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DEVELOPMENT OF GENE-SPECIFIC ACMG/AMP GUIDELINES FOR THE INTERPRETATION OF *APOB* AND *PCSK9* VARIANTS IN FAMILIAL HYPERCHOLESTEROLEMIA

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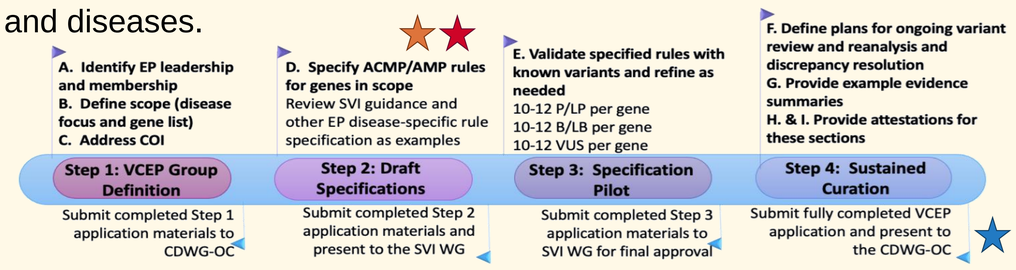
Background and Aims

The general ACMG/AMP guidelines for standardized variant interpretation provide a critical framework for determining pathogenicity but require adaptation to specific genes and diseases.



While ***LDLR***-specific guidelines for familial hypercholesterolemia (FH) have been in use since 2020, similar adaptations for ***APOB*** and ***PCSK9*** are needed to address the unique characteristics of these genes in FH diagnosis.

Methods

The Clinical Genome Resource (ClinGen) consortium's FH variant curation expert panel (FH VCEP) expanded its efforts to develop tailored guidelines for *APOB* and *PCSK9*. A panel of international FH experts proposed and voted on specifications for these genes, based on current evidence. These adaptations were compared to *LDLR*-specific criteria for consistency and refined based on gene-specific attributes.

Results

The proposed guidelines address the unique features of *APOB* and *PCSK9* variants (in **orange** and **red**, respectively). Although several criteria were specific for each gene, some of the criteria were considered to be applicable in the same way for the 3 FH genes, *LDLR*, *APOB* and *PCSK9* (in **green**).

LDLR

PVS1: loss of function

PS3/BS3: Functional studies
Level 1: Heterologous cells, whole cycle, quantification of mRNA, luciferase assays
Level 2: Hmz patient cells, minigene splicing assay
Level 3: Htz patient cells, RNA, high-throughput assays

Variant frequency in gnomAD
PM2: PopMax MAF ≤0.02%; **BA1**: PopMax FAF ≥0.5%; **BS1**: PopMax FAF ≥0.2%

Segregation
PP1_S: co-seg in ≥ 6 informative meiosis;
PP1_M: in 4-5; **PP1**: in 2-3
BS4: lack of seg in ≥2 families with ≥2 informative meioses each

Protein specific
PM1: missense in exon 4 or alter Cys residue
PM4: in frame indels
BP3: N/A

Cis/trans variants
PM3: variant in a patient with Hmz phenotype and only 1 pathogenic variant
BP2: Htz phenotype and already 1 pathogenic var

BS2: identified in ≥3 Htz or ≥1 Hmz normolipidemics

PP3/BP4/BP7: *in silico* prediction
REVEL (≥0.75 0r ≤0.5) and MaxEntScan for splicing

APOB

PVS1: N/A - LoF not mechanism for FH

PS3/BS3: Functional studies
Level 1: level 2 + level 3 assays, concordant
Level 2: binding and uptake assays with normal LDLR and patient LDL particles, 2 level 3 assays
Level 3: proliferation assays with patient LDL particles, ELISA assays

Variant frequency in gnomAD
PM2: PopMax MAF ≤0.03%; **BA1**: PopMax FAF ≥0.5%; **BS1**: PopMax FAF ≥0.2%

Segregation
PP1_S: co-seg in ≥ 6 informative meiosis;
PP1_M: in 4-5; **PP1**: in 2-3. only affected relatives
BS4: N/A

Protein specific
PM1: missense in relevant residues for structure and binding in exons 26 and 29
PM4: N/A
BP3: N/A

Cis/trans variants
PM3: variant in a patient with Hmz phenotype and only 1 pathogenic variant
BP2: N/A

BS2: N/A

PP3/BP4/BP7: *in silico* prediction
N/A

PCSK9

PVS1: N/A - LoF not mechanism for FH

PS3/BS3: Functional studies
Level 1: Biochemical study of PCSK9 concentration
Level 2: ELISA assays
Level 3: high-throughput assays

Variant frequency in gnomAD
PM2: PopMax MAF ≤0.001%; **BA1**: PopMax FAF ≥0.02%; **BS1**: PopMax FAF ≥0.01%

Segregation
PP1_S: co-seg in ≥ 6 informative meiosis;
PP1_M: in 4-5; **PP1**: in 2-3
BS4: lack of seg in ≥2 families with ≥2 informative meioses each

Protein specific
PM1: N/A
PM4: N/A
BP3: repetitive Leu residues

Cis/trans variants
PM3: variant in a patient with Hmz phenotype and only 1 pathogenic variant
BP2: N/A

BS2: identified in ≥3 Htz or ≥1 Hmz normolipidemics

PP3/BP4/BP7: *in silico* prediction
N/A

LDLR, APOB and PCSK9

PS1/PM5: New missense at the same codon as pathogenic variant

PS2/PM6: *de novo* occurrence, follow SVI guidance

PP4: identified in an index patient with clinical FH criteria

Not applicable

PP5/BP6: Previous report;
BP1/BP5: Other mechanisms;
PP2: Missense in gene with low rate of missense variants

Identified in unrelated index cases: Established clinical criteria = 1 point, DLCN 3-5, LDL>95th percentile = 0.5 points, included in FH cohort = 0,25 points.
PS4: ≤10 points, **PS4_M**: 6-9 points, **PS4_P**: 2-5 points

Conclusions

Following ClinGen approval, these gene-specific recommendations will enable high-confidence classification of *APOB* and *PCSK9* variants in ClinVar, enhancing the accuracy of FH diagnosis and supporting personalized management strategies for patients worldwide.

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