



General Variant Classification Assertion Criteria

General Information

When evaluating a variant for pathogenicity, GeneDx reviews information and evidence that includes, but not limited to, the following:

1. Gene and disease information (e.g. disease mechanism, pathogenic variant spectrum, disease incidence, inheritance pattern, penetrance, expressivity, functional domains etc.). Summaries of this information are available on our testing information sheets on our website.
2. Population frequency and state (homozygous/hemizygous) data
 - a. dbSNP (use discontinued after release of 1000 genomes and NHLBI ESP)
 - b. 1000 genomes (<http://www.1000genomes.org/>)
 - c. NHLBI Exome Sequencing Project (ESP) (<http://evs.gs.washington.edu/EVS/>)
 - d. Exome Aggregation Consortium (<http://exac.broadinstitute.org/>)
(Limited Use: The Exome Aggregation Consortium (ExAC) provides a beta-version of a set of exome sequencing data from a variety of large-scale sequencing projects. A peer-reviewed publication and final release of data are currently pending. The data includes low coverage and poor confidence calls which affect the data quality. The ExAC set includes data from individuals who were recruited for disease-specific studies, including cancer and cardiovascular diseases. Therefore, GeneDx does not routinely consider data from the ExAC browser in variant interpretation at this time but will continue to evaluate the utility of this data set in a clinical setting and will include ExAC data once a peer-reviewed publication is available and the full data set is released.)
 - e. Internal exome data
3. Clinically significant literature
4. Gene specific variant databases
5. Type of variant relative to disease mechanism
6. *In silico* Prediction algorithms: Prediction algorithms in use are listed in Table 2 of the "Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation from the American College of Medical Genetics and Genomics and the Association of Molecular Pathology." (Richards et al. *Genetics in Medicine*, 2015). In general, three prediction algorithms are assessed for missense and splice site predictions.
7. Familial testing or segregation studies
8. Contextual evaluation with other testing results (e.g. seen in cis/trans with other pathogenic variants)

Variant Classification

GeneDx classifies sequencing variants into five primary categories:

1. Pathogenic
2. Benign
3. Likely Pathogenic
4. Likely Benign
5. Variant of Unknown (Uncertain) Significance

Variants are scored into each of these categories based upon the concordance of the evidence. Note that evidence in the literature can render any predicted evidence as obsolete.



1. **Pathogenic Variants**

A pathogenic variant is known to be causative for a given genetic disorder based on previous reports or predicted to be causative based on the loss of protein function or expected significant damage to protein or protein/protein interactions.

Known Pathogenic

Variants “known” to be causative must have sufficient support relative to the type of variant. Evidence supporting pathogenic includes:

- a. known pathogenic variant in a specific population based on evidence in the literature (i.e. founder pathogenic variants)
- b. presence of variant in multiple affected individuals with distinct clinical presentations
- c. variant segregation with disease in multiple families
- d. functional studies in the literature demonstrating:
 - a. reduced or loss protein function (loss of function)
 - b. aberrant protein function (gain of function)
 - c. aberrant splicing in an appropriate functional assay
- e. reported loss of function variants (e.g. include frameshift, nonsense, canonical splice junction (at positions +1,+2, -1 and -2 in an intron)) with clear clinical correlations

Expected Pathogenic

Variants with a predicted loss of function include frameshift, nonsense, canonical splice junction (at positions +1,+2, -1 and -2 in an intron), and change in an initiation codon, but are not currently “known” to the field to be pathogenic. The effect of potential residual functionality of a truncated or abnormal protein product is eliminated by comparison to similar known, published pathogenic variants with the same predicted effect. In this category, loss of function must be an established disease mechanism for the disorder. Novel missense variants occurring at the same residue as known pathogenic missense variants may also be considered expected pathogenic in the absence of conflicting evidence.

2. **Benign Variants**

A benign variant has no known clinical significance supporting it to be causative of a given genetic disorder.

GeneDx evaluates population data relative to the disease incidence as stand-alone data for classifying a variant as benign. In general, any variant with an allele frequency >1% for an autosomal dominant or X-linked recessive disease or >3% for an autosomal recessive disease in populations with >400 individuals is considered benign unless contradictory evidence of pathogenicity is indicated. Any conflicting evidence is evaluated



in the context of the gene and disease information. For rare disorders, proportionally lower allele frequencies are accepted as stand-alone criteria relative to the disease incidence.

Silent and intronic variants beyond the canonical splice junction (at positions +1,+2, -1 and -2 in an intron) are also considered benign in the absence of stand-alone population data if all available evidence supports or predicts (*in silico* splicing algorithms) benign impact.

3. Likely Pathogenic Variants

A likely pathogenic variant is predicted to be pathogenic for a given genetic disorder based on the information and evidence of the variant relative to other known pathogenic variants.

Variants in this category have multiple lines of evidence supporting pathogenicity that can include, but is not limited to, the following:

- a. Located in functional domain or mutational “hot spot” as supported by nearby known pathogenic variants or literature
- b. Located in a residue with other known pathogenic variants described
- c. Internal familial-based segregation studies or presumed/known to be *de novo* events consistent with disease
- d. High evolutionarily conserved nucleotide or residue as indicated by consensus of *in silico* predictions indicating pathogenic
- e. No or extremely low allele frequency that is not consistent with it being a benign variant
- f. Variant found in *trans* with another pathogenic variant for an autosomal recessive disease in patient with gene-specific phenotype or other supportive testing (e.g. biochemical testing)

4. Likely Benign Variants

A likely benign variant is predicted to be benign for a given genetic disorder based on the information and evidence of the variant relative to other known benign variants.

Variants in this category have multiple lines of evidence supporting benign that can include, but is not limited to, the following:

- a. Located in region of the protein that lacks known pathogenic variants or is indicated in the literature as tolerant to variation
- b. Failure to segregate in internal familial-based segregation studies as consistent with disease
- c. Not in an evolutionarily conserved nucleotide or residue as indicated by consensus of *in silico* predictions indicating benign
- d. Low allele frequency consistent with particular ethnic group lacking significant representation in population databases



- e. Variant found with another known molecular basis for the disease
- f. Observations of variant in presumed healthy individuals relative to the disease information
- g. In-frame deletions or insertions in repetitive regions without a known function

5. Variants of Unknown (Uncertain) Significance

A variant of unknown or uncertain significance has insufficient or significant conflicting evidence to indicate it is likely benign or likely pathogenic for a given genetic disorder.

Insufficient or significant conflicting evidence includes, but is not limited to, the following:

- a. Multiple functional assays indicating opposing results
- b. Conflicting segregation studies, especially with common phenotypes
- c. Low allele frequency data in disorders with unique considerations (e.g. reduced penetrance, non-Mendelian inheritance, etc.)
- d. Located in regions not functionally well-established
- e. Lack of or inadequate clinical information of cases with variant
- f. Lack of sufficient data/evidence in original publications linking variant to clinical phenotypes