

## Ambry Genetics® General Variant Classification Scheme

Combination Rules For Classification	ACMG Code	Criteria	
Pathogenic Variant 1A 4B 3B+2C	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 <i>de novo</i> 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 <i>in vivo</i> functional data	
	Variant, Likely Pathogenic 3B 2B+2C 1B+4C	PM1	Located at a position or in a region critical for protein function
		PM2	Rarity in general population databases
		PM3	AR disorders, detected <i>in trans</i> 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
		PM5_PTC	Truncating VLP/P variant downstream of the PTC
		PP1	Cosegregation with disease in affected family members
	VUS	PP2	Missense Constraint - missense variant in a region of the gene that has a low rate of benign missense variation
PP3		<i>In silico</i> model predicts deleterious	
A_PP6		Alteration identified in the absence of any other coding sequence VLP/P ascertained in a highly unbiased cohort	
Insufficient or Conflicting Evidence			
Variant, Likely Benign 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	
	BP5	Co-occurrence with VLP/P in different gene providing alternate molecular basis for disease	
Benign Variant 1F 2D 1D+2E 4E	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	<i>In silico</i> 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 (1A-1C), Benign (1D-1F)

Codes denoted "A\_" have been added as Ambry Genetics specific codes following the ACMG numbering.

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.