

AGNES GINGES CENTRE FOR MOLECULAR CARDIOLOGY

VARIANT ASSESSMENT & ASSERTION CRITERIA

In our laboratory, variant assessment and determination of pathogenicity involved a multidisciplinary team composed of clinicians, clinical geneticists, genetic counsellors, research fellows, and lab technicians. Current guidelines from the American College of Medical Genetics and Genomics (ACMG).^{1,2} were modified to develop a more focused criteria applicable to the observed phenotype and correlation with specific genes. The clinical significance of variants was assessed using a systematic approach including, but not limited to:

- Search of existing data to help determine likelihood of pathogenicity (e.g., literature, population databases, communication with collaborators)
- Clinical and genetic evaluation of family members to determine if the variant segregates with disease
- Interpretation of experimental data including *in vivo* and/or *in vitro* models
- Revision of conservation and computational predictions by multiple *in silico* tools

Determination of variant pathogenicity was discussed at formal meetings and the evidence was weighted to reach an overall conclusion. Classification of variants was assigned using a five-tier terminology system (pathogenic, likely pathogenic, uncertain significance, likely benign, benign). All variant classifications are reviewed as new guidelines and/or additional information becomes available.

1. Richards CS, Bale S, Bellissimo DB, Das S, Grody WW, Hegde MR, Lyon E, Ward BE. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. *Genet Med.* 2008 Apr;10(4):294-300.
2. Bahcall OG. Genetic testing: ACMG guides on the interpretation of sequence variants. *Nat Rev Genet.* 2015 Apr 9.



BENIGN

- Frequency >5% in general population

LIKELY BENIGN

- Observed in population at frequency higher than expected disease
- Not a conserved region
- Multiple lines of computational tools predict no impact on gene
- Lack of segregation in informative family members
- Found in case where there is an alternate genetic cause

UNCERTAIN SIGNIFICANCE

- Limited or conflicting data

LIKELY PATHOGENIC

- Very rare or absent in general population
- Moderate segregation with disease
- A different amino acid substitution at same location has been well characterised as pathogenic in the same disease
- Functional evidence
- Suspected *de novo* (paternity not confirmed)
- Reputable source reports as pathogenic

PATHOGENIC

- Predicted null variant where LOF is known mechanism of disease
- Well-characterised pathogenic mutation
- *De novo* variant (paternity confirmed)
- Strong segregation with disease