Ambry Genetics® | Ambry Classifi™

# Ambry Genetics® Variant Classification Scheme

The Ambry Classifi™ program is the way we actualize our promise to provide genetic test results of unparalleled quality. A cornerstone of the Classifi program is the Ambry Genetics Variant Classification Scheme, which is a points-based framework that allows for a clear understanding of a variant's classification. Ambry is dedicated to routinely updating our variant classification scheme to reflect published recommendations and scientific data to drive accurate variant interpretation and deliver high-confidence classifications.

| Classification Score | | | | |
|---|---|---|---|---|
| **Benign**<br>**≤ -4 points** | **Likely Benign**<br>**-3 to -2 points** | **Uncertain Significance**<br>**-1 to +5 points** | **Likely Pathogenic**<br>**+6 to +9 points** | **Pathogenic**<br>**≥ +10 points** |

| | Point Range | ACMG Code | Criteria |
|---|---|---|---|
| CLINICAL | 0.25 to 10.00+ | PS2 & PM6 | ***De novo* Variant**<br>(with confirmed or unconfirmed parental relationships) |
| | 4.00 | PS4_CC | **Case-Control Studies**<br>(significant disease association) |
| | 0.25 to 10.00+ | PS4_Fhx | **Family History Data**<br>(internal data modeling) |
| | 0.25 to 10.00+ | PS4_PC | **Proband Counting**<br>(unrelated affected individuals without an alternate molecular basis for disease) |
| | 1.00 to 6.00 | PP4 | **Proband *in vivo* Functional Data** |
| | 0.25 to 10.00+ | PM3 | **Pathogenic Co-occurrence in Recessive Disorders**<br>(in *trans* or homozygous) |
| | 1.00 to 6.00 | PP1 | **Co-Segregation**<br>(with disease in affected family members) |
| MOLECULAR IMPACT | 1.00 to 10.00 | PVS1 | **Loss-of-Function Variant**<br>(nonsense, reading frameshift, 3' truncations, elongations, gross deletions, gross duplications, and initiation codon) |
| | 1.00 to 6.00 | PVS1 | **Loss-of-Function Splicing Variant**<br>(canonical splice sites or last nucleotide of exon) |
| | 4.00 | PS1 | **Same Amino Acid Change**<br>(as an established pathogenic variant at the same position; regardless of nucleotide change) |
| | 1.00 to 8.00 | PS3_RNA | **Functionally-validated Splicing Variant** |
| | 1.00 to 10.00 | PM4 | **In-frame Insertions and Deletions** |
| | 2.00 to 4.00 | PM5 | **Different Amino Acid Change**<br>(as an established pathogenic variant at the same position) |
| | 2.00 to 4.00 | PS5_RNA | **Different Splicing Variant**<br>(as an established pathogenic variant at the same splice site) |
| | 1.00 to 4.00 | PM1 | **Protein Structure** |
| | 1.00 | PP2 | **Missense Constraint**<br>(missense variant in a region of the gene that has a low rate of benign missense variation) |
| | 1.00 to 4.00 | PP3 | ***In silico***<br>(model predicts deleterious) |
| FUNCTIONAL | 1.00 to 10.00 | PS3 | **Functional Assay(s)**<br>(significant altered protein function in appropriate assay(s)) |

| Classification Score | | | | |
|---|---|---|---|---|
| Benign<br>≤ -4 points | Likely Benign<br>-3 to -2 points | Uncertain Significance<br>-1 to +5 points | Likely Pathogenic<br>+6 to +9 points | Pathogenic<br>≥ +10 points |

| | Point Range | ACMG Code | Criteria |
|---|---|---|---|
| CLINICAL | -10.00 to -1.00 | BS2 | **Observed in Unaffected Individual(s)** |
| | -3.00 to -1.00 | BS4 | **Lack of Segregation**<br>(with disease in affected family members) |
| | -3.00 to -1.00 | BP2 | **Same Gene Co-Occurrence**<br>(with pathogenic variant providing alternate molecular basis for disease) |
| | -1.00 | BP5 | **Different Gene Co-Occurrence**<br>(with pathogenic variant providing alternate molecular basis for disease) |
| | -3.00 to -1.00 | A_BP9 | **Case-Control Studies**<br>(no significant disease association) |
| | -10.00 to -1.00 | A_BP10 | **Family History Data**<br>(internal data modeling) |
| MOLECULAR IMPACT | -3.00 to -1.00 | BS3_RNA | **RNA Analysis**<br>(intronic or synonymous alteration with no splicing impact) |
| | -3.00 to -1.00 | BP1 | **Mechanism of Disease**<br>(inconsistent with known cause of pathogenicity) |
| | -2.00 to -1.00 | BP3 | **In-frame Insertions and Deletions** |
| | -3.00 | BP4_Ref | **Reference Amino Acid**<br>(consistent with other species) |
| | -3.00 to -1.00 | BP4 | ***In silico***<br>(model predicts benign) |
| | -1.00 | BP7 | **Synonymous or Specified Intronic Variant Qualifying for BP4** |
| | -1.00 | A_BP8 | **Protein Structure** |
| FUNCTIONAL | -10.00 to -1.00 | BS3 | **Functional Assay(s)**<br>(significant intact protein function in appropriate assay(s)) |
| FREQUENCY | -8.00 to -3.00 | BS1/BA1 | **Population Frequency**<br>(general population or subpopulation frequency too high to be pathogenic based on disease prevalence and penetrance) |

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..

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

Codes denoted “A_” have been added as Ambry specific codes following the ACMG numbering.

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