GeneDx
an OPKO Health Company

General Variant Classification Assertion Criteria

General Information

Data analysis and variant classification at GeneDx is a multi-step, standardized process involving technical data analysis, literature and database review, clinical review, final review, reporting, and variant follow-up. We systematically assess evidence to classify sequencing variants based on the ACMG/AMP standards and guidelines for the interpretation of sequencing variants (PMID 25741868), which were co-authored by one of our founders, and we incorporate criteria-specific and gene-specific modifications to refine the criteria (e.g., PMIDs 31534211; 30192042; 29493581; 29300372). We evaluate and classify copy number variants using a framework that is congruent with the Technical Standards for the Interpretation and Reporting of Constitutional Copy-number Variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) (PMID 31690835). Variant interpretation at GeneDx combines automated algorithms utilizing a broad array of information from public resources and internal databases, machine-learning approaches, and in-depth evaluation by a large team of skilled professionals. The approach takes advantage of our vast internal database of genotype and phenotype information collected over 20 years.

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

1. Variant, gene and disease information
    a. Type of variant correlated to disease mechanism (PVS1, PS1, PP2, BP1, BP7)
    b. Disease incidence (BA1, BS1, PM2)
    c. Inheritance pattern (PS2/PM6, PM3, BA1, BS1, PM2)
    d. Other genetic factors impacting affected status, e.g., penetrance and expressivity (PM2, BA1, BS1, BS4)
    e. Function and structure of protein (PS3, PM1, PM4, BS3, BP3)
2. General population frequency and state, e.g., homozygous/hemizygous (BA1, BS1, BS2, PM2)
    a. Genome Aggregation Database; http://gnomad.broadinstitute.org/
    b. Internal exome data from >120,000 exomes representing affected individuals and unaffected relatives
3. Clinically relevant evidence from the literature and internal testing
    a. Functional studies (PS3, BS3)
    b. Clinical cohort of probands (PS4, PP4)
    c. Segregation studies (PP1, BS4)
    d. Contextual evaluation of variant with other testing results, e.g., whether seen in cis or trans with other pathogenic variants (PM3, BS2, BP2, BP5)
4. Gene-specific variant databases (PP5, BP6)
5. *In silico* prediction algorithms (PP3, BP4)

When applying evidence, GeneDx may increase or decrease the strength of the ACMG-AMP criteria to refine criteria based upon factors such as the amount of data available and whether it has been replicated over time, the phenotypic fit, and whether the disease mechanism is well-established or not. Additionally, we alter the strength of certain criteria based upon gene-specific information. GeneDx also regularly works with external gene experts to gather additional evidence to improve and solidify classifications. We actively participate in the discrepancy resolution processes through both the ClinGen Sequence Variant Inter-Laboratory Discrepancy Resolution group (e.g. PMID: 28301460) as well as directly with other clinical laboratories (e.g. PMID: 27843123). GeneDx staff also leads or participates in expert panels aimed at refining variant classification and publishing SVI-Approved Expert Panel Specified ACMG/AMP Variant Interpretation Guidelines for several disorders/groups, such as *Cardiomyopathy Expert Panel, RASopathy Expert Panel, PTEN Expert Panel,* and *TP53 Expert Panel* (see also https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/).

Variant Classification

GeneDx classifies sequencing variants into five primary categories:

1. Pathogenic
2. Likely Pathogenic
3. Benign
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. Causative *v*ariants must have sufficient support relative to the type of variant. Evidence supporting pathogenic includes but is not limited to:

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 or confirmed *de novo* events 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

2. *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 are 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)

3. *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% in populations with >2,000 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.

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 are 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

References:

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