Interpretation and classification of genetic variants

Gunnar Douzgos Houge MD PhD

ESHG
EUROPEAN SOCIETY OF HUMAN GENETICS

Take home message # 1

Clinical information is important for variant classification

- **and even more for variant reporting** - or not

SolveRD: Pick-up rate increased from 50% to 70% by 2-level expert review (data analysis task force + data interpretation task force)

Two general systems for variant classification

On step system

ACMG/AMP – clinical importance likelihood

P – Pathogenic	**5**
LP – Likely Pathogenic	**4**
VUS – Variant of Unknown Significance	**3**
LB – Likely Benign	**2**
B – Benign	**1**

Classification does not consider penetrance and is not a severity scale

Two step system (A+B) + standard comment C

ABC step A – functional effect likelihood

FE – Functional Effect
LFE – Likely Functional Effect
HFE – Hypothetical Functional Effect
LNF – Likely Normal Function
NF – Normal Function

Step A grading only considers gene- or protein function – not clinical information or importance

The ACMG/AMP system: Criteria based (29)

P	(5) - Pathogenic
LP	(4) - Likely pathogenic (>90% / >95% for cancer)
VUS	(3) - Variant of unknown significance
LB	(2) - Likely benign (>90% / >95% for cancer)
B	(1) - Benign

The system is well suited for dominant single-gene disorders of high penetrance.

Hypomorhic alleles and **low penetrant variants** are often classified as a VUS – despite the significance being well known.

How can a known pathogenic variant be classified as a VUS? Because the black box says so...

Genetics inMedicine | ORIGINAL RESEARCH ARTICLE

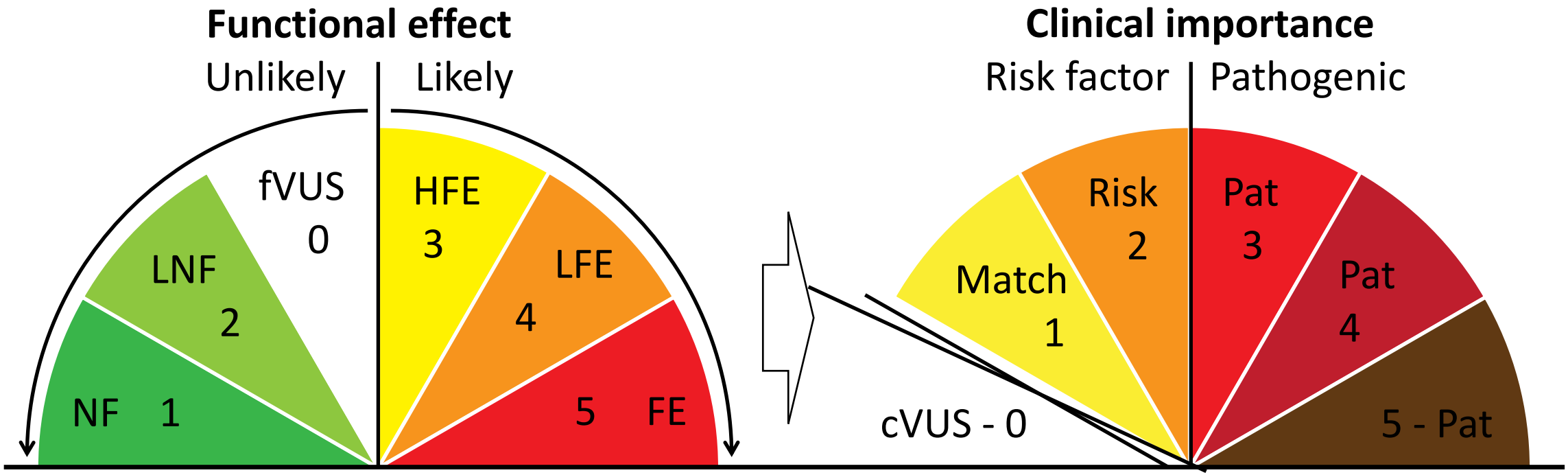
Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework

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)*

Unlike ACMG, the ABC system can classify any type of variant



Step A: Functional grading

1 - NF = Normal Function
2 - LNF = Likely Normal Function
0 - fVUS = functional VUS

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

Step B: Clinical grading

0 - cVUS = clinical VUS
1 - Match = right type of gene for this phenotype
2 - Risk = known risk factor / variant-of-interest

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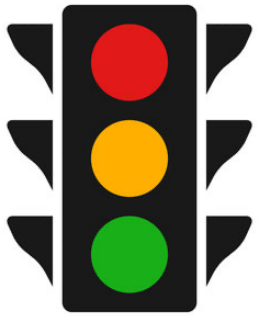
3 - Pat = pathogenic variant,
4 penetrance-graded when known
5

Severity classification:
Combined grade (A+B) classifies the variant from F to A:

Class		
0	Step A 0-2 + no step B = 0-2	Not reported – and clinical grading unnecessary
F	Step A + step B = **3**	Not reported
E	Step A + step B = **4-5**	Variant-of-interest (VOI) group, reporting optional
D	Step A + step B = **6-7**	Risk factor (RF) group, reporting recommended if clinical match
C	Step A + step B = **8**	Pathogenic (P), unknown or low (lifetime <20%) penetrance
B	Step A + step B = **9**	Pathogenic (P), moderate penetrance (lifetime 20-40%)
A	Step A + step B = **10**	Pathogenic (P), high penetrance (lifetime >40%)
X	Step A + step B = 5-10	Secondary/incidental/unsolicited/opportunistic finding

Based on F to A class, a standard comment is picked in step C:



ABC step C

standard variant comments adapted to the clinical question

A+B grade 0-2:

0 NORMAL findings

A+B grade 3-7, i.e. class F (3), E (4-5) and D (6-7):

1 NORMAL findings – no pathogenic or likely pathogenic variants were detected

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

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

4 VOI – A genetic variant of potential interest was detected

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

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

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

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

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

10 PATH – Likely compound heterozygosity for recessive pathogenic variants was detected

11 PATH – Homozygosity for a recessive pathogenic genetic variant was detected

A+B grade 8-10, i.e. class C (8), B (9) and A (10):

12 PATH – Heterozygosity for a dominant likely pathogenic variant was detected

13 PATH – Heterozygosity for a dominant pathogenic variant was detected

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

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

Incidental/unexpected findings (class X):

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

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

Heterozygosity for the *F5* Leiden «mutation»

F5(NM_000130.4):c.1691G>A, Arg506Gln **Monoallelic variant**
gnomAD MAF up to 5%
Functional assay: LoF allele due to resistance to activated protein C (APC resistance)
Literature: DVT associated, increases thrombosis risk ˜3 times

ACMG: PS3 PS4 PP1 BA1 = a VUS

ABC: A-5 (FE) + B-2 (risk) = 7 (class D).
Step C is either C-RF if relevant clinic or C-NORM if incidental finding.

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ESHG

ARTICLE

Check for updates

Stepwise ABC system for classification of any type of genetic variant

Gunnar Houge[1] · Andreas Laner[2] · Sebahattin Cirak[3] · Nicole de Leeuw[4] · Hans Scheffer[4] · Johan T. den Dunnen[5]

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Suggestion for integration of ACMG criteria in the ABC system:

Step A

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

Step B

cVUS	BS4, no clinical match, or no clinical Information	
VOI	gene fits phenotype	
RISK FACTOR	PM3 PP4 PP5	1 criterium is enough to grade as RF
PATH	known pathogenic (AR or AD)	
PATH	known pathogenic (AD, mod.)	
PATH	known pathogenic (AD, high)	

Points to remember

Variant classification (A+B grade) does *not* depend on the clinical question

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 above step B grade 2 (= RF - risk factor)

Standard variant comment selection (step C) depends on the clinical question

This allows reporting of a hypomorphic or low penetrant allele when relevant

– otherwise not

The ABC system is also well suited for rare diseases

Rare diseases may be associated with

- *De novo* variants in a disease-associated or novel gene
- Runs-of-homozygosity (ROH)
- Biallelic variants in a disease-associated or novel gene
- Monoallelic variants in a known recessive gene
- Chromosomal aberrrations (CNVs, SVs)
- Epigenetic changes

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: 121 Mb of ROH (runs of homozygosity) >5Mb divided on 10 chromosomes.

ACMG: Cannot be classified

ABC: A-3 (HFE) + B-2 (RF) = class E and a C-Risk Factor that was reported.

If the phenotype had been normal (e.g. a screening test of a fetus), the comment would also have been normal, i.e. no findings.

Same patient:

Homozygous NM_001382273.1(*TNK2*):c.622G>C p.(Ala208Pro)

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

Finding: Homozygous missense change to prolin of a conserved Ala in a conserved exon encoding the kinase domain of a cytosolic tyrosine kinase with high brain expression.

gnomAD pLI = 0, z = -0.2, but no homozygous LoFs in gnomAD.

ACMG: PM1 + PM2 + PP3 = VUS. Report?

ABC: A-4 (LFE) + B-1 (match) = class E and a C-VOI that was database registered and reported.

Heterozygous NM_018249.6(*CDK5RAP2*):c.3890del p.(Gly1297Valfs*6)

Clinic: Boy 3y with ASD, progressive microcephaly (- 4.7 SDS), deafness and right-side aortic arch. Brain-MRI: Small but otherwise normal brain.

Finding: Nucleotide deletion causing frameshift and predicted LoF of *CDK5RAP2,* a recessive microcephaly gene. gnomAD pLI = 0, z = -0.37.

ACMG: PM2 (not in gnomAD) = VUS. Report?

ABC: A-4 (LFE) + B-2 (risk) = class D and a C-RF that was reported because the recessive disease was a good match.

WGS later revealed a 7 kb deletion including the first *CDK5RAP2* exons but not the variant.

46,XX.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 with high brain expression. Nothing in databases (gnomAD, DGV etc), but a de novo duplication of *TLE4* registered in DECIPHER without phenotype data. gnomAD pLI = 1, z = 3.6.

ACMG: 1A (contains a gene) 2H (HI gene) 5F (unknown inheritance) = 0.15

ABC: A-3 (HFE) + B-1 (match) = class E and a C-VOI that was reported because the phenotype could be a match.

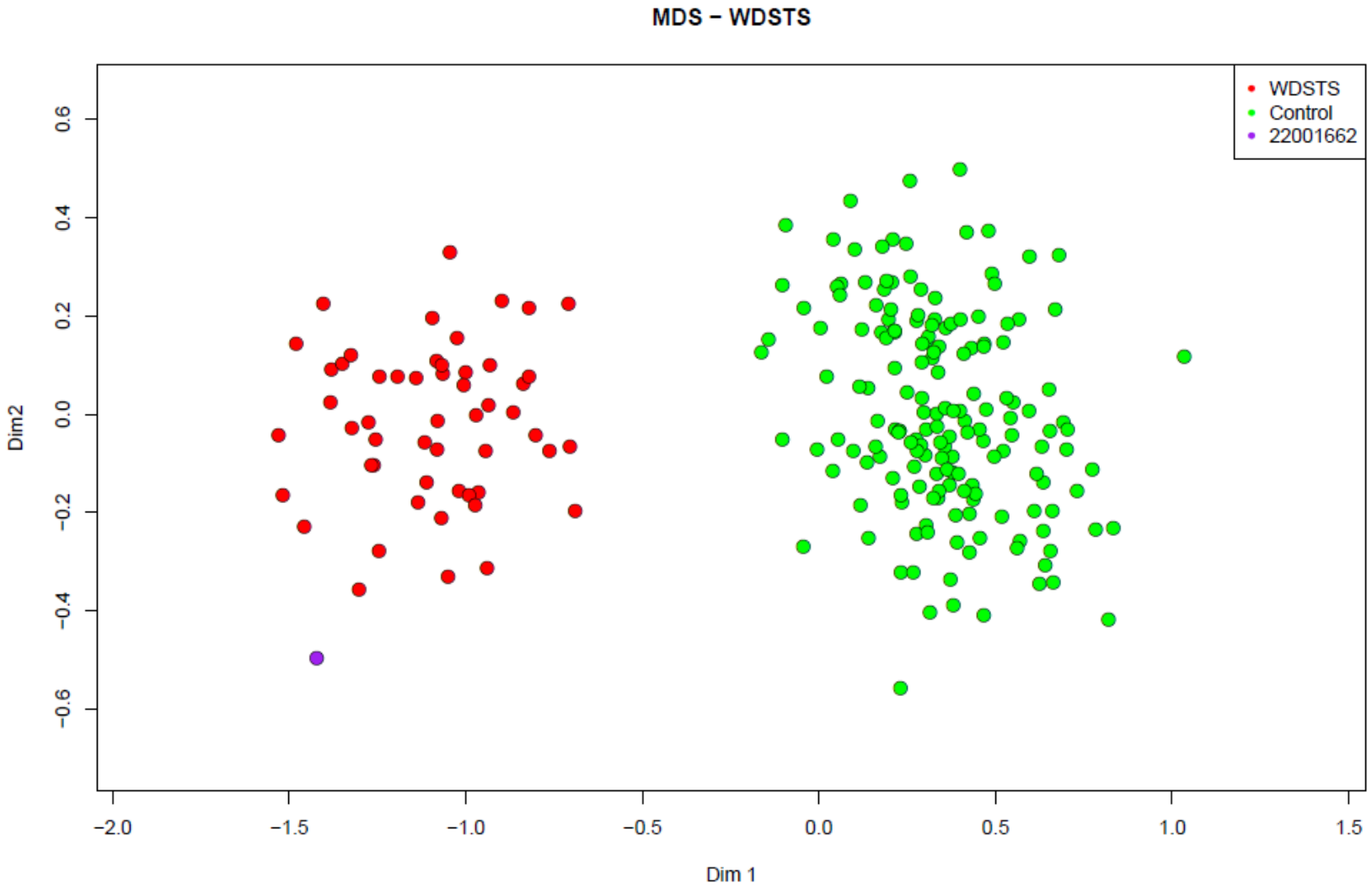
EpiSign methylation signature

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

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

ACMG: Cannot be classified.

ABC: A-3 (HFE) + B-1 (match) = class E and a C-VOI that was reported because of good clinical match.



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 diagnosis

Campbell Christopher
Banka Siddharth
Manchester University NHS FT

ABC system

- Intuitive use with a logical two-step grading (A+B leading to class A-F)
- Can classify all types of genetic findings
- Hypomorphic alleles are not labelled as a «VUS»
- Findings that are not pathogenic are not labelled «pathogenic»
- Can accomodate gene-specific or ACMG criteria if desired
- Provide a list of standard comments adapted to the clinical question
- Classification can be done by one (CLG/MD) or two (CLG+MD) persons