphisms in MTHFR were not associated with any difference in efficacy parameters during methotrexate treatment. Further prospective studies including new genetic polymorphisms and larger group of patients will be necessary in order to precisely assess drug efficacy and its toxicity.

P1195. Mutation patterns of mtDNA: empirical inferences for the coding region

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Human mitochondrial DNA (mtDNA) has been extensively used in population and evolutionary genetics studies. Thus, a valid estimate of human mtDNA evolutionary rate is important in many research fields. We analysed a portion of the coding region of mtDNA between positions 3230 and 4331 ($tRNA^{Leu}$, ND1 and $tRNA^{Ile}$ genes), using individuals belonging to extended families from the Azores Islands (Portugal) with the main aim of providing empirical estimations of the mutation rate of the coding region of mtDNA under different assumptions, and hence to better understand the mtDNA evolutionary process. Heteroplasmy was detected in 6.5% (3/46) of the families analysed. In all of the families the presence of mtDNA heteroplasmy resulted from three new point mutations, and no cases of insertions or deletions were identified. Major differences were found in the proportion and type of heteroplasmy found in the genes studied when compared to those obtained in a previous report for the D-loop. Our empirical estimation of mtDNA coding region mutation rate, calculated taking into consideration the sex of individuals carrying new mutations, the probability of intra-individual fixation of mutations present in heteroplasmy and, to the possible extent, the effect of selection, is similar to that obtained using phylogenetic approaches. Based on our results, the discrepancy previously reported between the human mtDNA coding region mutation rates observed along evolutionary timescales and estimations obtained using family pedigrees can be resolved when correcting for the previously cited factors.

P1196. Allele frequencies for 150 genetic markers of oxidative stress and myocardial infarction in Lithuania

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Myocardial infarction is a complex multifactorial and polygenic disorder, therefore large scale association studies that examine many polymorphisms simultaneously are required to allow reliable prediction of the genetic risk for myocardial infarction (MI).
After the large scale analysis of literature and bioinformatic databases 150 single nucleotide polymorphisms (SNPs) of 89 candidate genes, mostly involved in oxidative stress regulation and oxidative homeostasis, were selected to develop a microarray for arrayed primer extension (APEX) resequencing technology (Asper Biotech, Estonia). Most of the selected SNPs were in promoter regions or exons that might be expected to cause changes in the function or level of expression of the encoded protein.
The group of 49 persons from Lithuania having offspring(s) with myocardial infarction were initially analyzed to detect the allele frequencies for the SNPs of the candidate genes in MI. Chi square analysis was carried out to test deviations of genotype frequencies from Hardy-Weinberg equilibrium.
38 SNPs were not polymorphic in the studied group. The genotype frequencies of SNPs of *APOA5*, *ADRB2*, *LGALS2*, *GP1BA*, *ITGB3*, *PDE4D*, *GNG12*, *GPX4* and *NFKB1* genes deviated significantly from those predicted by the Hardy-Weinberg equilibrium ($p<0,05$). The small sample size could cause this deviation. The estimated allele frequencies of tested SNPs were similar to those detected in other European populations. In conclusion 112 SNPs were selected to be informative for further association studies.

P1197. How a mutation associated with Myotonic Dystrophy Type 1 migrates in Bashkortostan (Russia)

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Myotonic Dystrophy is a disorder with an autosomal dominant mode of inheritance. Anticipation phenomenon is well known in families with Myotonic Dystrophy type1 (DM1). This means that a half of the offspring of an affected parent could get this mutation and have more chances to suffer from a more severe clinical condition than their parents. Prenatal testing for DM1 is still not available in Bashkortostan (Russia) as a routine procedure. The territory of Bashkortostan is 143.600 square kilometers and the population is over 4 million people. We were interested to find out how population (and their genes) migrates within our Republic and to predict the mean distance for migration of DM1 mutation per generation. We performed a study of places of birth of inhabitants of Bashkortostan for calculating the coefficient of diffusion. We analyzed 875 births in our Republic and calculated the distances between places of birth of parents and their children. Our mathematical model shows that mutation associated with DM1 in Bashkortostan migrates for 120 km per generation.
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P1198. Genetic Variation Controlling Cellular Traits related to Down Syndrome: Reactive Oxygen Species

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Understanding the genetic basis of susceptibility to complex disorders is one of the major aims in medical genetics today. The availability of the genome sequence and variation allows now to study quantitative cellular phenotypes closely related to clinical manifestations. Production of reactive oxygen species (ROS) may be involved in a number of human disorders including Down Syndrome (DS). We measure ROS production with an enzymatic assay in lymphoblastoid cell lines (LCL) from several human populations, either as families or unrelated individuals, to assess the genetic architecture of this trait.
There was substantial inter-individual variation in cell lines from unrelated CEPH individuals and heritability estimates were 45% as assessed with 10 CEPH families. Genome-wide linkage analysis on these families showed 2 significant linkage signals on Hsa12 and Hsa15. In a genome-wide association analysis, we measured ROS production in Caucasian HapMap individuals (N=58) and associated this trait to 2.2 million SNPs from HapMap. Results confirmed previously detected linkage signals; in addition 8 new significantly associated loci were detected. We repeated a genome-wide association analysis in an independent population of LCL of healthy German individuals (KORA project). Preliminary analysis of the first 87 individuals confirmed the locus on Hsa15 and replicated two previously associated loci on Hsa4 and Hsa6. The comparison of ROS production of DS individuals (N=34) to either HapMap or KORA populations revealed a significant ROS decrease.
Cellular phenotypes could be used as proxies for complex disorders, and the approach described here may contribute to the genetic dissection of these traits.

P1199. Epidemiology of oculo-aruiculo-vertebral spectrum (OAVS): a registry-based study on European population

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Oculoariculovertebral spectrum (OAVS) is a phenotypically and genetically heterogenous disorder grouping together different conditions thought to be caused by impaired development of the first and second branchial arches including Goldenhar syndrome, facioauriculovertebral syndrome, hemifacial microsomia, and otomandibular dysostosis. We present the results of the population-based epidemiological study on the severe end of OAV spectrum.

The data were extracted from the database of EUROCAT (European Surveillance of Congenital Anomalies), a large European network of birth defect registries that use the same epidemiological methodologies. Based on data collected during the 1980-2004 period, we found the prevalence of the severe OAVS cases to be 2.63/100 000 births or 1/38022. The most frequently associated congenital malformations were major ear malformations that accounted for 30% of cases (68/224). Vertebral anomalies were reported in 30% of cases (67/224), and cardiac defects were present in 25% of cases (57/224). Severe central nervous system involvement was rare (17/224 - 8%). Prenatal ultrasound examination in the period 2000-2004 detected abnormalities in 15% (16/111) of cases. Live born infants with OAVS have a high first week survival (98.5%). Maternal and paternal ages do not seem to be risk factors for OAVS. Almost 35% of patients, born after the 37th week of gestation, weighed less than 2500 g. Among 272 patients, consanguinity of parents was registered in 5 cases. OAVS among sibs was found in 4 cases, while family history for OAVS was positive in additional 10 cases. No evidence of exposure to consistent teratogenic agents including maternal diabetes was noted.

P1200. Bone mineral density variation related to islet amyloid polypeptide (amylin) haplotypes in young and elderly women in Southern Sweden

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BACKGROUND AND AIM: The candidate hormone islet amyloid polypeptide (IAPP or amylin) is predominantly expressed by the pancreatic beta cells and cosecreted with insulin in response to food intake. Several studies have implied a physiological role for IAPP in bone remodeling. For example, IAPP knockout mice exhibit increased numbers of bone-resorbing osteoclasts and develop an osteporosis-like phenotype in adulthood. However, the importance of IAPP in bone remodeling in humans is not clear. In the present work we have investigated whether different IAPP haplotypes are associated with bone mineral density (BMD) in young women at peak bone mass and elderly women at high risk of fracture. MATERIALS AND METHODS: 1005 young women from the Malmö Peak-study (age 25±0.1 yrs, BMI 23.0±3.7 kg/cm2) and 1044 elderly women (Malmö OPRA-study; age 75±0.1 yrs, BMI 26.2±4.2 kg/cm2) were recruited. The primary phenotype was BMD assessed by DXA. Body composition data, calcaneus ultrasound estimates and fracture data (OPRA-study) were also available. Short nucleotide polymorphisms (SNPs) in the vicinity of the IAPP gene were retrieved from the International HapMap Genotype Database and genotyped by PCR using the ABI SNP genotyping assay. RESULTS AND DISCUSSION: Obtained data and an update of this study will be presented.

P1201. Model free Lods for linkage analysis of large complex pedigrees

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Parametric linkage analysis is a powerful tool for mapping genes for complex traits. When mode of inheritance is unknown, it was suggested to use model free Lod score for linkage analysis. The statistical properties of different parametric linkage tests have been compared for small pedigrees, but remain unknown for large extended pedigrees.

We compared model based and model free Lod score statistics for multipoint linkage analysis in a large complex human pedigree with multiple loops. We simulated quantitative traits with dominant, additive or recessive major gene effect and genotypes for a set of five-allelic markers. Three linkage statistics were compared: Lod, using true underlying QTL model parameters; PLod, based on the estimations of model parameters coming from complex segregation analysis and MFLod, estimating model parameters and the QTL position simultaneously. We used approximate likelihood calculation based on the loop breaking (Stricker et al., 1995).

For all analyzed models average values of the test statistics and the portion of experiments in which the test was >3, were highest for MFLod and lowest for the PLod. However, it is known that the distribution of multipoint Lod score under H_0 depends on frequencies of alleles, mode of inheritance, and so on. We analyzed type I errors for used linkage statistics and demonstrated that they are similar for Lod and PLod, but higher for MFLod. Nevertheless linkage power calculated on the base of empirical threshold level was higher for MFLod than for Plod or Lod.

P1202. Combinatorial genetic analysis of physical performance in athletes

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It has been shown physiologically that for rowing at least 70% of the energy requirement comes from aerobic metabolism; the remainder comes from anaerobic sources. Accumulating evidence suggests that polymorphisms of *ACE*, *ACTN3* and *PPARA* genes are strongly influence human physical performance. *ACE* I and *PPARA* G alleles are supposed to be favorable for endurance-oriented athletes, whilst *ACE* D, *PPARA* C and *ACTN3* R alleles are thought to enhance power performance. The purpose of this study was to determine genotype and allele frequencies of *ACE* I/D, *ACTN3* R577X and *PPARA* G/C polymorphisms in rowers and to detect genotype combinations that are prevalent in elite, sub-elite and non-elite rowers compared to controls. 173 male and female Russian elite, sub-elite and non-elite rowers and 842 controls were recruited for the study. Genotyping was carried out by RLFP. We found that frequencies of *ACE* D and *PPARA* C alleles were the lowest in elite level rowers compared to other rowers and controls. *ACTN3* X allele frequency tended to decrease with athletes' improvement of elite status. When all endurance-associated alleles were combined, we determined common endurance alleles frequencies in different groups. The highest value of endurance alleles frequency was observed in male elite (72.2%) and female sub-elite rowers (70.5%), compared to male (66.0%) and female controls (66.1%), respectively. The prevalent combination of genotypes in all groups was ID-RX-GG. The frequency of this combination was highest in elite rowers (28.6%); the lowest was found in controls (17.3%).

P1203. POLG disease mutations in Europe, Australia, New Zealand, and the USA explained by single ancient European founders

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Mutations in the catalytic subunit of the mitochondrial DNA polymerase gamma (POLG) have been found to be an important cause of neurological disease. We and collaborators have identified the POLG W748S mutation as the underlying cause of mitochondrial recessive ataxia syndrome (MIRAS) and found it to be among the most common

genetic causes of inherited ataxia in Finland - with a carrier frequency in the general population of 1:125. The characteristic clinical features in our patients included ataxia, peripheral neuropathy, dysarthria, mild cognitive impairment, involuntary movements, psychiatric symptoms, and epileptic seizures. Here, we show that the W748S mutation has a common ancient founder for all the disease chromosomes in Australia, New Zealand, Finland, Norway, United Kingdom, and Belgium. Furthermore, we show that a second common POLG mutation, A467T, also shows common European ancestry: patients from Australia, New Zealand, and the United States share a common haplotype with European patients. These data of ancestral haplotypes indicate that the *POLG* locus is quite stable and that the recessive W748S and A467T mutations have occurred once in history. They have effectively spread to populations of European descent with carrier frequencies up to 1% in several populations. Our data predict that these mutations are common causes of ataxia and Alpers disease in the Western world.

P1204. The analysis of the nuclear genes polymorphism's in Kazakhs

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The polymorphic nuclear locus in human genome are the objective genetic markers for the analysis of hereditary polymorphism of the modern human populations and evolution. A total of 324 unrelated healthy Kazakh people were studied. A portion of ACE, TPA25, PV92, eNOS3, APOA1, YaNBC148, YaNBC27 genes and deletion 32 b.p. of CCR5 gene from genomic DNA was amplified by PCR and analyzed on a 6% polyacrylamide gel. Each fragment was classified according to genotypes. Genotypes and allelic frequencies were revealed: for ACE gene - 27,2% (II), 21,3% (DD), 51,5% (ID), 52,9% (I), 47,1% (D); for TRA25 gene - 22,8% (II), 26,9% (DD), 50,3% (ID), 47,8% (I), 52,2% (D); for PV92 - 26,2% (II), 23,5 (DD), 50,3% (ID), 51,4% (I), 48,6% (D); for eNOS3 gene - 1,9% (AA), 18,2% (AB), 79,9% (BB), 11,0% (A), 89,0% (B); for APOA1 gene - 58,6% (II), 14,8% (DD), 26,5% (ID), 71,9% (I), 28,1% (D); for YaNBC148 gene - 8,3% (II), 46,9% (DD), 44,8% (ID), 30,6% (I), 69,4% (D); for YaNBC27 gene - 9,9% (II), 53,4% (DD), 36,7% (ID), 28,2% (I), 71,8% (D); for CCR5 gene - 0,62% (D/DEL), 92,3%(N/N), 7,1% (N/DEL), 4,2% (D), 95,8% (N). The distribution of the empirical genotypes and allelic frequencies and the indexes of heterozygosity of all genes were completely conformed to theoretical deviation of Hardy-Weinberg ($p>0,05$), except for APOA1 gene ($\chi^2=37,2$; $p<0,001$).

P1205. Association between platelet GPIIb-IIIa polymorphism (PlA1/A2) and atherosclerosis obliterans in diabetes mellitus type 2

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Epidemiologic studies show that thromboembolic diseases are the result of complex interactions between genetic factors and the chronic influence of the environment. Diseases like diabetes may worsen this picture and enhance the appearance of thromboembolic diseases. Thus, isquemic events like atherosclerosis obliterans are among the major causes of morbidity and mortality in patients with diabetes mellitus type 2. Recent studies suggest that platelet polymorphisms may act like risk factors for the development of such events.

The aim of this work is to study the association between platelet GPIIb-IIIa polymorphism PlA1/A2 and atherosclerosis obliterans in patients with diabetes mellitus, type 2.

Allelic frequencies will be studied in 3 different groups. 1 group of patients with diabetes that suffer isquemic events, 1 group of patients with diabetes that did not suffer isquemic events and 1 normal group. Relationship between allelic frequencies and clinicopathologic variables will also be studied.

Results and discussion of the results aren't available yet. However, results of, at least, 20 samples for each group will be accessible at the time of the congress.

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P1206. *ACTN3* and *ACE* genotypes in Greek elite athletes

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Only a few attempts have been made to shed light upon the influence of genes in making an Olympic champion. The aim of our study is to elucidate the genetic differences among a group of 101 elite Greek power-oriented track and field athletes and a random representative sample (181) of the Greek population by analyzing *ACTN3* and *ACE* genotypes. Athletes were defined as elite and included to the sample if they had represented Greece at the international level. Standard molecular genetic methodologies were followed. Genotype and allele frequencies were compared between elite athletes and controls by the Chi-squared test using the statistical package GENEPOP V. 3.4. Preliminary results for *ACE* locus indicated that the gene frequencies in the Greek elite athletes are similar to other northern European populations. Furthermore, concerning the *ACTN3* locus, it was showed that *ACTN3* genotype and allele frequencies in the top power-oriented athletes were statistically significantly different from those in the random sample of the Greek population: the frequency of the RR *ACTN3* genotype in power-oriented athletes vs. the general population was 47.94% vs. 25.97%. The difference was even more prominent for comparison of the subgroup of sprinters to controls. The results suggest an overall strong association between the presence of the RR genotype and elite power performance. Therefore, the *ACTN3* gene might be used as a molecular genetic marker to at least partially predict an athlete's ability to achieve peak power and sprinting performance.

P1207. Patterns of genetic diversity of populations of the Caucasus, Volga-Ural, Central Asia, and Siberia

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A total of 1049 individuals from Volga-Ural region of Russia (Turkic speaking Bashkirs, Tatars, and Finno-Ugric speaking Komis, Maris, Mordvins, and Udmurts), Central Asia (Turkic-speaking Kazakhs, Uzbeks, and Uighurs), the North Caucasus (Turkic speaking Karachays, Kumyks, Kuban Nogays, and Karanogays), and Siberia (Turkic speaking Yakuts, Tungusic speaking Evenks, and Mongolic speaking Kalmyks) were analyzed using eight Alu loci (ACE, ApoA1, PV92, TPA25, NBC27, NBC102, NBC148, and NBC182). Genetic differentiation in various regions of the world was fairly substantial. Basing on 8 Alu loci data Gst value for the world dataset was 0.089. Geographic divide between Europe and Asia, e.g. the Ural Mountains and the Caspian Sea, can also be considered as a genetic boundary. The North Caucasus populations demonstrated genetic pattern which is very close to Near East populations. European populations reflect neither geographic nor linguistic relationships. The data indicates that the populations of the two boundary regions between Europe and Asia, the Volga-Ural region of Russia, and populations of the North Caucasus are more similar to European than to Asian populations. Siberian and Central Asian populations are genetically closely related to each other. The fact that populations of the four regions analyzed fit the genetic variation throughout Eurasia attests that they were involved in the same major demographic processes that took place within the continent no matter what genetic differences or similarities the populations demonstrate.

P1208. Regional differences in the genetic variability of Finno-Ugric speaking Komi populations

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The Komi (Komi-Zyryan) people are one of the most numerous ethnic groups belonging to the Finno-Ugric linguistic community. They occupy an extensive territory in north Russia to the west of the Ural Mountains, in the northeast of the East European Plain. This is an area of long-term interactions between Europeans and North Asians. Genetic variability was evaluated in two geographically distinct populations, the Izhemski and Priluzski Komi. We searched for polymorphisms of the *TP53* gene

(a 16-bp duplication in intron 3 and three RFLPs: for *Bsh*1236I at codon 72, for *Msp*I in intron 6 and for *Bam*HI in the 3´ flanking region) and for variable number tandem repeat (VNTR) polymorphisms of locus *D1S80* and of the 3´ untranslated region of the gene for apolipoprotein B (*ApoB)*. Some data from our previous studies of *TP53*, 3´*ApoB* and *D1S80* variability were involved in the comparison of Komi with other Eastern European populations. Multidimensional scaling analysis of genetic distances was used for the evaluation of genetic relationships between populations. The results revealed some affinity between Priluzski Komi and Eastern Slavonic populations, and significant segregation of Izhemski Komi from other ethnic groups studied. The unique genetic features of Izhemski Komi may have been determined by their ethnogenesis or the pressure of environmental factors, such as special nutrition and adaptation to extreme climatic conditions.

P1209. Mapping quantitative trait loci (QTL): Is it worth investing in repeated phenotype measurements to improve genotype-phenotype correlations?

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Aim. In any attempt to identify alleles associated with human quantitative traits (QT), the investigators will use measurements of phenotype of interest in the recruited set of examinees and their individual genotypes. The power of any association study to detect human QTL will rapidly diminish if initial phenotype measurements are not accurate and repeatable.

Materials and methods. We investigated the 14 QT's in a sample of people from an isolated population of Croatian island of Vis. The measurements were performed by the same field crew using the same methods in February 2002 and May 2003, in total of 74 examinees. Phenotypic measurements were analyzed with paired parametric or non-parametric statistical tests, depending on the data distributions.

Results. Most traits exhibited significant differences between two measurements: urate (P=0.004; paired t-test), systolic blood pressure (P<0.001), diastolic blood pressure (P=0.001), creatinine (P=0.001), cholesterol (P<0.001), triglycerides (P=0.018), HDL (P=0.039), LDL (P<0.001), and glucose (P=0.002; all Wilcoxon). Only three traits did not exhibit significant differences; height (P=0.319), weight (P=0.055), and body mass index (P=0.784; all paired t-test).

Conclusion. Despite of assumed reduced genotypic and environmental variance in an isolated island population, measuring a single phenotypic value does not seem to be very reliable method for mapping QTLs. This study raises the issue of whether increasing sample size or repeating (some) phenotypic measurements is better research option in budget-restrained settings.

P1210. A population-based epidemiological and genetic study of X-linked retinitis pigmentosa

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PURPOSE: To perform a nation-wide elucidation of the prevalence and the mutation spectrum in X-linked Retinitis pigmentosa (XLRP), and to make genotype-phenotype comparisons.

METHODS: Our study comprised 96 affected males and 150 female carriers from 42 families representing all identified XLRP individuals in the Danish population (5.4 million inhabitants). RPGR and RP2 were screened for mutations, and the medical files of the patients were scrutinised and phenotype data extracted.

RESULTS: Prevalence of affected males was estimated to be 1:24.500 and 1:18.000 of female carriers. Cumulated life-risks for developing XLRP between 0.22 and 0.59 per 10,000 males were calculated. Molecular analysis of RP2 and RPGR uncovered 28 different mutations in 33 of 34 index cases analysed. 12 patients carried a mutation in RP2, 12 in exons 1-14 and 9 in ORF15 of RPGR. Males with RP2 mutations tended to have higher degrees of myopia, lower visual acuities, and better preserved visual fields than males with RPGR mutations at the same age.

CONCLUSIONS: A very high mutation detection rate in familial cases makes genetic testing a highly valuable clinical tool for genetic counselling and prenatal testing which is regularly demanded by Danish XLRP carriers. The proportion of RP2-mediated XLRP in the Danish population is higher and the proportion of RPGR-ORF15 is lower than reported in other studies. Thus, strategies for diagnostic procedures should take into account the population-specific mutation spectrum.

P1211. Genomic sequence analysis reveals a complex segmental duplication superstructure on human chromosome 17q arm

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Human chromosome 17 is associated with several types of cancer and genetic diseases, such as neurofibromatosis and breast cancer. Chromosomal instability is one underlying cause of these disease manifestations. A major mechanism resulting in chromosomal instability is the non-allelic homologous recombination (NAHR). It has been shown that the chromosome 17 is enriched with inter- and intra-chromosomal segmental duplications.

We conducted a genome-wide sequence alignment using expressed transcripts mapping within the previously identified duplicated domains to determine the full extent and architecture of the duplicated segments on chromosome 17q. This analysis identified a duplication superstructure (SDS) on 17q arm, consisting of 13 discrete domains, which share sequence homology. A similar structure was found on the syntenic chimpanzee chromosome.

Analysis of the genomic sequences of the duplication domains revealed the most highly copied sequences to be retrotransposable sequence elements. Twelve retrotransposable mRNAs and their sequence copies were found almost exclusively within the SDS, both in the human 17q and the chimpanzee 19q. The highest numbers of sequence alignments were observed with two transcripts, AK125814 and AK125932. Interestingly, the AK125814 transcript was found to be expressed in 5/6 healthy human and two chimpanzee PBMC samples. Sequencing of the rt-PCR products showed that AK125814 was preferentially expressed from one of the duplicated locations, which varied between individuals.

The complex duplication architecture on 17q may predispose to chromosomal instability via NAHR and possibly lead to disease causing copy number variation. The potential functional role of the actively transcribed, SDS-associated retrotransposable elements is an intriguing question.

P1212. Segregation patterns of CAG repeats in the MJD locus: a study in normal families from the Azores Islands (Portugal)

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Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder of late onset, caused by an expansion of a CAG repeat motif in the coding region of *ATXN3* gene. In the Azores, MJD reaches the highest worldwide prevalence (1/103). Previous studies devoted to segregation ratio distortion (SRD) in the MJD locus are controversial both in patients and in normal individuals. The aim of this work is to investigate the existence of SRD upon transmission of wild-type MJD alleles, in order to contribute for the understanding of the dynamics of triplet repeat loci. The number of the CAG repeats at the *ATXN3* gene was determined for 69 normal Azorean sibships (total of 364 transmissions). Sixteen allelic variants were observed, varying between 14 and 39 repeats. Alleles with 23 and 14 repeats were the most frequent, in agreement with previous population studies. From segregation analysis, our data suggest the occurrence of SRD, with distortion towards the transmission of the smaller alleles in paternal (p=0.037), but not in maternal transmissions. Furthermore, the difference in the number of repeats between the two alleles that constitute the transmitters' genotype (A_L-A_S), seems to have an effect on this distortion. With the exception of cases with A_L-A_S=1 or A_L-A_S≤2, the ten-

dency is for preferential transmission of the smaller allele or absence of preference. Excluding such cases, SRD becomes significant in both paternal and maternal transmissions. This evidence suggests that the transmitters' genotypic composition may act as a confounding factor in studies of SRD for wild-type MJD alleles.

P1213. Analysis of the population heterogeneity in East Slovakia using fifteen STR markers.

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The presented study has shown that population analyses in East Slovakia can be of great importance from the viewpoint of the examination of population differentiation. This study provides additional population genetic data of the four East Slovakian populations (Slovak Caucasians, Romanies from region Spiš, Olachian Romanies and Romanies called Labances) on the thirteen CODIS core STR loci with the highest proportion in the case of the Slovak population-Romanies Labances pair (all analysed 15 loci) and with the lowest portion in the Romanies from the Spiš region-Romanies Labances pair (4 out of 15 loci). In addition, considerable heterogeneity was detected between the Romany populations where ten out of fifteen STR markers showed significant differences in the allele frequency distributions. The high F_{ST} values detected between ethnic groups with different origin or between isolated populations can be explained the diverse genetic history and the isolation of the Slovakian subpopulations.

P1214. A case control study: no association between SLC6A3 (DAT1) with schizophrenia

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According to the dopamine hypothesis of schizophrenia' schizophrenia is associated with increased activity in dopaminergic neurons. Dysfunction of central dopaminergic neurotransmission has been suggested to play an important role in the etiology of schizophrenia.
The Na^+_Cl^- dependent dopamine transporter (DAT1) is a central regulator of the time and course and synaptic concentration of released dopamine by rapid reuptake of dopamine into synaptic terminals and mediating synaptic re accumulation of dopamine. In this study we thought to determine the possible association between the SLC6A3 gene (DAT1) core promoter diallelic polymorphic site -67 A/T and schizophrenia in western south of Iran.We use the case control study to determine possible association between -67A/T SNP and schizophrenia. Fifty unrelated male patient affected with schizophrenia were recruited for the study from golestsn and salamat hospitals in Ahwaz. Also fifty unrelated male controls were randomly selected as control group. Subsequently the allele and genotype frequencies of the polymorphism in two groups were studied. The genotype frequencies in the patient group were as follows: AA 68%, AT 24% and TT 8% versus the genotype frequencies in the control group were AA 78%, AT 10% and TT 12%. Based on this data alleiic frequency were 83% for A, 17% for T in control group and 80% for A, 20% in patient group. According to this data and x^2 test there is no association between DAT1 and schizophrenia.

P1215. Tracing the Origins of Romanies in Central Europe: preliminary mitochondrial and Y chromosomal data of Romani populations in Slovakia

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Since many linguistic and genetic studies placed origin of Romanies to Indian subcontinent, some attempts have been made to characterize mitochondrial and Y-chromosomal DNA variability, date exodus from homeland, trace migration routes, and estimate source and extent of non-Indian component within Roma populations. In order to contribute completing the outlined picture we analyzed 193 samples from Slovak Romani individuals for mitochondrial control and coding region markers as well as for Y-chromosomal SNP and STR markers. The mtDNA haplotypes detected in this sample fall into the common Eurasian mitochondrial haplogroups (H, U, K, J1, X, I, W, HV, T, C, M5, and M35) except of African L2a haplogroup (2.1 %), with the most frequent haplogroups M (24.4 %), H (22.3 %), and I1 (16.6 %). For Y-chromosomal DNA analysis haplogroup H1 of Indian origin has been found as most common (35.8 %). Other common haplogroups included E3b1 (13.7 %), J2 (18.9 %), R1a (13.2 %), and I (10 %), less frequent haplogroups were R1b3, R2, J1, G, and N. Variability of haplogroups and diversity found were lower comparing to European populations. Coalescence times calculated using HVS1 and 17 Y-chromosomal STR loci for paternal (H1 - 3035 ± 1115 YBP), and maternal (M5 - 3100 ± 1400 YBP) lineages of probable south-Asian origin indicated earlier isolation within indigenous inhabitants of Indian subcontinent. AMOVA using haplogroup frequencies of analyzed European Romanies populations suggested recent gene flow and low differentiation of Roma subpopulations.

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P1216. DHCR7 mutation carrier rates in Europeans - Significance for the Prevalence of the Smith-Lemli-Opitz Syndrome

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Smith-Lemli-Opitz Syndrome (SLOS, MIM270400) is a mental retardation and malformation syndrome with variable clinical severity. SLOS is caused by mutations in the delta7sterol-reductase gene (DHCR7, E.C.1.3.1.21), which impairs cholesterol biosynthesis.
The prevalence of SLOS has been estimated to range from 1:20.000 to 1:60.000 in European populations. Mutational spectra analysed in SLOS patients were different across populations with frequency maxima of common mutations in East-Europe (p.W151X, p.V326L), North-West-Europe (g.IVS8-1G>C), and South-Europe (p.T93M).
In order to compare prevalence and incidence of SLOS we estimated carrier frequencies of most common DHCR7 mutations in European Populations.
Nearly 8.000 chromosomes of Austrian, British, Czech, French, German, Greek, Italian, Polish, Spanish and Turkish origin were screened for the frequencies of the common mutations c.IVS8-1G>C, p.W151X, and p.T93M.
For the null mutations c.IVS8-1G>C and p.W151X a carrier frequency of about 1:60 was found, varying from 1:30 (Turkey) to 1:200 (Spain). These two null mutations plus the missense mutation p.T93M yield a carrier rate of 1 in 54 in Europeans. This results in an expected incidence for SLOS of 1:2.940 Europeans, varying from 1:1500 (Austria) to 1:9217 (Spain).
The discrepancy between the expected incidence and the prevalence is most likely due to neonatal and infancy death of the most severely affected children with SLOS and ascertainment bias of mild and atypical cases. SLOS syndrome may be responsible for a high number of miscarriages.
Thanks to all collaborators for population DNAs.

P1217. Frequencies of spinocerebellar ataxia subtypes support admixture with Indian population in Thailand

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Thailand.
Background: While there are strong genetic evidences linking the population in the Indochina peninsula with the Chinese, this was not the case with the Indians despite the proximity of the region to both populations. Using the heterogeneity of the spinocerebellar ataxias, a group of neurological disorders with different subtype frequencies among different populations, we looked for the evidence supporting relationship between the Thai population and both the Chinese and Indian populations.
Methods: We searched for spinocerebellar ataxia (SCA) type 1, SCA3 and SCA6 mutations in 340 patients from 182 families, in which at least one person had a clinical diagnosis of SCA, using GeneScan analysis. We analysed the relative frequencies of SCAs subtypes on a family basis, and compared these to the data in the related populations.
Results: SCA3 was the commonest, with a relative frequency of 17.0% (Agresti-Coull 95% C.I.: 12.2%-23.2%), followed by SCA1 at 10.4% (6.7%-15.8%). SCA6 was found in three families, with a relative frequency of 1.6% (0.3%-5.0%). When compared to the related populations, the Thai SCA3 frequency was less than that of the Chinese while higher than that in most of the Indian studies. The reverse is true for the SCA1. A similar study in Singapore, where the pattern of population admixture was clear, also showed somewhat similar results, although more inclined toward the Indians.
Conclusion: Our results supported some degree of admixture with the Indians in Thai population, and should encourage further study in the area.

P1218. Population study at fifteen short tandem repeat loci in the representative sample of Bosnia and Herzegovina residents - contemporary data

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In our previous population studies of B&H human population, we used 17 autosomal and 12 Y-chromosomal STR loci, as well as 28 NRY bi-allelic markers to generate referent database. Wishing to test our database in order to obtain specific results in various DNA analysis, we have decided to analysis additional individuals, firstly at fifteen autosomal STR loci (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, FGA). Therefore, we have tested additional group of 100 unrelated healthy individuals born in B&H. Qiagen Dnaeasy™ Tissue Kit was used for DNA extraction from buccal swabs and bloodstains and *PowerPlex 16® System* for amplification and detection. The total volume of PCR reaction was 5µl and PCR amplifications were carried out in PE GeneAmp PCR System Thermal Cycler. Electrophoresis of the amplification products was preformed on an ABI PRISM 310 genetic analyzer. The raw data were compiled and analyzed using Genemapper® v3.2. Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination and power of exclusion were calculated. Statistical differences between the new set of data and the data at same loci published earlier (based on 100 tested individuals) indicated the advantage of using recently obtained frequencies, especially as it pertains to the forensic-genetic statistical analysis. In addition**,** we compared B&H data with the data obtained from geographically closer European populations. The results of this study will be used as guidelines in additional improving of investigation of recent B&H human populations, initiated in our previous studies.

P1219. Possible common origin for the Tibeto-Burman and Austro-Asiatic speaking populations of India: a Y-chromosome study

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The geographic contiguity and the morphological similarity of the Tibeto-Burman speaking populations with the Austro-Asiatic speaking groups of India provide hints for the origin of the two linguistic families from a common stock in the remote past. However, this intriguing aspect of genetic relationship between the Tibeto-Burman and Austro-Asiatic speaking populations of India is little investigated and warrants further discussion through molecular genetics.
In this regard, we analyzed 38 biallelic and 20 short tandem repeat polymorphisms on the Y-chromosome in 293 male individuals of 22 different populations belonging to Tibeto-Burman (12) and Austro-Asiatic (10) linguistic families, inhabiting northeastern, eastern and central regions. Among the 14 haplogroups observed, subclades of haplogroup O were predominant (~83.96%) of which O2a, showing the highest frequency of ~ 55%, was the major common subclade present in both Tibeto-Burman (41.3%) and in Austro-Asiatic (73%) populations. AMOVA results indicated a higher degree of genetic differentiation (31.95%) among populations within the two linguistic groups than among the two groups (13.10%). The neighbor-joining tree and the PCA plot shows clustering of some Austro-Asiatic populations (mainly from Jharkhand) with few Tibeto- Burman populations from Arunachal Pradesh and Mizoram suggesting possible shared common ancestry of some Tibeto-Burman with a few of the Austro-Asiatic populations. STRUCTURE analysis is also in congruence with the genetic commonality between the studied linguistic groups. To get further insight into the issue, a median-joining network was constructed based on the haplotypes that fall into haplogroup O2a and the time of divergence for the haplogroup was also estimated.

P1220. Thiopurine S-methyltransferase (TPMT) pharmacogenetics: genotype-phenotype comparison and haplotype analysis in Estonian population

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Thiopurine methyltrasferase (TPMT) is a cytoplasmic enzyme catalysing the S - methylation of thiopurine drugs. Thiopurines exert cytotoxicity after metabolism to thioguanine nucleotides and they are turned into inactive metabolites by TPMT. To date, at least 23 single nucleotide polymorphisms/mutations have been reported in TPMT gene, 22 of them are or may be associated with low enzyme activity.
The aim of the present study was to measure erythrocyte TPMT activity in Estonian population, to genotype Estonian population using PCR-RFLP method for known alleles (TPMT*2, *3A, *3B, *3C, *3D, *8) and to sequence the whole coding region of TPMT (exons 3-10) to find novel sequence variants. Of 253 healthy subjects, 15 were heterozygous for TPMT*1/*3A (3%), two for TPMT*1/*3C (0.4%) and two for TPMT*1/*2 (0.4%). No subjects were detected with TPMT*3B, *3D and *8. Several others previously described intronic and exon mutations/polymorphisms were found via sequencing. Three novel mutations T30A in exon 3, A10G in intron 3 and A145G in intron 10 were detected. Association and haplotype analysis was carried out to find SNP differences between low (enzyme activity £58.8 ng/ml/h), intermediate and high (enzyme activity ³130 ng/ml/h) metabolizers. Four SNPs were present together (T114A, T94A, G460A, A719G) and were more significantly ($P < 0.001$) represented in low metabolizers compared to intermediate and high ones.
According to our study several markers predict low enzyme activity, but any specific markers were not correlated with high enzyme activity.

P1221. Analysis of three Microsatellite Markers (vWA31A, CSF1PO and TPOX) of two Romanian population groups and their genetic relationships to European populations

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In this research 3 different DNA-polymorphisms (Microsatellites: vWA31A, CSF1PO and TPOX) have been detected and analysed in a sample of 110 individuals from Prahova Valley (Carpathian Mountains) as well as in a sample of 200 individuals from the Romanian capital, Bucharest. Further, we investigated the extent of genetic influences of European neighbour populations on the Romanian genetic pool.
The results reveal that there were not any significant differences in the allele frequencies in the three Microsatellite markers from the pan-

mictic population of Bucharest and the partial isolated population from Prahova Valley.
Genetic distance analysis showed a close genetic relationship with Italian population as well as with Greek population groups.
Historically this could be the result of intense trading activities of Thracian tribal groups and Greek population groups who established trading colonies at the west coast of the Black Sea (actually East-Romania) during the 7th- 8th century. The most of the Italian influence is thought to be the result of the occupation of the Danube region during the time of the Romanian Empire. A small Slavic influence was also found. The genetic distance between Romanian and German, Croatian, as well as Hungarian populations were more significant.
The results can also be used for paternity and forensic analyses in the Romanian population.

P1222. Beta Thalassemia in Iran: Genetic admixture, new theories for origin and migration of some mutations

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Background: Iran as one of the Middle East country consists of multiethnic groups that have been influenced by various invasions and migration throughout history. In the eastern Mediterranean region, Iran is one of the major centers for the prevalence of β- Thalassemia. This review is an attempt to study the origin of β- Thalassemia mutations in different parts of Iran.
Methods: Through review and discussion of research literature and referred patients to our center, we compared the frequencies of β -globin mutations in different regions of Iran with those derived from neighboring countries.
Results: The analysis provided evidence of new theories about the origin and migration of some mutations like -25bp del, CD36/37, CD30 and introduction of IVSI-5 to outside, as well as, the remarkable genetic classification of the Iranian people and ethnic groups.
Conclusions: This review represents reflection of historic events due to genetic admixture and roles of invasions and migrations in this phenomenon. Also distribution of β- globin gene mutations in Iran showed high heterogeneity among the population in comparison with other population that this heterogeneity has been derived originally from a population with an ethnic Aryan background.

P1223. A Novel β-thalassemia mutation in the distal promoter of the β-globin gene

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A patient with β-thalassemia intermedia of Moroccan descent was found to be compound heterozygous for a novel β-thalassemia mutation G→A at position -190 5' to the β-globin gene and a previously described β^0-thalassemia allele frame shift codon 6 (-A). The novel mutation was found to be associated with the Mediterranean β haplotype IX [- + - + + + +].
The mother, who has the -190 G→A mutation as the sole abnormality of the β-globin gene, had normal hemoglobin, red blood cell indices and HbA_2 levels. Thus the -190 G→A mutation is silent.
The possibility that the novel -190 G→A substitution is a simple polymorphism in the β-globin gene was considered, but is unlikely given its presence in a region (-50 to -300) where other promoter elements important for differential control of gene expression have been described and the absence of this substitution in 80 other normal chromosomes. Although functional tests of the cloned gene in suitable expression assays will be needed to prove that the change at position -190 is a mutation rather than a simple polymorphism.

P1224. Allele frequencies of two novel tandem repeat polymorphism of the 5'-untranslate region of the human *CYP2E1* gene

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The ethanol inducible human cytochrome P4502E1 (CYP2E1) plays a key role in the metabolic activation of procarcinogens like aniline, vinyl chloride as well as acryl amide. CYP2E1 activity depends on inter-individual genetic polymorphisms as well as on interactions at translational level. Homozygotes for *CYP2E1*1D* allele have got an increased enzyme activity compared to the *CYP2E1*1C* allele.
In order to examine the relationship between pollutant -induced clinical outcomes and genetic polymorphisms 1003 Caucasians of the LISA epidemiological study were genotyped for enzymes of biotransformation. Regarding CYP2E1, a repeat in the 5'-untranslated region at -2178 to -1945 bp has been analyzed in more detail by allele specific PCR. Besides the expected alleles *CYP2E1*1A* (5 repeats), *CYP2E1*1C* (6 repeats, wt) and *CYP2E1*1D* (8 repeats) with a frequency of 0.4%, 97.4% and 2.1%, two novel alleles could be described by the present work.
The first novel allele (Acc-No. AM503355) contains 7 repeats and appears with a frequency of 0.05%. This allele is heterozygous expressed with the *CYP2E1*1C.*
The second novel allele contains 8 repeats and exclusively appears concomitantly with the *CYP2E1*1D* allele (frequency 2.1%), which is heterozygous expressed mainly with the *CYP2E1*1C* or rarely with the *CYP2E1*1A* allele. At the moment it is not clear whether the novel polymorphisms of the 5'-flanking region alter the CYP2E1 expression.

Po08. Genomics, technology, bioinformatics

P1225. Human APP overexpression causes neurodegeneration and synaptic loss in neural cells in transgenic *Drosophila.*

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the loss of neocortical and hippocampal synapses that precedes amyloidosis and neurodegeneration and closely correlates with memory impairment. Mutations in the amyloid precursor protein (APP) cause familial AD and result in the increased production of amyloid-β-protein (Aβ). However, it is not still clear how Aβ contributes to synaptic abnormalities in AD.

In our study, transgenic *Drosophila melanogaster* was established as a model to analyze AD pathology caused by APP and Aβ. *Drosophila* has α- and γ-secretases and does not have activity of β-secretase (BACE). Therefore, flies do not generate Aβ. We expected that overexpression of human APP in neural tissues could induces specific effects of APP independently from Aβ secretion. In contrary, double transgenic flies expressing human APP and BACE should generate Aβ and Aβ-specific phenotypes. To induce expression of APP in neural cells, we used elav-GAL4c155 driver and UAS-APP695 expressing system. As expected, only BACE-containing lines displayed Aβ. Orexpression of APP resulted in morphological changes and formation of abnormal behavioral fly phenotypes independently from Aβ generation . Most these phenotypes has been characterized by progressive neurodegeneration with numerous vacuoles in the cortex and neuropil and loss of synaptic density detected by decreased accumulation of presynaptic protein synaptotagmin in mushroom bodies. Thus, we observed a classical AD pathology in the absence of Aβ. We suggest that impairment of cellular functions of APP and secretion of neurotoxic forms of Aβ may independently contribute to the pathogenesis of AD.

P1226. Performance Comparison of BigDye® XTerminator™ Kit & Conventional DNA Sequencing Purification Methods

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BigDye® XTerminator™ is a new post-DNA sequencing reaction purification kit that removes unincorporated dye-labeled terminators and salts from completed DNA sequencing reactions prior to electrophoretic analysis. Adequate removal of these components is crucial in maximizing high-quality basecalls and useable data. Compared to conventional purification methods such as ethanol precipitation or spin columns, DNA sequencing reactions purified with BigDye XTerminator exhibit very few artifacts from residual dye-labeled terminators (dye blobs). Read lengths using the BigDye XTerminator kit are also comparable. Here, the performance of BigDye XTerminator will be compared to that of other purification techniques across a wide variety of DNA sequencing reactions.

P1227. Easy-to-use bioinformatic tools for individualized analyses and data mining in disease gene identification

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Our aim is to create comfortable tools for performing and interpreting linkage and association analyses, for data mining and expression studies. Steps that are repeated in several projects are (semi)automated so that they can be performed by the biologist, student or technician in charge.

Automated single-/multipoint linkage analysis for microsatellites and large-scale SNP data: easyLINKAGE Plus is an interface for automated setup and performance of linkage analyses. We implemented Allegro, Merlin, SimWalk, GeneHunter, SuperLink, FastSLink, and versions for several species. Results are given as text files and graphical outputs.

Web-based exploration of genomic association: Genome-wide association studies are a challenge to graphically display and interpret the results. AssociationDB allows interactive graphical exploration of the results integrating related gene information, tissue-specific expression and microRNAs. Association results can be imported from other programs or get calculated within the database.

Automated collection and integration of gene information from multiple databases: The user can enter a region of interest or a list of genes. OMIM reports, expression, SNPs etc. are displayed for all candidate genes at once on a single HTML page or PDF. Results are ready to be read and discussed with a cup of coffee.

Automated generation of primer sequences for *in situ* probes: This program creates primers for either transcript-specific or universal probes and checks specificity by BLAST.

P1228. "VALAPODYN: Validated Predictive Dynamic Model of Complex Intracellular Pathways Related to Cell Death and Survival."

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VALAPODYN is a research network funded by the European Commission (6th Framework Program) which is developing an original systems biology approach focused on the development of multidisciplinary functional genomics related to complex biological processes and cellular networks. The aim is to generate an innovative approach to model the dynamics of molecular interaction networks (MIN) in relation to cell death and survival to detect new therapeutic targets to treat human brain diseases. The project consists of fundamental genomics research which is integrating statistical data analysis with real biological data in order to functionally annotate genes and proteins. Specialized genomics and proteomics databases for MIN modeling are being used along with leading microarray and proteomics platform systems to investigate protein-protein interactions and regulation networks. This will help to identify and validate biological targets in complex intracellular pathways to cure multifactorial diseases. Dynamic modeling specifically addresses the systems biology of complex cellular pathways and transcriptional networks. The novel dynamic models will be validated by testing the selected drug targets on innovative *in vivo* and *in vitro* models of CNS pathologies.

As opposed to most current biological data analysis methods, VALAPODYN develops a dynamic and quantitative analysis method for new therapeutic targets through MIN dynamic models. It will provide a cutting-edge highly accurate *in silico* tool for identifying novel and effective therapeutic targets in a much faster, more efficient and more economical way than it is possible today. (www.valapodyn.eu).

P1229. Evaluation and introduction of new techniques in the genetic testing service by EuroGentest Unit 5.

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At present, the field of genetics is witnessing a fast expansion of new technologies that could have a strong potential for application in genetic testing. Unfortunately, implementation of these novel technologies is often hampered by the lack of complete evaluation of their technical performance in a diagnostic setting. EuroGenTest (EUGT) is a European Network of Excellence aiming at harmonizing genetic testing services throughout Europe. One key objective is to bridge the gap observed in the chain of new technology-transfer from research into implementation and ultimate accreditation in genetic diagnostics.

EGT-Unit 5 is specifically involved with the coordination and guidance of activities required for complete technical evaluation, validation and subsequent implementation of emerging technologies into diagnostic application.

In this respect we recruit new techniques in genetic testing at our EUGT-Unit 5 Website with our "call for technology" and present them at our Satellite-meeting during the ESHG-conference. As a follow up

several new approaches in genetic testing are selected for further evaluation and validation in collaboration with their manufacturers and inventors.Currently we are evaluating several new techniques including High-Resolution Melting-Curve Analysis (HR-MCA) and Conformation Sensitive Capillary Electrophoresis (CSCE), two fluorescent heteroduplex-based mutation scanning-methods in collaboration with Idaho Technologies and NGRL-Wessex together with Applied Biosystems respectively and Pyrophosphorolysis Activated Polymerization (PAP), a very sensitive detection method for the presence of low mutation levels in the presence of excess wt-allele, in collaboration ServiceXS. More details on ongoing and new evaluation projects will be shown also at the EUGT-Unit 5 Satellite-meeting during this conference.

P1230. Comprehensive human genetic analysis using 454 Sequencing™ technology

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454 Life Sciences has developed a revolutionary technology that can produce up to 100 megabases of sequence data on a single instrument. Many biologically meaningful and complex regions of the human genome can now be analyzed with this system without the time or cost constraints of conventional DNA sequencing methods.

We will demonstrate how this technology can be applied to the study of the cancer genome through ultra-deep sequencing of genes involved in oncogenesis. The technology also allows researchers to conduct studies of human genetic variation (SNPs, indels and rearrangements) from a complex population. We will also discuss applications in the study of gene regulation through sequencing of microRNA, transcriptome and gene copy numbers.

The large sequencing capacity of the 454 Life Sciences technology platform, combined with the flexibility and versatility of sample handling and data output makes it a method of choice for human molecular genetics.

P1231. Characterization of a human fetal cartilage EST library focussing on transcription factors

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Skeletogenesis in part takes place in the growth plate where chondrocytes undergo a coordinated and highly regulated process of cell proliferation, differentiation and apoptosis at which stage vascular invasion brings osteoblasts that replace the cartilage extra cellular matrix with trabecular bone. Disruptions to these extremely coordinated processes result in a group of genetic bone diseases. These highly diverse groups of disorders have a complex aetiology. Some of the involved signalling pathways and gene interactions are known but many molecular mechanisms that might explain the pathophysiology of numerous skeletal dysplasias that result from mutations in genes important for normal bone development still remain unknown. While the sequence of the human genome has been completed, many genes are still uncharacterized. Despite the huge number of ESTs in the public domain, there are still genes to be identified in rare tissues that are under-represented. To find cartilage-specific genes a human cartilage cDNA library was generated and 5000 ESTs are sequenced, computationally characterized. We and others have already characterized novel and tissue specific genes. Based on *in silico* motif searches 49 putative transcription factors have been identified. The temporal and spatial expression patterns of these transcription factors will be determined via *in situ* hybridization. Those transcription factors that are predominantly expressed in developing cartilage will be further characterized to elucidate their role during cartilage and bone development. This way we hope to identify new genes that are important for bone development and deregulation of those genes might be responsible for causing skeletal dysplasias.

P1232. The human CDK5R1 3'UTR contains distinct subregions affecting transcript stability

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Human CDK5R1 encodes for p35, a neurone-specific activator of CDK5, which is involved in neuronal migration and differentiation during CNS development. CDK5R1 has been implicated in neurodegenerative disorders and proposed as a candidate gene for mental retardation. The remarkable size of CDK5R1 3'UTR suggests a role of this region in the control of CDK5R1 expression by post-transcriptional regulatory elements modulating mRNA stability or translation efficiency. Bioinformatic analysis showed a high conservation degree in mammals and predicted several AU-Rich Elements (AREs). CDK5R1 3'UTR was cloned in pGL4.71 at the 3' end of the Renilla luciferase reporter gene to perform Dual Luciferase assays: the construct showed a decreased luciferase activity in six transfected cell lines. The quantitative analysis of luciferase mRNA suggests that CDK5R1 3'UTR affects mRNA stability. We identified five 3'UTR subregions reducing the luciferase activity in some instance with a cell line-dependent way. A region showed a significantly low halflife, suggesting an accelerated mRNA degradation. We also identified, by deletion analysis, a type I ARE displaying a stabilizing effect in two neuroblastoma cell lines. Our findings evince the presence of both destabilizing and stabilizing regulatory elements in CDK5R1 3'UTR. We are now attempting to identify, by REMSA and immunoprecipitation assays, stabilizing neuronal proteins that specifically bind the type I ARE, with the final aim of verifying the functionality of this element. The fine tuning of CDK5R1 expression by 3'UTR may play a role in CNS development and functioning, with potential implications in neurodegenerative and cognitive disorders.

P1233. Cells to Ct: Direct gene expression analysis from cell lysate

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Real-Time reverse transcription (RT)-PCR is a robust, simple and quantitative method for gene expression analysis in biological samples. Traditionally, RNA has been isolated from the sample to remove genomic DNA, RNases, and reverse transcriptase inhibitors before being used as substrate for RT-PCR. RNA isolation is fairly time-consuming and it can lead to loss of RNA in flow-through or incomplete elution with small samples. Direct cell-to-Ct gene expression analysis enables reverse transcription and real-time PCR analysis of RNA from 10-10^5 cultured cells without RNA isolation or purification. By eliminating the RNA isolation step, gene expression analysis of cultured cell line is substantially expedited and simplified. The unique Cells-to Ct lysis procedure allows the concurrent preparation of cell lysate and removal of genomic DNA in less than ten minute. The lysis procedure takes place at room temperature, allowing simple scale-up to robotic platforms for high-throughput applications.

P1234. Theoretical and functional analysis of expressing region of ceruloplasmin pseudogene

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Previously using computer analysis we showed that human (NG_001106) and chimpanzee (XM_516813) pseudogene of ceruloplasmin (pCp) contain region encoded Cp-like protein. The MitoProt program predicted that putative protein consists from N-terminus (66 aa) corresponding to signal peptide for mitochondrial protein import (SPMPI). Remaining region corresponds to domains 5 and 6 of blood Cp. In HepG2 and HuTu80 cells, pCp transcripts were detected by RT-PCR. pCp polypeptide was found in mitochondrial matrix by Western-blot.

To check the biological significance of predicted SPMPI-sequence it was cloned in vectors pEGFP-N3. HEK293 cells were transfected with pEGFP-N3-pCpSPMPI construct. The confocal microscopy data

showed that GFP was localized in the same intracellular compartments as Mito-dsRed. Also HepG2 cells were transfected with this construct. The cells were collected and subcellular fractions were isolated by differential centrifugation. Western-blot analysis with antibodies to GFP revealed GFP in mitochondria. Population comparative analysis of Cp gene 5'-UTR and translated pCp region in DNA isolated from 117 human blood samples by PCR with following PRLF analysis using of restriction enzymes set was carried out. The insertions or deletions as well as mutations in restriction sites in pCp gene amplicons were not found. At the same time a lot of changes were detected in Cp gene promoter. These data suggest conservatism of pCP region. In rat, expression of mitochondrial Cp has tissue specific pattern, not expresses in newborn and adult animals with copper deficiency, induced by Ag-feed. The biological role of putative protein product of pCp in copper metabolism is discussed.

P1235. Functional interactions of conserved non-coding (CNCs) sequences with other CNCs using circular chromosome conformation capture (4C)

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The comparison of human chromosome 21 (Hsa21) with the mouse syntenic regions led to the identification of roughly 3500 regions displaying an identity of >70% over a length of a least 100 nucleotides of ungaped alignment. About 65% (~ 2300) of these are conserved non-coding sequences (CNCs). Very little is known about the function of most CNCs. We speculated that a functional CNC would interact with its genomic target (i.e. an enhancer would bind to it's cognate gene promoter). Thus, the identification of any part of the genome that interacts directly with a CNC could provide clues on the function of the latter. We have generated libraries of CNC-interacting DpnII fragments by circular chromosome conformation capture (4C) whose identity is determined by subsequent sequencing. We have generated initial results concerning crosslinking of 18 Hsa21 CNCs with DNA fragments mapping hundreds of kilobases away from the "bait" on the same chromosome, or with fragments on other chromosomes. A total of 87 such potentially interacting DpnII DNA fragments have been identified. Interestingly, the median distance from the cloned DpnII fragments to the nearest conserved region is 661bp with a pvalue < 0.0003 when compared to the distribution of the median of the distances of 3000 random samples of 87 fragments. These results provide initial evidence that the function of CNCs is mediated by their interaction with other conserved regions.

P1236. EM algorithm for gene copy number estimation using TaqMan® Assays

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Recently, multiple studies have discovered an abundance of copy number variation of DNA segments ranging from kilobases (kb) to megabases (Mb) in size. Copy number variations can cause disease, as in microdeletion or microduplication disorders, or confer risk to complex disease traits such as HIV-1 infection and glomerulonephritis.
TaqMan® gene copy number assays have been developed for accurate detection of genetic variation at gene level using primers and probes designed for genomic DNA. Each well is duplexed with two assays, a FAM™ dye-based assay designed to detect the genes-of-interest and a VIC® dye-based assay for reference gene. In this study, we present an algorithm for gene copy number estimation based on EM algorithm for mixtures of normal distributions. The algorithm finds maximum likelihood estimates of parameters in probabilistic models, where the model depends on unobserved samples copy number of the gene-of-interest. Under current protocols, we are capable of distinguishing up to 8 copies of the gene of interest with at least 95% confidence.
To evaluate this algorithm, we present experimental results for 5 important drug metabolism genes (CYP2D6, CYP2E1, CYP2A6, GSTM1 and GSTT1) on 270 individual samples from International HAPMAP Project representing 4 different populations. Copy number analysis for these genes shows perfect consistency for sample duplicates. Copy number variations are observed for these genes, with significant differences between these populations. Furthermore, combining this data with SNP data, we demonstrate that departures from diploidy can cause apparent genotyping failure and give inaccurate genotyping.

P1237. A computational method to test for genetic relatedness in unrelated individuals

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Discovering susceptibility loci in complex disease requires a large number of individuals characterized by several thousand markers. An association between gene and disease may be incorrectly estimated if the allele frequencies differ among cases and controls depending on factors other than gene-phenotype correlation, i.e. inbreeding, genotyping errors, unrecognized population stratification, etc.
We present a computational method - available in the Jenoware library (http://medgen.univr.it/jenoware/) - designed to compute the probability of genetic relatedness in pairs of individuals. The program computes the likelihood of a pair of individuals to be unrelated over the likelihood to be relatives of I, or II degree respectively, conditional to the genotype. The program works with microsatellites and/or SNPs. False positive rate and power were assessed by simulation in unrelated individuals and in pedigrees. E.g. in order to estimate the support for I degree relatedness (power 80%, and false positive 5%), 10 microsatellites having heterozygosity >= 70% or 25 SNP having heterozygosity >= 15% are needed. We are extending the method to associated DNA markers that can be included as inferred haplotypes in the models to be tested.

P1238. Genome-wide analysis of copy number variations in patients with mental retardation by single nucleotide polymorphism arrays

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We investigated 67 children with unexplained mental retardation who had additional, but often mild symptoms. All children had inconspicuous high resolution banding analysis. The DNAs were analyzed using the Affymetrix GeneChip 100K arrays. Data analysis was performed with median normalization and genotype-specific dosage calculation using R-scripts and revealed 12 copy number variations (CNVs), 9 deletions and 3 duplications, that are most likely causative for mental retardation because they either arose de novo or are larger than known polymorphisms. One of those was a maternally inherited 1.4 Mb duplication in Xp22.31 in a male patient which includes the STS gene. The chromosome carrying the duplication was non-randomly inactivated in the mother. Two of the CNVs were de novo deletions affecting only a single gene. All CNVs were confirmed by quantitative PCR. They varied in size from 200 kb to 8 Mb. Five of the CNVs were flanked by low-copy number repeats. Three of them were known microdeletion syndromes. The fourth one is a deletion on chromosome 15q25.2 that was not described before. The fifth one is the duplication in Xp22.31. We compared different array types. The signal-to-noise ratio (SNR) of the 500K Affymetrix array was lower than the SNR of the 100K Affymetrix array. The SNR of the 300K Illumina arrays was in between. The increase of the number of features and a new design should improve the resolution and allow the reliable detection of gain and losses of single genes.

P1239. Multiplex Amplicon Quantification, a novel method for PCR based, high-throughput copy number variation analysis.

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Recently, a striking abundance of copy number variation (CNV) was discovered throughout the human genome. Although it has been sug-

gested that CNVs contribute significantly to the occurrence of disease phenotypes, analysis of CNVs has been hampered so far by the lack of suitable methods. Indeed, none of the currently available methods for quantitative DNA analysis combines flexible assay design, user friendliness and high throughput with robustness and cost effectiveness.
Here we present such a method, designated Multiplex Amplicon Quantification (MAQ), that essentially consists of the simultaneous PCR amplification of fluorescently labeled target and control amplicons in a 'one step, closed tube' reaction, followed by fragment analysis. The PCR primers for MAQ assays are designed straightforwardly using proprietary software allowing a very high (> 40) multiplexing degree. The resulting amplification products, covering genomic regions, genes or exons of interest, are subsequently analysed using standard fragment analysis equipment. The comparison of normalized fragment peak areas obtained from test individuals and from reference individuals results in a dosage quotient indicating the copy number of the target amplicon. To automate the analysis step we developed a program for straigthforward data analysis, MAQs, which calculates and visualizes the dosage quotients of each amplicon starting from the chromatogram files.
The combination of fast assay development, quick experimental procedure and data analysis, sensitivity and robustness makes the MAQ technology the method of choice for large scale, high thoughput analysis of any discovered or hypothesized CNV, either for diagnostic or for research purposes.

P1240. Determination of copy number changes in the MHC gene region and in genes associated with drug metabolizing enzymes using multiplexed oligonucleotide ligation assays

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Genome copy number variations (CNVs) are far more frequent than originally expected, and many of them affect gene copy numbers. It has been suggested that CNVs could have functional significance in complex disease, however because of technical limitations, the extent to which such contribute to phenotypic variations is only slowly coming into focus. We previously developed the SNPlex™ Genotyping System to address the need for accurate genotyping data, high sample throughput, study design flexibility, and cost efficiency. The system uses oligonucleotide ligation/polymerase chain reaction (OLA/PCR) and capillary electrophoresis (CE) to analyze single nucleotide polymorphism (SNP) genotypes. We have extended this system to permit the analysis of CNVs by comparing the intensity of signal ratios encoded through the ligation reaction between test and reference regions. This allows us to simultaneously address at least 72 loci in a single multiplex assay and to ensure high quality data normalization and assay controls are included. Here, we demonstrate feasibility of this method by addressing CNVs within the MHC region. By analyzing cell line derived DNAs with known copy numbers, CNV genotypes are determined for the RCCX module. Additionally the assay was able to detect differences in copy numbers between the highly homologous C4A and C4B genes. We also analyzed copy number variations in drug metabolizing enzyme (DME) encoding genes that have been previously shown to affect phenotype. Here we will show correlation in observed copy number variations between the multiplex OLA/PCR assay and single tube real-time PCR assays with TaqMan® probes.

P1241. Copy Number Variation Analysis Using Quantitative TaqMan® Copy Number Assays

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Recent whole-genome studies have identified 1447 CNV regions (CNVRs) that cover about 12% of the human genome. Some of CNVR may contain disease loci/genes, whose copy number changes could impact gene activity and disease susceptibility. Copy number changes are also detected in microdeletion/microduplication syndromes, which are associated with genomic disorders. Although array-based technologies are powerful for large-scale CNV discoveries and microdeletion/microduplication syndrome screening, more quantitative technologies with higher sample throughput are required to validate newly identified CNVs and to detect deletions/duplications for a large sample size in candidate regions/genes. To meet these challenges and demands, Applied Biosystems has developed TaqMan® based real-time quantitative copy number assays. Here, we report the development of the TaqMan® copy number assay design pipeline and validation of TaqMan® copy number assays. We used this proprietary pipeline to design assays targeting the chromosomal regions associated with genomic disorders and CNV-associated OMIM genes. The assays were tested with DNA sets for validation, HAPMAP DNA collection as well as samples with known deletions/duplications. The TaqMan® copy number assay is a duplex reaction with a FAM™-assay targeting the gene of interest and a VIC®-assay targeting the reference gene (two copies per diploid genome) in the same well. The copy number is determined by relative quantification using a reference sample known to have two copies of the gene of interest. Our validation data demonstrate a high success rate of assay design and excellent assay performance. TaqMan® copy number assays are quantitative and robust, with high reproducibility, specificity, and sample throughput.

P1242. In silico search for cRSS near exon/intron borders of human genes

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The recombination signal sequences (12RSS, 23RSS) are unique structures of Ig, TCR genes of jawed vertebrates. They are located on the borders of V, D, J segments and are target-sites of RAG1/2 proteins of the V(D)J recombination system. Nowadays, there is widespread that RSSs were derived from terminal inverted repeats of ancient transposons (Transib, etc) which were accidentally inserted into a gene ancestral to Ig and TCR.Our research of human genome in silico shows that structures which can be possible targets of RAG1/2 proteins (cRSSs) are very widespread near exons (±5 bp from 3' and/or 5' boarders) of proteincoding genes. In the structure of 11 % genes (2586), we have revealed 1949 12cRSS and 1393 23cRSS. 483 genes have cRSSs flanking different exones from 2 to 7, which can theoretically participate in the formation of intragenic deletions (using 12/23-bp spacer rule) in 29 of such genes. Only 2 % cRSSs are the structural elements of repetitive DNA sequences. Having compared the experimental data to the results of theoretical research, we have reached a conclusion that cRSSs in the considered intragenic areas were derived from random nucleotide combinations. Consequently, we can suppose that RSSs of Ig and TCR genes weren't derived from terminal inverted repeats of ancient transposons. The original RSS-like sequences in the precursor of Ig and TCR genes are more than likely to have appeared at random long before the appearance of RAG1/2 genes themselves.

P1243. Investigating human disease using mouse mutant phenotypes in the Mouse Genome Informatics (MGI) Database

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Mammalian models of human disease are critical to increasing our understanding of disease mechanisms and discovering potential new therapies. The laboratory mouse is an exceptional model of disease processes in humans due to the wealth of available genetic tools. The Mouse Genome Informatics Database (MGI, http://www.informatics.jax.org) is a free resource that provides integrated access to data on the genomics, genetics, gene expression, phenotype, and biology of the laboratory mouse. Via this extensive mutant data set, MGI becomes a natural tool for comparative phenotyping. Mining of MGI data in the context of human disease can reveal new or suggestive causative mutations for testing in each species.
In MGI, models of a disease are annotated to the Online Mendelian Inheritance in Man (OMIM) term for that disease. Additionally, a detailed phenotypic description of the model is entered using the Mammalian Phenotype ontology, a vocabulary of phenotypic traits. These human disease relationships can be searched by phenotypic or OMIM disease terms or by browsing the vocabularies. Information on mouse

models includes genes involved, their function, site and timing of expression and sequence data. Over 1,900 mutant phenotypes currently are associated with human disease phenotypes, representing approximately 12% of OMIM terms with one or more associated mouse models. These data and new mouse model data that continue to accumulate provide fertile ground for selecting appropriate experimental mice, as well as identifying potential mouse targets that may be used to explore new models.
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P1244. Specific features of dermatoglyphics problems in different diseases

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This paper is an application of the concept of a Genetic Algorithm, modified to approximate certain functions. The classical problem of the dermathogliphic recognition has a settlement in different modes existing at this time very strong real time applications.
What we want to bring something new in this article is a possible solution of the dermatoglyphic recognition even if they are damaged by some typical diseases or lightly damaged by will.
The application has been built on a certain method of recognition of some essential items in the symbolism of the human dermatoglyphics those through genetic approximation techniques we complete those if there is the case and that follows a search in the data base to identify the owner.

P1245. Development of TaqMan® SNP and DME Genotyping Assays on TaqMan® Low Density Arrays

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Applied Biosystems' TaqMan® Low Density Arrays (TLDAs) are 384-well micro fluidic cards that provide a convenient low- to medium-throughput platform for TaqMan® Gene Expression Assay panels. TLDAs are pre-loaded with assays, which greatly reduces experiment preparation time and eliminates the need for liquid-handling robots or multi-channel pipettors. Towards the goal of providing TaqMan® DME Genotyping Assay panels on TLDAs, we conducted benchmark tests to compare the performance of genotyping assays run on TLDAs and on conventional 384-well plates. TLDA cards contained 8 x 48 distinct TaqMan® Validated SNP and Drug Metabolism (DME) Assays, which were previously validated on sample sets including 45 Caucasian and 45 African American Coriell genomic DNAs. These same sample sets were run on the TLDAs and the resulting genotyping data were compared to data from the 384-well plate assay validation studies. Assay performance was found to be equivalent between platforms: all assays performed successfully on TLDAs (100% pass rate), call rate was greater than 99.0%, and accuracy was greater than 99.6%. Experiments are underway to define the lower limit number of DNA samples required for sample clustering and accurate genotyping in the absence or presence of control DNA samples. An interactive data analysis software tool, Autocaller™ software, is also in development. This software tool enables overlaying and viewing cluster plots from multiple plates or cards and is used to facilitate analysis of genotyping data from TLDA cards. Genotyping DME SNPs using TLDAs will streamline studies of drug metabolism and response variation among patients.

P1246. Fast, reliable and very cost-effective method for DNA extraction from bioptic specimen

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Procedure of DNA isolation is a basic step for molecular-genetics research as well as for archiving for future reference. Therefore, the protocols that guarantee optimum quality and quantity should be used in order to ensure long-term storage of DNA samples.
Three diferent protocols for DNA extraction from tumor tissue specimen obtained in biopsy, have been compared trough evaluation of following parameters: time-consumption, feasibility, DNA quality and quantity and cost of procedure.
Protocol for DNA isolation using salting out method by Miller et al. (1987) that was modified in our lab showed to be the fast, reliable in terms of DNA quality, quantity and long-term stability as well as highly cost-effective and applicable for archiving of DNA for future research using large-scale genetic analysis technology.

P1247. CpG islet methylation in the human genome

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DNA methylation of CpG islands is widely studied in higher eukaryotes and has been found to regulate gene expression, imprinting and cancer progression. Yet within the human genome, only 8% of all CpG dinucleotides reside within a CpG island. As DNA methylation is the addition of a methyl moiety to cytosine residues within a CpG dinucleotide, a large pool of genomic methylation resides outside of CpG islands and remains uncharacterised. CpG islets have the same DNA sequence characteristics as CpG islands in terms of GC-content, but are shorter in length and are thus more abundant within the genome (Wong et al, 2006). We have recently shown that methylation of these islets can vary in association with modified chromatin states, however the extent to which CpG islet methylation plays a role in other epigenetic phenonmenon such as gene regulation or X-inactivation has yet to be determined. To start to address this, we are investigating the relationship between the methylation status of CpG islets associated with known genes and expressed sequence tags (ESTs) and gene expression level. Preliminary results implicate CpG islet methylation in the regulation of some gene regulation, thereby suggesting an essential role for CpG islets within the mammalian genome.

P1248. Detection and mapping of partial trisomy and partial monosomy of HSA21 using BAC tiling arrays

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Down syndrome (DS) is one of the most frequent congenital birth defects, and the most common genetic cause of mental retardation. Affected individuals share certain clinical features such as mental retardation, congenital heart disease and characteristic facial and physical apparance. In most cases, DS results from the presence of an extra copy of chromosome 21. A major goal of understanding the molecular pathology of DS is the identification of HSA21 genes or other functional genomics elements that contribute to specific aspects of the phenotype. Rare cases of partial trisomy 21 could identify genomic regions associated with specific DS phenotypes.
In order to establish high resolution mapping of pathogenic partial aneuploidies and unbalanced translocations involving HSA21, we constructed a BAC microarray covering 21q. The array consists of 411 HSA21 BACs with a mean overlap of 85 kb giving an approximatively 2-fold tiling path.
Our study includes identification and mapping of 45 pathogenic chromosomal aberrations of chromosome 21 including 8 complete HSA21 trisomy and 32 partial aneuploidies for different segments of HSA21. In each case, the size of the segmental aneuploidy has been estimated and in 20 cases confirmed by real-time quantitative PCR. The breakpoints have been mapped to within 200kb on average. We also tested 5 cases with a normal karyotype on the basis of clinical findings indicative of a Down syndrome phenotype. Correlations of partial 21q duplication and monosomies with phenotypic features will be presented and contribute in the understanding of genotype-phenotype correlations in Down syndrome physiopathology.

P1249. Impact of Histone Modifications on Gene Expression and Differentiation

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Histones together with DNA form the nucleosome that provides the scaffold for the compaction of DNA. Histone tails are susceptible to a wide variety of modifications and the type of histone modification contributes to the degree of DNA accessibility and gene transcription. However, it is not clear whether histone modification marks are primary or secondary to transcription, whether histone modifications act synergistically to form a histone code and to what extent they function as signaling marks for the recruitment of effectors.

We investigated the relationship between transcript levels and four histone modifications (H4ac, H3ac, H3K4me2, H3K4me3). We performed ChIP-chip and expression array analysis in two independent mouse cell lines (C2C12, HL-1), and C2C12 in two differentiation stages.

Our results revise previous findings, based on univariate data, which had led to the belief that all of these modification marks are associated with elevated expression levels. The apparent positive effect of histone-3-lysin-4-dimethylation is caused by its correlated co-occurrence, and an effect that disappears when it is present by itself. Our results suggest that histone modifications form a code, as their combinatorial composition is associated with distinct read-outs. We show that modifications are highly dynamic during differentiation. Upregulation of transcripts is associated with a gain of histone-4-acetylation, but many modification conversions occur without a change of transcript levels.

This indicates that histone modifications precede transcription, rather than being a consequence of it, and primarily function as signalling marks for specific effectors, but are not by themselves a sufficient driving force for transcription.

P1250. DS5: Biological storage and retrieval: Bringing e-Science to embryo bioinformatics resources.

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The current technologies for storage, accession and manipulation of biological data have focused on efficient management, curation and analysis of data in terms of a centralised resource, by enabling large-scale data-download for analysis. Bio-resources that require a more distributed approach (e.g. for shared material, inter-institute collaboration or large volume data-analysis) are currently unacceptably low in power for the tasks required, or suffer limitations affecting usability and usefulness. These types of requirements are a central concern of development in e-Science and therefore, as part of the FP6 funded DGEMap (Developmental Gene Expression Map) project we are assessing the current architectures to improve on restrictions experienced. This is a collaborative effort between the e-Science Institute, the Institute of Human Genetics and the MRC-Wellcome Trust Human Developmental Biology Resource. The three main areas of focus have been: a) a database for the storage and access of internal experimental details, specimen records and users details, with an emphasis on upgrading the current technology to utilise web portal access, b) 3D mapping and visualisation software to increase accuracy and automation for data mapping, including inter-stage and inter-species analysis, and c) establishing a web presence equipped with search, analysis and data-mining tools, for the dissemination of data to the wider community.

P1251. Development and validation of the "chromosome X exon-specific array" that enables identification of copy number changes in genes of the X chromosome

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The aim of this study was to design and produce a specialized and novel chromosome X exon-specific oligonucleotide microarray for screening of all X-chromosomal genes and detection of copy number changes. Identification of genetic alterations is extremely important for research and clinical purposes. Recent studies have indicated that microdeletions and microduplications occur at a high frequency in the human genome, while advances in high-density oligonucleotide arrays have paved the way with unparalleled resolution. The new "chromosome X exon-specific array" contains about 22,000 60mer oligonucleotides covering more than 92% of all chromosome X exons and miRNA regions, as well as control regions from other chromosomes. Two known abnormal control samples, one with a well-characterized isochromosome X and another with a Xp22.2 duplication were interrogated on the array to test its efficiency and identify probes that do not perform efficiently. Modifications were carried out and the final "chromosome X exon-specific array" was used for screening of 20 XLMR families from the EURO-MRX Consortium. One out of the twenty families was identified by array-CGH as having a 1.78-kb deletion involving all exons of one gene and a second family was found to carry a 27-kb deletion involving all exons of two contiguous genes. The above genetic changes were confirmed with PCR. The "chromosome X exon-specific array" could be utilized to detect genetic changes in X-linked disorders and other chromosome X abnormalities. The "chromosome X exon-specific array" will become available from "Oxford Gene Technology" (OGT) biotechnology company within the next months.

P1252. Validation of a commercially available PCR kit for diagnostic testing for Fragile X CGG repeat

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Almost all cases of Fragile X syndrome are caused by an expansion of a CGG repeat in the 5' UTR of the FMR1 gene to more than 200 repeats. Molecular testing is usually performed by both PCR of the CGG repeat (up until 100 repeats) and Southern blot analysis.

We are currently validating a new kit from Abbott. With this kit it should be possible to detect large expanded CGG repeats. By calculating a peakheight ratio of the CGG repeat and of a control X fragment, information is gained about the status "heterozygous or homozygous" in female samples. We tested 39 known samples (13 female and 26 male) including full expanded alleles, homozygous normal females, and premutations.

Of the 13 females analysed, two homozygous and two full mutations were confirmed. Of the 26 males, one showed a full mutation and two had premutations. Of the 3 full mutations, 2 were visible in raw data.

Using the Abbott kit in Fragile X diagnostic testing is fast and only a small amount of DNA is necessary. There are no discrepancies in all 39 samples. Two of three full mutations were visible in the raw data. However, interpretation of peak ratios is sometimes difficult because the reliability of the ratios is dependent on the size of the normal allel. At this moment the size standard is not suitable for full mutation sizing, so checking the raw data is necessary.

P1253. A PCR-based assay detecting pre- and full mutations of the *FMR1* gene

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We evaluated the Fragile X PCR Assay (Abbott Molecular, Inc.) effectiveness in distinguishing the size of *FMR1* alleles. The assay is based on fluorescent multiplex PCR where the CGG repeat sequence is co-amplified with undisclosed gender-specific targets. We tested 122 samples, whose *FMR1* status had been ascertained by Southern blotting (HindIII/EagI digestion , hybridization with probe Ox1.9). The samples included 93 females (31 wt homozygotes, 24 wt heterozygotes, 20 premutations, 18 full mutations) and 29 males (10 wt, 7 premutations, 3 full mutations, 2 mosaics wt/full mutation, 8 mosaics premutation/full mutation). Results obtained with the PCR assay coincided with those obtained by Southern blotting. The determination of female zygosity was done through the calculation of the TR/X ratio, comparing the height of the peak corresponding to the CGG sequence with that of the gender-specific target. A ratio>1 indicates wt homozygosity. Of 31 homozygous females, 23 (74%) had TR/X>1, 7 (23%) TR/X<1 and 1 (3%) TR/X 0.9-1 (intermediate zone). Therefore TR/X

ratio does not appear to be entirely reliable and needs refinement. However, in all cases with expanded alleles a peak was visible after capillary electrophoresis. The largest allele amplified was about 300 CGGs, although the resulting peak was small and difficult to interpret. The amplification products could also be separated on a 2% agarose gel. In conclusion, although some cases will require Southern analysis (e.g. very large expansions and methylation mosaics), this PCR assay is capable of resolving most cases referred to the laboratory.
Partially supported by Abbott Molecular, Inc.

P1254. EURExpress, a web-based transcriptome atlas of the developing mouse embryo

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Genome-wide expression analyses have a crucial role in functional genomics. RNA *in situ* hybridization (ISH) provides an accurate spatio-temporal description of the distribution of transcripts at cellular resolution. The EU-funded EURExpress consortium is generating a transcriptome-wide acquisition of expression patterns by means of ISH with non-radioactive probes and using this data to establish a web-linked, interactive digital transcriptome atlas (www.eurexpress.org). The goal of EURExpress is to generate the expression data of > 20,000 genes on sagittal sections from E14.5 wild type murine embryos. EURExpress has generated over 9000 expression patterns, including the entire set of ENCODE genes, which have been thoroughly annotated using a special interface for high-throughput annotation. This interface includes 1420 anatomical structures and correlative trees regarding ontological (embryological) and topological relations allowing advanced queries. The analysis of the data produced so far has determined that 30-40% of genes show a specific/regional pattern of expression at E14.5. Interestingly, 20% of these are unknown genes of which a large percentage show restricted expression patterns in single tissues such specific structures of the CNS, eye, skin, liver, skeletal muscle and salivary glands. The potential impact on these data on the study of human development and disease is enormous allowing to identify tissue specific markers to characterize disease phenotype, to evaluate disease prognosis, to measure therapeutic benefits and to help identifying genes whose mutations lead to disease phenotypes. Future plans include studying other developmental stages and analyzing the mutant mice resources generated by the mutagenesis projects in USA and Europe.

P1255. Gene expression changes in rat brain under the action of peptide drug “Semax”, an analogue of ACTH(4-10)

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The heptapeptide “Semax”, an analogue of the N-terminal adrenocorticotropic hormone fragment (4-10) (ACTH4-10), has been shown to exert a number of neuroprotective effects. There are some investigations that connected these effects with the increase of neurotrophin NGF and BDNF gene expression under the peptide drug application in neuronal and glial cell cultures and in rat brain in vivo. In this work, we examined the action of “Semax” on rapid gene expression of 84 key gene, representative of 18 different signal transduction pathways. Male Wistar rats were treated for one hour with “Semax” (50 μg/kg, single intranasal application) and gene expression in rat brain hippocampus was analyzed by RT Profiler PCR Array Rat Signal Transduction PathwayFinder. It was found that under “Semax” treatment a significant (at least two-fold) changes in gene expression observe for twenty seven genes from sixteen signal pathways. Nine genes was up-regulated and eighteen was down-regulated. Most significant changes was found in Wnt pathway (down-regulation of peroxisome proliferator activated receptor gamma, vascular endothelial growth factor. cyclin D1, cadherin 1 genes), JAK-STAT pathway (up-regulation inducible NO synthase and Cxcl9 chemokine ligand genes and down-regulation of matrix metallopeptidase 10 gene). Also significant (almost five-fold) up-regulation was found for heat shock 27kDa protein. Thus, “Semax” induces rapid and wide changes in expression of key genes of some signal transduction pathways and it may be related to neuroprotective effects of “Semax”.

P1256. Ultra-Deep Digital Analysis of the Transcriptome

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We have used the Illumina Genome Analysis System, based on Solexa sequencing technology, to study very deep digital profiles of messenger RNA in a variety of biological samples. This approach allows us to study the transcriptome with unparallel depth, specificity, and sensitivity. For instance we show examples in human brain and the Universal Human Reference (UHR) RNA where more than 30 million sequence tags have been analyzed in a single experiment. Our approach can be used as a quantitative profiling tool, and also as a powerful discovery tool to find evidence of novel transcription. Unlike microarrays, the digital approach does not require *a priori* knowledge of the genome, so it can be used to study complete expression in any organism.

P1257. Genetic Algorithm in anthropological pattern recognition

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In this paper we have proposed to build a connection among the fields of bioinformatics, anthropology and artificial intelligence on the base of our research in this area. We have created a pattern that gives the possibility to extend a Genetic Algorithm like an optimization method and to turn it into an approximation method.
The program constructed on the base of these concepts gives an optimization of the anthropological human structures making a characteristic function for every individual or community partly. Having at our disposition an extended database that contains the characteristics of every individual of the stock community we can determine a few characteristics of a new individual. The application is to support the anthropological research through the intelligent interrogation of some individual-data versus a database-community to interpret more and more efficiently the appartenance of those data.
A special feature of this application is the construction in the background of a neuronal network for the continual learning and upgrade of the database.
Key words: genetic algorithm, pattern recognition.

P1258. The Serbian National Mutation frequency database

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The National and Ethnic Mutation databases (NEMDBs) are continuously updated mutation depositories, which contain extensive information over the described genetic heterogeneity of a population or ethnic group. These resources have recently emerged, mostly driven from the need to document the varying mutation spectrum observed for any gene (or multiple genes), associated with a genetic disorder, between different population and ethnic groups. We report the construction of the Serbian National Mutation frequency database (http://www.goldenhelix.org/serbian), derived from an academic effort to provide high quality and up-to-date information on the underlying genetic heterogeneity of inherited disorders in the Serbian population. The Serbian NEMDB Database contains brief summaries of the various genetic disorders prevalent in Serbia and studied for the Serbian population, namely beta-thalassemia, cystic fibrosis, phenylketonuria, thrombophilia, hereditary persistence of fetal hemoglobin and Y-linkled spermatogenic failure. Database core engine has been built and maintained online using the specialized *ETHNOS* software (Patrinos et al., *Hum Mutat*, 2005; 25:327-333, Kleanthous et al., *Hum Mutat* 2006; 598-599). An easy-to-use query interface provides instant access to the list and frequencies of the different mutations responsible for the inherited disorders in the Serbian population. Furthermore, numerous links to the respective Online Mendelian Inheritance in Man (OMIM) entries fruitfully integrate the database’s content into a single web site. This database can serve as a valuable online tool for molecular genetic testing of inherited disorders in Serbia and could potentially motivate further investigations of yet unknown genetic diseases in the Serbian population.

P1259. Estimating the predictive value of genomic profiling in modeling studies: a proof of principle

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Genomic profiling is anticipated to improve prediction of complex diseases and lead to personalized medicine, in which preventive and therapeutic interventions are targeted to the individuals´ genetic risk profiles. Whether profiling can yield the required predictive value is to be investigated in clinical-based empirical studies. We developed a modeling approach that estimates the predictive value based on literature data (Janssens et al. Genet Med 2006). Our simulation model constructs a dataset based on epidemiological information about the frequencies and effects of single genotypes and the population risk of disease, and calculates measures of predictive value in this dataset. We investigated the validity of the model by replicating two published empirical studies on the discriminative accuracy of genomic profiling. Discriminative accuracy is the degree to which genomic profiles can discriminate between subjects who will and who will not develop disease, assessed as the area-under-the-receiver-operating-characteristic-curve (AUC).

The first study was a large case-control study in type 2 diabetes reporting that the combined AUC of *PPARG*, *TCF7L2* and *KCNJ11* was 0.55 (Weedon et al. PLoS Med 2006). Our model estimated a similar AUC of 0.56. The other investigated *IL-6*, *ICAM1* and *SELE* for the prediction of myocardial infarction after cardiac surgery and found an AUC of 0.695 (Podgoreanu et al. Circulation 2006), compared to 0.691 using the modeling approach. Our findings suggest that the discriminative accuracy of genomic profiling may well be estimated in simulated data. Estimating the predictive value of genomic profiling prior to data-collection could guide translational research in promising directions.

P1260. A GPCR brain map using Taqman Low Density Arrays

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The G Protein-Coupled Receptors (GPCRs) are a broad class of transmembrane proteins. A large percentage of successful small molecule drugs specifically target proteins in this class. Our study is designed to generate an expression map of 368 GPCR genes in the human brain. We used the Applied Biosystems Human GPCR Panel Taqman® Low Density Array (TLDA) and RNA samples from 29 different regions of the brain to generate this map. A total of 62 TLDA cards, 23,808 individual qRT-PCR reactions, were run in this study. To make it possible to run this large number of qRT-PCR assays from limited starting samples, we used a multiplex preamplification strategy (Taqman® PreAmp Master Mix & TLDA matched oligos). We present a performance analysis for the GPCR TLDA and preamplification methodology as well as the GPCR brain map. Several genes show interesting tissue distributions constituting potentially interesting drug targets.

P1261. MicroRNAs in the developing inner ear

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MicroRNAs (miRNAs) are 17-23 nt double-strand RNAs that can inhibit the translation of target mRNAs and affect, directly or indirectly, the expression of a large part of the protein-coding genes. miRNAs are expressed differentially in different tissues. During the last years, miRNAs have been discovered as having important roles in development and disease of plants and animals. The vertebrate ear expresses specific miRNAs, as was suggested by recent studies in zebrafish and mice. Our goal is to identify miRNAs that contribute to the development and function of the mouse inner ear and may be involved in hearing and deafness in mammals, as well as their target mRNAs.

We have used bioinformatics and other prediction tools to identify potential miRNAs that may control inner ear development or function. Using expression microarrays and real time qRT-PCR to profile the miRNAs of the mouse inner ear, we deciphered the expression of miRNAs in cochleae and vestibules at various ages. Although most of the inner ear-specific miRNAs are expressed similarly in the cochlea and vestibule from the same age, some miRNAs have a different expression pattern. The differential expression of miRNAs in the cochlea and vestibule may be responsible for some of the differences in the cochlear and vestibular transcriptomes and functions. MiRNAs with expression levels that changed over time or were different in cochleae and vestibules were selected for further study, including localization by *in situ* hybridization and a search for their targets, using bioinformatics tools and mRNA microarray expression data.

P1262. Mutation scanning using high-resolution melting on the LightCycler® 480 Real-Time PCR System

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Established methods for PCR-based genotyping using dye-labeled probes only allow the detection of expected sequence variations. To screen for unknown mutations across DNA amplicons, sequencing is state of the art. Since sequencing is still expensive and not well-suited for high throughput analysis, other methods (*e.g.,* dHPLC, DGGE) have been established for pre-screening samples in order to identify those that contain sequence variants.

We describe a novel, homogeneous post-PCR method based on high resolution melting (HRM) of amplicons that enables pre-screening. Prerequisites to use this method are:

- a high-end realtime PCR instrument (*e.g.,* the LightCycler® 480 System)
- a target-specific pair of PCR primers
- PCR reagents containing an optimized intercalating dye
- a special software for HRM data analysis.

After PCR, sample-derived amplicons (up to 600 bp) are subjected to high resolution melting. For amplicons with sequence variations the melting curves differ in shape. A specific software algorithm analyzes these small differences and groups samples of similar curve shape. Using samples with known sequence as standards, other samples in the same group can be assigned to this sequence.

The new method thus enables detection of any sequence variation in the amplified DNA section. It is cost-effective, needs little optimization and hands-on time, and allows for high throughput screening (96/384 samples).

The LightCycler® 480 System now offers PCR and HRM-based mutation scanning on an integrated platform in multiwell plates. It also provides a master mix containing a novel fluorescent HRM dye.

P1263. High resolution melting curve analysis for CFTR alleles using the LightCycler©480 instrument

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High resolution melting curve analysis (HRM) is a fast method to detect sequence variants in human genomic DNA.

In order to asses the feasibility of HRM screening in CFTR genotyping, we have set up PCR protocols for CFTR exons 4, 7, 10, 11, 14b, 19, 20, and 21 allowing the analysis of the most prevalent CFTR mutations in Germany.

While heterozygous CFTR mutations (R347P, F508del, I507del, G542X, R553X, G551D, 1717-1G>A, 2789+5G>A, W1282X, N1303K) could easily be detected by standard PCR conditions, homozygosity for F508del and heterozygous R117H alleles escaped detection.

Therefore we decided to spike our PCR assays with “unlabeled probes”, short oligonucleotides complementary to the R117H and the F508del loci, respectively. This greatly enhanced the sensitivity of the PCR-melting system and allowed unequivocal calls of all pathological CFTR alleles tested so far.

In conclusion, we can demonstrate that HRM on the new LightCycler©480 instrument is a fast and accurate method to screen for the most prevalent CFTR mutations in Germany. Analysis time could be reduced to less than 2 hours (PCR amplification and melting curves).

P1264. Embryonic expression of *OTX2* and *DMBX1* in human brain

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Genes of the homeobox superfamily encode a common 60 amino-acid homeodomain motif. Most homeobox genes act as transcription factors, regulating gene expression during development. Mutations can lead to severe alterations in the genetic programmes of a wide range of organisms, including humans. Among homeobox genes, the

orthodenticle group comprise the *Drosophila orthodenticle* (*Otd*) and the vertebrate *Otx1* and *Otx2* genes. These play major roles in the specification and regionalization of the anterior neuromeres. The *Otx2* expression domain includes the forebrain and midbrain neuroectoderm, and marks a sharp boundary at the midbrain-hindbrain junction. This constitutes a cardinal signalling centre for brain patterning. In concordance with their expression pattern, inactivation of *Otd* and *Otx2* in *Drosophila* and mouse embryos, respectively, leads to total loss of the anterior part of the brain. Furthermore, experimental mouse models show that the Otd and Otx2 proteins are functionally equivalent, in spite of the different CNS architectures between *Drosophila* and mouse. However, this equivalence is still subject to differential transcriptional and translational control. In vertebrates, *Otx* gene duplication and corresponding modification in genetic control may have resulted in novel morphogenetic pathways, including the modification in shape and size of different brain areas. The analysis of the developmental expression of *OTX* genes in human embryos may therefore reveal possible innovative roles in brain organogenesis and potential links with neurological disorders. This may be particularly relevant in the cases of *OTX2* and *DMBX1*, the latter of which functions as a transcriptional repressor.

P1265. The importance of bioinformatics and DNA Banks for Human Genetics

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Over the past few decades, major advances in the field of molecular biology, coupled with advances in genomic technologies, have led to an explosive growth in the biological information generated by the scientific community. This deluge of genomic information has, in turn led to an absolute requirement for computerized databases to store, organize and index the data, and for specialized tools to view and analyze the data.

Bioinformatics is a new scientific discipline that combines biology, computer science, mathematics, and statistics into a broad-based field that will have profound impacts on all fields of biology.

bioinformatics presents mathematical models along with biological problems and computer science tools necessary to cope with data. bioinformatics applications in genomics and proteomics and later-generation techniques, etc are important, which attempts at linking genetic information with structure and function of molecules, metabolic processes and whole cells.

DNA bank is a global life sciences centre that creates and develops innovative product-driven biotechnology ventures.

Moreover, Establishment of DNA banks will help approved researchers to develop new and better ways of preventing, diagnosing and treating different illnesses.

The establishment of DNA banks and the development of bioinformatics are the most Important strategies for the future of Human Genetics. It should be said that different DNA banks have now been established in our country. We have established a human genomic DNA bank for working on human genetic diseases. In the context of this human genomic DNA bank, we further developed new bioinformatics software in Imam Hussein University.

P1266. Genomic biomarkers for Huntington's disease

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The aim of our study was to validate previously published [1] gene expression signatures in blood as a biomarker set for Huntington's disease (HD) and to test its potential clinical utility. By QRT-PCR we tested 61 Slovenian patients and 30 healthy controls and showed that 11 out of 12 candidate genes were significantly overexpressed in HD samples ($p<.05$) with a fold change ranging from 1.40 to 1.91.

Additionally, 24 patients with Parkinson's disease (PD) and 10 patients with fresh cerebrovascular insult (CVI) were included in the study. To estimate diagnostic performance of the 12 genes biomarker set we used k-nearest neighbor (kNN) classifier. The validation of the model was done using 10-fold cross-validation. Table 1 presents the performances of the experiment in terms of sensitivity, specificity and F-measure.

We conclude that we confirmed overexpression of 11 out of 12 genes of the proposed HD biomarker set, however the differences in expression were too small to reach an acceptable diagnostic performance.

Table : Performance measures of the kNN algorithm.

	Sensitivity	*Specificity*	*F-measure*
HD group (*N=61*)	.71	.77	.72
PD group (*N=24*)	.75	.88	.67
CVI group (*N=10*)	.70	.98	.74
Control Group (*N=30*)	.60	.90	.62

[1] Borovecki F, Lovrecic L, Zhou J, Jeong H, Then F, Rosas HD, et al. Genome-wide expression profiling of human blood reveals biomarkers for Huntington's disease. Proc Natl Acad Sci U S A. 2005 Aug 2;102(31):11023-8.

P1267. Evaluation of *in silico* splice tools for decision-making in molecular diagnostic

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It appears that all type of genomic nucleotide variation can be deleterious by affecting normal pre-mRNA splicing via disruption/creation of splice site consensus sequences. Since it is neither pertinent nor realistic to perform functional testing for all these variants, it is important to find out which ones could lead to a splice defect in order to restrict transcript analyses to the appropriate cases. Web-based tools aiming at providing such predictions are available. We evaluated the performance of 5 of them (Splice Site Prediction by Neural Network, Splice Site Finder, MaxEntScan, ESE Finder and RESCUE-ESE) using 36 unrelated retinoblastoma patients without a clear deleterious mutation but carrying different *RB1* variants (28 intronic and 8 exonic). These patients were screened for abnormal splicing using puromycin-treated cell lines and the results were compared to the predictions.

As expected, 18 variants impacting canonical AG/GT splice sites were correctly predicted as deleterious. Eighteen variations occurring at loosely defined positions (+/- 60 nucleotides from an AG/GT site) lead to a splice defect in 16 cases and 13 of them were classified as deleterious by at least one tool. In other words, 3 variants escaped detection and the remaining 2 were correctly predicted as neutral.

Overall our results suggest that a combination of complementary *in silico* tools is necessary to take the most appropriate decision (balance between the time and cost needed by RNA analysis and the risk of missing a deleterious mutation) because the weaknesses of one *in silico* tool may be overcome by another one.

P1268. Protein profile analyses indicate possible implication of terfenadine in Ca++ channel inhibition

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Drug safety prediction with biomarker is essential in drug evaluation as well as drug development. Terfenadine, a nonsedating histamine H_1 receptor blocker, cause a well-known adverse effect related in cardiac arrhythmia by prolonged QT interval, unlikely fexofenadine, its metabolic derivative. We found that terfenadine suppressed calcium channel current upto 90% at 3uM as well as delayed rectifier potassium channel. In contrast, fexofenadine was almost 100 times less effective in suppressing channel currents by conventional electro-physiology study. Cardiomyocytes were treated with 0.5, 1, 5, 10, 50uM of terfenadine and fexofenadine for 24hrs. Then, each cell lysate was subjected to 2-DE analysis to detect markers for the adverse effect caused by terfenadine using fexofenadine as a control. The proteins with more than 3 times up/down expression in each drug concentration were subjected to peptide ID. The expression of proteins involved in the calcium transporting/signaling were mainly changed by terfenadine treatment, on the other hand, the expression of proteins involved in the potassium transporting were changed by fexofenadine treatment. According to our results, terfernadine treatment has major effect on the expressions of calcium channel-related protein rather than potassium channel-related protein whereas fexofenadine affected mostly potassium channel-related protein expressions. This observation might shed light

on the inhibitory effect of terfernadine on calcium channel, besides its well-known effect on HERG channel. Further investigation on the inhibitory effect of terfenadine might help elucidating the mechanism of cardiac adverse effect caused by some antihistamine drugs.

P1269. Impact of arachidonic acid and moderate and high docosahexaenoic acid formulas on hepatic gene expression profiles in baboon neonates

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Long chain polyunsaturated fatty acids (LCPUFA), DHA and ARA, are recent dietary components in infant milk formula but vary in concentration because optimal levels have not yet been established. In this study, we utilized high-density Affymetrix oligonucleotide arrays ("U133 plus 2.0" with >54,000 probesets) to monitor changes in hepatic mRNA expression levels in baboon neonates, supplemented for twelve weeks with DHA at two levels (0.33% and 1.0%) and ARA at one level (0.67%). Twelve baboon neonates were randomized into three groups (n=4 per group): Control (C, no DHA-ARA); 1 x DHA (L, 0.33% DHA-0.67% ARA); 3 x DHA (L3, 1.00% DHA-0.67% ARA). Significance analysis (P<0.05), identified differential changes in gene expression among 1726 probe sets (ps). Gene ontology annotations assigned differentially expressed ps to a broad spectrum of biological processes, including lipid metabolism and transport, cell proliferation, signal transduction and development. Genes associated with PUFA metabolism, namely SCD1 and FADS1, were significantly downregulated. Most significantly upregulated was TOB1, a novel multifunctional protein which acts as a tumor suppressor in the liver. Transcripts (PPARA, FABP1, ACACA and ACSL3) regulating lipid metabolism, transport and oxidation were subtly downregulated, whereas, PPARD is upregulated. Ingenuity pathway analysis (IPA) identified six highly significant transcriptional networks associated with functions implicated in cellular growth and proliferation, tissue development, tissue morphology, cell to cell signaling, cancer and gene expression. Dietary DHA and ARA within normal ranges found in human and baboon breastmilk altered mRNA expression of a wide array of genes, including many genes associated with PUFA metabolism.

P1270. The UMD Central system: a tool to address genetic heterogeneity from LSDB

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Recent progress in molecular genetics led to the identification of thousands of mutations associated with human genetic diseases. To analyze this flow of information, Locus Specific Databases (LSDB) have been created. They are maintained by curators (experts of a specific gene or disease), who perform a meticulous annotation of genotype and phenotype associated with each mutant. Today, hundreds of LSDBs have been created worldwide and generic softwares have been designed to set up LSDBs and standardize the information description. One of these, the UMD software (http://www.umd.be), is widely used. LSDB are frequently referred as 'inch wide and mile deep' as they deal only with a single gene in opposition to Central databases 'mile wide and inch deep', which collect mutations for almost all the genes but with minimal annotation for each mutant. To fill the gap between these two approaches, we developed the UMD central system (http://www.umd.be:2200). This tool is able to query simultaneously various UMD-LSDBs and to display a summary of any relevant information from these various databases. This is particularly useful to address the genetic heterogeneity (one disease - several alternative genes). The user can select one or more symptoms and one or more genes and UMD-central will display for each gene, the number of mutants associated with these phenotypes. This tool can also be used for mutation distribution analysis, mutational events studies or insertions deletions and splice mutations analysis. This tool should be very useful for diseases involving different genes such as myopathies, breast cancers, cardiovascular diseases...

P1271. 3D modelling and gene expression mapping in the developing human brain

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Human brain architecture and function are linked, and defined by distinct classes of neurons and support cells. Embryonic brain development is characterized by dramatic changes in shape and size. Detection methodologies such as in situ hybridisation (ISH) and computer-based image manipulation help define the gene expression patterns underlying these tremendous changes. A set of three-dimensional (3D) models have been generated using optical projection topography (OPT), which serve as the framework onto which expression patterns are mapped with custom designed software (MAPaint).

In order to visualise and interpret developmental changes, the expression patterns of two genes WNT1 and FGF8, were compared at Carnegie stage 15 (CS15; 33 days post conception). WNT1 and FGF8 are key signalling components of the isthmic organiser, and are involved in structural differentiation, patterning and organogenesis. WNT1 is expressed in the midbrain and the midbrain/hindbrain/ boundary. FGF8 is expressed in the isthmus (the hindbrain/ midbrain boundary) and the apical ectodermal ridge of the fore and hind limbs. Physical sections stained by ISH for WNT1 and FGF8 were mapped against digital sections of the CS15 OPT model. Gene expression patterns, and selected structures or boundaries, could be visualised either independently or in combination.The 3D models and the application of complex software proved an invaluable tool in the visualisation of gene expression patterns during early human brain development. These techniques can be applied further, in order to study gene expression during different developmental stages, or the development of other complex structures such as the heart.

P1272. Identification of altered promoter methylation in non-small cell lung cancer

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Aberrant promoter methylation of tumour-related genes is a relatively common and stable finding in various malignancies, including non-small cell lung cancer. It is believed that some of the methylation changes, altering gene expression, occur already before the cancer may be clinically found. This makes methylation an attractive biomarker for early detection.

We have performed the retrospective analysis of 48 genes in 60 surgically treated lung adeno- and squamous cell carcinoma patients, using oligonucleotide microarrays as the screening platform. The lung cancer samples were analysed for promoter methylation, as well as the corresponding gene expression changes. The analysis included known and putative tumour suppressor genes controlling cell growth and differentiation, antiangiogenetic factors, genes participating in cell to cell and cell to extracellular matrix connections and metabolic detoxication processes.

During the setup and validation of microarrays, series of in vitro generated methylated and unmethylated sequences were used for the selection and calibration of oligonucleotide probes. A panel of tumour-free lung tissue was used as the negative control to distinguish the tumour-related methylation changes from the possible tissue-specific methylation.

The study will be further expanded to include a larger patient group from a different clinical centre.

P1273. Mutation screening in the human β-globin gene using a flow-through microarray platform

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The panoply of human globin gene mutation detection methods has become significantly enriched with the advent of microarray-based genotyping platforms. So far, a handful of microarray-based methods have been described for mutation screening in the human globin genes region, namely a microelectronic array, arrayed primer extension-based (APEX) systems and microarrays involving tagged single-base extension (SBE) with hybridization to universal glass microarrays. We report here a new flow-through microarray for human β-globin gene mutation detection. This microarray has a porous surface and uses Al_2O_3 as a solid support, which allows not only diffusion-independent binding kinetics, but also monitoring of hybridization of the extended primers in real-time by repeated cycling of the sample through the array. The hybridization process is therefore fully reaction-rate limited and completed within a few minutes. The microarray is read by a dedicated instrument (PamStation™), while data are analyzed using specialized software. The microarray is being developed to screen for both causative β-globin gene mutations leading to β-thalassemia and associated SNPs for haplotype analysis. For this reason, 20 different β-globin gene mutations and 15 SNPs located in the human β-globin locus can be currently addressed using this platform (Phase I) with the ultimate goal to expand the microarray's capacity to screen for a total of 108 causative mutations and 30 SNPs (Phase II). Taken together the low analysis costs (i.e. 50-70€ per patient), this platform might develop into an option for numerous potential applications from human molecular diagnostics to pre-implantation genetic diagnosis and non-invasive prenatal diagnosis of hemoglobinopathies.

P1274. Microarray analysis of bone metabolism genes in patients with osteoporosis

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Osteoporosis is a complex desease with a strong genetic component. Various genes encoding bone associated proteins, cytokines and receptors, have been shown to contribute to genetic basis of the disease. For analysis following loci were selected: Col1a1 (-1997 G/T, 1245 G/T), BGP (-198 C/T), IL-6 (-174 C/T), TNFα (-308 G/A), VDR (-3731 A/G, 61968 C/T), ER (36627 C/T, 36673 A/G) and GR (N363S A/G). These loci feature significant genetic polymorphism, and are thought to be associated with genetic predisposition to osteoporosis. A genetic study of predisposition to the osteoporosis is believed to extend our knowledge about the disease and to determine the optimal terms for antiosteoporotic therapies.
We have started to introduce in practice a new molecular approach, developed with using of multiplex PCR followed by allele-specific hybridization on biochip for SNPs detection. Amplified DNA with fluorescent labels was hybridized with oligonucleotide DNA probes immobilized in gel pads on a biochip. The efficiency of the protocol was tested in our laboratory. The results showed 100% concordance between the biochip-based approach and the established PCR protocol.
The genotyping procedure, which is faster, reliable and can be used for rapid screening on the biochip is suggested for the analysis of genetic predisposition to multifactorial diseases, including osteoporosis.

P1275. A new method for separation and characterization of small RNA by On-Chip Electrophoresis

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MicroRNAs have just been recognized to play important roles in regulation in the genomes of animals and plants. To understand and describe their functional roles many questions still have to be answered. A major drawback for current experiments is the lack of adequate analytical methods for the analysis of small RNA samples and understanding on how RNA integrity and different purification protocols affect its qualitative and quantitative analysis.
Here we describe a novel Microfluidic assay that is able to perform very sensitive high resolution analyses of small RNA samples on a commercial lab-on-a-chip platform commonly used for RNA QC analysis. The assay delivers information about size and concentration of small RNA species like miRNA, siRNA, t-RNA etc, in the range from 10 to 150nt. Purified or enriched small RNA fractions, as well as total RNA samples with miRNA concentrations down to 50 pg/µl can be analyzed.
Verification of quality and quantity of miRNA after extraction protocols is a major application for this Assay. Capability for separation and efficient detection of both single- and double strand nucleic acids widens the range for sensitive analysis with this On-Chip assay.

P1276. Functional assessment of DNA loop repair in human nuclear extracts

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Introduction: Mismatch repair (MMR) corrects small base mismatches and insertion/deletion loops that arise within microsatellite repeats. Current repair assays predominantly rely on cloning techniques. We have designed an alternative functional assay for MMR.
Methods: Heteroduplex DNA molecules containing an insertion loop were created by hybridisation of purified complementary single strands of DNA from PCR products of the *DMPK1* trinucleotide repeat locus from separate homozygous samples. A nick was introduced 5' to the heterology to direct the repair to a specific strand. Heteroduplex constructs were exposed to nuclear extracts from HeLa (MMR efficient) and LoVo (*MSH2* deficient) cells to repair the insertion loop. Using semi-quantitative fluorescence analysis, the ratio of fluorescence from the nicked strand and the complementary un-nicked strand could be determined (R-value). Repair efficiency was assessed by comparing R-values before and after exposure to nuclear extracts.
Results: Exposure of heteroduplexes to HeLa nuclear extracts resulted in change in the R-value, indicative of repair. Repair was independent of loop size (2, 21 and 24-bases) and nick directed. Little or no repair was detected for 2-base loops in absence of a 5'-nick, however, for larger loops repair was nick independent. Change in the R-value was proportional to the duration of exposure to nuclear extracts. LoVo nuclear extracts showed little repair for large loops and no repair was detected for the 2-base loops.
Conclusion: This construct is easily generated and modified to vary the size of the insertion loop. The same approach can be used to assess repair of base-base mismatches.

P1277. The mitochondrial DNA content per cell in human cord blood leukocytes gradually decreases during gestation

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Most diseases in premature neonates are secondary to immaturity of various organ systems. In addition, the inadequate capacity of mitochondrial energy production may play an important role in the neonatal morbidity. Isolated human cord blood leukocytes (HCBL) contribute very little to the overall metabolic turnover, but they may serve as easily available marker cells for studying the changes of mtDNA amount during fetal development. Therefore the aim of our study was to analyze the amount of mitochondrial DNA in HCBL during the gestation.
HCBL were isolated from blood samples of 107 neonates born between 25th and 41st week of gestation. Blood samples were obtained after the delivery from placental part of umbilical cord. The mtDNA amount was analyzed by the real-time PCR method on the instrument Chromo4 (Bio-Rad) using SybrGreen I.
The significant negative correlations were found between the relative mtDNA amount in HCBL and gestational age ($r = - 0,54$; $p < 0,01$) and birth weight ($r = - 0,43$; $p < 0,01$), respectively.
The results revealed that the mtDNA content per cell decreases in HCBL with onward fetal development. This may be explained by gradual shift of the hematopoiesis from fetal liver to bone marrow during the second half of pregnancy presumably accompanied by decreasing cell volume of HCBL as it was shown similarly in red blood cells.
This work was supported by grant GACR 303/03/H065.

P1278. Multiplex Ligation-dependent Probe Amplification (MLPA), a new assay for the molecular diagnosis of Duchenne and Becker muscular dystrophies

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Duchenne muscular dystrophy (DMD) is one of the most common and severe inherited neuromuscular diseases, affecting 1 in 3500 live born males.
Both of the DMD and BMD (Becker muscular dystrophy), a milder and less prevalent form of the disease, are X-linked recessive disorders caused by mutations of the dystrophin gene located at Xp21.
Mutation detection in the DMD gene defective in these diseases is a problematical trial complicated by the large size of the gene, which consists of 79 exons and 8 promoters spread over 2,2 Mb of genomic DNA. Hence, a molecular diagnosis should be proposed to the families in order to detect the carrier women and to suggest an antenatal diagnosis.
On this aim, we developed a protocol which is based on the semi-quantitative technique of diagnosis; the MLPA (Multiplex Ligation-dependent Probe Amplification) and the linkage analysis by tripled primers PCR (Reaction of polymerization in chain). These 2 techniques allowed us to study 13 cases including 7 clinically suspected DMD/BMD patients, 4 antenatal diagnoses and 2 girls suspected to be carrier.
The results showed that 5 of these cases have dystrophin's anomalies (38,4%); in four of them it is a deletion and only one is carrying duplication.
The maternal profile was studied in 9 cases. Three of them showed heterozygous deletions and only one, showed duplication.
This protocol allowed us the identification of homozygous and heterozygous deletions and duplications of dystrophin gene in only one step.

P1279. Multiplex Ligation-dependent Probe Amplification (MLPA) - an interlaboratory collaborative validation study

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The EU Network of Excellence EuroGentest aims to improve and harmonize the overall quality of genetic services throughout Europe. The thorough validation of new methods is one of many contributions to accomplish this, and Multiplex Ligation-dependent Probe Amplification (MLPA) is the first technology to be validated in this context.
MLPA is a relatively new method to determine the copy number of nucleic acid sequences by hybridisation of specific MLPA probe pairs that bind to adjacent sites and are joined by a ligation reaction before PCR.
A plethora of popular MLPA kits for different genetic diseases are available from the manufacturer. But even if their performance was already established in different molecular diagnostic laboratories, so far no major nor interlaboratory collaborative programs have been undertaken to fully validate MLPA.
Therefore, a validation study was set up, including 13 international diagnostic laboratories. Questionnaires were answered by the participants to evaluate the variability concerning MLPA protocols and analysis tools, with the remarkable outcome that the majority had adapted the original protocol. Furthermore, the P002 BRCA1 MLPA kit (MRC-Holland) was used on 10 wildtype DNA samples and 3 deletion or duplication samples to evaluate the performance and the precision of the method itself. Finally, a data set of 89 MLPA files for DMD was analysed by different software tools for the evaluation of performance and internal quality control.
We will present an analytical and diagnostic validation report and a generic standard operating procedure (SOP), and propose guidelines for diagnostic laboratories to successfully implement MLPA.

P1280. MS-MLPA (Methylation Specific Multiplex Ligation-dependent Probe Amplification) in the diagnosis of Angeman Syndrome

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Angelman Syndrome (AS) is a complex neurobehavioral disorder associated with loss of function of differentially expressed imprinted gene at the region 15q11-q13. It is a rare disorder. It occurs in one of 20 000 live births. It is due either to a microdeletion in the 15q11 locus (65-75%), or to uniparental disomy (5%), or to UBE3a gene mutations (10%), or to an anomaly of printing center (5%). In the 10% remainders cases, the cause still unknown.
The molecular study of the patients appear with a great importance with an aim of being able to conclude about the parental origin and, therefore, about the disease.
In this work, we developed an experimental protocol able to ensure the molecular detection of this pathology. It is based on the MS-MLPA technique (Methylation Specific Multiplex Ligation-dependent Probe Amplification), which is able to detect abnormal methylation of imprinted genes as well as deletion occurring at 15q11-q13 locus. The study of STRs markers of chromosome 15 allowed us to distinguish between uniparental disomy and microdeletion when abnormal methylation was detected.
These two complementary techniques allowed us to study 8 suspected AS patients. Only one of them showed a deletion in the 15q11-q13 region. This anomaly was confirmed with FISH (Fluorescent In Situ Hybridysation).
The combination of these two techniques, in the diagnosis of AS, represents a very good strategy to find more than 85% of causative anomalies. It can easily be used in the majority of laboratories because of its low price and simplicity.

P1281. Evidence for a degenerating X chromosome in the rodent species Ellobius lutescens

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The mole vole *Ellobius lutescens* is an exceptional mammal with an odd karyotype of 2n = 17,X with a single X chromosome in both sexes. We postulate that the single X of *E. lutescens* will degenerate in consequence of its lacking partner for recombination. In analogy to the fate of the Y chromosome of male mammals we suggest an accumulation and fixation of non-deleterious mutations that will finally lead to the inactivation of X-chromosomal genes. This proceeding accumulation of mutations may cause the extinction of the species *E. lutescens.*
To test this hypothesis we compared sequences of essential (*Zfx, Atrx, Flna*) and non-essential (*Opn1mw, Nr0b1, Xist, Mecp2*) X-chromosomal genes to orthologous sequences of other mammals; as control for the mutation rate of recombining chromosomes we analysed regions of the autosomal genes *Sfrs3* (essential) and *App* (non-essential). We compared the ratio of accumulated mutations in essential versus non-essential X-chromosomal genes to the ratio of autosomal essential versus non-essential genes.
The results confirmed our hypothesis of a degenerating X chromosome. A comparison of the sequence conservation of the studied essential X-chromosomal genes between *E. lutescens* and *E. fuscocapillus* showed a highly significant accumulation of mutations in non-essential X-chromosomal genes ($p = 1.95 \cdot 10^{-9}$) whereas the analysis of autosomal genes did not show significant accumulation of mutations ($p = 0.73$). So we expect a progressive decay of the single X chromosome and as a consequence the extinction of the species *E. lutescens.*

P1282. Identification of primate-specific non-coding RNAs at human chromosome 15q11.2

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Non-coding RNAs play important roles in many cellular processes rather than serving as templates for protein synthesis. Computational and experimental approaches have predicted thousands of ncRNAs in human genome, but most of them are yet unknown and only a small fraction have been partially characterised in terms of function or expression pattern. We observed that the chromosomal region 15q11.2 share characteristics with other regions containing ncRNAs: repetitive elements in tandem, imprinted locus, and signals of instability. We have performed a computational analysis of the target one-megabase

sequence searching for putative ncRNAs at this region. Among 86 stable hairpin candidates, of 100 nucleotides and not overlapping with repeats, we selected 30 to check their expression by northern blot and primer extension and we identified 21 primate-specific new ncRNAs. Two correspond to new microRNAs and 19 to a new class and family of ncRNAs. Computational analysis based on conservation of the secondary structure supports that 15 of them are real ncRNAs. Most predicted genes targeted by these two miRNAs were common to both, and 11% of the targets encode proteins that participate in the development of the central nervous system, suggesting a role of 15q11.2 miRNAs in neurological functions and disorders

P1283. High throughput genomics using Applied Biosystem's SOLiD™ System

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Massively parallel sequencing systems are making genetic analysis cheaper and enabling experiments capable of answering increasingly complex biological questions. The SOLiD™ system, is a new platform using either fragment or mate paired libraries to generate >1-2 GB of data/run with >99.99% consensus accuracy. We will present a summary of the current chemistry of the SOLiD™ system together with data generated to demonstrate sequence quality and coverage of a number of increasingly complex targets, including bacteria, yeast, *C. elegans* and mammals. The SOLiD™ chemistry and instrumentation can be readily adapted to a number of applications by modification of the ways in which the input nucleic acids are prepared and the output data is analyzed. Some of the applications under development with collaborators will be presented as well as data demonstrating the utility of the system

P1284. Next generation sequencing - Opportunities and constraints in pooling and quantitative sequencing

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Next generation sequencing promises to address a range of applications including: identification of somatic mutation profiles, gene expression by tag counting, and measurement of allele frequencies in pooled samples of cases and controls in association studies. We developed a model to simulate digital sequencing with pooled samples, in the presence of error. We discover that for pooling and quantitative sequencing, the number of samples that can be pooled and the minor allele frequency of variants that can be detected is critically dependant on the threshold for SNP calling, which in turn is strongly influenced by the measurement error rate. As next generation sequencing platforms typically produce short reads (25-35bp), coverage needs to increase over 20x to compensate. Increasing the coverage improves the estimate of the error rate, but cannot overcome problems with detecting very low frequency variants with large numbers of pooled samples. We validated this model through empirical sequencing by oligonucleotide ligation and detection (Applied Biosystems SOLiD(TM) system) of 81 PCR amplicons from exons of EMS-mutagenized C. elegans worms encompassing ca. 25kb of sequence with over 1500x coverage. Amplicons were pooled down to a 1:100 ratio (1:200 ratio for alleles). The results were compared with di-deoxy sequencing data carried out independently. Our results suggest that even if coverage needs to increase significantly when using short reads as compared with di-deoxy sequencing, low platform error rate is the most critical factor for detecting allele variants in pooled samples or mixtures by next generation sequencing platforms.

P1285. Measuring the quality of computer tools used in diagnostic genetic testing

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As part of the EuroGentest project (www.eurogentest.org) we investigated quality measurement of computer tools used within diagnostic laboratories. A literature survey highlighted the need to provide specific information, such as what the tool does, who it is for, etc.; information about data used by the tool; information about tool operation; and information on its performance, such as sensitivity and specificity, or the completeness of its data coverage. A survey of laboratories found that most tools used are either for particular pieces of equipment or are databases. It also showed that quality assessment is often missing or unstructured. Furthermore, there is no trustworthy source to validate the quality of the clinically most critical features of the tools. We propose that tools can be categorised according to their purpose, with a specific list of features developed for each category. Assessment of performance requires standardised tests for parameters specific to each tool category. Expert groups for each tool type are needed to propose and review the features and performance measures. One important category of tool that we found in laboratories was sequence analysis tools. We have used previous work to propose a list of specific features and performance measures for these tools. We have investigated presentation of this data using a 'wiki', i.e. a web site editable by its users. This allows groups of experts to be formed for the particular tool category and to collaboratively enter and review data about the tool.

P1286. In search of tissue specific regulators in periodontium - a bioinformatic approach.

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Tissue specific gene expression can be regulated by tissue specific promoters, enhancers, silencers, transcription factors, differential methylation, tissue specific alternative splicing, as well as other transcriptional and post-transcriptional factors. The methods used for studying the regulatory elements are multiple, however, they are mostly useful in cases where some information about the promoters active in a given tissue is available.

While the tooth development is well described, the regulation of gene expression in the tissues maturing after the tooth development is complete is unclear. Periodontal ligament tissue (PDL) is essential for structural support of the teeth (attaches root to the bone). The understanding of what makes it so special is essential for the development of regenerative treatments of this tissue.

Expression profiling data of the primary cell cultures of periodontal ligament tissue and outer gum tissue (gingiva) was performed using Affymetrix HU133A arrays. The analysis has identified 333 genes differentially regulated in these tissues. This set of genes was then subjected to promoter analysis to identify the CpG islands and promoter binding sites. We have used a number of tools, such as Promoter-Express, TRES, TFSEARCH, PAINT, CpGProD, CpG islands searcher, Methylator and MethCGI to generate an overview of the promoters of the differentially regulated genes. As a result we identified signature promoter features of these differentially expressed genes.

Currently, we are analyzing the role of the differential methylation between the two tissues and the role of some selected transcription factors identified in this screen.

P1287. Direct Sequencing Quality Control: a Novel Software Approach to Reducing Variant Review Time and Labor

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With the completion of the Human Genome Project, the shift from de novo sequencing to direct sequencing (resequencing) has created the need for more accurate variant detection for medical research and clinical diagnostics. The bottleneck in the workflow (from DNA extraction to result data analysis) has been cited as taking up to 70% of researcher's time per project, due to manual review of individual nucleotide bases. This review has been required due to the necessity of having confidence in the variant result.

Increasing confidence can come from applying diligent quality control metrics, including use of Quality Values for DNA trace value and confidence values for variant validity. Based on Applied Biosystems experience, this system will filter out low quality data. The software will then direct users to review only low confidence variants.

A flexible workflow based system is being built to enable researchers to obtain their high confidence results in less time. Methods for filtering low quality data based on optimal settings and quality visualization tools will be integrated into the system along with simpler variant review and reporting tools to allow researchers to quickly analyze their data.

P1288. RNA Integrity database: A web repository for RNA isolations

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Lab-on-a-Chip devices are broadly used for RNA integrity analysis on gene expression analysis. This creates the need and opportunity for users to screen and validate RNA traces for relevance and troubleshooting. Here we describe the design of the backbone as well as examples of use for a new RNA profile web database. The database aims to host a large variety of sample types spanning different genus, tissues and sample treatments, although the database is initially limited to contain bioanalyzer traces. Each electropherogram is annotated with sample source details as well as analytical data like UV, Ribosomal ratios, RIN and more. They also include details on the RNA extraction and the downstream experiment.

By design the database is open to the scientific community: free querying and curated contributions for individuals as well as large batch uploads from core labs. Individuals are able to compare their own results with those of others with similar samples and protocols. Advanced filtering also allows comparison of alternative RNA isolation methods based on its resulting electrophoretic traces or other criteria.

P1289. Easily obtain high quality RNA from human blood samples for gene expression analysis: Tempus™ blood RNA tubes and RNA isolation systems

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When blood is drawn into standard evacuated collection tubes, the expression profile of gene targets can continue to change. Applied Biosystems has designed Tempus™ Blood RNA Tubes for the collection of human whole blood samples and the stabilization of RNA expression profile. After the sample collections, researchers can then choose from two streamlined protocols to isolate RNA. Recent real-time PCR and microarray data using the RNA isolated from the Tempus™ Blood RNA Tubes and the Tempus™ Blood RNA Isolation Kits have shown the gene expression profile is stable for up to five days when the tubes are situated at room temperature or for up to seven days at 4°C.

P1290. A rapid and sensitive method for RNA quality determination on capillary electrophoresis systems.

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RNA quality is directly correlated to the success of various applications, such as microarray or real time qPCR-based gene expression analyses, cDNA library construction, Northern analyses, and RNAse protection assays, which utilize RNA samples from various organisms, tissues, cell lines, and precious biological samples. We present a novel method for examining RNA integrity and purity using capillary electrophoresis that is more cost-effective and scalable than current standard methods. This highly sensitive method uses less material, uncovers impurities following RNA purification, and detects degradation resulting from nuclease contamination. Total RNA and cRNA, derived from various tissues and cell lines, were stained with the dye YOYO-1 and run through a custom polymer formulation on a capillary electrophoresis platform. Using downstream analysis software, we resolved RNA species and their relative quality based on parameters such as size, profile, peak area, and peak height. Our results highlight the potential for high-throughput capillary electrophoresis as a much more discriminatory and cost-saving method in evaluating RNA quality.

P1291. Collection, storage and processing of clinical samples for molecular analysis.

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The collection and preparation of clinical samples for molecular analysis is important for expanding applications in genetic identification, drug discovery, predictive medicine and pharmacogenomics. To simplify sample processing we have developed two chemically treated devices which are useful for dried clinical sample collection, sample archiving and, most importantly, DNA preparation for amplification. Nucleic acid preparation from samples collected on a treated matrix is simple, rapid and automatable. Blood and buccal cell samples collected on FTA®, a surfactant modified matrix, and FTA® Elute, a chaotropic salt modified matrix, can be stored for over 10 years under ambient conditions as demonstrated by STR analysis. We have demonstrated the use of DNA from these samples for genetic identification, real time PCR, DNA sequencing, and allele specific hybridization methods.

Increasing demand for nucleic acids from archived samples dictates that systems and devices should be able to support whole genomic amplification. We have examined the recovery of DNA from samples archived on treated matrices and evaluated its' suitability for whole genomic amplification. We have also measured the inter-sample variability and correlated this with nucleated cell counts, hematocrit and sample age. It is clear that blood and buccal samples dried on chemically modified matrices are stable and provide an excellent source of nucleic acids for future studies.

P1292. Computational prediction of SNPs of schizophrenia candidate genes that interfere with normal function of ESE

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Despite the ability of genetic association studies in assigning schizophrenia susceptibility genes, results have been quite spurious. SNPs (single nucleotide polymorphisms) are the most common genetic variation in schizophrenia association studies. Most of these variations are located in noncoding part of genome, previously thought to have no particular function. In recent years regulatory function of these SNPs has attracted much attention. SNPs which are located on regulatory elements might interfere with mechanisms that regulate gene expression such as splicing. Exonic Splicing Enhancers (ESEs) are one of the most important splicing regulatory elements which can stimulate splicing and seem to be particularly relevant for regulating alternative splicing. ESEs appear to be very prevalent and may be present in most exons, if not all. We have computationally analyzed SNPs of 16 candidate genes for schizophrenia, to find whether they interfere with the normal function of ESE. In total, 9335 SNPs were analyzed by S/R rich protein binding site prediction algorithm. 1.12% of SNPs can potentially interfere with ESE function. These SNPs might have regulatory function thus are interesting candidates for schizophrenia association study. Following table shows the results of this study.

Number of SNPs after Genetic Footprinting	Number of SNPs Located at ESE	All analyzed SNPs	Analyzes Genes
18	19	257	COMT
0	5	363	DTNBP1
2	9	638	NRG1
5	5	29	RGS4
2	2	559	GRM3
0	8	1367	DISC1
0	1	120	DAOA
2	2	87	DAAO
5	5	283	PPP3CC
11	11	287	PRODH2
8	8	125	Akt1
2	3	296	MUTED
3	4	597	MRDS1
6	6	3923	ERBB4
15	15	173	GAD1
3	3	231	FEZ1
82	106	9335	Sum

P1293. Low Volume Dispensing for DNA Sequencing at the Ernest Gallo Clinic and Research Center, University of California San Francisco

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The Ernest Gallo Clinic & Research Center (EGCRC), a part of the Neurology Department of the University of California San Francisco (UCSF), studies basic neuroscience and the effects of alcohol on the brain. The Genome Center at the EGCRC is involved in a large re-

sequencing project that consists of sequencing candidate genes for alcoholism from a set of approximately 1000 people, identifying rare variants of interest and tracking those variants through extended families. The genomics facility produces about one million sequences per year utilizing two 3730xl instruments, (Applied Biosystems, Foster City CA), two Biomek FX robots and one Equator GX8 (Deerac Fluidics).
Miniaturization of sequencing reactions was undertaken to save in reagent costs by reducing volumes and to save in consumables by removing the need for expensive disposable tips. The genome sequencing community uses quality metrics based on PHRED scores with 20 being the base line for regular sequencing. In the Gallo genome core, heterozygous variant detection especially for very rare variants requires that sequences must yield a PHRED score of 30 before proceeding to analysis. It was essential to keep these quality metrics while at the same time reducing volume.
Here, the miniaturization process is described, along with the integration of the Equator GX8 into the sequencing pipeline resulting in a high level of data integrity and a significant reduction in operating costs.

P1294. Single Nucleotide Polymorphism (SNP) analysis in XX and XY sex reversal

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Introduction: The sex determining genes *SRY* and *SOX9* are required to initiate/maintain testicular development. However, the genetic interactions controlling the earliest steps in gonadal development remain poorly understood. Molecular abnormalities underlying a high proportion of human XY gonadal dysgenesis, XX maleness and XX true hermaphroditism remain undiscovered. The use of high density SNP array in sex reversal patients could be useful to characterize the etiology of the abnormal gonadal development and provide new molecular insights into the normal regulatory network in the testis and ovary development.
Aim: Evaluate gene dosage effect in 8 XY females and 8 *SRY*-negative XX males using a Single Nucleotide Polymorphism (SNP) chip.
Methods: Affimetrix 500,000 SNP array for screening over 80% of the genome within 10Kb of a SNP and a median inter-marker distance of 3.3 Kb was used.
Results: Seven XY patients have been studied. The hybridization conditions were optimal obtaining a call rate around 95%, data analysis was performed with Affymetrix gene chip software. We finished the analysis in 3 XY females, showing the presence of duplicated and deleted SNPs in all the cases. We found 3 shared regions in all the cases, in chromosome 2 and 14, where two and one possible candidate genes were present. In the meeting we will present the analysis of the rest of XY population and the preliminary results in XX-SRY negative males.
Conclusions: These results suggest that gene dosage participates in gonadal development, where threshold levels of expression control entry into the male or female pathway.

P1295. Splicing Sequences Finder: a bioinformatics resource to identify sequences involved in splicing

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Today thousands of intronic or exonic variations potentially resulting in splicing defects are identified yearly in molecular diagnostic laboratories. To assess the pathogenicity of these mutations, we develop the Splicing Sequences Finder (SSF) tool (http://www.umd.be/SSF), which allows the identification of the various splicing elements from a crude sequence. These elements include:
- The impact on the well-characterized acceptor and donor splice sites motifs.
- The identification of branch point sequences (BP) whose key role in splicing can also explain the pathogenicity of some intronic mutations.
- The ESE and ESS (exonic splicing enhancer or silencer), ISE and ISS (intronic splicing enhancer or silencer), which are now recognized as targets for exonic missense or even nonsense mutations.
The identification of these last motifs was mainly based on bioinformatics and functional analyses. Therefore, many matrices are today available to predict these auxiliary-splicing sequences and to evaluate the pathogenic impact of mutations.
We designed the SSF software as a single resource that includes the various bioinformatics algorithms necessary for an exhaustive evaluation of the various splicing elements. We also developed a new algorithm to identify BP sequences. It was confronted to all mutations known to involve a BP sequence and was found reliable in all cases.
The SSF tool could give valuable information for molecular diagnostic laboratories to evaluate the pathogenicity of a mutation. It can also be used to identify potential targets for antisens oligonucleotides to silence ESE or BP and therefore lead to exon skipping.

P1296. Interactome of the Sushi-Repeat protein SRPX2 that causes brain disorders of the speech areas

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The rolandic and perisylvian brain areas are responsible for speech production and association of rolandic epilepsy (RE) with oral and speech dyspraxia (OSD) is well known.
We have identified the first gene, SRPX2 (Sushi Repeat Protein, X-linked 2) that is mutated either in RE with OSD, or in RE with bilateral perisylvian polymicrogyria*. More recently, evolutionary study on SRPX2 identified only one human-specific mutation (p.R75K) that has appeared since the human-chimpanzee split. Three-dimensional modeling of the first SRPX2 sushi domain located K75 in the same critical loop as where the pathogenic mutation p.Y72S has also occurred.
Sushi domains have been shown to sustain protein-protein interactions and are frequently found in complement control proteins and in the selectin family of adhesion proteins. However, the actual function of SRPX2 remains unknown. We have undertaken a systematic SRPX2-interactome strategy in order to understand the role(s) of SRPX2 through the protein(s) it interacts with, and to identify novel candidate genes. The actual interactions of SRPX2 with its 18 putative partners as identified by yeast two-hybrid experiments, is currently being assessed using in-cell co-transfection followed by co-immunoprecipitation experiments, and on-chip (BIAcore) experiments. A first SRPX2 interactor has now been firmly identified, providing exciting insights on the possible molecular mechanisms driven by SRPX2.
Generally, SRPX2 represents a first key point towards the identification of molecular pathways associated with the functioning and the development of the brain speech areas, in both pathological and physiological conditions.
*Roll et al., Hum. Mol. Genet. 2006

P1297. GENIUS: A New Universal Stripassay Analyzer Software

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Molecular genetic diagnostic applications have been widely used worldwide. Reverse hybridization based stripassays helped dissemination of these applications. Evaluation of the stripassays is a subjective process and reporting of the results is not easy unless there is a medical genetic specialist who can interpret the results. An automated system which can scan, evaluate and archive stripassays and give a standardized report is essential for accurate reporting.
There was not an automation system in the market for different type of stripassays produced by different vendors. GENIUS was developed to automate analysis, comment and reporting steps of the stripassay usage. Today GENIUS can analyze over 20 different stripassays from different vendors. Some of these tests are CVD (Cardio Vascular Diseases Susceptibility), Cystic Fibrosis, FMF (Familial Mediterranean Fever), Thalassemia, Haemochromatosis, Gaucher, etc.

Application has a very strong image processing mechanism and flexible database architecture. Medical staff puts tests into scanner and GENIUS gets image from device. Then application checks the test type. After the identification of test, it searches mutation areas and other critical check points on the stripassay. By using analysis results, GENIUS refers to database and generates comments about the results. Adding a new test to system takes approximately 2-4 hours depending on the number of mutations on the stripassay. So it is very easy to adapt GENIUS for different markets which use different stripassays.
Oracle 10g is the database of the GENIUS. Addition to this Oracle BI EE is being used for analytic reporting.

P1298. Comparison of two fluorescent dsDNA binding dyes SYBR Green I and EvaGreen for Melting Curve Analysis

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One of the basic detection systems for real-time PCR is based on dsDNA binding fluorescent dyes, from which SYBR Green I is the best known. However, the SYBR Green I is not the only dye for these applications. Because of their unique feature (enhanced fluorescence when bound to the dsDNA) SLDs (SYBR Green Like Dyes) are frequently used in detection of specific PCR products by melting curve analysis (MCA). Recently, a new fluorescent dye EvaGreen has appeared which shows (by the manufacturer's information) better properties for real-time PCR than SYBR Green I. To investigate the more appropriate dye for our MCA applications we decided to compare some properties of these two dyes such as photostability, detection limit with diverse range of dye concentration and PCR product concentration and some parameters of the final peak in MCA. Since the molar concentration of each dye is different in the 1x working solution our measurements were done by the basic working concentrations and by equal molar concentrations too. We also compared the accuracy of the Tm values obtained from a larger set of each sample. In our study we used 4 PCR products (for the detection of the 35delG, W24X, R127H mutations and a polymorphism GJB2-SNP1 in GJB2 gene). According to our findings EvaGreen has better properties (the peaks obtained from MCA by EvaGreen are generally higher and sharper and the Tm values are more accurate) however it shows lower photostability than SYBR Green I. Detailed results will be presented on our poster.

P1299. Association study of therapeutic response and SERT, MDR1 and 5-HT2c gene polymorphisms in female schizophrenic patients

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Interindividual differences in treatment response to second generation antipsychotics point out that genetic factor may be relevant. The aim of this study was to investigate the relationships between variants of serotonin transporter gene (SERTPR and SERTin2), serotonine receptor (5-HT2c-759C/T) and multidrug resistant gene (MDR1-2667G/T, 3435C/T), and initial symptomatology and treatment response in 106 female schizophrenic patients treated with olanzapine for up to 3 months. Afterwards, we compared allele, genotype and haplotype distributions between those patients and 108 control female subjects.
Methods: Genotyping was performed by PCR-RFLP, and real-time PCR methods. To assess and evaluate therapeutic response, all patients were rated using PANSS. Overall, the presence of SERTPR-S allelic variant and SS genotype was associated with significantly more weight gain in subjects who were non-obese at the time of admission (p= 0.02). The presence of SERTPR-L variant was associated with significantly better treatment response measured with total PANSS and general PANSS subscale (p<0.04), while the presence of SERTin2-I variant determined better treatment response only in several items. We found significant associations with lower initial PANSS, and MDR1-2667G/T genotype. 2677T allele and TT genotype were associated with significantly worse treatment response. Also overrepresentation of G2677/3435T haplotype in schizophrenic female patients compared to controls was significant (p=0.025). Test result for linkage disequilibrium between two MDR1 loci was found to be significant. These findings identify genetic factors associated with olanzapine- treatment response in female schizophrenic patients.

P1300. Gene expression signature in peripheral blood detects thoracic aortic aneurysm

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Thoracic aortic aneurysm (TAA) is usually asymptomatic and associated with high mortality. Adverse clinical outcome of TAA is preventable by elective surgical repair; however, identifying at-risk individuals is difficult. We hypothesized that gene expression patterns in peripheral blood cells may correlate with TAA disease status.
Gene expression profiles of peripheral blood samples collected from 58 individuals diagnosed with TAA and 36 normal individuals (controls) were analyzed in this study. Significance Analysis of Microarray (SAM) analysis identified potential signature genes characterizing TAA vs. normal, ascending vs. descending TAA, as well as sporadic vs. familial TAA. A 41-gene classification model for detecting TAA was constructed using a training set containing 36 TAA patients and 25 controls. Testing this classification model on an independent testing set containing 22 TAA samples and 11 controls yielded an overall classification accuracy of 78%. These 41 classifier genes were further validated by TaqMan® real-time PCR assays. Classification on the 30 testing samples based on the TaqMan data resulted in similar classification accuracy (80%) as that from microarray data.
This study generated a distinct molecular signature in peripheral blood cells that can detect TAA patients from normal individuals. Validated by TaqMan® real-time PCR assays, the classifier genes identified in this study define a set of promising potential diagnostic markers, setting the stage for a blood-based gene expression test to facilitate early detection of TAA disease.

P1301. Universal array-based multiplexed test for polymorphism detection of genes associated with inherited thrombophilia

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Thrombophilia refers to disorders, which are associated with a persistent hypercoagulable state and a tendency towards thrombosis. They may be inherited, acquired or complex, when genetic factors interact with environmental influences. Besides acquired risk factors, such as hyperhomocysteinemia and high levels of factor VIII, there are single nucleotide polymorphisms in the prothrombin (20210G> A), *MTHFR* (677C> T), factor V Leiden (1691FVG> A), *PAI-1 (*5G> 4G), GPIIIA (196C> T), fibrinogene (455G> A) genes which result in hereditary thrombophilia. We have developed the fast and easily adapted method of detection mutations associated with thrombophilia, suitable for technological process at any standart molecular diagnostic laboratory. This convenient and reliable detection of mutations is based on multiplex PCR with subsequent hybridization on the biochip. Use of this technique open new opportunities for carrying out large-scale population researches of genetic predisposition to thrombophilia. The practical recommendations have been developed for the choice of therapy strategy in case of investigated mutations detection.

P1302. A homogeneous high-throughput assay for the primary screening of type 1 diabetes related HLA-DQB1 alleles

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We have developed a homogeneous genotyping system allowing simple, low-cost and rapid genotyping of thousands of samples. The method utilizing an asymmetric PCR and subsequent hybridization of allele specific probes with LNA additions for the genotyping of HLA-DQB1 alleles *02, *0302 and *05/6 can be used as the primary screen-

ing step in large-scale projects where subjects at high genetic risk for development of type 1 diabetes are identified. The method was validated by typing a set of 50 known samples. To promote simplicity in handling and storage the sample material of choice was blood dried on sample collection cards.
The developed homogeneous assay platform allows the typing of hundreds of samples within one working day; an assay can be run in approximately 4 hours with sample number limited only by the number of thermal cyclers available. The homogeneous assay gave correct genotyping result for 100 % of the samples used to validate the method. The costs of the assay are minimal and the reductions in hands-on time provide considerable improvements compared to the heterogeneous genotyping methods comprising of separate PCR and hybridization steps.
The presented assay system provides a functional approach to the rapid screening of thousands of samples at low cost, a general starting point for large scale screening studies.

P1303. The chromatin state of ultraconserved non-coding elements

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Vertebrate comparative genomics has proven to be an effective approach for uncovering functional elements in the human genome. Ultraconserved elements (UCEs) show extreme conservation in mammals and are defined by a perfect sequence identity between human, mouse and rat over at least 200 bp. Most UCEs map in the vicinity of genes that are key regulators of vertebrate development. A number of UCEs have been reported to possess enhancer activity and they are thought to be involved in the regulation these genes.
To further uncover the functions and mechanisms of action of UCEs we assessed their chromatin state in various human cell types (lymphoblastoid, fetal lung and neuroblastoid). We performed ChIP-on-chip experiments using antibodies against histone H3 modifications that mark active (K4-dimethyl,trimethyl) or repressed chromatin conformations (K27-trimethyl and K9-dimethyl). Custom tiling microarrays were designed to contain the sequences of 287 non-coding UCEs, as well as the 5' regions of the nearby genes.
Preliminary results show that ~20% of the UCE regions are strongly enriched for K4-dimethyl modification, out of which half are also enriched for K4-trimethyl. Many of these enrichments are present in all cell types, suggesting these modifications could be constitutive. These enrichments appear to be specific to UCE regions as they were not found in control regions. A very limited number of UCEs show a repressed chromatin conformation. Our results provide a framework for further understanding the potential functions of UCEs in the regulation of developmentally essential genes.

P1304. Analysis of USF1 and USF2 by chromatin immunoprecipitation and whole genome tiling arrays identifies candidate genes for familial combined hyperlipidemia

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USF1 is a transcription factor associated with familial combined hyperlipidemia (FCHL) and it binds as a heterodimer with USF2. Binding sites for USF1, USF2 and regions of histone H3 acetylation (H3ac) were mapped across the whole genome in HepG2 cells using ChIP and tiling arrays. Regions with signal significantly higher than negative controls were identified and subsequent Q-PCR in 48 such regions showed <1% false positives for each factor. Regions bound by USF1, USF2 and H3ac were characterized against a range of annotated genomic features. H3ac signal was found at 10.900 regions, 50% of them <1kb from transcription start sites (TSS) of protein coding genes (PCG). The footprint of this signal showed a bimodal pattern with a major peak downstream of TSS at +600-800bp and a smaller peak symmetrically located upstream of the TSS. The ChIP-chip signals were compared to the expression pattern of 18 000 genes and the height of the downstream peak was positively correlated with gene expression. USF1 and USF2 preferentially bound -300 to -100bp i.e. upstream of TSS of PCG. In bidirectional promoters USF1 and USF2 bind between the two TSS and the peak of H3ac is downstream of each TSS. USF1 and USF2 signal was found in promoters of over 1000 genes. Genes with promoters bound by USFs were analyzed using gene ontology classification and three biological pathways were identified that contained genes that are excellent candidates for FCHL.

Po09. Genetic counselling, education, genetic services, and public policy

P1305. EuroGentest Survey and Database of Quality Assurance in European genetic laboratories

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Although a number of public websites provide lists of medical genetic testing laboratories and of available, reliable public information about Quality Assurance (QAu) is sparse or even intentionally absent. To remedy this, EuroGentest has performed a long-term survey of QAu in European genetics laboratories, in collaboration with Orphanet. Over 1000 laboratories in Europe offering some form of genetic testing were identified and received an online survey, and approximately 350 labs have replied to date, from 32 countries.

To ensure the highest possible quality of the data, which might vary according to the identity of the respondent in a laboratory, replies were peer-reviewed and validated by comparison with EQA providers and accreditation bodies, prior to dissemination via the European QAu database.

Based on the evaluation of the QAu data for 2004-2005, half of diagnostic providers have a designated quality manager. Only 15% of laboratories are fully accredited and approximately 50% of laboratories participate in EQA schemes, distributed by 21 different providers. With the collaboration of the latter, we were able to confirm about 90% of EQA participation declared by laboratories.

With the new awareness of the central role of QAu, making this information available will benefit consumers, by facilitating informed choice of laboratory partners for performing tests, and genetics services, by facilitating selection of reliable partners for referral of tests which cannot be performed locally and by valorizing their efforts and investment in QAu. This study provides the first overview of the status of QAu in European genetics laboratories.

P1306. On the necessity of adult medical genetics services in developing countries

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Medical genetics service is often percieved as expensive, luxorious and unnecessary in public health in most developing countries. The situation is worse among adult medical genetic services that are almost completely neglected and limited to academic interest only. I performed a retrospective review of adult patients chart admitted at Chulalongkorn Hospital, a university-based hospital of Thailand to find the prevalence of genetic diseases and management. The main result is that the prevalence of genetic diseases in in-hospital patients is about 0.6% and the three main diagnoses are hypertrophic cardiomyopathy, thalassemia and familial adenomatous polyposis syndrome. This is still an underestimate because nearly all patients are not seen by medical geneticist and only obvious genetic disorders are recorded. This data is the first in our country to demonstrate the problem of adult-onset genetic disorders. The fact that the prevalence isn this study is lower than in western countries, especially in pediatric wards, indicates that more comprehensive prospective records of in-hospital patients and community-based should be done to increase the awareness of genetic diseases, and thereby change national policy in the future.

P1307. Predictive genetic testing of Alzheimer disease - A public perspective

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Alzheimer disease (AD) is the common cause of adult-onset progressive degenerative dementia and complex in its etiology. Genetic factors have a key contribution to development of AD. Although several vulnerability genes are identified and well characterized, some familial cases of this disease are due to rare mutation in other genes. The risk of AD appears to be heterogeneous in its nature with additional genetic risk factors interplay.

Several certified tests for prediction of AD are commercially available enabling publically accessible genetic testing. Its certainty is complicated by inability to assess all possible potent risk factors. Additionally, genetically based prediction of AD onset is questioned due to general ethical and social issues, concerning benefits but also potential harm. However little is known about public standpoints towards predictive genetic testing in neuropsychiatric disorders as AD. This presentation argues main issues related to predictive genetic testing for complex disorders supplemented with data from Bosnia and Herzegovina based on 1000 individual attitudes toward AD predictive genetic testing collected using pre-designed questionnaire. The statistical sample is representative for BH general population in terms of age, sex, social and demographic structure. Results are differentiated and structured among group of medical workers (familial with this issues) stratified by education level and general population subsample. There is a correlation between affirmative attitude and level of education, medical branch and urbanization. The most important obstruction in this type of survey is incomplete knowledge on methodology of genetic testing of AD and potential risk of its inadequate interpretation.

P1308. Assessment of a selection of patient information materials discussing genetic testing from across Europe

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AIM: To assess the quality of written information for patients and families about genetic testing, from a range of European countries. METHOD: Written information relating to genetic testing for 5 conditions was gathered from genetic departments across 7 European countries. 50 pieces in total were randomly chosen and assessed for the inclusion or omission of 14 key issues. These had been identified by pre existing tools as being important for inclusion when developing or assessing material relating to genetic testing. RESULTS: Whilst the majority of information discussed issues relating to the condition including background and effect (n=48, 96%), treatment and management (n=37, 74%) and heredity and risk (n=49, 98%), less than half the information discussed issues related to patient rights (n=12, 42%) and a discussion of benefits as well as potential risks of genetic testing (n=22, 44%). Only half discussed where to get additional information and support from (n=25, 50%). Benefits were more likely to be included (n=41, 82%) than any risks involved (n=24, 48%). The issue discussed least frequently was the possible psychological and social effects of genetic testing (n=9, 18%). Pre-written leaflets provided a far more comprehensive discussion of the key issues than personal letters did. Information relating to hereditary breast cancer scored far better than information on the other conditions. DISCUSSION: It is important that patients and families receive good quality written information about genetic testing. The development and translation of quality assessed prewritten information leaflets is one way to ensure this.

P1309. Regulating expression: the governance of human developmental genetics.

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In this poster we will present the findings from recent empirical research undertaken as part of the Developmental Gene Expression Map (DGEMap) design study. This EU funded project aims to establish the foundations of a pan-European infrastructure, integrating a currently fragmented research effort. Our role within DGEMap has been to report on the ethical, legal and social aspects of this area of research, which arise particularly from its requirement for tissue taken from embryos donated following legal elective termination of pregnancy.

The first results we report are from an e-mail survey of scientists, conducted during 2006, that included members of the ESHG. In our survey respondents were asked to offer their views on a range of issues concerning research using human embryos including; ethical oversight, ethics training and legislation at local, national and EU levels. One key finding was a strong desire for ethics training for scientists at all career stages. Detailed results will be presented and the potential implications for a common research governance framework explored.

Secondly, we will present the findings of a symposium of invited experts that was held in Newcastle in February 2007. The aim of the event was to highlight the range of concerns and considerations for

the governance of developmental genetics research in as diverse an entity as the EU. The plurality of views on the status of the human embryo and the resulting implications for collaborative science will be explored.

P1310. Principles of Bioethics in the Universal Declaration on the Human Genome and Human Rights

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Looking at the bioethical discussions from management perspective, it could be observed that those discussions are based on three policy principles put forward in the provisions of the above Declaration: inherent dignity of human beings , their fundmental unity and respecting their human rights and freedoms. Strategical principles of bioethics aim at the best interests of human beings and protection of public health while respecting common genetical heritage of humanity. To achieve the above objectives, independent, multidisciplinary and pluralist ethics committees must be established in order to first assess the ethical, legal and social issues raised by research on the human genome and its application and second, to identify the practices that could be contrary to human dignity, such as germ-line interventions.
Putting forward a comparative (Islamic-International) analysis of the above principles, our paper tries to show the possible directions that bioethics system of Iran can take. The paper finishes with proposals on how to guide the system towards the best possible direction.

P1311. The twilight zone between health and sickness: A qualitative exploration with asymptomatic BRCA1/2 mutation carriers

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Harboring a germ-line mutation in BRCA1/2 genes increases the lifetime risk for breast-ovarian cancer. Within the Ashkenazi population, three predominant mutations were identified in 2.5% of this population, resulting in the increased prevalence of hereditary breast-ovarian cancer to 20% and 40%, respectively. The current study is aimed at exploring the way in which healthy women at high-risk for developing breast-ovarian cancer experience and give meaning to the dialectic between being healthy and being at high risk.
Seventeen in-depth interviews with asymptomatic BRCA1/2 mutation carriers were conducted. Analyzing the narratives revealed several themes constructed around: Fears in Conflict - the continuous conflict between the threat inherent in being a BRCA1/2 carrier and the fear accompanying the prospect of a prophylactic surgeries in the absence of clinical findings; Family Clock - the definition of personal risk in accordance with the age of onset of their mother's cancer "I hope that nothing happens to me by the age my mother got sick."; Illusion of Control - the sense that knowledge is power: "I have always known that I am a carrier. At least now, there is something I can do."; The Inner Mother - the way in which the women's mothers coped with the cancer shaped their own story of coping.
With growing knowledge about the contribution of molecular genetics to multi-factorial diseases, these findings provide insight into individuals whose lives hang in the balance between health and sickness, and suggest ways in which geneticists can integrate family context into their counseling practice.

P1312. Cancer in the Family - see your Geneticist! Who will go and who won't.

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The National Health Service (NHS) Scotland prides itself of a health care system that is free for all at the point of delivery and aims to offer equal access to health provisions regardless of age, employment status or socio-economic class. There are four regional genetic centres in Scotland serving a population of 5 million and delivering an integrated clinical and laboratory genetic service. The Scottish Executive, the ministerial body for health issues, has taken the lead in providing guidelines for genetic counselling, testing and screening of individuals at risk of colorectal (CRC) and breast/ ovarian cancer (BOC). Referrals to the Scottish genetics centres are ever increasing, but questions remain whether individuals most at risk are adequately accessing the service provided. We therefore investigated the referral and attendance patterns of 4178 individuals from CRC and BOC families at the South-East Scotland Clinical Genetics Centre between 2000 - 2006 with a particular emphasis on age, sex and social deprivation indices. Patients from CRC and BOC families attending the genetics service had lower deprivation indices than the general population. Lower deprivation indices were more marked in the CRC group than in the BOC group ($p<0.001$). Effective education and communication strategies targeted at patients of all educational and socio-economic backgrounds need to be developed to address these inequalities in uptake of genetic services.

P1313. The first demonstration of genetic predisposition in all cancers using model of early onset breast cancer in Poland

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Aim. Search for combinations of cancer susceptibility markers detecting with 100% sensitivity early onset breast cancer patients.
Patients. 473 unselected Polish patients affected by breast cancers under age of 51 years and paired controls from families without cancers among first and second degree relatives matched for age and sex.
Methods. Frequency of all possible combinations between genomic markers identified as associated with cancer susceptibility in Poland have been composed between cases and controls.
Results. 17 combinations of 26 mutations or polymorphisms within 14 genes (BRCA1, BRCA2, CHEK2, p16, XPD, MC1R, CYP1B1 and others) have been found to cover all of breast cancer cases. The same genomic patterns were present only in 84.22% of controls.
Conclusions
Hypothesis:
1. All of cancers occur on the basis of increased genetic susceptibility.
2. Less than 50% of population has any risk of cancer of particular site.
Acknowledgments: Authors thank Ms Gibaszek R. and Putresza E. for excellent support in collection and analysis of registry data.

P1314. Baseline situation in Turkey about genetic consultancy for women who have risk of breast cancer

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BACKGROUND:Breast cancer is the most common cancer among Turkish women. The BRCA1 and BRCA2 tumor suppressor genes explain most of the disease in families. Consequently, there is a growing demand for genetic assessment services including provision of genetic risk information and genetic counselling with possible presymptomatic testing.
AIM:This study reports prospective baseline data about the multidisciplinary genetic and surgical assessment and counselling of familial breast cancer cases and their women relatives who have a risk.
METHOD:State University Hospitals are included in this cross-sectional descriptive study as they are accepted role models for the health service system. Data is collected by the same researcher with a structured questionnaire during face to face interviews with the head of the related clinics.
RESULTS:Among 58 Turkish State Universities, 36 have medical faculties and research hospitals. In 18 of the university hospitals there are "Outpatient Clinics for Breast Diseases". If there are at least 2 different disciplines working together on the issue is accepted as a multisciplinary clinic. Twelve of the outpatient clinics are multidisciplinary (2/3), 9 of them (3/4) declared that they have links with psychiatry department members but not as a continuous team member. Only in 4 of the clinics, genetic counselling or consultancy to the patients with familial

risk is given on standard algorithms.
CONCLUSION:Cancer clinics that deal with familial cancers will include genetic assessment programs. It must be remembered that patients have rights to receive breast cancer risk information and genetic testing consultancy before and after genetic testing .

P1315. Preconceptional CF and HbP carrier screening in the Netherlands: First results of a trial in a multi-ethnic society

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Objective: The reaction of couples planning a pregnancy was studied to a combined offer of ancestry dependent cystic fibrosis (CF) and haemoglobinopathies (HbPs) carrier screening.
Methods: 9453 people (20-35 years) were offered CF and/or HbP carrier screening at their general practitioner's (GP) office by either their GP (n=4720) or the Municipal Health Service (MHS) (n=4733). The target group was defined as having a partner and planning a pregnancy. Its size was estimated by means of a reply form (=respondents) and a telephone survey among a sample of non-respondents (n=201).
Results: 14% (1365/9453) responded. The target group was estimated to be 33%: 490/1365 respondents and 66/201 out of the 8088 non-respondents. Thirty-four percent intended to participate in carrier screening (166/490). Only 87 actually had a carrier test done together with their partner: 29% (25/87) and 71% (62/87) were invited by the MHS and their GP, respectively. The proportion participating non-Western immigrants (31%) was less than the proportion invited (50-60%), and people with lower education were underrepresented (only 10% of participants). The participation rate was ~ 3%. "Lack of time" (37%) was the main reason for non-participation. The majority supported offering CF and HbPs carrier screening to all couples planning a pregnancy: 77% and 71% of participants and non-participants, respectively.
Conclusion: Immigrants and people with lower education reacted differently to the offer of preconceptional CF and HbPs carrier screening. Furthermore, MHS' sending the invitations seems to be a barrier. Both lead to challenges for the implementation process.

P1316. Genetic Assessment of a Clinical Case of Congenita Myotonica

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In clinical practice there would be some cases of myotonia with clinical symptoms other than muscular ones who have been associated with mutations in the muscle chloride channel gene. Most cases reported to date show a recessive inheritance pattern, with loss of function of the corresponding protein.
Myotonia congenita is inherited both in an autosomal recessive type namely Becker disease and also in an autosomal dominant manner which has been called Thomsen disease; the same mutation may occur in families with both types of inheritance. In the autosomal dominant type of the disaese, the proportion of cases caused by *de novo* mutations is unknown. Perhaps each child of an individual with autosomal dominant myotonia congenita has a 50% chance of inheriting the mutation.
Here we report clinical and molecular findings on family members from the central region of Iran with dominantly inherited myotonia congenita as the two brothers in the family show characterized clinical picture of sustained contraction of the skeletal muscles which identified in Thomsen's myotonia. In genotype/phenotype correlations investigation these patients showed the typical EMG patterns of the dominant type resulted in a mild tendency for a discharge of repetitive action potentials to occur in response to electrical and mechanical stimulation.

P1317. Genetics in clinical practice: Competences for non-genetics healthcare staff

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Genetics impacts on the patient care provided by many different health professionals. What genetic activities are appropriate for health professionals outside specialist genetics services, how should these activities be carried out and how can we ensure a high quality service for patients?
In the UK, the NHS National Genetics Education and Development Centre collaborated with Skills for Health (the statutory body responsible for healthcare competences) to develop "competences for genetics in clinical practice for non-genetics healthcare staff". Appropriate genetic activities were identified by a large group of healthcare staff from many different professions. They identified nine activities which cover the pathway for patients with or at risk of a genetic disorder. For each activity, the health professionals described how the activity should be carried out - so that for the first time, a set of competences with performance criteria and underpinning knowledge and understanding have been defined for genetic activities for non-specialists.
The competences cover widely applicable activities - such as understanding genetics relevant to your practice, recognising possible genetic implications, taking an appropriate family history and referring patients - and more specialised activities such as assessing genetic risk or understanding genetic testing. Few health professionals will need all the competences, only those relevant to an individual's clinical role should be selected.
The competences can be used in job descriptions where genetic activities are part of a professional's role; in developing services or new roles; for assessing individuals' learning needs; and to inform the development of education and training courses.

P1318. Social support in the context of genetic testing of late-onset neurological disorders

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Introduction: In genetic counselling, the evaluation of the social worker has the aim to value if the individuals at-risk, when informed about their probability of being or not a career of the gene of neurological hereditary late-onset disease (Huntington disease, Machado-Joseph disease, familial amiloyd polineuropaty (FAP) ATTR V30M and CADASIL , are able to give an appropriate healthy answer (they dispose of resilient capacity) or an unsuitable answer not healthy, being this damaging for the individuals.
In our protocol of the genetic counselling for PST, the social worker values two groups of risk factors : (1) The vulnerability centered in the subject (the genetic predisposition, resources of the personality, cognitive resources, etc.) (2) The vulnerability connected with the inadequacy of the environment in which the individual is inserted (familiar unsuitable structure, the separation of the parents, death of the parents, chronic disease, economical fragility and / or poverty, social isolation, etc.)
The Social Intervention is carried out through: (1) Exchanges that hold emotional positive attitudes (Sluzki, 1996: 16); (2) The counselling (formal or informal) which allow the establishment of interactions that have the aim of sharing informations on the disease in question; (3) Material or instrumental support; (4) Technical support or of services; (5) The access to new contacts.
Conclusions :Contribution of the development, psycodynamics, cognitive and social models (strategies of resolution of problems, psychotherapy, stress and coping, personalities, and attitudes) add an important value to the program of genetic counselling fo PST

P1319. Inheritance patterns in coronary heart disease

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Introduction: Coronary heart disease (CHD) is the leading cause of death in developed countries. The prevalence of CHD is rising rapidly in the world due to increased exposure to CHD risk factors. CHD is the most common heart disease that is believed to be caused by multiple genetic factors, environmental factors, and interactions among these factors.
Material and method: 366 consecutive cases were studied by genetical analysis. Genetic counselling, pedigree analysis, clinical and Para clinical studies were done for patients.
Results: There were 282 male (77%) and 84 female (23%). Mean age

was 55.13±11.20. History of diabetes, hyperlipidemia, hypertension and obesity were seen in 22.4%, 51.9%, 32.1% and 42.3% respectively. Familial marriage was seen in 66 patients (18.03%). There was early onset CHD (diagnosis at less than 50 year of age) in 156 cases (42.6%). 82% of them had positive familial pedigree in the different familial patterns. 25.6% of cases with early onset CHD and 12.4% of cases with late onset CHD were born from consanguineous marriage (P=001).
Discussion: This study can assist physicians and genetic counsellors to realize the contribution of positive familial pedigree and inheritance pattern to cases with CHD. Identification of familial patterns and other risk factors will provide valuable information for prevention and control of CHD. These results can be used for next molecular analysis.

P1320. Female triple heterozygous CF allele delF508, MTHFR C/T, MTRR A/G with fetus 69,XXX

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Genetic counseling claimed by the healthy couples is very rare process in St.Petersburg and in all places of Russia. The couple consulted considers they are healthy. Proband female 27-yr-old is Ukrainian descent and her husband 34-yr-old is Lezghian. Proband has occupational hazard (contact with mineral dyes). Our clients are intensive smokers. Proband's gynecological history is advantageous. The couple denies congenital and inherited pathology among their relatives. The results of laboratory investigations are the following: couple karyotype 46,XX and 46,XY,21stk+; DNA investigation: PKU allele R408W is absent; proband is heterozygous for cystic fibrosis allele delF508, MTHFR C/T and MTRR A/G. She had higher level of blood homocystine (17.4 micromole/l vs. 5-15micromole/l). After medication during 3 months with Elevit (folic acid, VitB1 and VitB12) the level of blood homocystine was decreased to 8,9 micromole/l. Proband broke off medicine intake. She got pregnant soon after that. At 11th weeks of gestation oedema of fetus generalized, particularly of the back region of neck, was revealed with US investigation. The fetal material was obtained by biopsy of the chorionic villi. The fetus karyotype was found to be 69,XXX and this pregnancy was interrupted. We continue our genetic support of this couple.

P1321. Cross-cultural Communication in Genetic Services: Bridging the gap

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The two major communities (Greek and Turkish Cypriot) in Cyprus were separated by a border since 1974. The sharing of healthcare services, between the two communities became very difficult. Recently efforts of bringing the two communities together have been underway. Ethnic background and differences should not interfere with healthcare. Cross-cultural gaps and communication problems are often obstacles in the provision of healthcare services. Furthermore, the importance of a `multidisciplinary` team is known to be essential for the best care of patients with rare disorders as are genetic conditions. Communication between healthcare professionals, therapists and patients within such a team is fundamental. The multicultural composition of Cyprus creates need for a network to establish new and expand the existing channels of communication between such professionals.
Attempt to create a network in the care and referral of patients, living with or at risk of genetic or inherited conditions in both communities in Cyprus officially began in December 2006 although was in design since May 2006. Through this network, professionals will be able to collaborate, exchange ideas and assist each other with the common goal of assuring efficient and sustainable patient care. Also the patients are invited to share problems, experiences and hopes while contributing in building a network for better communication and multidisciplinary care.
The first steps of networking and bridging the gap will be presented as a poster. Furthermore the problems (practical, cross-cultural and others) we encountered as the professionals who originated this network and partnership will be discussed.

P1322. Technical validation of a system for cystic fibrosis newborn screening

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Newborn screening for cystic fibrosis has recently been implemented for the province of Alberta, Canada. We were assigned the task of developing a comprehensive cystic fibrosis screening system for the approximately 42,000 births per year in this province. We assessed the effectiveness of a process designed to maximize diagnostic sensitivity and specificity. Initial immunoreactive trypsinogen (IRT) measurement was carried out with the top 2% as a cutoff for cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation analysis. Validation of four DNA extraction methods from dried blood spots on newborn screen requisitions was followed by assessment of three CFTR mutation detection protocols. These mutation detection protocols were validated by analysis of 45 genotype controls and a test set of 5,000 newborn blood spot samples, 2,000 of which were screened in parallel at two sites, prior to initiation of the newborn screening program. From these assessments, a system has been developed that incorporates standardized extraction and targeted analysis of common mutations as a primary screen. Clinical assessment of mutation-positive cases includes confirmatory testing, as well as full CFTR coding sequence screening for point mutations and intragenic rearrangements.

P1323. Professional accounts of inheritance in Type 2 Diabetes and Coronary Artery Disease involving ethnic minority groups

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The incidence of common, complex disorders such as diabetes type 2 (T2D) and coronary artery disease (CAD) is known to vary between populations. We have examined the published literature and conducted interviews with a range of professionals - clinicians, laboratory scientists and public health specialists - to assemble and compare the explanations put forward to account for the higher incidence of these conditions among UK South Asians and aboriginal groups in Australia. There are potentially important consequences for health and social policy of the different explanations proposed, as well as implications for responsibility and blame at individual, family and community levels. In this presentation, we analyse interview data (from researchers, health professionals and support group representatives in the UK) using the methodology of rhetorical discourse analysis, by paying particular attention to how explanations of `inheritance' are framed and justified in these accounts. Our findings suggest that `inheritance' is articulated in multiple senses to include genetic, cultural and familial meanings, while allusion is made to lifestyle choices. This nuanced notion of inheritance is explored further by linking our analysis to notions of `agency', `responsibility' and `blame'. We also report patterns of difference across and within the professionals' accounts.

P1324. The reproductive choices made by South African mothers who have children with Down syndrome

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Down syndrome is the commonest cause of congenital developmental disability in industrialized countries, where it occurs in approximately 1.4 per 1000 live births. In South Africa, the birth prevalence of Down syndrome was documented as 1.8 and 2.09 per 1000 live births in urban and rural populations, respectively.
The aim of this study was to determine the reproductive choices of women with a child with Down syndrome, aged 1 year or older. The survey was conducted using a structured questionnaire. The sample consisted of 50 women; 36 African, 4 Asian and 10 Caucasian. The questionnaire assessed the mothers' knowledge of Down syndrome prior to diagnosis, what counselling was received and how this knowledge was utilised. Information was obtained on the use of family planning, the knowledge and use of prenatal medical genetic screening and diagnosis, and decisions for future pregnancies. None of the mothers had prenatal diagnosis in their pregnancy with their Down syndrome child, but 76% (38) would want prenatal diagnosis in future pregnan-

cies. Of the 50 mothers, 21 (42%) would terminate a pregnancy if Down syndrome was detected, 26 (52%) would not, and 3 (6%) were unsure what they would have done if faced with this decision. Of the Caucasian women, 40% (4) would opt for termination of pregnancy, 40% (4) would not and 20% (2) were unsure. Of the African and Asian women, 52.8% (19) and 75% (3) respectively would not terminate an affected fetus. The information gained has helped provide a more effective genetic counselling service.

P1325. DYSCERNE - A European Network of Centres of Reference for Dysmorphology

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Over 2,500 rare and difficult to diagnose conditions presenting with patterns of birth defects have been identified. The rarity of these dysmorphic conditions means that even in Centres of Reference, experience may be limited and a diagnosis might be delayed or not made at all. Making a correct diagnosis is the cornerstone of patient management, enabling clinicians to locate other patients with the same condition, share clinical experience, and increase individual and collective knowledge about rare conditions. For patients and their families, the importance of having a diagnosis cannot be over emphasised. It can help them come to terms with the condition, reassure them that they are receiving appropriate care, and may facilitate making contact with other affected individuals and families for support and advice.

DYSCERNE aims to raise current standards for the diagnosis, management, and information dissemination of rare dysmorphic syndromes. The Network is funded by the European Commission, Directorate General for Health and Consumer Protection, and comprises six designated Centres of Reference for Dysmorphology (UK, Belgium, France, Italy, The Netherlands and Poland). The lead partner, Manchester University, UK, will be the coordinating and managing centre for the Network.

To facilitate the project aims, a web-based electronic dysmorphology diagnostic system will be established, enabling clinicians to submit difficult to diagnose cases electronically for expert review. Project members will also develop guidelines for selected multi-system disorders which will be piloted and disseminated widely throughout the clinical genetics community. DYSCERNE will also serve as a demonstration project for future EU Networks.

P1326. Training non-MD medical geneticists: suggestions from Lithuania

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Translation of the achievements in human genetics and genomics into clinical practice results in increasing introduction of new diagnostic genetic tests and growing demand for highly qualified non-MD specialists (medical geneticists) performing such tests, interpreting and communicating their results. Nevertheless, adequate system for their training is still absent in a number of EU countries including Lithuania.

A three stage system for training non-MD clinical/laboratory geneticists is under development in Lithuania (introduction is expected in 2008). 1st stage: undergraduate (bachelor) studies programme ensuring background education. 2nd stage: graduate (master) studies programme ensuring basic knowledge in the disciplines essential for clinical/laboratory geneticists (molecular genetics, cytogenetics, biochemical genetics, genetic counselling, bioinformatics). 3rd stage: post-graduate studies programme (comparable to a residency studies programme for medical specialties) ensuring a high level knowledge and expertise in one of the basic specialisations: molecular genetics, cytogenetics, or biochemical genetics. Specialists graduating from the 3rd stage studies programme will be able to apply for certification by the Lithuanian Society of Human Genetics.

A system for non-MD medical geneticists' certification on the basis of a EU-level exam (e.g., similar to that already in action for laboratory chemists) is necessary. Such certification should enable including the non-MD medical geneticist into the EU Registry of clinical/laboratory geneticists (more exactly, a special section corresponding to the above-stated specialisation). Registered specialist should be able to work in any clinical/medical genetics laboratory across EU. At the same time EU-level certification would be an official document confirming specialist's qualification in his/her native country.

P1327. Genetic Education: Adapting Strategies for Secondary Schools

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The Genetics Service participates in an initiative of the National Agency for the Scientific Culture, witch allows Secondary School students to do an internship integrated in the assistance work of a Health Service**,** where the study of the genetic diseases and prenatal diagnosis allow them to apply and to deepen the knowledge acquired in school, qualifying them for the active life, not neglecting the exercise of the citizenship.

The name of this internship project is „Genetics Seen by the Young People". It has the duration of 1 week and was already attended by 66 students.

This internship is essentially practical but is strongly based in the genetic principles lectured in Biology Course. 80% of internship time is spent in laboratorial activities and the remaining 20% is spent in the Prenatal Diagnosis, Dismorphology and Preconception consultation, always with previous authorization by the patients. Then, all the students that worked as interns in the service since 2001 reply to an inquiry that had several goals, namely the importance of the internship in the professional choice. The students unanimously affirmed that this internship had enormous importance in the acquisition of new knowledge and in the opening of other work perspectives that they only imagined possible with a Medical Degree.

Our conclusion is positive and encouraging, and it suggests that a strong interconnection between the school and the business world can be the instrument that lacks, increasing the satisfaction and the self-esteem of the students, reducing deviant behaviours and school abandonment.

P1328. Methodology analysing decision making for multidisciplinary staff before antenatal diagnosis.

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We introduce a methodology analysing decision making in clinical ethics. It enables to assess the various reasons health professionnals refer to, without being clearly conscious of it, when a difficult decision has to be made.

The issue is :

- To define the ethical aspect in the decision through worldwide acknowledged principles: autonomy, beneficience and non-maleficience ([1])
- To work on the basic idea that emotions have to be used which we consider crucial in order to become aware of the ethical aspect of the decision

Thus we believe such worldwide valued concepts become clear to health professionnals through some emotional experiences ([2]). We are speaking here of respect, compassion and fear. Practitioners discover through these emotions how much they value these principles.

Feeling of respect reveals how much they value the principle of autonomy, feeling of compassion how much they value the principle of beneficience and feeling of fear the principle of non-maleficience.

To make our point clear, we will show how our methodology works in a difficult decision making situation : a request for termination of pregnancy after a Turner syndrome chromosomal anomality has been revealed on the fetal caryotype.

1. T.L Beauchamp et J. Childress. Principles of Biomedical Ethics, Oxford University Press, New-York/Oxford, 1994.

2. Livet P., Emotions et rationalité morale, PUF, coll. « Sociologie », Paris, 2002.

P1329. Glucose metabolism, lipid profile and inflammatory markers in healthy subjects in relation to family history of type 2 diabetes mellitus

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Population studies have shown that family history to type 2 diabetes mellitus (T2DM) is a risk factor for developing the disease, but also for T2DM predictors, including anthropometric and metabolic modifications.

Our study aimed to compare anthropometric data, lipid and glucose metabolism and inflammatory factors in healthy subjects with positive family history [Fam(+)] and negative family history [Fam(-)] of T2DM.

Subjects and Methods. A total of 139 healthy subjects were studied, the subjects whose first degree relatives suffered from T2DM were compared with those without T2DM among close relatives.

Weight, height, waist and hip circumference were determined, body mass index and waist-to-hip ratio were calculated. Fasting serum glucose and insulin were measured and used for calculation of insulin resistance index by homeostasis model assessment (HOMA IR). Serum TC, TG and HDLC levels were determined enzymatically, apolipoproteins A-I and B by Laurell's electrophoresis, C-reactive protein by a highly sensitive immunoprecipitation test and fibrinogen by the method of Clauss.

Results. Anthropometrical data as well as mean values of lipoprotein parameters, fasting serum glucose, insulin, HOMA IR, C-reactive protein and fibrinogen did not differ between the groups of positive vs. negative family history to T2DM. Conclusion. Positive family history of T2DM was not associated with impaired glucose and lipid metabolism in our cohort of healthy subjects.

P1330. Offering carrier screening for fragile X syndrome to non-pregnant women - a pilot study

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Population-based carrier testing for fragile X syndrome (FXS) remains controversial despite fulfilling many of the WHO criteria. Health professionals' concerns relate to perceived difficulties in community understanding of the complexities of this condition.

To inform policy around genetic screening, we conducted a three-phase pilot study to assess acceptability and feasibility of offering FXS carrier screening to non-pregnant women in an Australian family planning clinic.

In Phase 1, clinic staff and female patients attended an FXS information session and participated in focus groups. Their understanding, views, interest and concerns about offering FXS carrier screening were discussed. Overall, women and staff were positive towards screening. These qualitative data informed production of a brochure, two questionnaires and testing protocols, which underwent validation. Questionnaires included demographics, awareness and knowledge of FXS, attitudes towards carrier screening, decision-making, and anxiety. In Phase 2, larger number of women were recruited, completed a questionnaire, and were also offered FXS screening. The second questionnaire was completed one month later. A small number of women who had completed both questionnaires took part in follow-up interviews (Phase 3) about their experiences in participation

Of the 338 women recruited, 96% completed Q1, 54% completed Q2, to date, and 30 have been interviewed. Of the 20% (n=63) of women who were tested, three grey-zones and one pre-mutation were found. Preliminary analysis indicates that women were overwhelmingly in favour of the availability of FXS screening to all women, although fewer had screening for a variety of reasons. Women's understanding of FXS was reasonably good.

P1331. From legislation to societal learning. A new policy paradigm for genetics and insurance.

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Over the past years, one of the most contentious topics in policy debates on genetics has been the use of genetic testing in insurance. In the rush to confront concerns about potential abuses of genetic information in insurance, most countries throughout Europe have enacted genetics-specific legislation while the Genetic Information Nondiscrimination Act (GINA) is currently pending in the US Congress. In this presentation we want to reflect on the adequacy of genetics-specific legislation. We will give two main arguments why we consider this approach not to be viable in the long run. First, these laws reflect a "genetic exceptionalism", overemphasizing the role of genes and enhancing the reduction of our identity and life chances to genes. New developments in genomics therefore do not fit legal concepts. This means that there is a growing gap between legal relatively stable definitions and dynamic genomics practice. Secondly, genetics-specific legislation creates some unintended effects. By giving exclusive legal protection to the group of genetic risks, other *non*-genetic risk groups are unintendedly being under-protected. Given these drawbacks, we argue that it is time for a new policy paradigm, which stresses investing in social learning processes more than in introducing defensive legal walls. While genetics-specific legislation reifies a momentary relation between science and society, a social learning approach enables more continuous interaction between science and society. Just as genomics is enabling medicine to take a more prospective approach, policy making will similarly need to experiment with the genome sciences as they affect science, health and society.

P1332. Exploring of medical staffs' attitudes in genetic services provided to international spouses in Taiwan

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Global migration have challenge the health services in Taiwan. In recent 3 years, every one out of five newly married couples are international marriage. With this increased ethnic diversity, health professionals require more culturally competent approach especially in genetic counseling. The purpose of this study was to explore attitudes and needs among medical staffs who provide reproductive care and genetic services to international spouses.

In this survey, an assessment checklist with 5 scaled score consisted of 18 questions related to cultural competent care was used in the questionnaire. Of the 470 participants, 374(79.6%) responded. We found that (1) Lack of language appropriate information materials: For example, 90.2% staffs agreed that education materials displayed had to reflect cultural backgrounds of counselees; however, only 34% complied in practice. (2) In direct communication styles: 93.6% subjects considered themselves as unable to communicate directly with international spouse due to limited time during clinical encounter. (3) Stereotype found in attitudes: For example, 81.5% participants concerned that international spouses could not make their own reproductive decisions and had difficulties in caring of their children.

Conclusion: We found that there exist gap between concepts and practice during encounter with international spouses. Health professionals need to improve cultural competency to deliver appropriate trans-cultural genetic services.

P1333. Improving communication skills in genetic counseling - simulation based national project

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Background: Simulated patients (SPs) using play rolling actors are widely used for training and evaluation of health care professionals. In this study SPs based course aiming to improve communication skills in genetic counseling was developed and validated.

Methods: Based on authentic clinical situations rising important counseling dilemmas 8 SPs based scenarios were developed. Each scenario included - description of the clinical situation and detailed script

to the actors on the answers and behaviors involved in the counseling process. Following actors training, the scenarios were evaluated during the training of experts in human genetics becoming later the trainers in this program. 36 human geneticists (7 genetic counseling students, 20 genetic counselors, 4 fellows and 5 experts in human genetics) participated in 3 training days. Each participant was performing 2-3 scenarios as active participant in the "hot seat" and observed 3-4 other scenarios. Training was videotaped for debriefing and additional feedback was given by the actors.
Feedback: According to participants' feedback questionnaires (using 1-4 Likert scale) most participants indicated that - the scenarios represented realistic clinical cases (96%), training improved their understanding the counselees' needs (78%), improved their ability to give information (63%), and increased the awareness to ethical issues (58%). All participants recommended the use of SPs for further training in genetic counseling.
Conclusions: In this study SPs based training scheme in genetic counseling was developed and implemented. Feedback questionnaires supported the content validity of the scenarios and the potential contribution of such project in improving communication skills in this field.

P1334. Genetic counselling network in the Islamic Republic of Iran(1997-2007)

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Introduction & Aims: The importance of genetic and congenital factors which causes many diseases & disabilities is obvious with a glance at the facts and figures in the world. In Iran certain factors exist that worsen the situation, such as:
High percentage of consanguinous marriages & etc. This comprehensive program is planned with the aim of implementation of a genetic counselling network. The General Goal is to decrease the incidence and prevalence of genetic and congenital disabilities.
Material& Methods*:* For implementing this program the network has gradually expanded with about 125 genetic counselling centers throughout the country.
The first level are counseling centers in smaller cities(The staff are a general physician along with a professional nurse, midwife or geneticist); the intermediate level are counselling centers in the capital of each province(The staff are a specialist, a general physician, and one professional nurse, midwife, or geneticist).
The highest level is the „State Commission of Genetics" in Tehran (The staff are about 10 specialists).
The referral centers (The head of six Regional Offices) are located in the capital of six provinces(The staff in the provincial referral center are at least 2 specialists and 2 - 3 general physicians, with years of experience).
Results: The network has gradually expanded since the year 1997, and now we have 125 governmental and private genetic counselling centers.These centers have been offering services to approximately 220,000 clients until now.
Conclusion: The genetic counselling network offers good quality, low cost and easily available services to population at risk.

P1335. The evaluation of referral reasons for genetic counseling and prenatal diagnosis at a tertiary genetic center: a Turkish experience

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Genetic counseling is the process which assists individuals in understanding the nature of the genetic disorder, the probability of developing or transmitting it and the ways how to deal with the condition. With the advancements in human health field and particularly in genetics, it has been possible for the scientists and for the public to be aware of the mechanisms underlying genetic diseases. To what extend the scientific and communal evolution affected the genetic counseling sessions is the aim of this retrospective study.
We evaluated 5549 files of a tertiary genetic counseling and prenatal diagnosis center retrospectively throughout 1998-2006 years in Turkey. Among the subjects provided genetic counseling, the groups included: those whose referral reason is related to prenatal diagnostic tests, and those whose referral reason was other than prenatal diagnosis. In the first group the most common referral reason was advanced maternal age (45.01%), which is followed by high risk result on triple test (22.99%), previous child with congenital malformations or single gene disorder (9.57%), abnormal ultrasound finding (5.35%), parental anxiety (4.81%), genetic disease in the family members (4.22%), the presence of thalassemia trait in the parents (2.74%) and the others (5.31%). In the second group the most common reason for referral was recurrent miscarriages (32.99%), which is followed by previous child with congenital malformations or single gene disorder (25.76%), infertility (13.59%), unidentified genetic condition (9.82%), genetic disease in the family members (6.13%), amenorrhea (5.89%), consanguinity between couples (3.69%) and the presence of thalassemia trait in the parents (2.13%).

P1336. A qualitative investigation of the impact of DMD on South African families

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South African parents who have a son with Duchenne Muscular Dystrophy (DMD) are confronted with unique circumstances and barriers, including poverty and minimal access to resources. The aim of this study was to investigate the level of genetic knowledge of parents and the impact of DMD on the family.
A qualitative approach was selected as it aims to portray the richness and complexity of real-life events from the participants' perspective. Ten semi-structured qualitative interviews were conducted with parents who had sons with DMD between the age of 8 and 15 years, lived in the Cape Town metropolitan area and attended Red Cross War Memorial Children's Hospital (RCWMCH).
The level of understanding of the genetics of DMD was generally inadequate; this was related to socioeconomic status, level of education and influenced by cultural beliefs. Financial problems, difficulties with public transport, lifting, inappropriate wheelchairs and home care of the affected boy were among the most frequently mentioned problems of the participants. Emotional problems related to having a son with DMD were also mentioned.
The findings of this study will help healthcare professionals involved in the care of boys with DMD to better understand some of the barriers that South African parents face. Education and information are an important form of support. Therefore, the service at RCWMCH may be improved by having a dedicated genetic counsellor as a member of the inter-disciplinary team involved with families in which a diagnosis of DMD is made, to facilitate information giving and assist in providing psychosocial support.

P1337. Prenatal genetic counselling from the women's point of view

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In Italy prenatal genetic counselling and diagnosis are part of the routine antenatal care offered to women at increased risk of fetal chromosomal anomalies. However, counseling methods (individual, in group, use of a decision aid) and involved providers (medical geneticists, obstetricians, nurse/midwives) vary widely among centres. The aim of this study was to explore the pregnant women's genetic counselling experience and to compare the relative efficacy of different approaches to genetic counseling. Interviewed participants, all women of advanced maternal age (>= 35 years), can be divided in two main groups according to ethnic origin: Italian and immigrants mainly from Middle-East and Eastern-Europe. Overall, participants showed satisfaction with the content of genetic counseling, while knowledge, coping and state anxiety vary according to counseling methods and patients' demographic and socio-economic variables. Most women expressed a preference to be counselled by a nurse/midwive than by a "more technical" medical geneticist. Increased anxiety levels were reported by women experiencing a counselling approach with the administration of a pre-counselling genetic questionnaire.

P1338. Results of Genetic Counselling and molecular genetic testing of severe monogenic disorders in Hungary between 1993 and 2006.

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During the genetic counselling of the severe monogenic genetic diseases it is very important to establish the exact etiological diagnosis. The molecular genetic examinations play outstanding role in this diagnostic procedure. Molecular genetic diagnosis provides effective possibility for prevention of serious genetic disorders by prenatal diagnosis and possible termination of pregnancy whenever treatment of the diseases is unavailable. Moreover, genetic results provide accurate differential diagnosis and proper medical care for the patients.

Molecular genetic examinations on the following severe genetic disorders have been performed in our laboratory during last 13 years: Spinal Muscular Atrophy -in 78 families with 98 prenatal diagnosis; FRAXA - in 38 families with 2 prenatal diagnosis; Charcot-Marie-Tooth disease type 1A - in 14 families; Ducenne/Becker Muscular Dystrophy - in 12 families; Congenital Myasthenic Syndrome - in 1 family; Facioscapulohumeral Muscular Dystrophy - in 2 families; Limb Girdle Muscular Dystrophy - in 2 families; Angelman syndrome - in 5 families. The detailed results will be reported in our presentation.

By performing the molecular genetic analysis effective diagnosis and correct genetic counselling was established in our genetic unit.

P1339. The perspectives and experiences of genetic counsellors and linkworkers (interpreters) working together in clinical genetic consultations

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Language, ethnicity and culture affect many aspects of genetic counselling, and there is a need for contextually appropriate and accurate communication. Britain is a multicultural and multifaith society, and English is not the patient's first language in a proportion of consultations. This research provides an insight into the experiences and perspectives of genetic counsellors and linkworkers (interpreters) when they work together. The study was based on convenience sampling at one centre in the UK. Six genetic counsellors and five linkworkers participated. Exploratory semi-structured interviews were taped and transcribed, and the transcripts were analysed thematically. Both groups identified the following issues as having an important impact on joint consultations: differences in their professional roles and responsibilities; developing a working relationship; the language of genetics; and, training and support needs. All participants agreed that a partnership between the two professional groups is key to achieving the best outcome for patients. However, they also commented that minimal (if any) training is provided to enable them to work together effectively. Relationships are ad-hoc, and there is little opportunity to prepare for consultations together. This small study has identified some ways to improve the service offered to patients, including: training for genetic counsellors and linkworkers to raise awareness of each other's roles and to learn how to work together; the development of a common and consistent lexicon of key genetic counselling terms and concepts for translation into minority ethnic languages; and, ensuring there is time for pre- and post- consultation briefings to occur.

P1340. Prenatal and preimplantation diagnonis in Cancer Genetic: what's the role of Genetic Counsellors?

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In cancer genetic, most syndromes are dominantly inherited - which leads to a risk of transmission of 50%.

Prenatal and preimplantation diagnoses are options frequently chosen by affected patients who do not want to have a child with the same disease. They do not wish their children to go through the same suffering. In France, those medical interventions depend on ethical laws which request a "particularly severe and not curable affection". Prenatal and preimplantation diagnoses are not allowed to all the hereditary predispositions to cancer.

Genetic counsellors have newly been introduced in France. Before 2004, genetic counselling was only done by specialized physicians. Now that genetic counsellors have been introduced to the field, their missions have yet to be defined.

This work presents different propositions about how genetic counsellors could afford a comprehensive support through adapted consultations to couple with one partner affected by a dominantly inherited cancer. Their missions could be mainly focused on accompanying couples from the first consultation of genetic through their questions about having a baby, taking the risk of transmission or not, all the way to the birth, the renouncement, the prenatal diagnosis or the preimplantation diagnosis.

P1341. "Coping first, genetics second": A qualitative study exploring family communication of genetic information.

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Communicating genetic information in families can be problematic. Often important information about a person's own health or their reproductive health (or both) is **not** transmitted to all at risk relatives. Guidelines from peak bodies are general and fail to address how genetic health professionals should facilitate communication to at-risk relatives. The authors are investigating families' experiences of communicating genetic information and whether there is a greater role for genetic health professionals within this experience. Qualitative interview data from consultands and focus groups with genetic health professionals has revealed:

1) Consultands feel overwhelmed with information after genetic counselling and their priority is to manage the health and social aspects of the genetic condition, relegating telling family members to a lower priority.

2) Consultands usually communicate with first degree relatives, however often pass responsibility to their parents to tell other siblings and second and third degree relatives. Further decisions are made about whom to tell based on social bonds and a feeling of moral obligation. Hence communication occurs in an *ad hoc* manner, over time.

3) The accuracy of information and the message may be lost.

4) Both health professionals and families want assistance in facilitation of communication in families.

These data demonstrate the need for further evidence to inform best practice for genetic health professionals dealing with communication of genetic information in families.

P1342. Should Individuals be Informed about their Salt Sensitivity Status? First Indications of the Value of Counseling on Genetic Predisposition to Low-Risk Conditions

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The present study examined the possible pathways for positive effects of genetic testing for relatively 'low-risk' conditions by (1) exploring the impact of being tested for one's genetic predisposition on intention and (2) exploring and comparing the determinants of the intention to restrict salt intake with and without a genetic predisposition to being salt-sensitive. In a cross-sectional within-subjects design, patients being tested for genetic predispositions to salt sensitivity reported higher intentions to restrict their salt intake in case their blood pressure should prove to be salt-sensitive, confirming the value of genetic testing for low-risk conditions. In case the blood pressure should prove to be salt-sensitive, intention was observed to be significantly associated with general health, perceived severity, and self-efficacy, whereas the intention in case no salt-sensitivity was predicted by perceived severity, self-efficacy, and current stage of change. Overall, the results suggest that genetic testing for low-risk conditions has a positive impact on the motivation to engage in preventive behavior. Furthermore, genetic counselors should primarily focus on the severity of the genetic predisposition and the feasibility of the recommended preventive behavior.

P1343. Developing family route maps - A tool to help access information and services for families with genetic conditions

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AIM: To explore information and services currently available to families with six rare genetic disorders as the first stage in the development of *Family Route Maps* designed to signpost information and guide patients, families and carers through the available appropriate services within the UK.
METHODS: Focus Groups and supplemental interviews with patients belonging to Support Groups as well as interviews with health professionals who specialise in these conditions. An online patient questionnaire is also available to widen patient participation.
SUMMARY: Common themes were identified and seven clear categories emerged: Information;Communication; Education of Healthcare professionals; Diagnosis of rare genetic disorders; Empowering patients and parents/carers; Ethical, Legal and Social issues; and, Treatment & Surveillance of patients and families with rare genetic disorders.
In addition to providing future practical guidance to people with the named disorders, the project has: Produced a generic *Route Map* template which can be used for other genetic conditions; Identified several themes common to all the groups involved; Highlighted the *real* needs and concerns expressed by patients and their families; Raised awareness amongst clinicians of the necessity for clear care pathways to help patients living with rare and sometimes life-threatening conditions, with the aim of a protocol for support, monitoring and treatment.
DISCUSSION: The themes have far-reaching implications for health service provision for people with genetic disorders, sharing the findings with health care professionals as well as patients is essential.

P1344. The Italian External Quality Control (I-EQC) Programme for Beta Thalassemia molecular diagnosis: five years of activity

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β-Thalassemia (βT) is one of the most common single gene disorders and is prevalent in the Mediterranean region, the Middle East, the Indian subcontinent and East Asia. Frequency of the disease may vary widely, depending on the ethnic population. The highest incidences are reported in Cyprus (14%) and Sardinia (12%) (1).
Approximately 200 different molecular defects affecting the β--globin gene (*HBB,* 11p15.5 - OMIM 141900) have been reported.
Molecular genetic testing of *HBB* is available and may be useful for prediction of the clinical phenotype, presymptomatic diagnosis of at-risk family members and prenatal diagnosis.
The I-EQC Programme for βT is performed within the National Project for the standardization and quality assurance of genetic tests (2).
Five trials have been performed from 2001 to 2006 (one per year); 11/12, 14/14, 16/16, 16/16 and 18/18 are the respondent versus enrolled public laboratories (75% of Italian public laboratories) (3).
Genotyping results indicate a general good level of quality; 900 alleles were analysed in five years. About 98.8% of them was correctly assigned; 0.6% was wrongly genotyped.
Methods predominantly used to detect mutations were RDB, ARMS, DGGE and sequencing of DNA.
Reporting of results is still inadequate although a new form for the written reports was introduced in 2004.
Funded by: “Progetto nazionale per la standardizzazione e l’assicurazione di qualità dei test genetici”, Ministry of Health (Rome).
1. Cao and Galanello, *N Engl J Med* 347(15), 2002;
2. Taruscio et. al., *CCLM,* 42(8):915-21 2004;
3. Dallapiccola et al., *Analysis*, 2006

P1345. Definitions of Genetic Testing vary widely among international institutions and professional and other organizations (EuroGentest WP3.4)

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This work resulted from the very first of the EC Expert Group “25 Recommendations on the Ethical, Legal and Social Implications of Genetic testing”. It aimed at contributing towards the discussion of a consensus definition and its global applicability.
We collected 153 documents, from 77 European and international institutions, national health associations, regulating and research funding agencies, professional organizations, civic and ethics bodies, pharmaceutical and insurance industry.
Definitions ranged from being strictly synonymous to DNA-based testing, to any source that can provide unambiguous genetic information. They may or not bridge different applications, contexts, mutations and material analysed. Pharmacogenetics, somatic mutations, population screening, or even carrier testing and caryotyping were included only sometimes; while non-medical or research applications, identity testing and sources other than genetic material (other tests, physical exam, family history) are seldom covered. Some trends could be found when comparing different professional organizations or among countries and cultures (geneticists *vs.* pathologists, insurers *vs.* human rights and patient interest-groups, ethical bodies *vs.* commercial labs, Europe *vs.* USA). E.g., the ASHG definition covers only tests in DNA and specific gene-products, while the ESHG includes also caryotyping and all sources of genetic information.
We conclude that a consensus definition that could be globally developped and applicable is probably impossible, and that there should be instead several context-dependent and/or working definitions, according to the purposes aimed. While methodology is irrelevant, information content (genetic information) and/or the genetic material analysed (DNA-based and cytogenetic testing) may serve different motivations for genetic testing. This has practical implications in the scope and competences among different professionals, for oncogenetics, pharmacogenetics and forensic genetics, regulation of health activities, private labs, health economics and policies, insurance, reimbursement of costs and anti-discrimination policies. All this, in turn, will reflect on national and international legislation, either in practice or to be developed.

P1346. Patient’s and doctor’s attitude to genetic testing for common disease predisposition

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Developing tools in genetic testing for common diseases demand for careful examination of what people and health care professionals know and expect from new technologies.
100 doctors and 100randomly selected people from general rural population (Tomsk,Russia) were studied in respect to their attitude to genetic testing for diseases predisposition with the help of special questionnaires.
It was revealed that 78% of respondents would like to determine the risk for diseases with genetic predisposition. The main reasons to undergo such investigation were people’s concern about their health(43%), doctor’s recommendation(21%), and curiosity(20%). If the elevated risk for any of common diseases would be diagnosed than 88% of people would change their habits and only 12% would not change anything. The desire of people to evaluate their own risk of probable diseases did not depend from the level of education, but last seriously affected the people’s awareness about new genetic tools. Positive attitude to a suggestion to undergo genetic testing for disease predisposition can be influenced by current patient’s health status - most of people(75-80%) whom self-reported health was “excellent”, “good” or “normal”would like to be tested whereas in a group who defined their health status as “unsatisfactory” only 50% expressed their wish to be tested genetically.
93%of doctors considered future opportunities with genetic testing to be useful for the purpose of prophylaxis-33%, more accurate definition of patient’s diagnoses(47%), and in adjusting patient’s management(17%). 95% of responded doctors were sure that results of genetic testing would be useful to persuade their patients to change life style with the aim of prophylaxis.
The results of that study have shown the considerable interest of both patients and doctors for arising new genetic technologies in the sphere on common disease predisposition testing, positive attitude of both groups as well as the need of education in that sphere.

P1347. Information on genetic testing in Europe : successes and difficulties

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Genetic tests are now offered internationally, through both public and private sector genetic testing services. The physicians prescribing these test and the biologists receiving the samples need to know which tests are available, where they are offered and whether the identified laboratories meet quality standards. To meet this need, www.orpha.net started ten years ago to set up a database of clinical laboratories in the field of rare diseases. The data collection covered 1 country in 1997, 7 in 2001, 15 in 2003, and 22 in 2006. It will cover 35 countries in the coming two years. This major effort was made possible thanks to the support of the EuroGenTest NoE, with additional resources from the EC DG for public health. Currently, the database includes data from 1,166 laboratories offering 14,451 tests for 1,318 diseases. The data collection is done mainly at the country level through partnerships with key leaders in the field. The laboratories are requested to fill-in an extensive questionnaire (either online or by more traditional means). The response rate is about 80% after three reminders. All the information is updated yearly through an online questionnaire already containing the existing information, in order to minimize the workload for the laboratory. One of the identified problems is the large number of requests that laboratories receive to complete surveys. This information is widely used by our website visitors. Orphanet is accessed daily by over 20,000 users, of which 20% are looking for information on genetic testing.

P1348. Justice in delivering genetics: assessing the role of intellectual property law

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While it is believed that genetics could offer numerous opportunities for global health improvement and play an important role in meeting the UN *Millennium Development Goals* (MDGs), there is also a fear that existing global health inequalities will be amplified by the evolution of genetics. There are a plethora of normative, socio-economic and political obstacles to more equitable distribution of health and genetic related benefits that will be addressed in the course of this presentation to get a better understanding of how public policy should be designed. This evaluation is vital to ensure that legal regimes and adequate public policy assist in ensuring that this promising field develops in a way that improves people's health without leaving the most vulnerable outside of the process.

My paper attempts to determine how and if a justice theory of distribution translates into positive law. I chose to assess the patent system in referring to the standard of access through different lenses and to how underlying politics and economics matter. This leads me to realise that the application of strong patent rights in genetics is more compatible with the reduction of the public commons, the creation of health gaps associated with people's capacity to pay, and with an international basic structure established by a few powerful stakeholders. This evaluation is vital to ensure that public policy can assist in ensuring that genetics develops in a way that improves health without leaving the most vulnerable outside of the process.

P1349. Patient Empowerment In Clinical Genetics Services: A New Model Developed from Qualitative Research

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Measuring the benefits to patients from using clinical genetics services is problematic, with little agreement about appropriate outcome domains. The aim in this qualitative grounded theory study was to develop a theoretical framework describing how patients and their families benefit from using clinical genetics services. Seven focus groups and 19 interviews were conducted with patients, representatives from patient support organisations, and health professionals. Results suggest that the patient benefits from using genetics services could be summarised by a new concept of empowerment. Empowerment includes dimensions of decision-making, knowledge and understanding, future-orientation and instrumentality. Empowerment is a similar concept to that of perceived personal control (PPC), and a measure of PPC has been developed for use in evaluating clinical genetics services. However, empowerment includes some benefits not captured by PPC relating to the empowerment of other at-risk relatives, and future generations. The present study also identified what genetics services can do to empower patients and their families, such as providing information and counselling, and better guidance through the health and social care systems. Aspects of service process identified as contributing to empowerment included providing time to talk, open access and yearly follow-up. These findings will contribute to identifying a core set of outcome measures for clinical genetics services.

P1350. Decision models evaluating genetic testing in haemochromatosis: a multidisciplinary collaboration.

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It's recognised that evaluation of genetic technologies requires collaborations between geneticists, health service researchers, public health and health economics. We present the methods used in a Health Technology Assessment examining the clinical utility of DNA testing in haemochromatosis (HHC). The aim being to evaluate the use of DNA tests for detecting HHC in subgroups of patients suspected of having the disorder on the basis of clinical presentation and disturbed iron parameters, and in family members of those diagnosed with haemochromatosis. A clear distinction is drawn between diagnostic strategies in those suspected of having haemochromatosis and testing strategies in family members.

Objectives and Methods: To determine the clinical validity of DNA tests to diagnose HHC.

To summarise the evidence on the clinical utility of diagnostic strategies using DNA tests to detect cases for treatment or monitoring in terms of clinical- and cost effectiveness.

To compare costs and consequences by decision analytic modeling of diagnostic algorithms for HHC and family testing strategies with and without DNA testing in terms of cost per case detected.

The structure and data inputs of all the decision trees are informed by systematic literature reviews, the results of systematic searches and discussion with experts. Costs will be derived from primary data from previous studies, and national and local NHS unit costs. The outcome will be reported as cost per case detected.

Results: The structure of the decision models will be presented and the results of the reviews used to provide data to populate the models.

P1351. Two years Belgian experience in molecular diagnosis of Haemophilia.

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Deficiency or dysfunction of blood coagulation Factor VIII (F8) or factor IX (F9) causes Haemophilia A and B, the two most common bleeding disorders affecting one in 5000 or in 30000 males respectively. Clinically, the classification as mild, moderate or severe depends on the level of clotting factor which is strongly linked to the nature of genetic defect. The two responsible genes *F8* and *F9* are located on Xq28 and respectively encode a mature protein of 2332 and 415 amino acids. Since 2005, we performed mutational testing on 103 haemophilia A (61 severe, 42 moderate/mild) and 21 haemophilia B (9 severe, 12 moderate/mild) unrelated individuals from the Haemophilia Centre in the Cliniques Universitaires Saint-Luc. In the severe haemophilia A group, using a multistep strategy including mutation targeted PCR, Southern blot and gene dosage by MLPA technology (MRC-Holland), we observed one inversion of intron 1 (1%), 24 inversions of intron

22 (40%), 4 deletions and 1 duplication (8%). *F8* sequencing showed 68 additional mutations distributed throughout the coding exons. Of the 68 mutations, 17 (28%) predicted premature truncation in the severe group while the others are amino acid substitutions in both severe (11/61, 18%) and moderate/mild group (40/42, 95%). *F9* sequencing allowed the detection of all mutations (17 missense, 2 small deletions, 1 small insertion and 1 large deletion). In conclusion: 90 distinct mutations including 25 novel changes unreported so far were found in Belgian haemophilia individuals underlying the interest of genetic testing for carrier detection and prediction of antibodies production.

P1352. Development of molecular biology laboratory training courses for health care professionals at the North West Genetics Knowledge Park in the UK

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Techniques for analysing genetic variants related to disease and treatment are constantly advancing allowing more sophisticated and rapid analysis of patient samples. The consequence of greater access to these techniques is that many clinical disciplines have to embrace the new technology.

The North West Genetics Knowledge Park is developing a programme of training courses for health professionals in response to the expressed need for training in practical molecular genetics laboratory techniques. The first of these training courses - a national, recurrent two-day course for clinical cytogeneticists, accredited by The Royal College of Pathologists - was run in January 2007 with successful training outcomes. Course evaluation has provided useful insights and protocols for future development: it is intended that the developmental process for this pilot course could be replicated with other health professionals.

An opinion canvassing exercise was initiated within the cytogenetics profession. A questionnaire was sent to the leads of UK clinical cytogenetics laboratories via the Association of Clinical Cytogeneticists, to assess the need for a laboratory-based training course. Questions were asked about the optimum length of a course and the key laboratory techniques they would want to see included. The initial course content was based on this consultation. Techniques in the intensive two-day course included the Polymerase Chain Reaction (PCR), Quantitative Florescence (QF) PCR, Multiplex Ligation Dependent Probe Amplification (MLPA) and microarrays.

Further, specific training courses are being developed for professionals working in other clinical disciplines including general pathology laboratory staff, using the successful protocol used for cytogenetics professionals.

P1353. Health Perception in individuals requesting pre-symptomatic testing for late-onset neurological diseases

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Health perceptions are personal beliefs and assessments of general health and show us how people see themselves as being well or not. Health perceptions are a subjective concept and may reflect more individual feelings and beliefs than their current health state. Individual perceptions about their own health are very important for counseling in late-onset diseases (Huntington's disease, Machado-Joseph's disease and familial amyloid neuropathy).

Our aim is to evaluate the health perception of the individuals that come for pre-symptomatic testing and compare them with healthy people, not at risk for genetic disease.

HYPOTHESIS Individuals at-risk that come for pre-symptomatic testing may present a worst health perception and show more adverse indicators regarding their health, once they are pressed by the doubt of being or not being a carrier for an incapacitating disease.

RESULTS

• There are significant differences regarding factor "current health". The group at-risk presents a more positive perception about their current health.

• The group at-risk shows higher health perception indicators than the control group.

• The thinks that their current health is better than the control group and, more than this one, try to continue their life even when feeling ill; the group at-risk see themselves healthier and feeling well-being longer than the control group. The group at-risk is more reserved when feeling ill and is more worried with their health than the control group.

• The control group avoids that illness interferes in their lives, more than the group at-risk. The control group has more expectations about having a healthier life and suffered less delayed diseases.

P1354. The North West Genetics Knowledge Park (Nowgen): the first five years

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Nowgen was set up as one of six Genetics Knowledge Parks (GKPs) in the UK, funded by the Department of Health for five years in 2002.

The vision for Nowgen was to build on the achievements of one of the most comprehensive genetics services in the UK, to create multidisciplinary work programmes. This was served by the award of €6 million to build *The Nowgen Centre* in Manchester, providing a focus for all Nowgen's activities, including networking events to create links with industry, aimed at increasing innovation.

Nowgen has created a multidisciplinary health services research programme to improve delivery of genetic medicine whilst considering priorities for patients. Our innovative approach comprises: evaluation of clinical genetics services; economic evaluation of new services and technologies; an academic research programme on ethical and social dimensions; projects mainstreaming genetics into the NHS; a programme of professional education.

We place public engagement at the core of our activity, since we consider that informed choices about priorities in healthcare will be critical to the integration of genetics into mainstream medicine. Through its Public Programmes, Nowgen is highly regarded for its work in education and dialogue, empowering people to make decisions about health management.

Nowgen has established a coherent programme (of health services research, professional training, commercial events, and public engagement and education), strategic relationships and clear commercial opportunities for the future. As we reflect on achievements to date, we are revising our strategy to impact health and science policy and practice over the next five years.

P1355. Study on causes of hearing loss and patterns of inheritance of genetic deafness in Iran

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Hearing loss is one of the most frequent anomalies in some parts of the world. A deaf person suffers many problems such as social relationships, information acquisition from his/her environment, increased anger, and treatment costs.Through genetic counseling, we may decrease the chance of birth of deaf people.To do so, we need an accurate statistical data of causes of hearing loss and patterns of inheritance in genetic deafness in every population.

The aim of this study was to find out the causes of hearing loss and patterns of inheritance of genetic deafness among our referred patients.

We studied all the 7092 pedigrees of patients which referred to us for genetic counseling during the years 2001-2004. From 7092 pedigrees, 335 had hearing loss. Among these 335 pedigrees, 616 cases of hearing loss were found.Therefore, these 616 cases were studied according to the causes of hearing loss, patterns of inheritance, consanguineous and non-consanguineous marriages, and sex by using SPSS software. Our study showed that genetic causes was the main etiology of hearing loss (67.8%).The most frequent pattern of inheritance was autosomal recessive (86.7%),after that autosomal dominant (11.2%) and the last pattern was X-linked(2.1%).

In consanguineous marriages, the most frequent cause of hearing loss was genetic causes while in non-consanguineous marriages the most frequent cause was acquired and unknown causes. The pattern of

autosomal dominant was more frequent in non-consanguineous marriages than consanguineous marriages. Our study also showed that there was no relation between sex and cause of hearing loss and patterns of inheritance.

P1356. Intention towards ethnicity-based carrier screening referral for hereditary hemoglobinopathies among primary health care providers in the Netherlands

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In the Netherlands, carriership risks for hereditary haemoglobinopathies are particularly high in (descendants of) of immigrants from Surinam, the Antilles, Turkey, Morocco and western Africa, but general practitioners and midwives have no formal guidelines for informing their patients about carrier screening for these diseases. Patients too may be unaware of options for testing. The aim of this pilot was to explore attitudes and intentions towards haemoglobinopathy carrier screening on the basis of ethnicity, in primary health care providers.
Intention to refer patients for carrier screening was measured using structured questionnaires based on the theory of planned behaviour. Surveys were collected from 191 primary health care providers. Attitude towards patient-education programmes about the subject was positive, but they showed no clear intention to refer patients for carrier screening solely on the basis of ethnicity (on 7-point scales, general practitioners and midwives had respective median scores of 5.0 and 5.7 for attitude and 4.0 and 4.5 for intention). Regression analysis showed that social norm explained 43% of intention to refer for carrier screening. Colleagues' (supposed) opinions are thus a strong influence. Both professional groups do take ethnicity into account when diagnosing anaemic patients, as prescribed by existing guidelines.
Primary health care providers were also invited to attend an informational session about screening and testing for hereditary hemoglobinopathies. Attendees of such sessions (N=33) did not score differently than persons surveyed beforehand (N=166), on any of the psychological parameters. Thus, new measures are needed to change behaviour in carrier screening referral.

P1357. Molecular Characterization of the Diseases B-Thalassemia & Hemophilia-A amongst the people of Jammu & Kashmir State

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The present study on the Molecular Characterization of the Diseases B-Thalassemia & Hemophilia-A affecting the people of J&K State, India,has been carried out with an aim to characterize the types of mutations affecting populations of J&K State and also to identify the carriers amongst these populations. During this study the primers we used had already been used by the people in the adjoining states. Present study helped in detecting the common mutations and this has successfully been been used for the purpose of Genetic Counselling in the Human Genetic Research cum Counselling Centre, in Jammu. Details of the Primers used in the present study and the results obtained shall be discussed.

P1358. Presymptomatic testing for Huntingtons disease in Norway

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by an expanded number of CAG repeats in the HD-gene. The number of HD patients in Norway is estimated to 225 and there are approximately 1125 risk individuals. Empirically, most of these are aware of their increased risk. We present nationwide data of presymptomatic tests in Norway since 1990.
HD was mapped to chromosome 4p in 1983 and the HD-gene was isolated in 1993. Direct PCR-analysis of the gene has been available in Norway since then. Presymptomatic testing started at Ullevål University Hospital in Oslo in 1990 and at Haukeland University Hospital in Bergen in 2003. A standardized test procedure following the IHA/WFA guidelines has been used. In addition, there has been a possibility of an individual follow-up consultation in the department of medical genetics or follow-up in group sessions.
In the years 1990-93, 54 risk individuals underwent linkage analysis and in the years 1994-99, 108 risk individuals were tested directly. In the period 2000-06 180 risk individuals have been tested. Based on this number, we estimate that 16 % of Norwegian risk individuals chose presymptomatic testing in recent years. Of these risk individuals, 80 (44 %) were HD gene carriers. Up to 2006, a total number of 342 persons have completed presymptomatic testing for HD.
Conclusion: Sixteen percent of Norwegian HD risk individuals choose presymptomatic testing. This is in line with other Western European countries.

P1359. Time trends in incidence of cystic fibrosis in two European regions: Data from two newborn screening programmes.

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This study aimed to determine the incidence of cystic fibrosis (CF) and its time trends over a 16-year period (1990-2005) in two European regions which have a long practice of newborn screening for CF: Brittany (western France) and Veneto/Trentino Alto-Adige (northeastern Italy). The birth incidence of CF was 1/3153 in Brittany and 1/3540 in Veneto/Trentino, what did not differ significantly (p=0.2450). Time trends analysis using Poisson regression revealed that the birth incidence decreased significantly in the Italian area over the 16-year period (average annual percent change (AAPC): -4.7% - 95% CI: [-7.3; -2.0] - p=0.0008), whereas this trend was not observed in Brittany (AAPC: -0.6% - 95% CI: [-3.7; 2.5] - p=0.6909). The uptake of prenatal diagnosis (PD) appeared more common in Brittany. By incorporating in the calculations all the terminations of CF-affected fetuses made over the same period (79 for Brittany and 31 for Veneto/Trentino), the adjusted incidence of CF would be 1/2191 in Brittany and 1/3116 in Veneto/Trentino. This would correspond to a change in the incidence rates of 30.5% for the first region (highly significant - p=0.0002) and of 12.0% for the second one (not significant - p=0.1600). As the motive of each PD was registered (previous affected child, family testing, prenatal screening, echogenic bowel), the impact on the incidence of the various public health policies could easily be measured. This study illustrates how the incidence of a disease evolves according the region characteristics, the public health strategies implemented and the cultural attitudes towards PD.

P1360. A comprehensive and practical model to integrate Genomic Services in Iranian Primary Health Care (PHC) system, a pilot survey

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Background: Over the last 25 years, promotion level of public education along with a high range of health services in both rural and urban areas of Iran has lead to a remarkable improvement of health indices. In the meantime, a national screening program for thalassemia has also been set up in Primary Health Care (PHC), resulted in a 70% reduction in the expected annual birth rate of affected infants (Samavat A. et. al. 2004). In this survey, we are seeking a practical model to implement a wide range of genomic services in the PHC of Iran.
Method: There are different genomic testing strategies worldwide. Some are offered publicly such as in the UK and some are offered

privately such as in the USA, for instance.
Since 2004, a screening program has been set up in a well-defined population of Tehran, Iran's capital. Based on our experiments earned from referral/follow-up system within PHC, we designed 2 distinctive and comprehensive programs for the urban and rural areas.
We therefore proposed following 3 phases:
1-Mobilizing and organizing the community to active participation;
2-Implementation,
3-Assessment the program.
Conclusion: As a general condition of Iran, population of 68 millions, population age distribution mean which is 23 years, high rate of consanguinity, strength of national PHC, general public demand, and the human rights may direct us to offer a national extraordinary health services including genomic services.
This pilot survey may lead us to achieve valuable results; however, further studies are required to reach a comprehensive model.

P1361. Implementation of the Swiss Federal Law of Human Genetic Analysis

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The new Swiss Federal Law of Human Genetic Analysis (LAGH), applicable from April 2007, fixes the legal framework for prescribing and performing non-research genetic tests in humans. In the era of constantly-increasing genetic knowledge and of rapidly progressing laboratory techniques, the LAGH is designed to protect human dignity, prevent abuses and ensure a high standard of testing.
We will focus principally on the medical application of the Law, which also encompasses testing in legal, workplace and insurance contexts including DNA profiling for investigating parentage and for identification purposes in civil and administrative proceedings.
Non-directive genetic counselling and respect of the patient's right to autodetermination is required and the conditions are described in detail in the text of the Law.
Informed consent is required before performing any genetic testing, and must be obtained in writing before tests for presymptomatic, prenatal and "family planning" (reproductive choice) purposes.
Prescription of genetic tests is restricted to medical doctors and, in prenatal or presymptomatic settings, to those with specific competence in genetic counselling.
Licensing to perform molecular or cytogenetic testing is obligatory. The accompanying Ordinance on Human Genetic Analysis defines the criteria for the minimum acceptable standard of quality management, including the competence of the laboratory director and personnel, EQA and quality assurance requirements comparable to the ISO 15189 standard.
The obligations defined by the LAGH are closely based on pre-existing national and international standards and practices, yet Switzerland is one of the first countries in the World to transfer these into law.

P1362. Medical genetic service of Leningrad province: 2003-2005 years

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Medical genetics service is stationed at District Children Hospital. It works in close co-operation with the Department of Medical Genetics of the MAPS, Municipal Centre of Medical Genetics etc. Genetic service realized neonatal screening for PKU and CH, cytogenetic investigations for making diagnosis of chromosomal pathology (2840 samples), second trimester prenatal screening for congenital defects, double-test, (18000 pregnant women); confirmation of hereditary diagnosis, medical care, long term inpatient and outpatient care, dietary management, genetic counseling. 36589 of newborns(98,65%) were examined through neonatal screening. Five cases of PKU and five cases of CH were diagnosed. Cohort of children with PKU (23 cases) and with CH (46 cases) has special dietary and medicinal treatment. Ultrasound investigation was performed for pregnant women (25821of examinations). 225 of the fetuses were found to have congenital malformations. 160 of pregnancies were interrupted owing to congenital malformations or chromosomal diseases of the fetuses.

P1363. Sequencing and MLPA under diagnostic rules

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The field of molecular genetics is innovative and dynamic, resulting in continual upgrading or improvement of technology. Molecular genetic diagnostics therefore needs to be responsive to this to bring new or improved technology into clinical management of patients and families, but in the context of strict regulation and control. In Australia, the National Pathology Accreditation Advisory Council (NPAAC), the body responsible for setting guidelines and standards for medical laboratory accreditation, have released standards for validation of in-house developed assays for use in diagnostic testing. The detailed requirements include full documentation of the design phase, the production phase, the technical validation phase and the monitoring phase.
The design phase includes literature review and /or peer consultation process to identify alternatives and the experience of other as well as the clinical usefulness of the proposed assay. The production phase includes conforming to safety principals for patients and laboratory staff as well as assessment of the analytical performance and the clinical performance of the assay. The technical validation phase includes assessment of the assay sensitivity and specificity, measurement of uncertainty if applicable and medically relevant criteria. The monitoring phase includes assessment of the assay performance in internal QC, external QAP or sample exchanges with other laboratories. The presentation will encapsulate the above phases of validation for molecular genetic testing in hereditary nonpolyposis colorectal cancer (HNPCC), using a capillary sequencing assay to detect unknown sequence changes and the MLPA assay (multiplex ligation-dependent probe amplification) to detect intragenic or whole gene deletion mutations.

P1364. Myotonic Dystrophy in Yakuts (Russian Federation)

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In the Republic of Sakha (Yakutia) among Yakuts (430 thousand people) we have documented a high prevalence of DM1, that is is 21,3 per 100 thousand.
The purpose of this research was studying distribution among different ethinc groups, the clinical presentations of myotonic dystrophy and the distribution of the CTG-repeat in the indiginous populations of Yakutia. Two regions of accumulation of DM1 are established in the territories of Viluiskii and the Central group of uluses. In 35 Yakut families the classical form of myotonic dystrophy prevails (81,5 %), but we also encountered congenital and early childhood forms of the disease. The median age of patients with DM1 was 31,4±1,5 years, and the average age of onset was 16,8±1,1 years. The most common clinical presentations were, progressive muscular weakness, cardiovascular and endocrinological symptoms. Family studies revealed clinical variability and anticipation. We analysed the distribution of CTG-repeat alleles in the normal Yakut population(Federova et al., 2003) in the two main ethnic groups groups (Viluiskii, Central, Northern Yakuts - 192 individuals). We found 18 different allelic variants from 5 up to 29 CTG-repeats. The most common alleles had 12 and 13 repeats (combined prevalence 79 %). We found a low frequency (3-6 %) of unstable alleles with > 19 CTG repeats.
We suggest that one of the reasons for the accumulation of DM1 in the Yakut population is a founder effect of these unstable alleles. This needs to be confirmed in further studies. A method of preventive maintenance of DM1 in Yakuts is to provide effective medical-genetic consultation with prenatal DNA-diagnostics.

P1365. Implementing Personal Health Records for Neurofibromatosis Type 1 - Patients' and Health Care Professionals' Views and Experiences

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Personal health records (PHRs) are client held records. As part of our Service Development Project, we have developed a PHR for children

and adults with Neurofibromatosis Type 1 (Nf1).
Nf1 is a relatively common autosomal dominant condition characterised by wide variability in its clinical expression. Birth incidence is about 1 in 3,000 which means there are approximately 164,000 affected individuals in the EU. The course of the disease is unpredictable and affected individuals carry a lifelong risk of developing serious complications. Fortunately, most people affected with Nf1 will have skin signs only, but there is still a need for vigilance particularly during the first five years, and also during adolescence, when the risks of developing specific types of complications are greater. Because of this, regular clinical reviews and targeted screenings are important in the management of these patients, and it is hoped that the PHR will facilitate this, particularly where there is a high disease burden and patients are under the care of several specialists.
Nearly 200 PHRs have been issued to Nf1 patients attending clinics in Manchester, Liverpool and Newcastle. An evaluation exercise is underway which will look at the effectiveness of PHRs as clinical tools, and explore patients' and health care professionals' experiences using the records. This submission will present the results from this evaluation and will include feedback from a patient satisfaction survey, audit data regarding record use, and feedback from a questionnaire given to health care professionals working in a variety of roles and clinical specialities.

P1366. Exploring existing and deliberated community perspectives of newborn screening in Australia to inform policy standards in newborn screening and the storage and use of dried blood spots

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Since the 1960s newborn screening (NBS) for several rare and serious disorders has been in place across Australia and testing now enables the early detection of over 30 conditions. Policies across Australian States have diverged in some aspects of NBS, especially in the retention and further use of the dried blood spots collected as part of the screening. To date there has been limited empirical evidence of wider community attitudes to inform the deliberations of health professionals and policy makers towards national consistency.
Methodologies: Nine moderated small group discussions were held with 40 participants including young adults, recent parents and older parents. The groups were reconvened one week later for further discussion enabling considered deliberation of the issues raised and prompted by the stimulus materials provided.
Results: The findings suggest that there is limited community awareness of the public health importance of NBS and that resulting biological samples are stored for varying periods in different Australian States. Members of the wider community presented with opportunities to consider current procedures and policies surrounding consent, storage and further use of the dried blood spots appear reassured and to have high levels of trust. However there are clearly some groups who have concerns with the storage of dried blood spot specimens and perceive that these may be abused.
Conclusions: The findings are important for improving current communications about NBS, developing policies, maintaining public confidence and the development of State and National initiatives in genetic health.

P1367. The overlapping area between Osteogenesis Imperfecta and child physical abuse: a bibliometric analysis.

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Osteogenesis Imperfecta (OI) is a hereditary connective tissue disorder. Its manifestations are fragile bones, multiple bone fractures, bone deformities with a history of minimal or no trauma. This clinical picture may be observed in Child Physical Abuse (CPA). CPA is a relevant differential diagnosis when a child presents unexplained fractures. Aim is to quantify the weight and the trends of the worldwide literature on OI and CPA, in order to measure if and how the 2 topics overlap and are studied together. A retrospective bibliometric analysis was carried out. Through PubMed, literature was explored and 2 quoted phrases Osteogenesis Imperfecta (OI), Child Physical Abuse (CPA) were searched in all available manuscripts. Starting from retrieved papers, a descriptive analysis and a text mining search were performed (SAS software). Search retrieved 7,140 manuscripts, distributed per interval period (1950-2006) per each DB as follows: OI 48%, CPA 52%. English is the leading language of publication: OI 70%, CPA 93%. For both DBs, top Countries of publication are USA and UK. An overlapping area exists between OI & CPA: 70 manuscripts representing the 1% of the whole (1969-2006), written in English (84%) and published in the US and UK (45% and 29%). Further results on text mining search will follow. Results obtained show that English-written papers predominate and understanding English results fundamental to update oneself. The overlapping area is nearly absent despite the strong correlation of the two issues, it means that bibliographic search on CPA/differential diagnosis could be easily biased retrieving incomplete results, with the continuous risk of falling in a bibliographic cul-de-sac.

P1368. Psychological effects of parenting stress on parents of Down Syndrome children

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Down syndrome (DS) occuring in one out of 800 live births is the most frequent genetic cause of mental retardation and associated medical problems. Having a child with DS is an unexpected and stressful experience for most parents but they are the ones who have a key role to play in determining the extent of their child's handicap. In addition to this, cultural and social aspects play a great role in determining the behaviors of the mother and the father; usually differ in a great variety. The aim of the study is to evaluate the social, economical and individual problems of the parents who have a child with DS by using a questionnaire designed by our group. The questionnaire consisted of 18 questions assessing the families' characteristics, their relations with each other and the other people and their attitudes towards the DS child. We found out that DS children mostly spent their time by their mothers, and mothers reported higher levels of stress than fathers ($p<0.05$). Most of the parents tried to solve their problems by helping each other, however the rate of mothers who reported higher possibilities to divorce was significantly higher than the fathers ($p<0.05$). We focused on the effect of having a child with Down syndrome on the parents, factors causing problems on the family and the characteristics of a family with Down syndrome children. In conclusion genetic counseling and follow-up visits of those families need more specified and professional approach.

P1369. Are DNA patents hampering genetic testing? - An empirical study

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In the debate about DNA patenting and its impact on public health services and access thereto, empirical data are essential to demonstrate whether patents are hindering access to diagnostic testing or not. We provide a patent landscape analysis i.e. a quantitative and qualitative patent analysis related to hereditary diseases. We focus on patents related to human genes or their use in diagnostic testing in Europe. They include the most frequently tested hereditary diseases like hereditary breast and ovarian cancer, hereditary colon cancer, cystic fibrosis and Fragile X syndrome.
Quantitatively, the amount of patent applications and patents granted by the EPO and the USPTO were mapped. This is primarily based on internet searches of patent databases using specifically developed search algorithms. Qualitatively, the claims of relevant DNA patents were exhaustively analysed and classified according to their subject matter. In general, the data extracted from the DNA patents provide insight into several areas including the scope of patent protection sought or obtained, the identification of closely related patents, trends in DNA patenting for diagnostics, identity and activity of competitors active in the field, etc.
The outcome of this empirical study is an overview of DNA patents covering diagnostic practice. On this basis, it will become possible to

test to what extent the claim that a 'patent thicket' is emerging in the genetics field is valid and that patents are indeed hindering access. This study could contribute to policy formation in the field of DNA patenting and development of new licensing strategies.

P1370. Patient experiences and preferences of receiving genetic information.

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In 2006 we conducted a qualitative study of 27 people (with or at risk of genetic conditions or parents of children affected by a genetic condition) to explore their experiences and preferences of receiving genetic information. Telephone interviews focused on provision of information by healthcare professionals outside specialist genetics services and explored how and from where patients initially received information about the genetic basis of the condition and their views and preferences on such information provision.

The results indicate a perceived need for greater awareness of genetic aspects of conditions amongst healthcare professionals. Patients acknowledged that healthcare professionals cannot have detailed knowledge about all genetic conditions, but felt that identifying and referring patients appropriately was important. Whilst they recognised the value of information from specialist genetic services, some would prefer their specialty consultant, with whom they had established a rapport, to be more involved in the provision of genetic information. Family practitioners were viewed as the best group to provide ongoing support and co-ordination of information.

Patients stressed the need for healthcare professionals providing genetic information to do so in a non-judgemental manner; be mindful of their use of terminology; tailor the information provided to the preferences of individuals; and inform people where they can access further information. Awareness of the emotional impact of genetic information for individuals and the wider family was also considered important. These results have implications for the education of healthcare professionals if patient expectations are to be met.

P1371. Retrospective evaluation of the referral reasons of patients at a tertiary paediatric genetic center in Izmir, Turkey

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Genetic counseling is a service to help individuals and the other family members translate scientific knowledge into practical information which is possible before conception, during pregnancy, after birth, during childhood, or even in adulthood. Particularly families seek genetic counseling when their children have features suggestive of a genetic disorder. Our study aimed to review and evaluate the distribution of the referral reasons during the past eight years. A total of 2342 patients were referred to our outpatient clinic. The referral reasons included genetic disorders comprising mental retardation-multiple congenital anomaly and isolated anomalies in 1472 cases (62.85%), syndromes which may be associated with cytogenetic abnormalities in 634 (27.07%), suspected single gene disorders in 134 (5.72%), suspected microdeletion syndromes in 48 (2.05%) and the others in 54 (2.31%). Among the 1472 cases with genetic disorders comprising mental retardation-multiple congenital anomaly and isolated anomalies, dysmorphological facial features were detected in 821, isolated anomalies in 374, mental retardation in 351 and growth retardation in 244. In this study it was determined that 494 cases (21.09%) were referred because of clinical suspicion of Down syndrome, 124 (5.29%) Turner syndrome, 50 (2.13%) metabolic storage diseases, 38 (1.62%) neuromuscular disorders, 33 (1.41%) ambiguous genitalia. In conclusion, genetic disorders such as Down syndrome and Turner syndrome still account a considerable number of the referrals in our outpatient service. Although complex diseases are very common in the population, cases with those diseases were not referred as much as expected to the genetic counseling sessions.

P1372. Using a new package for drawing pedigrees for teaching medical genetics

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Recently we published a paper about our newly created package for drawing pedigrees (B.Veytsman, L.Akhmadeeva, The PracTeX Journal, Nov, 2006). This software suite could be useful for teaching medical genetics at the Universities. Every medical student has to get a skill of making pedigrees. Pedigrees are very helpful in confirming diagnosis and in estimating prognosis for people in the family. Using our new package could help students to draw beautiful genealogic trees. It makes them learn more about different symbols of a pedigree and about methodology, it saves much time which is precious during classes, it gives a chance to save all the versions and to add new family members easily.

P1373. Brazilian's case-study of Poverty and Deficiency

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Recent demographic studies are related to the growth of the relationship between poverty and the risk for deficiency (Fujiura & Yamaki, 2000; Kaye et al, 1996) which can be described as a "new morbidity" (Baumeister, Kupstas, 1996). The present study discuss the "coprevalence" of poverty in a *Social Rehabilitation Program based in Community* (PRC), from Francisco de Paula Municipal Foundation, in Rio de Janeiro/ Brazil (FUNLAR-RIO), specially focusing families with children and teenager from 0 to 24 years old. A qualitative research related to maltreatment and human rights of people with disabilities was done through 24 case-studies in profundity, using a triangulation method of case-discussions with team work, reflexive groups with families, interview with local leaderships and participant-observation with the financial support of Faperj and CNPq (2005-2006). Working with people in extreme social exclusion, PRC make the registration of the disabled people they encounter in different favelas, make an evaluation of their needs and try to create a network of different sources according to family needs and their disabled son or daughter (health promotion, education inclusion, social benefits for poor families, etc). Domestic Violence is another problem to be faced with protective solutions involving Brazilian social polices. In this study we can evaluate the impact of actions to promote human right and violence prevention with families of disabled children. We could describe changes in health conditions, work and school inclusion, leisure and recreational activities, physical and social environment and the importance of Brazilian social politics to protect their poor population.

P1374. Provision and quality assurance of preimplantation genetic diagnosis in Europe

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Although PGD is now a well-established treatment, no current, comprehensive independent data exist about the practice and provision of PGD in Europe. In response to this, a survey was developed in collaboration with EuroGentest and ESHRE, to assess the provision of PGD services in Europe, access by patients and the current state of quality assurance. Additionally, expert opinions were obtained through interviews conducted with professionals in specific countries.

The survey identified 53 centres offering PGD in 17 European countries, performing a total of 2-3000 cycles in 2005 (excluding PGS). The most widely-available testing was for familial chromosomal anomalies, followed by monogenic diseases, and HLA typing. Quality assurance of PGD testing was evaluated by several criteria. While half of the centres have designated quality managers, just 33% have or are prepar-

ing for accreditation or certification. Two-thirds of centres responded that they did not participate in EQA, a problem exacerbated by a lack of PGD-specific schemes. Approximately 19% of the centres do not keep data on accuracy; 9% do not even follow up until birth. Genetic counselling is provided and informed consent obtained in 94% of centres but follow-up after testing is very limited.
PGD is an expanding activity with an increasing international flow. We identified a potential need for improvement in the overall quality system of providers. In this respect, development of PGD-specific EQA is of particular importance. There is also a need to improve monitoring of PGD, especially long-term follow-up, through increased public funding and international co-operation.

P1375. The role of genetic counseling on women's informed decision making for invasive prenatal testing

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Genetic counseling is a unique psycho-educational process centered on providing very special genetic information, with the goal to facilitate clients' knowledge and ability for optimal decision making for invasive prenatal testing: understanding relevant information, informed choosing a course of action consistent with one's attitudes and resulting in minimal decisional conflict, guilt or regret.
This study was aimed to assess whether a special genetic counseling and decision aid, when compared to a leaflet or referral MD information, improve women's informed decision making and diminish uncertainty in the context of prenatal invasive testing. Pregnant women at 15-18 weeks gestation, referred for prenatal invasive testing, were asked to fill our questionnaire, before testing. They where divided into two groups: first group has been provided with special genetic counseling, and second group was provided with pamphlet or referral MD information. Knowledge, understanding relevant information, informed choice, and anxiety and decision conflicts were measured. Satisfactory level of knowledge was found significantly more frequent in decision aid group compared with the leaflet group. Decision aid group was more than twice better eligible for informed choice. Depression, worry and anxiety attitudes to the pregnancy/fetus were found more frequently in leaflet group. Decisional conflict was found very low in both groups.
Appliance of specify genetic counseling resource can produce an improvement in women's knowledge about the complexities of prenatal genetic testing. This resource can potentially play an important role in improving women's informed decision making.

P1376. An integrated programme of professional development in genetic medicine at the North West Genetics Knowledge Park

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It is widely recognised that there is a need for training in genetic medicine for people working in many medical disciplines. It is also vital that the general public is both informed and involved in decision-making about how genetic technologies are used within health services.
At Nowgen, the UK's North West Genetics Knowledge Park, we have exploited our position at the interface between clinical genetics, patients and the public, and our links with national professional development initiatives, to devise a comprehensive training programme that addresses these important issues. This comprises three main elements:

1. A comprehensive programme of training for health professionals, in order to ease the integration of genetics into mainstream health services, which will ensure that over 5000 training hours are delivered this year. Attendees are drawn from specialties such as cardiology, midwifery, paediatrics and oncology.
2. We have developed and delivered a programme of communication workshops, to help geneticists to communicate with non-specialist audiences.
3. As genetic medicine makes a greater impact on people's lives there are more demands on other professionals, such as school teachers, lawyers, journalists and ethicists, to understand the latest developments in genetic medicine. We have supported a diverse group of non-healthcare professionals through training events, to increase awareness of genetic medicine in society.

We shall continue this integrated programme, which will address the professional, public and policy implications of genetic medicine and ensure greater links between all stakeholders, essential for future delivery of modern health services.

P1377. An invasive prenatal diagnosis and termination for abnormal cytogenetic findings. Psychological experience for pregnant women following

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Background. The invasive prenatal diagnosis includes the application of early amniocentesis (16-18 gestational week) or cordocentesis (22-25 week of gestation) in a pregnancy with certain indications. Abnormal cytogenetic finding results in interrupting of a pregnancy. That causes one of the greatest psychological traumas that have its characteristics.
Purpose. Study analyzed the kind of psychological problems, somatic problems, the influence on marriage and the duration of these problems in women whose pregnancy was interrupted depending on the method of prenatal diagnosis that was used.
Methods. Based on the research carried out at the Institute of Mental Health, Department of Medical Genetics from 1999-2003, 146 pregnancies were interrupted because of the abnormal cytogenetic findings. The interruptions were performed in 20-27 gestational week. After pregnancy interruption women were filling form that tested the kind of problem that appeared (psychological, somatic or combined), divorce and the duration of psychological problem. Statistical method that was used was Chi square test.
Results. 83% of all pregnant women describe psychological problem (most frequently in 55% - emotional problem that persisted during the period of two years (38%)). Divorce was the less frequent (1%).
Conclusion. Statistically significant difference was not proven that there is correlation between the kind of the problem and the method used for prenatal diagnosis. Nevertheless, certain specificities exist and they will be discussed in the presentation.

P1378. Synthetic quality controls for Cystic Fibrosis testing, are they useful for a diagnostic laboratory?

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One of the necessities for laboratories to assure high quality in molecular genetic testing, is the use of appropriate positive quality control (QC) materials.
Laboratories mainly use patient samples as QC, which of course include a maximum of two mutations per sample. Bearing in mind that some assays can test for more than 100 mutations, multiplex QC materials could save valuable time and reagents.
The European Cystic Fibrosis (CF) Network enclosed a synthetic multiplex QC developed by MMQCI as an additional sample in two consecutive CF External Quality Assessment schemes. For 2005, the sample included R553X, I507del, R117H, 394delTT, 2183AA>G, R347H and 5T all in homozygote state. In 2006, again a synthetic sample was distributed, with partly the same variants: 3876delA, R553X, 394delTT, R117H, I507del, W1282X, 3905insT, F508C, 5T and 7T all in heterozygote state.
133 laboratories returned their results on the analysis of the synthetic blood sample in 2005. The multiplex QC material performed well in the majority of assays and laboratories. The most common error was the inability to find all mutations (20%). Seven laboratories encountered detection problems and five laboratories reported extraction failure. Mutations not present in the QC material were reported by 14 out of 83 laboratories.

For the scheme of 2006, results were submitted early 2007. A comparison will be made between the two trials. Furthermore, the evaluation will determine if there is any improvement for laboratories who participated in the trial twice.

P1379. Quality Management and accreditation of genetic testing services

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The EuroGentest network aims to improve and harmonize the quality of the genetic services in Europe. The network encompasses Biochemical, Clinical, Cyto- and Molecular Genetics. The EuroGentest Quality Management group is aiming to improve the organization and harmonization of External Quality Assessment (EQA) schemes, facilitate the development of guidelines and disseminate Quality assurance (QAu) information through a databaseas well as assisting laboratories in attaining and maintaining accreditation. In addition, this group is reviewing suitable quality control materials (QCMs) and providing documentation/SOPs on new technologies.

Since January 2005, the group has disseminated information on accreditation through five international workshops. A database on the current status of QAu in European genetic testing services will soon be publicly available. On the website, laboratories can find the EQA scheme most appropriate to their needs through discipline specific registers of schemes in Europe. All three laboratory disciplines have expanded their repertoire of EQA including a pilot European cytogenetics scheme, CEQA. Minimum quality guidelines have been published for cytogenetics and some biochemical analytes. Draft guidelines for microarrays will be published later this year. In collaboration with EMQN, best practice meetings will be organised in 2007 for Familial Breast Cancer, Spinocerebellar Ataxias and Maturity Onset Diabetes of the Young to generate consensus guidelines. Finally QCMs for Prader-Willi/Angelman syndromes are being developed and validation of MLPA, diagnostic CF-testing kits and DNA extraction methods are in progress through a core group of accredited laboratories with reports due this year.

P1380. Improving the quality system in our laboratories by sharing experiences

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Genetic services have considerably increased their activity in the past few years and quality assurance (QAu) is now essential in order to minimize potential errors and deficiencies. The introduction of quality standards in laboratories, and the growing interest in and requirement for accreditation, has made better understanding of quality management (QM) and QAu a priority. Consequently the EU project EuroGentest aims to improve and harmonize the overall quality of genetic services.

In a focus on training and education, key parameters in improving quality, a series of workshops has been held in 2005 and 2006 to aid laboratories in their processes of developing QM systems and working towards accreditation. The subjects varied from living with quality systems and comparing the different norms for accreditation, to specific topics like internal audit and IT support for QM.

An interactive approach combining brief expert presentations, case studies of concrete situations related to quality and group debates to exchange experiences and opinions form the basis of the workshops. A specialist in the "human side of change processes" participated actively in these workshops, helping to overcome the aspect of motivation and change, which is inevitable when implementing a quality system.

Approximately 60 different genetic laboratories from 20 different countries have participated in the first five workshops and 16 laboratories participated more than once. Tangible outcomes have included criteria for selecting an appropriate IT programme to support QM systems, a list of companies providing such software, and a concrete interpretation of all phases of an internal audit.

P1381. Collaborative Experience of the Romanian Prader Willi Association with Medical Specialists

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Introduction In the absence of a national governmental strategy for Rare Diseases (RD), an EU priority for Romania, the collaboration of local and national NGOs and medical specialists is essential. The aim of our paper is to focus on the encouragement of a collaborative effort between Higher Education Medical Universities, medical specialists, and NGOs serving beneficiaries in the RD sector through a multidisciplinary approach.

Results Families of children with RD have interacted with medical specialists and benefited by becoming more assertive and by achieving more developmental milestones. APWR has established contacts with a Genetic Lab and renewed the contact with Mauro Baschirotto, Institute for Rare Diseases and established new relationships with genetics specialist which helped us to diagnosed the patients in important genetics Institute and laboratories: Institute of Medical Genetics from Zurich, Institute of Human Genetics- Wurzburg, Institute of Clinical Genetics, Olgahospital-Stuttgart, Genetic Lab. Organized a training course for parents, genetic evaluating for children, a training course in genetics for family doctors, under auspicious of the Medical University, Timisoara.

Conclusions The health of people with disability and the social integration can be improved if they have every opportunity to enjoy family life, education, friendship, access to public facilities and freedom of movement. Action should be aimed at collaboration between medical specialists, families, and NGOs. Developing awareness about the needs of children with RD and engaging public in a shared strategy for the development of genetic services, will ensure a collaborative international approach in sharing of expertise and experience.

P1382. Recurrent spontaneous abortion and consanguinity

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An expected 15% of clinically established pregnancies abort spontaneously. When all known causes of fetal losses are ruled out, there remains a population of women (0.5-1.0%) who have recurrent fetal losses of unknown etiology. A important part of these recurrent spontaneous abortions may be due to primarily genetic causes. The significant characteristic of couples experiencing such fetal losses is the sharing of alleles between husband and wife. The suggestion developed here states that the sharing of recessive lethal genes could be caused by consanguinity. These recessive lethal alleles could not act alone to cause problem, but they could act with lethal alleles on other sister chromosome. This means both husband and wife were normal and usually their family did not have any genetic disorder, but these fetuses could not live with these alleles.

Present study reports the result of genetic counseling for couples with recurrent spontaneous abortion in Yazd Infertility Research and Clinical Centre. The results showed the coefficient of inbreeding of 4% of these couples was 1/8. In 25% it was 1/16 and in 14% was 1/32.

Karyotype was normal in 99% and remain 1% had inversion especially in chromosome 9.

Finding the lethal genes in most of these couples was not possible or very expensive, because no abnormalities were found in their pedigree. Abortion causes important psychosomatic problem for mothers, therefore using assisted reproductive technique such as egg or embryo donation were recommended in most of these couples especially after 5 or 6 abortion.

P1383. EuroGentest: Reference Systems for Genetic Testing

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The lack of well-characterised reference materials (RMs) for most genetic tests causes difficulties in validating and developing new assays and results in tests being run without proper quality controls. EuroGentest is addressing this issue by defining the present and future needs for RMs, setting priorities for and supporting the development of new RMs and building an enduring network involving all stakeholders in RM development.

Two International Symposia on Reference Materials for Genetic Testing have been organised, involving health care professionals, scientists, regulatory and industry representatives. The symposia led to international collaborations, enabled the development of a prioritization list of RMs needed, and gave an update and better awareness of the international situation.

In direct support for the development of RMs for genetic testing, field trials for new candidate RMs have been organised, research on the optimal stability conditions of DNA-based candidate RMs was performed, and NIBSC was awarded a sub-contract to develop RMs for Prader-Willi and Angelman syndromes. Furthermore, a guidance document on the use of RMs in genetic testing and the availability of new RMs for genetic testing on the market has been drafted.

Meetings have also been organised in order to clarify the role and interpretation of the IVD Directive 98/79/EC in the fields of genetic testing and RMs by bringing together regulatory authorities, industry and stakeholders in genetic testing.

These activities have raised awareness of the importance of properly-characterised RMs and reference methods in genetic testing, and will lead to availability of additional RMs in this field.

P1384. Prenatal diagnosis - evaluation of attitude, experience and choice of counseled families

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The aim of this study was focused on the influence of prenatal diagnosis on the emotional state of patients and the significance of medical and informational factors on the reproductive decision of the counseled families.

A total of 120 pregnant women: 30 after ultra-sonographic screening, 30 with family history for hereditary diseases, 30 in advanced maternal age and 30 healthy controls underwent an information interview, an anxiety questionnaire and a grief scale.

Most of the women have received information by obstetrician or clinical geneticist. Women with previous reproductive failure and those who had higher educational level proved to be well informed. High proportion of the participants considers reliable the diagnostic and prognostic potential of the prenatal diagnosis. The type of the diagnostic procedure influences insignificantly the reproductive decision.

On the base of this study we concluded: The reproductive decision depends on the quality of genetic counseling. The level of anxiety is determined by the kind of procedure and reproductive history. Age, previous reproductive failure and its type are decisive factors for coping with the loss.

P1385. Rett syndrome molecular diagnosis and implications in genetic counseling: report of a case

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Rett syndrome is a rare genetic disease with X linked dominant inheritance. RTT is one of the most frequent causes of mental retardation in females. We report a case of Rett syndrome in a 4 years old girl with hypotonia and hypotrophia. Stereotypic hand movements like clapping and tapping, upper limb spastisity, absence of object grabbing and eye contact were evident. She couldn't sit or walk. Deep Tendon Reflexes were diminished. Eye fundoscopy was normal. The parents had the impression that the infant had not been normal even in the first days of life. MRI showed a mild cerebral atrophy at 15 months of age. Familial history was unremarkable.

We found 502C>T nonsense mutation in *MeCP2*. Genetic analysis in parents showed no genetic alteration in wild type *MeCP2*. As the mother was pregnant, PND was performed and sequence analysis in CVS DNA showed no alteration.

Due to familial cases and germinal musaicism reported in some families, genetic counseling must include mutation detection in mother and PND if requested by parents. As mutations in *MeCP2* can be expressed by a spectrum of atypical phenotypes genetic analysis might be proposed in many cases which are not presented as classical RTT.

Recently mutations of *CDKL5* and *NTNG1* have been found in patients with RTT features. Further studies are needed to define clinical guidelines for their analysis in a MeCP2 unrelated Rett syndrome.

P1386. Risk Perception of Developing Inherited Diseases

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Huntington's disease, Machado-Joseph's disease and familial amyloidal neuropathy are progressive neuropsychiatric disorders which are inherited as an autosomal dominant trait. They are late onset diseases with symptomatic treatments but no cure. These three diseases result in different levels of disability, which may cause different risk perceptions. Although the risk of developing the disease decreases gradually with age, at-risk individuals are never entirely sure that they have escaped the disease.

Some at risk subjects who have not been tested are aware of their 50% risk, but some are not.

We studied 213 individuals who are at risk for late-onset genetic diseases, specifically with a 50% genetic risk for three diseases. These individuals were asymptomatic, aged 18 year or older and they had not been tested for the respective disease.

From a total number of 213 subjects, 193 answered the question regarding whether or not they were informed regarding their risk. Among the 193 subjects, 116 (54,5%) were aware of the 50% risk condition, and 77 (36,2%) were not.

The value of the subjective risk is greater (52, 41%) and closer to 50% when the subjects were aware of their 50% risk than when they were not (44, 79%).

There are significant statistical differences between the subjective risk of the subjects that were aware of their 50% risk and those who were not ($F(1,191) = 4.143$, $p<.050$).

The subjects who were aware of their 50% risk had a higher level of education ($F(4,188) = 4,918$, $p<.010$) than the subjects who were not.

Sex, age, disease and education level did not influence the choice of the risk perception value.

P1387. Professor Svetlana K. Klueva, founder of the Department of Medical Genetics of SPbMAPS

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In memoriam of Professor Svetlana Kliment'evna Klueva (1931-2005).

In Russia medical education has some specific features. It provides periodical training for certificated physicians at the institutes of advanced medical studies like MAPS, the oldest one, to improve their professional skills. Over a long period of time in the USSR genetics and medical genetics were prohibited and a lot of physician generations were not able to study human genetics. Now it is impossible to be successful physicians without knowledge of medical genetics and molecular medicine. Professor Svetlana Klueva was the founder of the Department of Medical genetics in SPbMAPS. She was obliged to carry on a longtime and exhausting struggle with officialdom to organize this department. It was formed in 1989 and its staff included 5 physicians certificated and highly skilled in medical genetics (a clinical geneticist highly skilled in human genetics and complex diseases, a clinical geneticist-pediatrician, a clinical geneticist highly skilled in biochemical genetics and inborn errors of metabolism, a clinical geneticist-neurologist and a clinical cytogeneticist. Two principal trends were based by S. Klueva: 1) training physicians for practical genetic service and 2) teaching medical genetics for all doctors with basic clinical specializations. The principal goal of S.K.Klueva's activities was forming

genetic thinking in the physicians independently of their specialization. Professor S. Klueva with her colleagues provided possibility to acquire knowledge of human genetics for more than 5000 physicians. The Department of Medical Genetics organized by Professor Svetlana Klueva is her “monumentum aere perennius”.

P1388. Genetic screening criteria in the age of Genomics

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In the domain of genetic screening insight in screening criteria is needed. They help weighing pros and cons when preparing decisions on new possibilities for screening generated by Genomics. Some genetic screening programs in the Netherlands have developed rapidly, such as cascade screening for familial hypercholesterolaemia, while others were introduced many years after implementation in other countries, such as prenatal Down syndrome screening.
We investigated long term developments in Dutch genetic screening policy by the means of a witness seminar. Most of the key players involved in decision making on genetic screening since 1970 attended the seminar. In addition we set up focus group meetings to explore attitudes and choices of potential users regarding genetic testing.
The witness seminar showed a remarkable confusion of tongues on terms like “prevention”, “responsible parenthood” and “population screening” as part of the Dutch screening debate in the 1970s and 1980s. Moreover there were frequent misunderstandings among policy makers and scientists on the ins and outs of informed decision making in the domain of reproductive screening. Screening was sometimes understood as obligatory, where informed decision making was intended. Policy aims to “protect citizens against the dangers of screening”. Hence, screening for disorders for which no treatment is available (including prenatal screening) is deemed problematic. However, focus group participants stated that they want to be able to make a personal choice as independent citizens, based on adequate information.
Governmental responsibility for quality control suits citizens present-day expectations better.

P1389. Supporting policy development for population-based genetic screening

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Jurisdictions around the world face complex decisions regarding the introduction or expansion of population-based genetic screening programs. Over 50 sets of criteria have been proposed to support decision-making.
OBJECTIVES: 1) to propose a systematic approach to genetic screening policy-making which enables more balanced and informed decisions and integrates multiple issues, types of evidence and perspectives; and 2) to initiate consensus-building among stakeholders around the decision-making criteria and process.
METHODS: A series of literature reviews and consultations were used to draw upon existing criteria and to analyse the decision-making process. Focus groups were conducted with geneticists, public health specialists, social scientists and citizens to explore the utility and acceptability of core criteria derived from the literature review. Discussions within a knowledge network and consultations with policy-makers and international experts contributed to refining the decision-guide.
RESULTS: The decision-guide consists of general principles, three decision-nodes, criteria for each node, and types of evidence to consider for each criterion. The logic of the decision-making process is reflected in the decision nodes addressing 1) the utility of the screening strategy for individuals and families, 2) the relevance of implementing the screening program for a particular target population, 3) and the judicious allocation of resources at the societal level.
DISCUSSION: The decision-guide makes explicit the reasoning and the information upon which policy decisions are based and presents a number of innovations over existing criteria, by clarifying the iterative nature of decision-making, balancing perspectives, comparing alternatives, considering context, and promoting the documentation of knowledge gaps and trade-offs.

P1390. The delay between pregnancy confirmation in primary care and antenatal sickle cell and thalassaemia screening: a population-based cohort study

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Background: Antenatal sickle cell and thalassaemia (SCT) screening programmes aim to facilitate timely informed choice for couples. In the UK, for example, the target for screening is ten weeks gestation. The objective that screening should be implemented early in pregnancy may be compromised if there is any delay between pregnancy confirmation in primary care and implementation of screening.
Objective: To determine the time between confirmation of pregnancy in primary care and antenatal SCT screening.
Design and setting: Cohort study of all pregnancies reported in 25 general practices in two UK inner city Primary Care Trusts with universal screening policies.
Participants: Anonymised data on all pregnancies reported to the participating general practices for a minimum of six months.
Main Outcome: Time from pregnancy confirmation in primary care to antenatal SCT screening.
Results: There were 1,496 eligible women whose SCT carrier status was not known and who intended to proceed with the pregnancy. The median (interquartile range) gestational age at pregnancy confirmation was 7.6 weeks (6.0-10.7 weeks). The median gestational age at screening was 15.3 weeks (IQR 12.6-18.0 weeks), with only 4.4% being screened by 10 weeks. The median delay between pregnancy confirmation and screening was 6.9 weeks (4.7-9.3 weeks). After allowing for practice-level variation, there was no association between delay times and maternal age, parity and ethnic group.
Conclusion: Reducing the considerable delay between pregnancy confirmation in primary care and antenatal SCT screening requires development and evaluation of methods of organising and delivering antenatal care that facilitate earlier SCT screening.

P1391. Language skills and narrative production in individuals with Smith-Magenis syndrome

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Smith-Magenis syndrome is a rare genetic disorder in which language abilities are dependant on different factors: mental retardation, speech delay, severe behavioral difficulties.
Language impairment and delayed language onset have been described, although not investigated in detail, in children with Smith-Magenis (17p.12 deletion syndrome).
The aim of the study is to investigate different areas of language: ability to tell a narrative, phonology, syntax and receptive vocabulary in a group of six girls and eight boys, aged 8 to 28 years, with 17p12 deletion syndrome. The mean range full-scale IQ was below 40 to 70 for children and 44 to 64 for young adults (see Table 1)
Four different language tasks were used: (1) a narrative task (Frog Story) (2) a repetition task including all 36 French phonemes in different positions (3) a picture vocabulary task and (4) a receptive syntactic task
Results revealed dramatically significant improvements in articulation tasks especially in young SMS. Young adults still made phonetic and syntactic errors and provide ambiguous references in the story. Their language performance was better in lexicon than in other components of language. Such findings suggest slow and asynchronous development in language and communicative skills. An implication of these results is the need for continuing speech and language intervention in individuals with Smith-Magenis Syndrome

Table 1. Demographic details for SMS

	Children n = 10	Young Adults n = 4
Sex		
Males Females	5 5	3 1
Age (years.months)		
Mean (SD) Range	9.7 (2.4) 8-14.11	22.10 (3.7) 20.11-28
IQ		
Mean (SD) Range	43 (10.7) below 40 to 70	55 (13.4) 44 to 64

P1392. Neuropsychological and Behavioural profile in adolescents and young adults with Smih-Magenis syndrome

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Smith-Magenis syndrome (SMS) is a multiple congenital anomalies and mental retardation syndrome associated with an interstitial deletion of chromosome 17 band p11.2. Six adolescents (3 boys and 3 girls aged 12 to 15 years) and 4 adults (3 boys and 1 girl aged 19 to 22 years) with SMS were administered a cognitive battery including a neuropsychiatric and a behavioural scale for measuring Intellectual efficiency (PEP-R, EDEI-R, WAIS) Neuropsychiatric Inventory (NPI), Social maturity and Autonomy (Vineland Adaptative Behaviour Scale).
The results demonstrate a mild-to-moderate range of mental retardation of all patients, (IQ for young adults ranged from 44 to 64), without any discrepancy between verbal and performance scales.
Adolescents show impulsive and unstable behaviors with agitation/ mood instability, auto and hetero-aggressivity which interfered with learning and social variables. Intellectual efficiency is underestimated. Sociocultural level is a strong factor to understand the pattern of their cognitive profile. Scores on logical reasoning and working memory are poor. Moreover, speed of information processing is very impaired.
Adults were so impulsive that they could not inhibit a contrary action. Concerning sexual behavior, desinhibition is very frequently reported by the families.
Motor instability remained important but the mood instability was more adaptive. 3 out of 4 young adults have episodes of fabulation but none have any depression.
Such results show evidence that stimulations for speaking and learning are crucial in adolescents and young adults with SMS and should be carried on. Their needs are still educational and have strong implications for their cognitive abilities and autonomy.

P1393. Molecular diagnosis of Egyptian patients with spinal muscular atrophy

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Childhood spinal muscular atrophy (SMA) is one of the most common autosomal recessive disorders. It is characterized by symmetrical muscle weakness and atrophy of limbs and trunk. It has an estimated incidence on 1:6000 live births and a carrier frequency of 1:50 individuals. Childhood SMA is classified into 3 types (I, II and III) according to the age of onset and the clinical severity.
This study aimed to characterize the molecular basis of SMA among Egyptian patients.
Eighty two Egyptian patients were clinically diagnosed as SMA, they were classified into 32 type I, 36 type II and 14 type III cases. Detection of homozygous absence of exons 7 and 8 of SMN1 gene was carried out using the PCR-restriction digestion method, whereas, deletion of NAIP exon 5 was detected through multiplex PCR.
Homozygous absence of SMN1 gene exon 7 and 8 or exon 7 only was found in 69.4% of all patients. Of these patients, exon 5 of NAIP gene was deleted in 78% of type I, 34.3% of type II, and 28.6% of type III.
The molecular basis of SMA in Egyptian is almost similar to that reported by other ethnic groups. The PCR-restriction digestion method is fast, accurate and reliable for direct diagnosis of SMA and it could be used efficiently for genetic counseling. Studying the presence of compound heterozygous and homozygous point mutations in SMN1 gene is recommended in the non-deleted SMA patients (approx. one third).

P1394. Cardiac genetic services - Ascertaining individuals at risk of a sudden cardiac death syndrome

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Purpose: An audit of referrals to the North West Regional Genetic Service (population 4.3 million) in 2004, showed that 24 patients had been referred to Clinical Genetics with a personal or family history of a Sudden Cardiac Death (SCD) syndrome e.g. long QT syndrome, Brugada syndrome or a cardiomyopathy. Statistically there are approximately 12,000 patients in Greater Manchester alone with these conditions. Clearly this patient group is under-represented in genetic services suggesting a large unmet need.
In order to determine the optimum method of increasing appropriate referrals to Clinical Genetics, a two year service development project was designed looking at three different referral methods, across the three cardiac tertiary centres in the North-West.
Findings: The project has demonstrated that the most effective way of increasing appropriate referrals has been the presence of the cardiac liaison nurse in cardiology clinics and on the wards. This has resulted in a 454% increase in referrals to 109 in the first year of the project. An audit of 61 referrals has shown an average of 5 surviving first degree relatives per pro band. The project has therefore identified approximately 545 at risk relatives.
The nurse has also been able to identify barriers to referrals and ways of overcoming them.
Conclusion: Increasing appropriate referrals to clinical genetics has enabled the identification of family members at risk of a SCD syndrome. This has facilitated the provision of genetic counselling, genetic testing and cardiac assessment of these family members, thereby reducing the risk of Sudden Cardiac Death.

P1395. Partnering to develop orphan drugs : evaluation of OrphanXchange services

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The OrphanXchange project started in the context of a FP6 European contract called OrphanPlatform. One of the aims of this project was the development of information tools to allow a comprehensive and integrated approach for addressing the set of factors that currently affects translational research in the field of rare diseases and orphan drugs. A database of ongoing research projects was set up in the context of Orphanet and a specific website, www.orphanxchange.org, was created to facilitate the formation of partnership activities between academic scientists and industry. The website provides free access to research projects that scientists wish to develop in partnership with industry. For industry, it is a database of business opportunities. Although minimal information is provided freely, more detailed information is only accessible after registration, which allows identification of the customers. Customers only have access to the scientist's details if they make a contact request. Currently, the database includes 129 projects. The site is visited 500 times per month. Half of the 213 registered users are from industry (biotech, big pharmas, consultants, investors), and the other half are from academia. These members are from 31 countries, the largest number of users coming from France, Japan, the USA, and Sweden. In two years, 113 requests to make contact with a researcher were received. Three partnership projects were successfully developed thanks to the service. The plan is to extend this service to cover requests for partnership between academic teams, and between industry teams (for example, small SMe and big pharma).

P1396. A procedure for validation, verification and follow-up of methods in an accredited genetics laboratory

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Test validation, defined as "confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled" is a necessary element of quality assurance and a requirement for laboratory accreditation.
Both new methods and modifications that may influence the quality of the results must be validated before diagnostic use. For practical reasons, the intensity of validation must be adapted for different procedures - laboratories should define for each test the elements to validate that are necessary and sufficient.
Validation, the more thorough procedure, requires test-based confirmation of precision (repeatability and reproducibility), sensitivity, specificity and ruggedness. It is applied for example to new technologies, or in case of major technical modifications to existing methods, or when the performance of installed methods is insufficient.
Verification (or "implantation validation") is a less intensive procedure, typically employed in case of minor technical modifications or additions, or when implanting a diagnostic kit that has been validated by an external company or laboratory. Examples include changing a primer sequence in a PCR reaction or an enzyme for digestion, or adding an additional exon to an existing sequence-based test.

Even when the new test is applicable, validation is never finished: it is essential to define appropriate internal quality control (IQC) to follow the performance of the test and detect possible problems.
We present detailed examples of validation/verification in our accredited laboratory and a new approach to IQC which reveals a continuous improvement in the ruggedness of our methods since accreditation.

Po10. Therapy for genetic disease

P1397. Neonatal lentiviral gene transfer in a knock-out mouse model for afibrinogenemia

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Congenital afibrinogenemia is a rare autosomal recessive coagulation disorder characterized by the complete absence of plasma fibrinogen. The fact that afibrinogenemia is caused by mutations in a single small gene, in most cases the fibrinogen alpha-chain gene (FGA), combined with the existence of a knock-out mouse model, encouraged us to design a gene therapy approach to correct the coagulation deficiency. The C57BL/6J Fga knock-out mice used in this study develop abdominal hemorrhagic events during the neonatal period. We observed an important variability in the severity of the bleeding phenotype and the survival rates in homozygous Fga -/- mice. Certain characteristic patterns of bleeding, notably navel bleeding, allow us to select candidates, before genotyping is possible, with good chances of survival post-injection. HIV-1-derived lentiviral vectors expressing GFP, human FGA or murine Fga cDNAs under the control of a ubiquitous or hepatocyte specific promoter have been produced in the laboratory. Considering the early onset and the severity of the phenotype, we opted for neonatal gene transfer by injection of lentiviral vectors in the temporal vein. Injections of lentiviral vectors expressing GFP revealed that newborn fibrinogen deficient mice survive the intra-temporal vein injection, with the efficiency of hepatic transduction estimated by immunohistochemistry at approximately 20%. Analysis of one knock-out mouse injected with lentiviral vectors expressing murine Fga revealed the presence of fully assembled fibrinogen hexamers in plasma. Investigations are ongoing to define the minimum therapeutic vector dose, and to evaluate the efficiency of correction of the bleeding phenotype, both short-term and long-term.

P1398. The role of c-myc in the pathogenesis of Autosomal Dominant Polycystic Kidney Disease Type 2

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Autosomal Dominant Polycystic kidney disease (ADPKD) is one of the most frequent genetic diseases in humans and results from mutations in the PKD1 or PKD2 gene. Some of the pathogenic features of PKD are dysregulated epithelial cell proliferation, apoptosis and differentiation.

Although the genes mutated are known, the mechanism by which cysts are formed remains unclear. Many proteins and pathways are dysregulated in polycystic kidneys. One of these is the proto-oncoprotein c-myc, found to be overexpressed in kidney epithelial cells in animal models and tissues from human patients. In addition, a c-myc transgenic mouse developed a PKD phenotype suggesting that c-myc contributes in the pathogenesis of PKD.

The purpose of this study is to determine whether c-myc plays a role in the PKD2-induced abnormal proliferation observed in epithelial cells. We generated stable clones overexpressing mutated PKD2 in HEK293 cells, to examine whether c-myc expression was altered in this system. Overexpression of WT or mutant PKD2 does not alter cellular proliferation or c-myc levels. As a result, we assumed that the increased expression of c-myc may be a secondary effect and is produced by the dysregulation of other pathways in the cells.

Using a transgenic rat model generated by overexpression of mutated PKD2 (1-703), we isolated kidney tubular epithelial cells and showed augmented levels of PCNA, accompanied by an increase in c-myc expression compared with WT tubular epithelial cells. Therefore, we decided to use this model to study whether downregulation of c-myc at the cellular level reverts the abnormal proliferation phenotype.

P1399. Bioethics, Human Cloning: Aspect of Theologians, Physicians and Geneticists in Iran

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Introduction: It appears that the nature and extent of public information and debate on cloning and its potential advantages and disadvantages in the area of human health vary around the world.Furthermore an oriental trend has shown in this field.The research team thinks that scholars are among the most powerful effectors that shape attitudes towards human cloning.This study illuminates how Iranian scholars in the field of medicine,genetic and theology think about the most important ethical consequences.

Methods: Ethical issues that arise in human cloning are discussed along with reasons susceptibility data may be offered to participants in the future.To develop a questionnaire,a series of the most important ethical considerations of human cloning were grouped under the four bioethical principles;autonomy,beneficence,nonmaleficence and justice. The method of filling that was face to face interview.

Results: One hundred and two physicians,34 geneticists and 65 theologians responded to the survey for a response rate of 75%.The overall main attitude of physicians and geneticists towards human cloning and stem cell research was 3.11 and the main attitude of theologians towards these issues was 3.21 out of 5.Age,sex and literacy grade has no significant effect on the attitude in different groups of professionals.

Conclusions: Biomedical and theological scientists in this study showed weak positive attitude towards the issues.Knowing many limitations we faced like lack of previous comparable method,possible selection bias,and intuitive nature of many conclusions,no generalization is wise and further research efforts especially qualitative ones are indispensable for unmasking the realities and making new theories in this respect.

P1400. Antisense-mediated exon 51 skipping restores local dystrophin expression in muscle of Duchenne muscular dystrophy patients

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Duchenne muscular dystrophy (DMD) patients suffer from a progressive, severe muscle-wasting disease due to frame-disrupting mutations in the dystrophin gene and a complete loss of functional dystrophin. By contrast, mutations that maintain the open reading frame give rise to internally deleted, partially functional dystrophins and are found in the less severe Becker muscular dystrophy patients (BMD). Antisense oligonucleotide compounds (AONs) have recently shown therapeutic promise for DMD patients. By inducing specific exon skipping during mRNA splicing AONs have been successful in repairing the open reading frame and thus restoring dystrophin expression in cultured muscle cells from patients, as well as in the *mdx* mouse model. As an essential step towards broad clinical studies and future applications, we here evaluated efficacy of a single, intramuscular dose of DMD AON PRO051. Four DMD patients with different mutations were included on basis of eligible mutation, adequate condition of the target muscle, and positive *in vitro* PRO051 skip-response. A dose of 0.8 mg PRO051, without any excipient, was injected into the tibialis anterior muscle and a biopsy was taken after 4 weeks. In each of the individual biopsies specific exon 51 skipping was observed on RNA level. This resulted in abundant dystrophin protein expression at the membrane, as demonstrated by immunohistochemical and western blot analyses. PRO051 was well tolerated and did not provoke serious adverse events in any of the patients: local treatment with PRO051 was safe and highly efficient. Our results provide strong basis for subsequent studies on systemic treatment of DMD patients.

P1401. Enzyme replacement therapy in 4 patients with mucopolysaccharidoses type-6

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The mucopolysaccharidoses (MPS) are a heterogeneous group of lysosomal storage disorders caused by the deficiency of enzymes involved in degradation of glycosaminoglycans. Mucopolysaccharidosis type-6 is characterised by osseous, corneal and visceral changes without intelligence impairment.

We started to enzyme replacement therapy in four patients with MPS type-6 on November 2006 with Naglazyme (Galsulfase). Our patients are a girl-7.5 years old and 3 boys-3.5, 6 and 9 years old. All patients have coarse facies, joint stiffness, corneal clouding, hearing loss, valvular heart dissease, dysostosis multiplex, hepatosplenomegaly and normal intelligence. One patient has also glaucoma, and one has adenoid vegetation.

These patients were diagnosed clinically and definitive diagnosis were made by enzyme analyses. Deficiency of Arylsulphatase B enzyme was found in all patients.

We assayed the patients with clinical examination,vital signs, 6 minutes walking test, echocardiography, measures of joint ranges, pulmonary function tests, ophtalmologic and hearing examinations before the therapy.

All patients received weekly intravenous infusions with 1 mg/kg Galsulfase. Because of the potentiallity of infusion reactions patients received antihistamines and antipyretics before the infusions. They hadn't any complication except two patient had vomits for two times.We aimed to treat the somatic symptoms of patients. When 3 months of the therapy were completed we reassay the patients clinically. Their complaints about sleep apnea and difficulties of daily activities were reduced.

P1402. Multi-exon skipping removing exons 45 through 55 of the DMD transcript could rescue up to 63% of Duchenne Muscular Dystrophy patients

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To collect and analyze mutation information, we developed a generic software called Universal Mutation Databases (UMD). This software includes a large set of analysis tools primarily created for molecular epidemiology studies and subsequently to address more complex topics such as genotype-phenotype correlations. With the development of innovative therapies for patients suffering from rare genetic diseases, it appeared that bioinformatics and LSDBs could play an important role.

Thus, we created specific routines to help researchers to design therapeutic strategies such as exon skipping whose archetype is the DMD gene associated with Duchenne (DMD) and Becker dystrophies (BMD).

Approximately two-third of DMD patients show intragenic deletions from one to several exons of the DMD gene leading to a premature stop codon. Other deletions that maintain the translational reading frame result in BMD. The opportunity to transform a DMD phenotype into a BMD phenotype appeared as a treatment strategy with the development of various technologies, which are able to induce an exon skipping at the pre-mRNA level in order to restore an open reading frame. Because the DMD gene contains 79 exons, thousands of potential transcripts could be produced by exon skipping and should be investigated.

By using UMD algorithms, we predicted that an optimal multi-exon skipping leading to the del45-55 artificial dystrophin (c.6439_8217del) could transform DMD phenotype into asymptomatic or mild BMD phenotype. This multiple-exon skipping could theoretically rescue up to 63% of DMD patients with a deletion while the optimal mono-skipping of exon 51 would rescue only 16% of patients.

P1403. Recovery of female Fanconi anemia fertility after chemotherapy, irradiation and bone marrow allograft/further evidence against oocyte regeneration by bone arrow-derived germline stem cells

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Mammalian oocytes are formed before or shortly after birth. However, recent controversial papers cast doubts on this paradigm by claiming that new ovarian follicles can be generated in the mouse by germline stem cells (GSCs) supplied by the bone marrow (BM). Here we consider an issue related to the potential existence of GCSs : the genetic origin of offspring after allogeneic hematopoietic stem cell transplantation in humans. To clarify this issue, we have examined a rare clinical situation in which a woman with Fanconi Anemia (FA) gave birth to a child after allogeneic BM transplantation. We have genotyped the mother (patient), the daughter, and the donor; several informative polymorphic microsatellites demonstrated the genetic relationship between the other and the daughter. The rare clinical situation described here is reminiscent of the observations described in the controversial papers. However, it leads to an alternative explanation. Our data show that recovery of fertility after BM transplantation can result from incomplete depletion of the ovarian follicular pool and not from its replacement by donor BM-derived GSCs.

P1404. The pharmacological chaperone AT1001 increases levels of mutant alpha-galactosidase A in Fabry patient cell lines and reduces GL-3 levels in a mouse model of Fabry disease

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Fabry disease is an X-linked lysosomal storage disorder caused by deficient α-galactosidase A (GLA) activity and accumulation of globotriosylceramide (GL-3) and related glycolipids. Over 400 Fabry mutations have been reported; ~60% are missense. Previously, the pharmacological chaperone, AT1001 (migalastat hydrochloride), was shown to increase R301Q GLA activity *in vitro* and *in vivo*. To evaluate the response of other Fabry GLA mutations, male patient lymphoid cell lines representing more than 50 different missense mutations were incubated with AT1001 (5 nM to 1 mM) for 5 days, and effects on GLA activity were determined. Basal enzyme activity ranged from 0% to 35% of wild type (WT). Significantly increased GLA activity was observed in over 34 cell lines (1.5- to 20-fold; post-treatment GLA activities ranged from 2.4% to 110% WT), with varying EC_{50} values: from 600 nM to >1 mM. Next, the effect of AT1001 was tested in GLA knockout mice that express a human R301Q transgene (R301Q Tg/KO). Daily oral gavage of AT1001 (30 mg/kg PO; 4 weeks) to male R301Q Tg/KO mice resulted in significantly increased GLA activity ($p<0.05$; n=6-7) and significantly reduced GL-3 levels in skin and heart ($p<0.05$; n=6-7). In kidney, GL-3 levels were reduced but did not reach statistical significance. In conclusion, GLA activity is increased in patient-derived cell lines after incubation with AT1001 for many mutant forms of the enzyme. Increased enzyme activity is also seen after oral delivery of AT1001 to Fabry transgenic mice, with a concomitant decrease in tissue GL-3 levels.

P1405. Safety of agalsidase alfa enzyme replacement therapy in a cohort of 1329 patients in FOS - the Fabry Outcome Survey

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Background: FOS was established in 2001 to monitor the long-term efficacy and safety of enzyme replacement therapy (ERT) with agalsidase alfa in patients with Fabry disease. This is a rare X-linked lysosomal storage disorder that results in progressive accumulation of the enzyme substrate globotriaosylceramide in cells throughout the body, leading to organ failure and premature death. The FOS database has recently been expanded to include patients from non-European countries. The present analysis provides demographic and safety data from a total cohort of 1329 patients enrolled in FOS as of January 2007.
Methods: The FOS database was analysed in terms of patient demography and safety of ERT.
Results: The 1329 patients in FOS have been recruited from 109 centres in 19 countries. Of those enrolled, 828 (62%) are receiving ERT. Approximately equal numbers of males and females are on treatment, and 154 (18.6%) are receiving treatment at home. More than 25 men have been given agalsidase alfa for at least 6 years, and > 50% of the 200 children in FOS are receiving ERT. Mild infusion reactions are the most common drug-related adverse events, reported in 11% of males and 4% of females and representing < 1% of the infusions given. Most reactions occurred between 3 and 6 months after the start of treatment and then declined in frequency. No IgE antibodies have been detected.
Conclusion: Long-term safety data from a large cohort of male, female and paediatric patients in FOS confirm that ERT with agalsidase alfa is very well tolerated.

P1406. A Gaucher disease e*x vivo* response study: the pharmacological chaperone AT2101 increases levels of glucocerebrosidase in patient-derived cells

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Gaucher disease (GD) is caused by a deficiency of lysosomal glucocerebrosidase (GCase). Deficient GCase activity leads to symptoms such as anemia, thrombocytopenia, hepatosplenomegaly, bone necrosis, infarcts and osteoporosis, and in some cases, neuropathic disease. To evaluate the effects of a pharmacological chaperone, AT2101, on mutant GCase levels, we conducted an *ex vivo* response study using macrophages and EBV-transformed lymphoblasts derived from peripheral leukocytes. Plasma was also screened for potential biomarkers associated with inflammation, bone metabolism, multiple myeloma and neurodegeneration. An interim analysis was conducted on samples from 40 patients enrolled at 5 sites in the United States. Results: The study included 21 males and 18 females with type I GD, and one male with type III GD. Patients ranged in age from 7 to 83 years, 38 of 40 patients were receiving enzyme replacement therapy (ERT) and blood was drawn prior to ERT infusion. Analysis of 40 potential markers in plasma showed elevated TRACP 5b, PARC, IL-8, IL-17, VEGF, MIP-1α and α-synuclein and reduced bone-specific alkaline phosphatase levels in most patients. Macrophages were successfully derived from 34 of 40 patients, of which 32 demonstrated a dose-dependent increase in GCase levels (average = 2.8-fold, range = 1.5- to 6.5-fold) when treated with AT2101 (5 days). Similar results were observed for 5 additional patient-derived lymphoblast cell lines. AT2101 significantly increased GCase levels in cells from patients with different genotypes including N370S/N370S (n=11), N370S/L444P (n=8), N370S/84insG (n=11), N370S/R163X, N370S/Y212H, L444P/del 136T, L444P/F216Y, L444P/L174F, G202R/R463C, and K79N/complex B exon 9/10 (type III GD).

P1407. Hyperbranched polylysines as vehicles for gene delivery into eucaryotic cells

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Efficient gene delivery into eucaryotic cells is an extremely critical step in gene therapy. Development of new non-viral DNA vectors seems to be a perspective approach for effective gene delivery into cells. This study was confined to the groups of hyperbranched polylysine vehicles (spLLs). Hyperbranched polylysine with its arginine or histidine surface modified derivatives was studied. Surface lysine/arginine or histidine ratio was 1/1 or 9/1. All vehicles were studied for their capacity to bind DNA and provide protection of plasmid DNA from nuclease degradation. Beta-galactosidase gene expression in HeLa cells indicated transfectional capacity of tested vehicles. All spLLs proved their capacity to condense and protect plasmid DNA. Transfectional capacity of arginine-modified spLLs had no distinguishable difference from that of unmodified hyperbranched polylysine. Histidine-modified polylysine compounds showed 7-20-fold increase of transfectional activity in comparison with unmodified vehicle. Transfectional activity of histidine-modified polylysines was not affected by glycerol treatment. Thus histidine-modified spLLs were found out to provide more effective DNA delivery into cells, compared to arginine-modified spLLs. We suggest that modification of polylysine surface with histidine results in increase of endosomolytic ability and augmented transfectional activity. Inclusion of endosomolytic peptide JTS-1 into DNA/histidine-modified spLLs complexes resulted in 4-fold increase of transfection efficiency. The level of transfectional activity was comparable to that of PAMAM-dendrimer Polyfect. The results demonstrate that polymer surface modification with histidine might be perspective for development of new effective gene delivery vehicles on the basis of hyperbranched polylysines. This work was supported with grants of CRDF (ST-012) and RFBR (06-04-08338).

P1408. Miglustat in Gaucher Disease Type 3

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Thirty patients (29 on ERT, one BMT) were randomized 2:1 to receive for 12 months either miglustat 200 mg t.i.d. or no treatment. All enrolled patients in the 12-month extension phase received miglustat.
Of the 30 patients who completed the first year of treatment, 28 entered the extension study. Mean age (SD) was 10.2 (4.8) years (60% female). The median exposure to miglustat was 729 days (6-802 days). The prevalence and severity of the clinical manifestations were heterogeneous. Comparison of 24 months versus 12 months of treatment did not show a significant difference in vertical saccadic eye movement velocity changes, the primary endpoint. Organ volumes and haematological parameters were stable. A decrease in chitotriosidase levels and an increase in pulmonary FVC were observed in some patients. The most frequently reported adverse events (AEs) were diarrhoea (72%), tremor (38%) and abdominal pain (34%). Diarrhoea was mild and its frequency decreased over time. None of the previous AEs led to study discontinuation. One patient had a confirmed polyneuropathy and withdrew from the study and 3 had sub-clinical neuropathy (1 poly- and 2 mono-neuropathy); one of the latter was included in the no treatment group. There were no deaths.
This 24-month miglustat trial in GD3 patients did not show an effect on the neurological endpoints assessed, but some effects on systemic disease parameters and lung function were observed. The tolerability profile of miglustat at 200 mg t.i.d. was comparable to that reported with the approved dose for GD1 (100 mg t.i.d.).

P1409. The Hunter Outcome Survey (HOS): clinical characteristics of patients with mucopolysaccharidosis type II

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Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is a rare, progressive, X-linked disorder of glycosaminoglycan (GAG) metabolism caused by a deficiency of the enzyme iduronate-2-sulfatase. This results in progressive GAG accumulation within multiple tissues and organs. In 2006, a global survey of MPS II patients - the Hunter Outcome Survey (HOS) - was established to enhance our understanding of the natural history of MPS II, monitor the safety and efficacy of enzyme replacement therapy, and provide the basis for the development of clinical management guidelines. As of October 2006, 100 patients had enrolled in HOS at centres in 11 countries. The median ages at enrolment, onset of symptoms and diagnosis of MPS II were 11.3 years, 1.5 years and 3.5 years, respectively. The prevalence of clinical features at the time of enrolment into HOS was variable; nasal obstruction was reported in 28% of patients, enlarged tonsils or adenoids in 68%, enlarged liver or spleen in 88%, joint stiffness in 87% and facial dysmorphia by 98%. Cardiovascular manifestations of MPS II were found in approximately 75% of patients. These included murmur in 77%, valve disease in 69%, and cardiomyopathy in 12% of patients. Life-table analyses indicated that by age 7 years, approximately 50% of patients with Hunter syndrome display some cardiovascular abnormalities, increasing to 95% by age 15 years. As enrolment increases, HOS will improve our understanding of MPS II, advance the assessment of the long-term impact of ERT, and allow the development of evidence-based clinical management guidelines.

P1410. Oxidative stress in Peroxisomal disorders

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Peroxisomal disorders are subdivided into peroxisome biogenesis disorders (PBDs) and single peroxisomal enzyme deficiencies. Many peroxisomal diseases exhibit excessive oxidative stress leading to neurological alterations and dysfunction. This study aimed to investigate whether oxidative stress is involved in the pathogenesis of peroxisomal disorders by estimating various oxidative stress parameters in cases suffering from peroxisomal disorders.
A total of twenty patients with peroxisomal disorders, their ages ranged from 6 months to 13 years (mean 5.9 ±3.2) were compared to fourteen healthy controls. All individuals were subjected to full history taking including three-generation pedigree analysis concerning parental consanguinity and similarly affected members in the family with meticulous clinical examination to detect any malformation or anomaly. VLCFAs and phytanic acid estimation was done to verify the diagnosis. Other investigations including brain magnetic resonant image (MRI), electric encephalogram (EEG), visual evoked potential (VEP), auditory potential and plain X- rays were done to asses the pathological condition of the patients. Oxidative stress parameters including nitric oxide (NO), malondialdehyde (MDA) and superoxide dismutase (SOD) were estimated in both patients and controls.
This study showed significant increase of both MDA and NO in PBDs. It was also demonstrated that SOD was significantly low in PDB more than controls.
Conclusion: Lacking functional peroxisomes leads to generalized increase in oxidative stress confirming the important role of peroxisomes in homeostasis of reactive oxygen species (ROS) and the implications of its disturbances in cell pathology. The study recommends supplementation with antioxidants besides other lines of treatments.

P1411. Activation of PPAR pathway stimulates mitochondrial oxidative phosphorylation and is a potential therapy in respiratory chain defects.

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The mitochondrial Respiratory Chain (RC) disorders are the largest group of inborn errors of metabolism and still remain without treatment in most cases. Here, we tested whether bezafibrate, a drug acting as a Peroxisome Proliferator Activated Receptor (PPAR) agonist, could induce a stimulation of RC residual capacities in four patient cell lines carrying mutations in the Fp (flavoprotein, complex II, CII), or BCS1 (complex III, CIII), or SURF1 (complex IV, CIV), or COX10 (CIV) genes. Exposure to bezafibrate (400µM, 72h) was found to increase (+38 to +50%) the CII, CIII and CIV activities in control fibroblasts, and immunoblots showed parallel increases (+82 to +150%) in Fp, Core 2, SURF1, COX2 and COX4 protein levels. A similar treatment by bezafibrate improved RC capacities in BCS1- and COX10-deficient fibroblasts, as indicated by the stimulation of CIII (+133%) and CIV (+71%) enzyme activity, and by the higher expression of representative proteins. This was related to a drug-induced augmentation in the mRNA abundance of the mutated BCS1 or COX 10 gene. Fp and SURF1 mRNA were also induced by bezafibrate, but without changes in RC residual capacities due to the mutated protein instability. The molecular mechanisms underlying these effects likely involved PPARδ and the co-activator PGC1α that could induce the expression of genes encoding structural subunits or ancillary proteins of the OXPHOS apparatus, leading to stimulate the activity and protein levels of RC complex. These effects could find applications for the correction of some moderate RC disorders due to mutations in nuclear genes.

P1412. Growth Hormone is Effective in Treatment of Short Stature Associated with SHOX Deficiency: Two-year Results of a Randomized, Controlled, Multi-Center Trial

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SHOX encodes a homeodomain transcription factor responsible for a significant proportion of long-bone growth. Patients with mutations or deletions of SHOX, including those with Turner syndrome [TS] who are haploinsufficient for SHOX, have variable degrees of growth impairment, with or without a spectrum of skeletal anomalies consistent with dyschondrosteosis.
Fifty-two prepubertal subjects (24 male, 28 female; age 3.0-12.3 years) with a molecularly-proven SHOX gene defect and height below the 3rd percentile for age and gender were randomized to either a growth hormone (GH)-treatment group (n=27) or an untreated control group (n=25) for 2 years. To compare the GH treatment effect between subjects with SHOX-deficiency (SHOX-D) and those with TS, a third study group, comprised 26 pts with TS aged 4.5-11.8 years, who also received GH. Between-group comparisons of height velocity, height standard deviation score (SDS) and height gain (cm) were performed using analysis of covariance accounting for diagnosis, sex and baseline age.
The GH-treated SHOX-D group had a significantly greater first-year height velocity than the untreated control group ($p<0.001$) and similar first-year height velocity to GH-treated subjects with TS (8.9±0.4 cm/y, $p=0.592$). GH-treated subjects also had significantly greater second-year height velocity ($p<0.001$), second-year height SDS (-2.1±0.2 vs. -3.0±0.2, $p<0.001$) and second-year height gain (16.4±0.4 vs. 10.5±0.4 cm, $p<0.001$) than untreated subjects.
In summary the efficacy of GH treatment in subjects with SHOX-D was equivalent to that seen in subjects with TS. We conclude that GH is effective in improving the linear growth of patients with various forms of SHOX-D.

P1413. Treatment of SMA cell line using 5-NENI-amiloride

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Spinal muscular atrophy (SMA) is a common inherited neuromuscular disorder caused by homozygous loss of the survival motor neuron 1 (SMN1) gene. All SMA patients carry at least one copy of a nearly identical SMN2 gene. However, a critical nucleotide change in the exon 7 of SMN2 results in alternative RNA splicing and exclusion of exon 7 in the majority of SMN2 mRNA, thus producing a low level of functional SMN protein. Increasing SMN protein production by promoting SMN2

exon 7 inclusion and/or SMN2 gene transcription, therefore, could be a therapeutic approach for SMA. It has been shown that cellular pH microenvironment can modulate pre-mRNA alternative splicing in vivo. In this study, we tested whether inhibitors of the NA^+/H^+ exchanger can modulate the exon 7 splicing of SMN2 mRNA. We found that treatment with a NA^+/H^+ exchanger inhibitor, 5-(N-ethy1-N-isopropy1)-amiloride (NENI-amiloride), significantly enhances SMN2 exon 7 inclusion as well as SMN protein production in SMA cells. In addition, NENI-amiloride increases the number of nuclear gems in SMA cells. We further explored the underlying mechanism and our results suggest that NENI-amiloride may promote SMN2 exon 7 inclusion through up-regulation of the splicing factor SRp20 in the nucleus. In conclusion, our finding that NENI-amiloride, an inhibitor of the NA^+/H^+ exchanger, can increase SMN protein production in SMA cells provides a new direction for the development of drugs for SMA treatment.

Bold presentation numbers indicate the presenting author

PRESCRIBING INFORMATION: Replagal (agalsidase alfa) 1mg/ml concentrate for solution for infusion. Please read Summary of Product Characteristics (SmPC) before prescribing. **Presentation:** Concentrate solution for intravenous infusion: vials of 3mls (containing 1ml of concentrate) and 5mls (3.5ml of concentrate). **Indication:** Replagal (agalsidase alfa) is indicated for long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry Disease (α-galactosidase A deficiency). **Dose:** Replagal is administered at a dose of 0.2mg/kg body weight every other week by intravenous infusion over 40 minutes. **Contraindications:** Life-threatening hypersensitivity (anaphylactic reaction) to the active substance or any of the excipients. **Warnings and precautions:** 13.7% of patients treated with Replagal in clinical trials have experienced idiosyncratic infusion-related reactions. Overall, the percentage of infusion-related reactions was significantly lower in females than males. The most common symptoms have been rigors, headache, nausea, pyrexia, flushing and fatigue. Serious infusion reactions have been reported uncommonly; symptoms reported include pyrexia, rigors, tachycardia, urticaria, nausea/vomiting, angioneurotic oedema with throat tightness, stridor and swollen tongue. The onset of infusion-related reactions has generally occurred within the first 2-4 months after initiation of treatment with Replagal although later onset (after 1 year) has been reported as well. If mild or moderate acute infusion reactions occur, medical attention must be sought immediately and appropriate actions instituted. The infusion can be temporarily interrupted (5 to 10 minutes) until symptoms subside and the infusion may then be restarted. Mild and transient effects may not require medical treatment or discontinuation of the infusion. In addition, oral or intravenous pre-treatment with antihistamines and/or corticosteroids, from 1 to 24 hours prior to infusion may prevent subsequent reactions in those cases where symptomatic treatment was required. As with any intravenous protein product, allergic-type hypersensitivity reactions are possible. If severe allergic or anaphylactic-type reactions occur, the administration of Replagal should be discontinued immediately and appropriate treatment initiated. The current medical standards for emergency treatment are to be observed. As with all protein pharmaceutical products, patients may develop IgG antibodies to the protein. A low titre IgG antibody response has been observed in approximately 24% of the male patients treated with Replagal. These IgG antibodies appeared to develop following approximately 3-12 months of treatment. After 12 to 54 months of therapy, 17% of Replagal treated patients were still antibody positive whereas 7% showed evidence for the development of immunologic tolerance, based on the disappearance of IgG antibodies over time. The remaining 76% remained antibody negative throughout. No IgE antibodies have been detected in any patient receiving Replagal. The presence of extensive renal damage may limit the renal response to enzyme replacement therapy, possibly due to underlying irreversible pathological changes. In such cases, the loss of renal function remains within the expected range of the natural progression of disease. **Paediatrics:** Experience in children is limited. Studies in children (0-6 years old) have not been performed and no dosage regimen can presently be recommended. Limited clinical data in children (7-18 years) do not permit recommendation of an optimal dosage regimen presently. As no unexpected safety issues were encountered in a six-month study with Replagal administered at 0.2mg/kg, this dose is suggested for children between 7-18 years. **Pregnancy and lactation:** Very limited data on pregnancies exposed to Replagal (n=3) have shown no adverse effects on the mother and newborn child. Animal studies do not indicate direct or indirect harmful effects with respect to pregnancy or embryonal/foetal development when exposed during organogenesis. It is not known whether Replagal is excreted in human milk. Caution should be exercised when prescribing to pregnant or nursing women. **Interactions:** Replagal should not be co-administered with chloroquine, amiodarone, benoquin or gentamicin since these substances have the potential to inhibit intra-cellular α-galactosidase activity. As α-galactosidase A is itself an enzyme, it would be an unlikely candidate for cytochrome P450 mediated drug-drug interactions. In clinical studies, neuropathic pain medicinal products (such as carbamazepine, phenytoin and gabapentin) were administered concurrently to most patients without any evidence of interaction. **Side effects:** The most commonly reported undesirable effects were infusion associated reactions, which occurred in 13.7% of patients treated with Replagal in clinical trials. Most undesirable effects were mild to moderate in severity. The following AEs have been reported: peripheral oedema, headache, dizziness, dysgeusia, neuropathic pain, tremor, hypersomnia, hypoesthesia, paraesthesia, parosmia, lacrimation increased, tinnitus, tinnitus aggravated, tachycardia, palpitations, flushing, hypertension, cough, hoarseness, throat tightness, dyspnoea, nasopharyngitis, pharyngitis, throat secretion increased, rhinorrhoea, nausea, diarrhoea, vomiting, abdominal pain/discomfort, acne, erythema, pruritus, rash, livedo reticularis, angioneurotic oedema, urticaria, musculoskeletal discomfort, myalgia, back pain, limb pain, peripheral swelling, arthralgia, joint swelling, sensation of heaviness, rigors, pyrexia, pain and discomfort, fatigue, fatigue aggravated, feeling hot, feeling cold, asthenia, chest pain, chest tightness, influenza like illness, injection site rash, malaise, corneal reflex decreased, oxygen saturation decreased. **Overdosage:** No case of overdose has been reported. **Pharmaceutical precautions:** Store in a refrigerator (2°C – 8°C). **Legal category:** POM. **Pack sizes, Product Licence Numbers and Cost:** 1ml of concentrate for solution for infusion in a 3ml vial. Pack sizes of 1, 4 or 10 vials. 3.5ml of concentrate for solution for infusion in a 5ml vial. Pack sizes of 1, 4 or 10 vials. EU/1/01/189/001-006. Price of £356.85 for 3ml and £1161.57 for 5ml vials. **Further information available from Product Licence Holder:** Shire Human Genetic Therapies AB, Rinkebyvägen 11B, SE 182 36 Danderyd, Sweden.

Adverse events should be reported to the Yellow Card Scheme. Information about adverse event reporting via this scheme can be found at www.yellowcard.gov.uk. Adverse events should also be reported to Shire Human Genetic Therapies on + 44 (0)1223 422707 or faxed on +44 (0)1223 424666.

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