



## Versiti Diagnostic Laboratories: Hematology Genetics Variant Classification Assertion Criteria

Variants are interpreted in the course of clinical germline genetic testing performed on samples submitted to our specialty hematology reference laboratory. All reported variants are reviewed by a multidisciplinary team of nationally and internationally recognized clinical experts in the non-malignant hematologic disease states (coagulation disorders, neutropenia, thrombocytopenia, platelet function disorders) that are the focus of the Versiti Hematology Genetics diagnostic laboratory service. The team consists of scientific laboratory directors, molecular pathologists, variant interpretation scientists, genetic counselors, bioinformaticians, and clinically practicing academic hematologists. In addition, research and clinical faculty of Versiti's Blood Research Institute (BRI) consult on cases as appropriate. Variants are classified based on the totality of available evidence, which includes not only published medical and scientific literature, but also clinical history, family history, and laboratory phenotype provided for incoming cases through active partnership with referring providers, to optimize interpretation of testing results.

### General Information

When evaluating a variant for pathogenicity, Versiti reviews information that includes, but is not limited to, the following:

1. Gene and disease information such as disease mechanism, disease incidence, phenotypic specificity, expressivity, penetrance, inheritance pattern, functional domains, known hotspots, amino acid conservation etc.
2. Population frequency data, such as:
  - a. gnomAD (<https://gnomad.broadinstitute.org/>)
  - b. ExAC (<http://exac.broadinstitute.org/>)
  - c. dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>)
  - d. Exome Variant Server (<https://evs.gs.washington.edu/EVS/>)
  - e. 1000 Genomes (<https://www.internationalgenome.org>)
  - f. Variant Viewer (<https://www.ncbi.nlm.nih.gov/variation/view/>)
  - g. Internal sequencing data
3. Peer reviewed publications
4. Disease-/gene-specific databases
5. *In silico* prediction tools, such as:
  - a. Mutation Taster
  - b. Polyphen
  - c. Condel
  - d. SIFT
  - e. Human Splice Finder
  - f. REVEL
6. Internal laboratory patient cohort
7. Correlation with reported clinical history
8. Family testing and/or segregation data



9. Evaluation of other genetic testing results (e.g. variant seen in cis/trans with another variant or large deletion analysis)
10. Correlation with in-house laboratory results of non-genetic testing (hemostasis testing, platelet/neutrophil/antibody testing, etc.)
11. Domain-/disease-specific knowledge or expertise

### **Variant Classification**

Results are classified and reported in accordance with 2015 ACMG standards and guidelines for the interpretation of sequence variants (PMID: 25741868) and current ClinGen Sequence Variant Interpretation Working Group recommendations and publications (<https://clinicalgenome.org>). Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (<http://www.hgvs.org>).

Versiti classifies variants into one of the five following categories:

1. Pathogenic: Conclusive evidence demonstrates that the variant directly causes or contributes to disease
2. Likely Pathogenic: Strong evidence supports that the variant causes or contributes to disease
3. Uncertain Significance: Current evidence regarding the identified variant is insufficient or inconclusive
4. Likely Benign: Strong evidence supports that the variant does not cause or contribute to disease
5. Benign: Conclusive evidence demonstrates that the variant does not cause or contribute to disease

#### **• Pathogenic and Likely Pathogenic Variants**

A pathogenic variant is a variant known to be causative of disease based on previous reports or published literature or predicted to be causative based on loss of protein function, expected significant protein damage, or nonsense mediated decay.

A likely pathogenic variant is a variant that is predicted to be pathogenic for a given disorder or disease based on the information and evidence relative to other pathogenic variants.

Variants classified as likely pathogenic or pathogenic must have sufficient cumulative support based on the following evidence:

- Known pathogenic variant in a specific population based on literature evidence (founder variant)
- Presence of variant in multiple affected individuals with specific phenotypic or clinical presentations
- Presence of variant in multiple affected family members with a specific clinical picture and where the variant segregates according to the disease specific inheritance pattern
- Functional studies in the literature demonstrating loss of function, gain of function or aberrant splicing
- Reported loss of function variants including frameshift, nonsense, and canonical splice junction variants with a clear and specific clinical presentation



- Located in a “hot spot” based on distribution of nearby pathogenic variants with lack of benign variants in that area
- Located at a residue where other pathogenic variants have been described
- Presumed or known *de novo* variant
- Highly conserved nucleotide and predicted to be pathogenic by several *in silico* tools
- Absent or extremely low allele frequency, consistent with disease frequency, in population databases
- Variant found in *trans* for an autosomal recessive disease where the patient has a gene-specific phenotype

Variants expected to be pathogenic would be variants with a predicted loss of function (frameshift, nonsense, canonical splice junction) but are not currently published as pathogenic. In this case, loss of function must be a known disease manifestation where similar loss of function variants have been published as pathogenic. Novel missense variants may be considered pathogenic or likely pathogenic if there is sufficient additional evidence, as above, supporting its pathogenic nature.

- **Variants of Uncertain Significance**

A variant of uncertain significance has insufficient or conflicting evidence to classify the variant as likely benign or likely pathogenic for a given disorder.

Insufficient or conflicting evidence can include:

- Multiple functional assays with conflicting results
- Low allele frequency data
- Location in regions that are not functionally established
- Lack of or inadequate clinical information
- Lack of or conflicting evidence in publications linking the variant to clinical phenotypes

- **Likely Benign Variants**

A likely benign variant is predicted to be benign for a given disorder based on information and evidence of the variant relative to other benign variants but does not have enough supporting evidence to definitively rule it as benign.

Variants in this category have multiple lines of evidence supporting benign:

- Failure to segregate with disease when disease is highly penetrant
- Not an evolutionary conserved nucleotide or residue by *in silico* predictions
- A higher allele frequency than disease incidence would indicate based on mode of inheritance
- Observation of variant in presumed healthy individuals
- Variant found with another known pathogenic variant fully explaining phenotype in question



At Versiti, variants classified as likely benign more than six months prior to observation in a current case are re-evaluated to determine if any additional evidence alters the classification.

- **Benign Variants**

A benign variant has no clinical evidence supporting it to be causative of a highly penetrant Mendelian disorder or has definitive evidence to demonstrate that it does not cause or contribute to such disease. Versiti uses an allele frequency  $>5\%$  as stand-alone evidence for a benign variant. Other considerations such as the number of homozygotes or hemizygotes in public population databases will suggest that the variant is benign.

Silent or intronic variants beyond the canonical splice site can be considered benign without stand-alone population data if other evidence supports benign classification.

*Versiti, January 2020*