The ABC of variant classification is to ask the right questions

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Disclaimer

This work has not received any commercial support

and the resulting **ABC system** for variant classification is free to use,

see www.eshg.org under News for the most updated version.

Suggestions for improvements are highly appreciated.

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Background

In 2018, during my ESHG presidency, a task force was created to make a variant classification system

to guide variant reporting

and

to classify any variant or finding

this resulted in the

the ABC system

a system that can integrate any other system or IT/AI tool

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Variant found…
What is the question?

ACMG/AMP classification → Is the variant **pathogenic?**

ABC classification → Is the variant **clinically relevant?**

Option of not reporting? … Can we at a later time point ***risk being sued*** ?

Are we asking the right questions?

ACMG asks about **pathogenicity** – which is a difficult question because:

- The penetrance is reduced: Many carriers of the variant remain healthy
- The condition is recessive: Most carriers of the variant remain healthy
- Clinical information and/or clinical knowledge could be lacking

ABC asks about **clinical relevance** – which a lab may struggle to answer because:

- Clinical information and/or clinical knowledge could be lacking

But: Clinical relevance is an easier question to answer that pathogenicity

The ACMG/AMP system

tries to answer the question of pathogenicity

P	- Pathogenic
LP	- Likely pathogenic (>90% / >95% for cancer)
VUS	- Variant of uncertain significance
LB	- Likely benign (>90% / >95% for cancer)
B	- Benign

which is fine for dominant monogenic disorders of high penetrance, but difficult for **risk alleles** and **low-penetrant** variants. System for these are being elaborated, but will probably be different (i.e. more complexity)

Using IT-based classification, variants of well-known clinical significance can be labelled a VUS

Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework

Sean V. Tavtigian, PhD[1], Marc S. Greenblatt, MD, PhD[2], Steven M. Harrison, PhD[3], Robert L. Nussbaum, MD[4], Snehit A. Prabhu, PhD[5], Kenneth M. Boucher, PhD[6] and Leslie G. Biesecker, MD[7];
on behalf of the ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI)

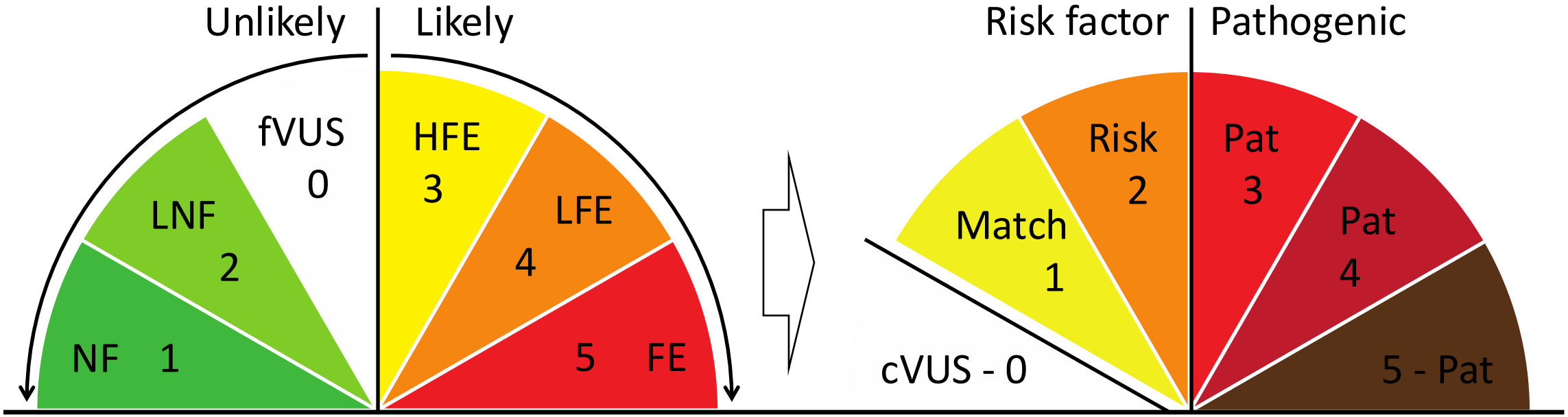
Beware that

1) *The a priori likelihood that a variant is causative is defaulted to* ***10%***
2) *The odds-of-pathogenicity is the square root of the value above:* ***350 – 18.7 – 4.3 – 2.08***
   (= very strong / strong / moderate / supportive)

The ABC system answers 3 questions and can classify any type of finding

1. Does the variant affect gene function?

2. Has the variant clinical relevance?



ABC step A: Functional grading

NF	= Normal Function
LNF	= Likely Normal Function
fVUS	= functional VUS

HFE	= Hypothetical Functional Effect
LFE	= Likely Functional Effect / hypomorphic allele
FE	= Functional Effect (e.g. LoF or GoF)

ABC step B: Clinical grading

cVUS	= clinical VUS («GUS») or no clinical information
Match	= VOI: right type of gene for the phenotype
Risk	= Known RISK FACTOR

Pat	= PATHOGENIC variant, penetrance-graded (3-5) when known

3. Should the variant or finding be reported?

ABC step C:

Standard but flexible variant comments based on joint **A+B** class **A to F**

and adapted to the clinical question

A grade 0-2 (no step B grading):

NORMAL findings

A+B grade 3 (class F):

NORMAL findings – no pathogenic or likely pathogenic variants were detected

A+B grade 4-5 (class E) and 6-7 (class D):

NORMAL findings – no pathogenic variants that could be related to the phenotype were detected

NORMAL findings – no pathogenic variants that could explain the phenotype were detected

VOI – A genetic variant of potential interest was detected

VOI – Heterozygosity for a recessive genetic variant of potential interest was detected

VOI – Hemizygosity for a genetic variant of potential interest was detected

VOI – Homozygosity for a genetic variant of potential interest was detected

RISK FACTOR – A genetic variant that increases susceptibility for this phenotype was detected

RISK FACTOR – Heterozygosity for a recessive genetic variant of interest was detected

PATH – Likely compound heterozygosity for recessive pathogenic variants was detected

PATH – Heterozygosity for a dominant likely pathogenic variant was detected

A+B grade 8 (class C), 9 (class B) and 10 (class A):

PATH – Homozygosity for a recessive pathogenic genetic variant was detected

PATH – Heterozygosity for a dominant pathogenic variant was detected

PATH – Heterozygosity for a dominant pathogenic variant of moderate penetrance was detected

PATH – Heterozygosity for a dominant pathogenic variant of high penetrance was detected

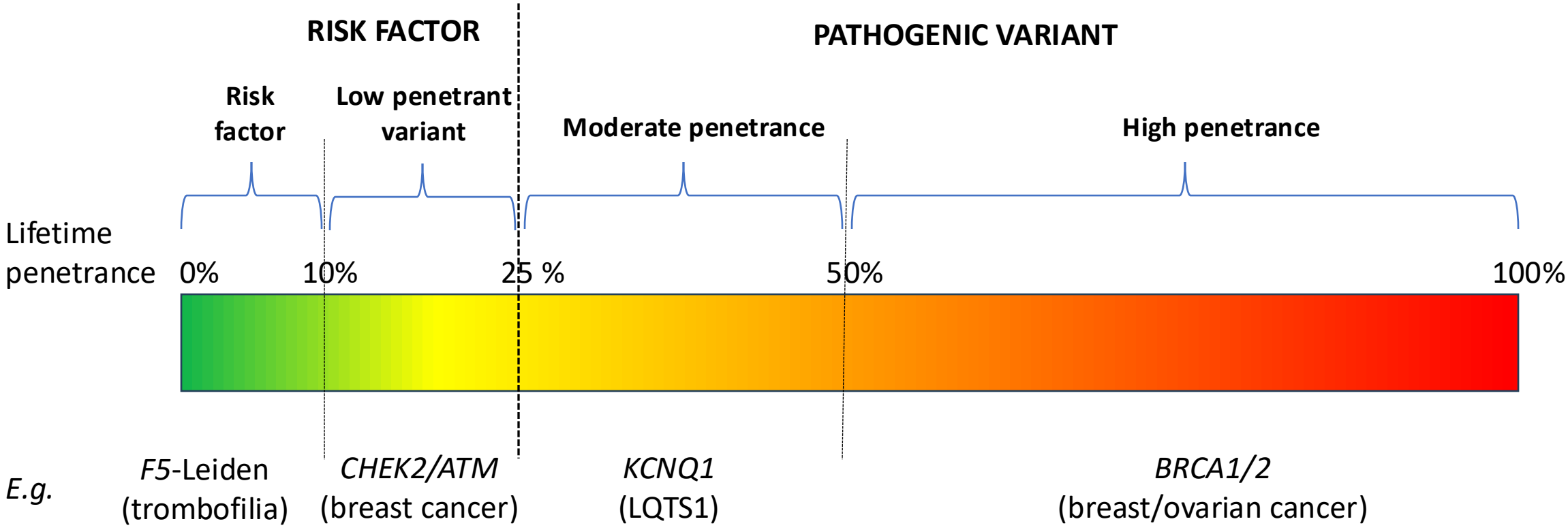
Incidental/unexpected findings and A+B grade 7-10 (class X):

IF – A genetic variant unrelated to the clinical question was detected

IF – No obvious match between genotype and phenotype. Further clinical investigations necessary

When is a variant a risk factor, a low penetrant variant, or pathogenic?

No consensus exists, but my suggestion is:



ABC points to remember

Steps A+B: Grades are clinical question independent – classifies a variant from F to A:

A known *hypomorphic allele* is by default step A grade 4 (= LFE)

A de novo unknown is never lower than step A grade 3 (= HFE)

A single recessive allele is not pathogenic in step B, maximum a RISK FACTOR

Step C: Standard comments are clinical question dependent:

This allows reporting of a hypomorphic or low penetrant variant when clinically relevant – otherwise not

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ACMG criteria can be integrated into the ABC system, preliminary suggestion:

Step A

5 - FE	PVS1 PS1 PS3	1 criterium enough to grade
4 - LFE	PP1-Strong PM4 PM5	2 criteria or more: upgrade to FE
3 - HFE	PS2 PS4 PM1 PM2 PP1 PP2 PP3 PP5	3 criteria or more: upgrade to LFE
0 – funct VUS	not enough data to classify	
2 - LNF	BS1 BS2 BS3 BP1 BP2 BP3 BP4 BP5 BP6 BP7	
1 - NF	BA1	

Note: One “pathogenic” ACMG criterium is enough to grade. Known hypomorphic alleles are by default grade 4 - LFE.

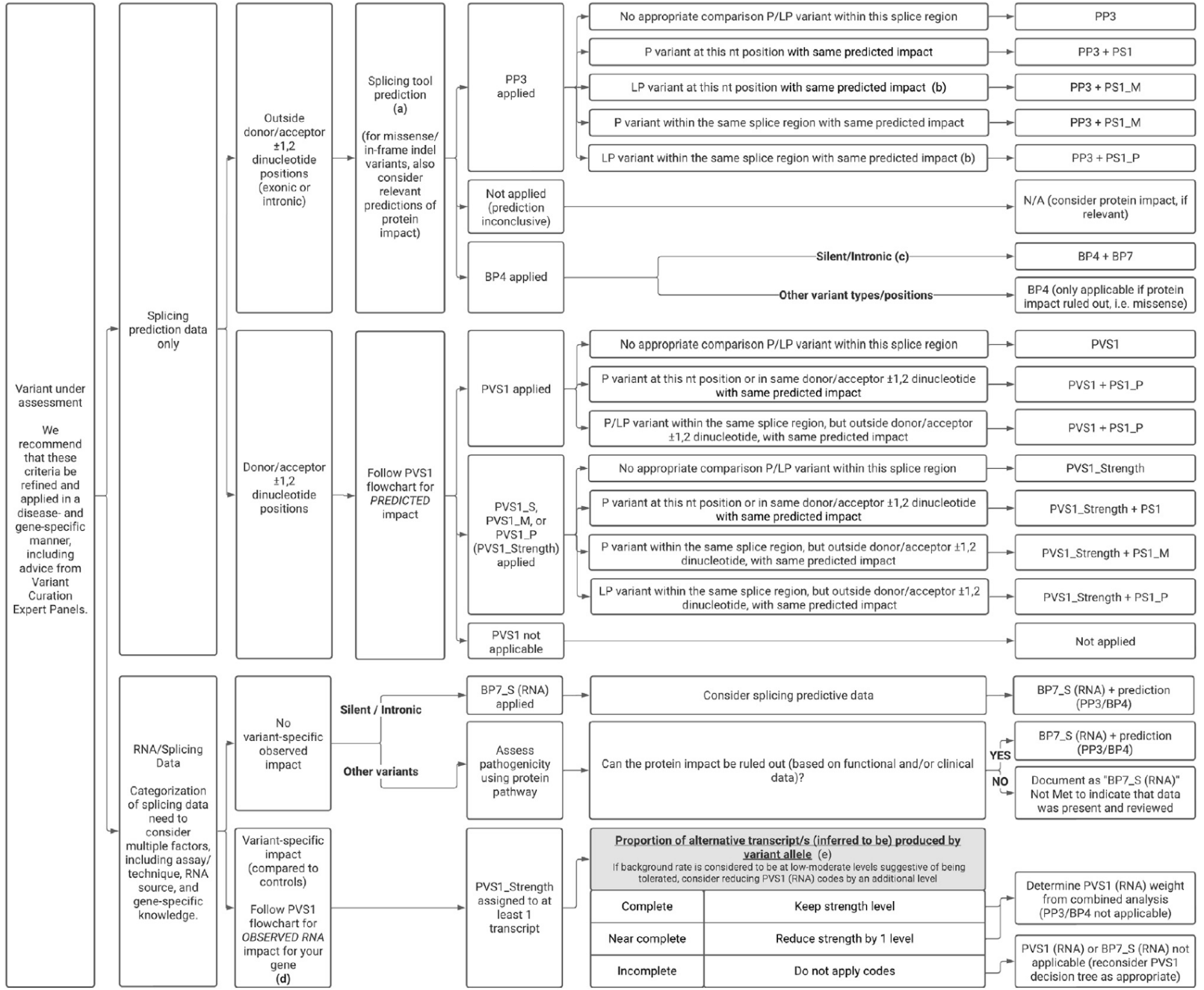
Step B

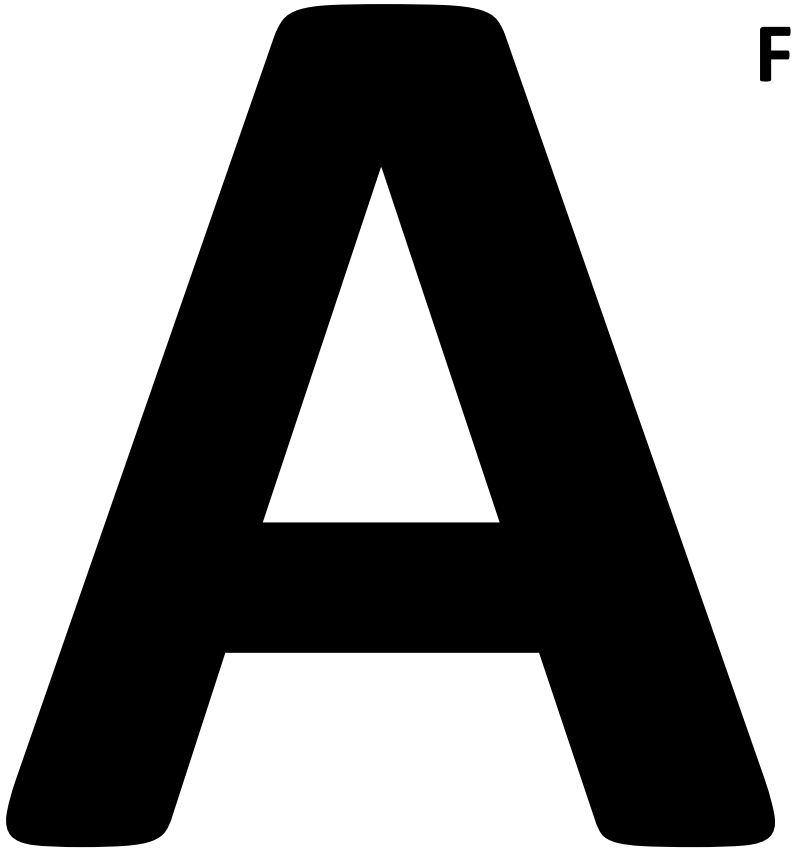
0 – clin VUS	BS4, no clinical match or clinical Information	
1 - VOI	gene fits phenotype	
2 - RISK FACTOR	PM3 PP4	1 criterium is enough
3 - PATH	known pathogenic (AR or AD)	
4 - PATH	known pathogenic (AD, moderate penetrance)	
5 - PATH	known pathogenic (AD, high penetrance)	

Splice variant evaluation

The variant classification system (regardless which) should integrate other systems (like ACMG points, REVEL, spliceAI and AlphaMissense) - just beware of double counting.

E.g. the suggested ClinGen SVI subgroup's flow-chart for splice variant evaluation →





Functional effect

Yes	5
Likely	4
Hypothetically	3
Not likely (LNF)	2
No (NF)	1
Unknown	0

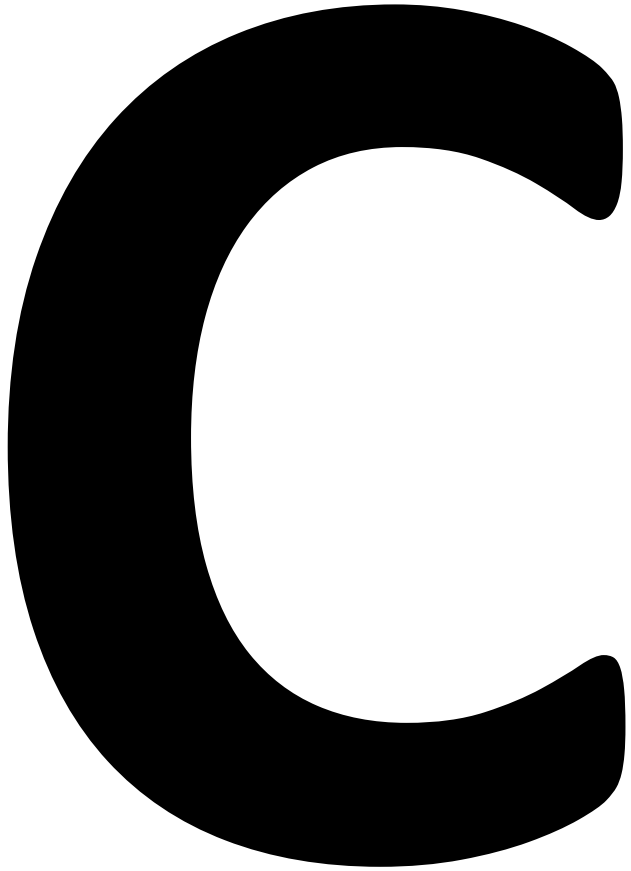
Use all available computer tools to help,
including AI tools and point-based ACMG grading.
By default: Hypomorhic alles are 4, de novo min. 3

B

Clinical match

No – or unknown	0
Hypothetical	1 – VOI
Known	2 – RISK FACTOR
Pathogenic (AD)	3 – PATH
	4 – PATH mod.
	5 – PATH high

Indication and some clinical information needed.
Use Exomizer and similar HPO tools to help.



Pick a standard comment

NORMAL (4 alternatives)

VOI – Variant-of-Interest (4 alternatives)

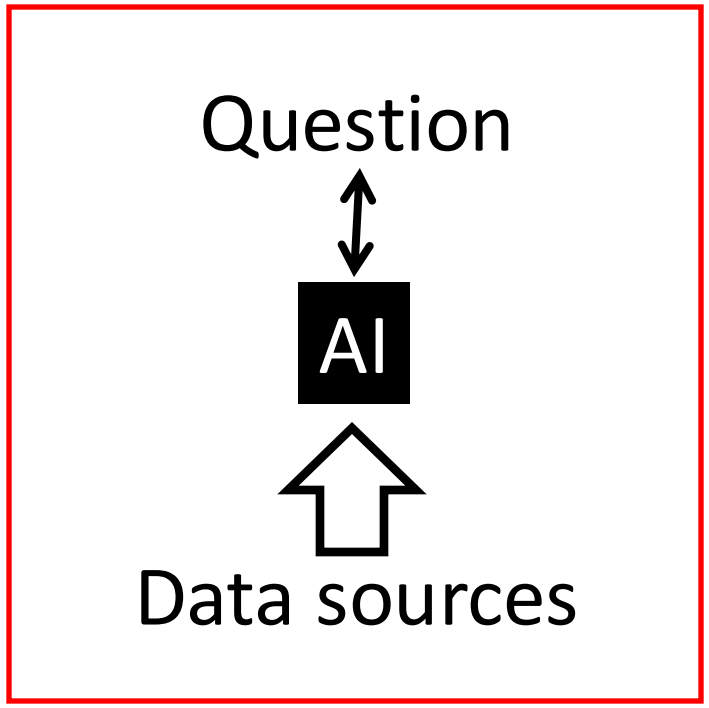
RISK FACTOR – known (2 alternatives)

PATH – pathogenic (6 alternatives)

IF – incidental finding (2 alternatives)

Why is the clinical question so important?

- It could determine if a variant or finding should be reported
- It could help the lab to look in the right place
- It is crucial for successful use of AI tools to classify variants!



The quality of the question is just as important as the quality of the input*

*Geir KF Sandve, professor in informatics and AI researcher

An ESHG study of

ACMG and ABC classification comparison of ten challenging cases

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Table 1: Result of variant classification: Average grading and percentage of variant reporting.

Case #	Gene	Variant[1]	Clinical fit with variant	ACMG[2] grade	ABC-A[3] grade	ABC-B[3] grade	% reported after ACMG Incl maybe	% reported after ACMG excl maybe	% reported after ABC	
1	*CHEK2*	p.(Ile200Thr)	Good	3,5 - VUS	4,3 - LFE	2,3 - RF	83	71	91	← 83 / 91
2	*CACNA1A*	p.(Arg1437Gln)	Good	3,2 - VUS	3,1 - HFE	1,9 - RF	95	81	95	
3	*ADAMTS18*	p.(Arg246Ter)	Excellent	4,3 - LP	4,4 - LFE	3,1 - P	95	95	100	
		p.(Arg573Pro)		3,3 - VUS	3,1 - HFE	2,0 - RF				
4	*HUWE1*	p.(Glu4315Lys)	Good	3,3 - VUS	3,4 - HFE	1,9- RF	95	74	100	
5	*COL5A1*	Thr915Met	Good	2,9 - VUS	2,6 - HFE	1,5 - VOI	80	54	79	
6	Deletion of *ANK2*	1 Mb de novo deletion	? Normal fetus	3,8 - LP	3,6 - LFE	0,8 - VOI	67	57	65	
7	Duplication of 16p11.2	227 kb duplication	Moderate	3,1 - VUS	3,0 - HFE	1,5 - VOI	71	60	79	
8	*PTPN11*	p.(Gly268Ser)	Poor	4,2 - LP	3,9 - LFE	1,2 - VOI	64	50	67	← 64 / 67
9	*TNFRSF1A*	p.(Arg121Gln)	Moderate	2,6 - VUS	3,1 - HFE	2,0 - RF	64	40	81	
10	*ABCA4*	p.(Asn1868Ile)	Excellent	2,7 - VUS	3,6 - LFE	1,8 - RF	67	50	86	← 67 / 86

1) For variant details, see Supplementary File S1. Abbreviations: LP = likely pathogenic; VUS = variant of unknown significance; LFE = likely functional effect; HFE = hypothetical functional effect; RF = risk factor; VOI = variant of interest; P = pathogenic.
2) The ACMG grades are the average of the grades given by all participating laboratories, see Supplementary file S2 for details. Please note that to be able to calculate this, ACMG classes were converted to numbers: P=5, LP=4, VUS=3, LB=2 and B=1.
3) ABC grades are the average of ABC-A and ABC-B grading, see Supplementary file S2 for details.

Case 8: no clinical match, but known Noonan-associated *PTPN11* variant

Finding	NM_002834.5(*PTPN11*): c.802G>A, p.(Gly268Ser) Not in gnomAD 4.0, ClinVar 9x P/LP, Literature: Reported several times as a cause of Noonan syndrome. No functional tests, never reported as de novo.
Clinic	Incidental finding in young man with rhabdomyolysis (and high CK) after strong physical exercise.
ACMG	PS1 (established pathogenic) + PM2 (not in gnomAD) results in class LP. Report?
ABC	Step **A** (functional) grade 3 (HFE - hypotetical functional effect) Step **B** (clinical) grade 1 (VOI – variant of interest), leads to A+B = 4 and Step **C** class E - and a standard comment in line with local/national/international guidelines can be picked (personally, I would not have reported it)

ESHG study: 2/3 or labs would report - regardless of classification system used

Case 1: Well-known *CHEK2* variant

CHEK2(NM_001005735.2) c.599T>C, p.(Ile200Thr) **Monoallelic variant**
gnomAD MAF 0.49%, pLI = 0
ClinVar: ~20x LP/P, 10x VUS
Functional assay (good lab): LoF allele
Literature: Many articles mentioning the variant as cancer associated

Clinical information: Female with breast cancer age 41, maternal aunt breast cancer age 38, paternal sister breast cancer age 36. No other finding upon extensive testing.

Survey result among 41 laboratories:
ACMG classified from VUS to P, mostly VUS (average 3.5).
ABC classified as HFE to FE in step A, average LFE (4.3) and in step B as RISK FACTOR, gives joint grade 3+2=5 or class E - that one may choose to report or not.

Case 10: Well-known hypomorphic *ABCA4* allele

ABCA4(NM_000350.3) c.5603A>T, p.(Asn1868Ile) **Monoallelic variant**

ABCA4 is the only known causal gene of Stargardt-type macular dystrophy

gnomAD MAF 4.2% (364 homozygous), pLI = 0
ClinVar: 9 times B/LB, 4 times VUS, 3 times LP
Functional testing: No data
Literature: Definite Stargardt-disease associated hypomorphic allele (PMID 28446513)

Clinical information given: Man 40 years with poor vision and strong clinical suspicion of Stargardt-type macular dystrophy

Survey result among 41 laboratories:
ACMG classified from B to VUS, mostly VUS (average 2.7)
ABC classified from VUS to LFE, mostly LFE (average 3.6), and in step B as RISK FACTOR

Case 10: Example of wide-spread and non-concordant ACMG criteria selection

Carrier of *ABCA4* Asn1868Ile and no second variant in a 40 years old male patient with classical Stargardt disease

Lab#	PS1	PS3	PS4	PM1	PM3	PM5	PP2	PP3	PP4	PP5	BA1	BS1	BS2	BP4	BP6	#
1					1				1	1		1				4
2																0
3		1					1			1	1			1		5
4																0
5							1	1				1		1	1	5
6											1					1
7				1				1			1					3
8												1				1
9																0
10								1				1	1			3
11		1					1			1	1		1			5
12				1				1	1			1				4
13											1					1
14								1		1		1				3
15								1	1	1						3
16										1		1	1	1		4
17		1					1			1	1					4
18									1			1	1			3
19			1					1	1							3
20									1		1					2
21																0
22								1			1					2
23												1		1		2
24				1				1				1				3
25				1				1								2
26									1	1		1				3
27		1					1			1	1			1		5
28						1	1						1			3
29								1	1			1				3
30								1	1			1				3
31																0
32								1	1			1				3
33																0
34		1			1											2
35																0
36																0
37			1		1							1				3
38		1					1			1	1			1		5
39																0
40	1				1		1				1			1		5
41																0
42									1							1
43		1		1						1	1			1		5
SUM	1	7	2	5	4	1	8	13	11	11	12	15	5	8	1	2,419 n=33
		0,21		0,15	0,12		0,24	0,39	0,33	0,33	0,36	0,45	0,15	0,24		0,27 (0,12-0,39)
	0,03	0,21	0,06	0,15	0,12	0,03	0,24	0,39	0,33	0,33	0,36	0,45	0,15	0,24	0,03	0,21

On average 27% corcordance

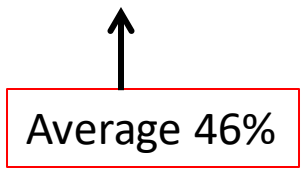
No single criteria used by >50% of the laboratories

Table 2: ACMG criteria used by 33-36 of the 43 laboratories. Concordance rates were calculated as number of laboratories that selected a given criterium divided by the number of laboratories that responded, see Supplementary file 3 for details and calculations.

Case #	Gene	Variant	Clinical fit	ACMG concordance in criteria selection (%)[1]	Number of ACMG criteria used in >50% of laboratories
1	*CHEK2*	p.(Ile200Thr)[2]	Good	32	3
2	*CACNA1A*	p.(Arg1437Gln)	Good	44	2
3	*ADAMTS18*	p.(Arg246Ter)	Excellent	72	2
		p.(Arg573Pro)		50	2
4	*HUWE1*	p.(Glu4315Lys)	Good	50	3
5	*COL5A1*	p.(Thr915Met)	Good	37	1
8	*PTPN11*	p.(Gly268Ser)	Poor	63	4
9	*TNFRSF1A*	p.(Arg121Gln)[2]	Moderate	37	2
10	*ABCA4*	p.(Asn1868Ile)[2]	Excellent	27	0

[1] Criteria selected by less than 10% of the laboratories (3 or less) were excluded from the calculation.

[2] Known low-penetrant variant / hypomorphic allele.



Case 10:
Should it be reported?

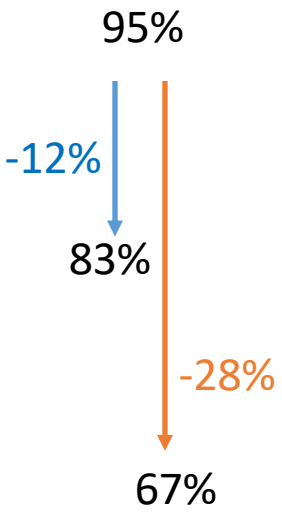


Table 3: Bayesian likelihood for Stargardt disease

Presence of juvenile macula dystrophy	**TRUE**	**FALSE**	**NOT REPORTED**
Prior probability for Stargardt disease:			TRUE/FALSE
Case A: Clinical picture fits with Stargardt disease in man 40 y	0.95	0.05	0.95/0.05
Case B: Other cause found for reduced vision in man 40 years	0.01	0.99	
Conditional probability [1] for Stargardt disease:			
1. An *ABCA4* hypomorphic Asn1868Ile variant detected	0.10*	0.08**	
2. A 2nd *ABCA4* loss-of-function variant NOT detected	0.20*	0.99	
3. No ABCA4 variant reported (despite finding Asn1868Ile)			0.10*/0.91
Joint probability:			
Case A	0.0190	0.0040	0.095/0.046
Case B	0.0002	0.0784	
Posterior probability:			
Case A: 0.0190 / (0.0190 + 0.0040)	0.826	0.174	
Case B: 0.0002 / (0.0002 + 0.0784)	0.003	0.997	
Case if no report of Asn1868Ile (0.095 / (0.095 + 0.046)			0.67

[1] Conditional probability data based on *Zernant et al (see ref's) and **gnomAD (2 x minor allele frequency)

Carrier of the *F5* Leiden «mutation»

F5(NM_000130.4) c.1691G>A, Arg506Gln **Monoallelic variant**

gnomAD MAF 5% (Europeans), i.e. ˜10% are carriers
Functional assay: GoF variant due to resistance to activated protein C (APC)
Literature: DVT associated, heterozygosity increases thrombosis risk ˜3 times.

ACMG: PS3 (function) PS4 (prevalence) PP1 (co-segregation) BA1 (gnomAD) = a VUS

ABC: A-5 (FE) + B-2 (risk factor) = class D (6-7),
Report step C: RISK FACTOR and report if relevant clinic / normal if incidental finding

.arr[GRCh37] 9q21.31(82125508_83332721)x1

Clinic: Girl 11y with feeding difficulties and learning problems. Mother and father also have learning problems, not tested (yet).

Finding: 1,2 Mb deletion removing one gene, *TLE4,* encoding a trancriptional repressor. Nothing in databases (gnomAD, DGV etc), low statistical LoF tolerance: gnomAD pLI = 1, o/e = 0.09.

ACMG:
CNV: 1A (contains a gene) 2H (HI gene) 5F (unknown inh.) = 0.15
SNP: PVS1 (deletion) PM2 (gnomAD) = LP, but the GUS makes it a VUS.

ABC: A-5 (FE) + B-1 (right type of gene) = C class D and a VOI comment.

Extensive IBD in first child of first cousins

Clinic: Girl 2y with severe NDD with hypotonia, bad epilepsy, dysmorphic face, normal HC and brain-MRI.

Finding: SNP array: 180 Mb of ROH (runs of homozygosity) >5Mb / 10 chromosomes.

ACMG: Cannot be classified

ABC: A-3 (HFE) + B-2 (risk factor) = C class E and a RISK FACTOR comment, further clinical evaluation/laboratory testing (NGS) could be indicated.

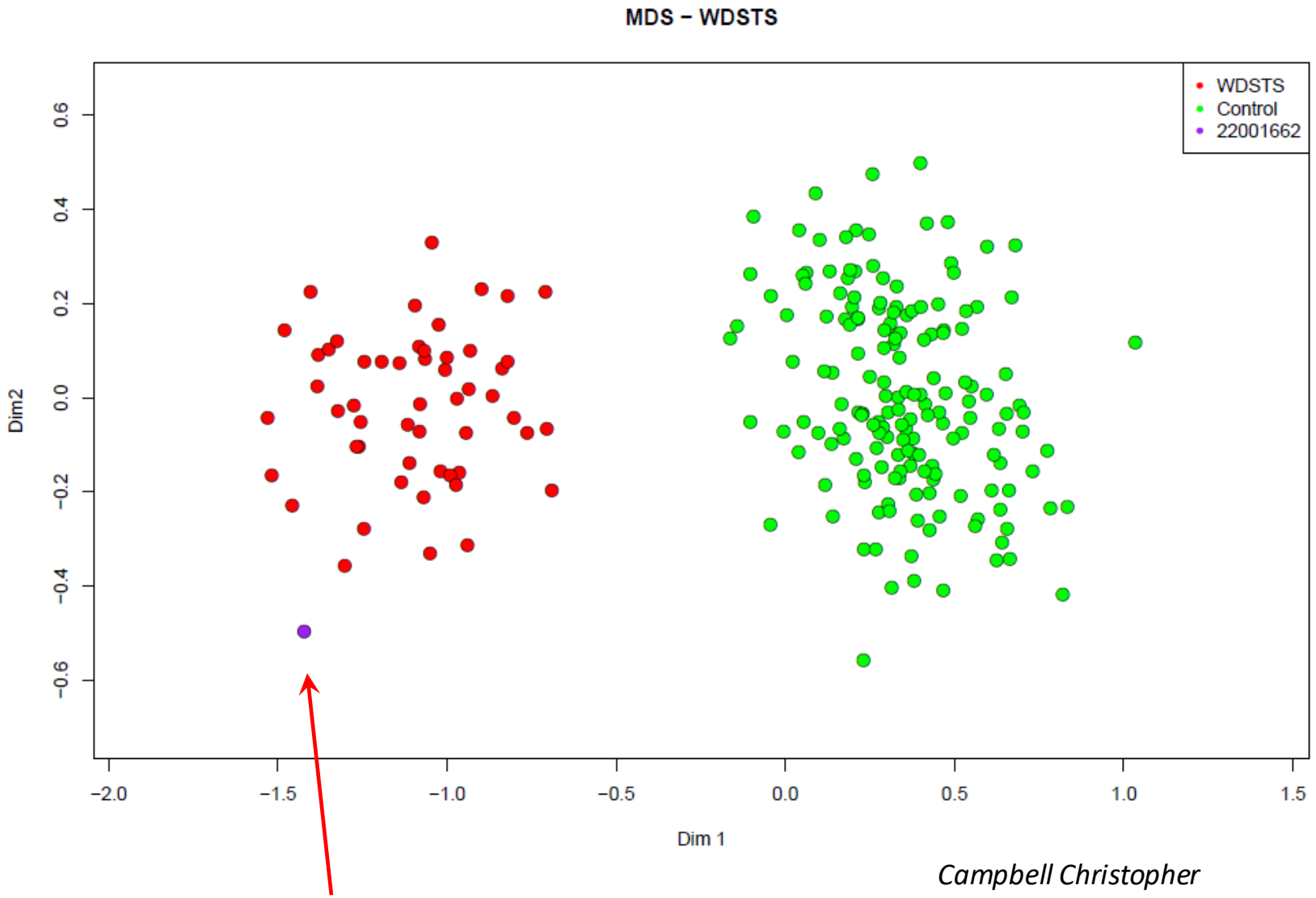
EpiSign methylation signature

Clinic: Boy 8 mo with feeding difficulties (needed PEG), NDD with hypotonia, and short stature.

Finding: High-resolution copy number array and TRIO-WES normal. EpiSign methylation profile suggested Wiedemann-Steiner (WSS) syndrome.

ACMG: Cannot be classified.

ABC: A-3 (HFE) + B-1 (match) = C class E and pick of a VOI comment.



After the EpiSign result, inspection of the NGS BAM file revealed a de novo

NM_001197104.1(*KMT2A*) c.3648dupA p. (Glu1217Argfs*5)

confirming the WSS diagnosis

Campbell Christopher
Banka Siddharth
Manchester University NHS FT

The ABC classification system of variants/findings

- A logical, two-step **A+B grading**
- Any type of (epi)genetic finding can be classified
- Hypomorphic and low penetrant alleles are not labelled as «VUS»
- Can incorporate ACMG points and gene-specific criteria (like VCEP recommendations)
- Can incorporate all desired computer/AI-based systems, but avoid double counting
- **In step C** a standard comment adapted to the clinical question is picked
- Findings that are not pathogenic are not labelled pathogenic
- Classification can be done by one (CLG), two (CLG+MD/GC) or many (MDT) persons

Take home message # 1

Clinical information is essential for variant classification because

1) it provides the question – that could guide variant reporting
2) it increases variant pick-up rate

SolveRD: Pick-up rate increased from 50% to 70% by 2-level expert review:
First molecular, then clinical (data analysis + data interpretation task forces)

Take home message # 2

The use of the word pathogenic should correlate with the actual risk of developing disease.

Variant classification and reporting in the future

A

CLG does
Functional grading

As computerized as possible:
ACMG scoring (points)
AI-based tools
Other prediction tools

Grade 0 to 5
(the 0's will mostly be outside the exome)

B

CLG/GC/MD/MDT does
Clinical grading

Clinical fit?
Right type of gene?
Tools linking genotype to phenotype (Exomiser etc)

Grade 0 to 3 (sometimes 4-5)
NO match (or unknown)
VOI - Variant-of-Interest
RISK FACTOR
PATH – Pathogenic

C

CLG/GC/MD/MDT selects
A standard comment

Dependent on clinical question

Not the same as a clinical report

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European Journal of Human Genetics (2022) 30:150–159
https://doi.org/10.1038/s41431-021-00903-z

ACKNOWLEDGEMENTS

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ARTICLE

Check for updates

Stepwise ABC system for classification of any type of genetic variant

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Received: 22 July 2020 / Revised: 13 February 2021 / Accepted: 22 April 2021 / Published online: 13 May 2021

Now in EJHG**: Comparison of the ABC and ACMG systems for variant classification**
- a truly collaborative effort of 49 authors from 43 laboratories (Open Access)

See www.eshg.org under News for an updated version, including presentations