

## Ambry General Variant Classification Scheme

COMBINATION RULES FOR CLASSIFICATION	ACMG CODE	CRITERIA
<b>Pathogenic Variant</b> 4B 3B+2C  <b>Variant, Likely Pathogenic</b> 3B 2B+2C 1B+4C	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 assumed de novo alteration
	PS3	Deficient protein function in appropriate functional assay(s)
	PS3_RNA	Functionally-validated splicing variant
	PS4 & PP4	Detected in individual satisfying established diagnostic criteria for classic disease without a clear VLP/P
	PS4	Significant disease association in appropriately sized case-control study(ies)
	PM1	Located at a position or in a region critical for protein function
	PM2	Rarity in general population databases
	PM3	AR disorders, detected in trans with a VLP/P or homozygous in affected individuals
	PM4	In-frame insertions/deletions in a non-repetitive region
	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
	no code	Alteration identified in the absence of any other coding sequence VLP/P ascertained in a highly unbiased cohort
<b>VUS</b>	<b>Insufficient or Conflicting Evidence</b>	
<b>Variant, Likely Benign</b> 1D 2E	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
<b>Benign</b> 1F 2D 1D+2E 4E	BP5	Co-occurrence with VLP/P in different gene providing alternate molecular basis for disease
	BP3	In-frame insertions/deletions in a repetitive region without a known function or association with disease
	BP4	In silico model predicts benign
	BP4	Amino acid seen as reference in majority of species
	BP7	A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site
	no code	No disease association in case-control study(ies)

Weight range: Pathogenic (1A-1C), Benign (1D-1F)

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.