Ambry Genetics®

Ambry Genetics® General Variant Classification Scheme

Classification Minimum Threshold	ACMG Code	Criteria
Pathogenic Variant 5B	PVS1	Alterations impacting or resulting in nonsense, reading frameshift, 3' truncations, elongations, gross deletions, gross duplications, and initiation codon
	PVS1	Canonical donor/acceptor splice sites (+/- 1, 2) or Last nucleotide of exon
	PS1	Same amino acid change as VLP/P regardless of nucleotide change
	PS2 & PM6	Confirmed or unconfirmed *de novo* alteration
	PS3	Significantly altered protein function in appropriate functional assay(s)
	PS3_RNA	Functionally-validated splicing variant
	PS4_PC	Proband Counting for unrelated affected individuals without a clear VLP/P
	PS4_CC	Significant disease association in appropriately sized case-control study(ies)
	PP4	Proband specific phenotype *in vivo* functional data
Variant, Likely Pathogenic 3B	PM1	Located at a position or in a region critical for protein function
	PM2	Rarity in general population databases
	PM3	Recessive disorders, detected *in trans* with a VLP/P or homozygous in affected individuals
	PM4	In-frame insertions/deletions in a non-repetitive region or Protein length changes in non-LOF genes
	PM5	Different missense variant at same amino acid position as VLP/P
	PM5_RNA	Different splicing variant at same splice site as VLP/P
	PP1	Cosegregation with disease in affected family members
	PP2	Missense Constraint - missense variant in a region of the gene that has a low rate of benign missense variation
	PP3	*In silico* model predicts deleterious
VUS	Insufficient or Conflicting Evidence	
Variant, Likely Benign 1D	BA1 & BS1	General population or subpopulation frequency is too high to be pathogenic based on disease prevalence and penetrance
	BS2	Observed in unaffected individual(s) for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder
	BS3_RNA	Intronic alteration with no splicing impact by RNA analysis
	BS3	Intact protein function observed in multiple appropriate functional assays
	BS4	Lack of segregation in affected members of a family
	BP1	Mechanism of disease is inconsistent with known cause of pathogenicity
	BP2	Co-occurrence with VLP/P in same gene providing alternate molecular basis for disease
	BP5	Co-occurrence with VLP/P in different gene providing alternate molecular basis for disease
Benign Variant 1F	BP3	In-frame insertions/deletions in a repetitive region without a known function or association with disease
	BP4_Ref	Amino acid seen as reference
	BP4	*In silico* model predicts benign
	BP7	A synonymous or specified intronic variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site
	A_BP8	Not predicted to impact specific critical structural or functional features
	A_BP9	No disease association in case-control study(ies)

Weight range: Pathogenic (5B-1C), Benign (1D-1F)
Codes denoted "A_" have been added as Ambry Genetics specific codes following the ACMG numbering.

The criteria in the classification scheme are to be applied to variants in genes with moderate, strong, or definitive Gene-Disease validity.

The variant classification scheme is not intended for the interpretation of alterations considered epigenetic factors including genetic modifiers, multifactorial disease, or low-risk disease association alleles and may be limited in the interpretation of alterations confounded by incomplete penetrance, variable expressivity, phenocopies, triallelic or oligogenic inheritance, or skewed X-inactivation.

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