Specification of ACMG/AMP guidelines for variant interpretation in Familial Hypercholesterolemia

Joana R. Chora^{1,2}, Michael A. Iacocca³, Alain Carrié^{4,5}, Sarah E. Leigh⁶, Lukáš Tichý⁷, C. Lisa Kurtz⁸, Tomas Freiburger^{9,10}, Eric J. Sijbrands¹¹, Robert A. Hegele³, Joshua W. Knowles^{12,13}, Mafalda Bourbon^{1,2}.

¹Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal. ²Biosystems and Integrative Sciences Institute, Lisbon, Portugal. ³Western University, London ON, Canada. ⁴Hôpitaux Universitaires Pitié-Salpêtrière/Charles-Foix, Paris, France. ⁵Sorbonne Université, Paris, France. ⁶Genomics England, London, United Kingdom. ⁷Center of Molecular Biology and Gene Therapy, University Hospital Brno, Brno, Czech Republic. ⁸University of North Carolina, Chapel Hill NC, USA. ⁹Centre for Cardiovascular Surgery and Transplantation, Brno, Czech Republic. ¹⁰Masaryk University, Brno, Czech Republic. ¹¹Erasmus University Medical Center, Rotterdam, The Netherlands. ¹²Stanford University, Palo Alto CA, USA. ¹³FH Foundation, Pasadena CA, USA.

Background and Aims

The general ACMG/AMP guidelines for standardized variant interpretation in Mendelian disorders are a great asset in determining variants' pathogenicity, but need to be adapted to each specific gene and disease.

Methods

In 2016, the Clinical Genome Resource consortium (ClinGen) familial hypercholesterolemia variant curation expert panel (FH-VCEP) was created with the goal to specify the guidelines for *LDLR*, *APOB* and *PCSK9* in FH. The panel of international FH experts proposed and voted on a draft of specifications, which were tested on known variants. Final adjustments were made based on the pilot study data.

Population data

For MAF values, use the population with the maximum value in the Genome Aggregation Database (gnomAD).

- MAF > 0.005 (0.5%) → **STAND-ALONE (BA1)**
- MAF > 0.002 (0.2%) → **STRONG (BS1)**
- MAF < 0.0003 (0.03%) → **MODERATE (PM2)**

Variant identified in a panel of well-phenotyped normolipidemic controls (minimum of 100 alleles) → **STRONG (BS2)**

Protein specific

MODERATE (PM1), Mutational hot spot

All variants in exon 4, except synonymous **MODERATE (PM4)**, Protein length changes

In-frame indel smaller than whole exon, plus all whole-exon duplications. Variant must meet PM2 criterion.

Cis/trans variants

When case-level data is available, the identification of a novel variant in a patient:

- With homozygous FH phenotype (untreated LDL-C ≥ 13 mmol/L or ≥ 500 mg/dl) but only one heterozygous proven pathogenic variant in *LDLR*, *APOB* and *PCSK9*. Novel variant must meet PM2 criterion → **MODERATE (PM3)**
- With heterozygous FH phenotype (untreated LDL-C < 13 mmol/L or < 500 mg/dl) and one heterozygous proven pathogenic variant in *LDLR*, *APOB* and *PCSK9* → **SUPPORTING (BP2)**

Not applicable

Previous report (**PP5/BP6**); *in silico* in synonymous (**BP7**); repetitive regions (**BP3**); missense in gene with low rate of missense variants (**PP2**); other mechanisms (**BP1/BP5**)

With thanks to the EAS for support in the form of a Young Investigator Fellowship. The contents of this poster are presented on behalf of the ClinGen FH Variant Curation Committee. JR Chora is funded by SFRH/BD/108503/2015. ClinGen is primarily funded by the National Human Genome Research Institute (NHGRI) (grants U41HG006834, U41HG009649, U41HG009650). ClinGen also receives support for content curation from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) (grants U24HD093483, U24HD093486, U24HD093487). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Same amino acid

Novel missense variant at the same codon as a pathogenic (FH-VCEP) variant and leads to:

- same amino acid → **STRONG (PS1)**
- different amino acid → **MODERATE (PM5)**

De novo

De novo in a patient with the disease and no family history:

- maternity and paternity confirmed → **STRONG (PS2)**
- Assumed *de novo* → **MODERATE (PM6)**

In silico prediction

If both missense and splicing prediction are applicable, only one is needed for **PP3** but both are needed for **BP4**. Do not apply if PVS1 is given.

For missense prediction: Use REVEL

- scores ≥ 0.75 → **SUPPORTING (PP3)**
- scores < 0.5 → **SUPPORTING (BP4)**

For splicing prediction: Use MaxEntScan (MES) and Human Splicing Finder (HSF):

- i) authentic splice site strength
 - variant/wt score in MES < 0.8 AND in HSF < 0.96 → **SUPPORTING (PP3)**
 - variant/wt score in MES AND HSF ≥ 1.0 → **SUPPORTING (BP4)**
- ii) exonic *de novo* splice site strength
 - *de novo* site/authentic site score in MES AND HSF ≥ 0.9 → **SUPPORTING (PP3)**
 - *de novo* site/authentic site score in MES AND HSF < 0.8 → **SUPPORTING (BP4)**

Results

Here we present the final FH-VCEP proposed guidelines for standardized FH variant interpretation, although some criteria are still under discussion (!). After debating the pilot study results and the three genes different characteristics, it was decided to focus the full FH-specific guidelines on *LDLR* alone and set some recommendations on how to interpret *APOB* and *PCSK9* variants.

Functional data

STRONG (PS3/BS3)

- Study of *LDLR* whole cycle in true homozygous patient cells or heterologous cells transfected with the mutant plasmid.
 - < 70% of wild-type in expression, binding OR internalization → **PS3**
 - > 90% of wild-type in expression, binding AND internalization → **BS3**
- RNA studies (RNA from patient's cells) with aberrant transcripts quantification. Aberrant transcript is proved by sequencing and is:
 - > 67% of wild-type transcript → **PS3**
 - < 33% of wild-type transcript → **BS3**
- Luciferase studies. Transcription levels:
 - < 25% of wild-type → **PS3**
 - > 90% of wild-type → **BS3**

MODERATE (PS3_Moderate)

- Study of only part of the *LDLR* cycle in true homozygous patient cells or in heterologous cells transfected with the mutant plasmid.
 - < 70% of wild-type in expression, binding OR internalization → **PS3_Moderate**
- Minigene splicing assay. Aberrant transcript is proved by sequencing and:
 - no wild-type transcript present → **PS3_Mod**
- Variants with Level 3 functional studies performed by two or more independent labs, with concordant results (must be different assays)

SUPPORTING (PS3_/BS3_Supporting)

- Study of *LDLR* cycle in heterozygous patient cells
 - < 85% of wild-type in expression, binding OR internalization → **PS3_Supporting**
 - > 95% of wild-type in expression, binding AND internalization → **BS3_Supporting**
- RNA studies without transcript quantification
 - an aberrant transcript is identified by sequencing → **PS3_Supporting**
 - no aberrant transcripts are identified by sequencing → **BS3_Supporting**
- High-throughput microscopy; Multiplex Assays of Variant Effect (MAVE); deep mutational scanning assays.

Phenotype

Variants identified in FH patients, diagnosis based on any validated clinical criteria, ex. Dutch Lipid Clinic Network (≥6), Simon Broome (possible/definite), MEDPED; after alternative causes of high cholesterol are excluded → **SUPPORTING (PP4)**

Conclusions

After ClinGen approval of these final guidelines, the FH-VCEP will curate all FH variants in ClinVar at a 3-star level, contributing to standardized FH diagnosis worldwide and ultimately improve patient management and prognosis.

Loss of function

VERY STRONG (PVS1)

- (1) deletion of full gene;
- (2) Null variants predicted to undergo NMD: i) nonsense/ frameshift; ii) GT--AG 1,2 splice sites that lead to out of frame consequence; iii) deletion of single or multiple exons that lead to out of frame consequence; iv) intragenic duplications proven to occur in tandem, that lead to out of frame consequence

STRONG (PVS1_Strong)

- (1) Remaining null variants: i) nonsense/ frameshift; ii) GT--AG 1,2 splice sites; iii) deletions of single or multiple exons;
- (2) intragenic duplications presumed to occur in tandem, that lead to out of frame consequence predicted to undergo NMD.

MODERATE (PVS1_Moderate)

- (1) variant in initiation codon

Prevalence

Variants in unrelated cases with FH diagnosis Preferably, case data from ClinVar. Variant must meet PM2 criterion

STRONG (PS4)

- 15 or more unrelated cases

MODERATE (PS4_Moderate)

- 6 to 14 unrelated cases

SUPPORTING (PS4_Supporting)

- 2 to 5 unrelated cases

Segregation

Co-segregation of the variant with the phenotype in families. Co-seg = affected relatives (LDL-C > 75th percentile) are carriers and unaffected relatives are not carriers

STRONG (PP1_Strong/BS4)

- Co-seg in > 7 informative meioses → **PP1_Strong**
- Lack of co-seg in > 2 informative meioses (Beware of alternative causes) → **BS4**

MODERATE (PP1_Moderate)

- Co-seg in 5-6 informative meioses

SUPPORTING (PP1)

- Co-seg in 3-4 informative meioses