General Variant Classification Assertion Criteria

General Information

Variants identified by Whole Genome Sequencing are evaluated for pathogenicity based on evidence including, but not limited to:

1. Overlap of gene-associated phenotype(s) with the primary phenotype reported in the proband submitted for clinical testing
2. The known mechanism of disease such as loss of function vs. gain of function, dominant negative etc. Additional considerations include penetrance and variable expressivity reported in the literature.
3. Population frequency of variant as represented in gnomAD (https://gnomad.broadinstitute.org/), other population specific variant databases (i.e.-GME Variome; igm.ucsd.edu/gme/), or our internal NYGC database
4. *In silico* predictions of pathogenicity (Provean, SIFT, CADD, REVEL, MaxEntScan, etc)
5. Presence of a variant in disease associated variant databases including ClinVar, HGMD, and LOVD
6. Survey of available literature for evidence including identification of affected individuals in which the variant has been identified, the presence of the variant *in trans* with a pathogenic variant (for genes associated with autosomal recessive disease), functional studies, and additional evidence for or against variant pathogenicity
7. Location and type of variant compared to other variants reported in the gene
    a. Type of variant – missense, nonsense, frameshift, canonical splice, synonymous, deep intronic, etc compared to the variants that have been identified in affected individuals in the literature
    b. Location of variant in specific domain – determination of location within a domain from available literature as well as online databases (UniProt, Pfam, etc)
8. Co-segregation of a variant with the disease within the family submitted for clinical testing
9. Presence of other potentially pathogenic variants identified in the proband submitted for clinical testing

Variant Classification

Variants are classified as Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign, or Benign based on "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology" (Richards, et. al., 2015; PMID:25741868) and additional recommendations published for specific criteria (Tayoun et al, 2018; PMID:30192042 and others available at https://clinicalgenome.org/working-groups/sequence-variant-interpretation/)

1. Pathogenic – A variant known to be causative for disease. Pathogenic variants fulfill multiple evidence criteria for pathogenicity (PMID:25741868, 30192042) and typically have been observed in

multiple affected individuals in the literature, have functional evidence for pathogenicity, and/or segregation studies supporting pathogenicity, as well as additional supporting evidence.

2. Likely Pathogenic – a variant predicted to be pathogenic, but lacking enough evidence to meet Pathogenic. Likely pathogenic variants are often rare nonsense, frameshift, canonical splice variants in genes with a known loss-of-function mechanism, but without previous report in affected individuals in the literature or only identified in a single or small number of individuals in the literature, or missense variants in known functional domains, *de novo* variants and/or variants with supportive segregation data.
3. Variants of Uncertain Significance – rare variants without sufficient evidence, or conflicting evidence of pathogenicity. Typically, these are rare variants with little or no additional supporting evidence of pathogenicity.
4. Likely Benign – variants with evidence against pathogenicity. Typically, variants with population frequency higher than expected for disease, not reported in affected individuals in the literature, and/or meet additional criteria for a benign variant.

Benign – variants with strong evidence against pathogenicity. Benign variants often have significant population frequency, which is higher than expected for disease causing variants, have functional evidence showing no reduced or altered protein function, and/or other evidence strongly supporting lack of pathogenicity.