

A GUIDE TO INTERPRETING SEQUENCE VARIANTS

Molecular genetic testing involves determining the significance of changes or variations in a DNA sequence, including single nucleotide variants, insertions, deletions, inversions and other rearrangements. For some variants, the clinical consequences of the sequence change are well understood and they have been characterized in published scientific literature and/or databases; these variants can be classified as definitively pathogenic or benign. A pathogenic variant (also called a mutation), is a change that alters the normal function of a gene product and results in a genetic condition. A benign variant is a change that is known to be neutral (not causing a genetic condition). When the effect of a variant is not known, or the scientific evidence available at the time of reporting is not sufficient to make a definitive classification, the variant may be considered either “likely pathogenic,” a “variant of uncertain significance (VUS),” or “likely benign.”

HOW DOES THE LABORATORY INTERPRET VARIANTS?

In order to assess the clinical significance of a variant, we use databases describing previously reported variants such as the Human Gene Mutation Database (HGMD) and ClinVar, as well as relevant published scientific literature. We also consider the frequency of a variant in control populations from sources including dbSNP, 1000 Genomes Project, Exome Variant Server (EVS), and the Exome Aggregation Consortium (ExAC). Additionally, we use software programs (PolyPhen2, SIFT, Align GVGD and MutationTaster) that provide *in silico* predictions about the effect of missense changes that result in the translation of a different amino acid at that position. These programs consider the physiochemical difference between two amino acids, if the change is within a functional protein domain, and if it is evolutionarily conserved. It is important to note, however, that *in silico* prediction tools are not always accurate, and therefore only aid in providing supporting evidence to prioritize variants.

CATEGORIES OF SEQUENCE VARIANTS

The five categories of variants used by the Molecular Genetics Laboratory have been adapted from the variant interpretation recommendations by the American College of Medical Genetics and Genomics (ACMG). The table below provides an overview of the criteria used to classify a variant within each category:

Pathogenic	Likely Pathogenic	VUS	Likely Benign	Benign
<p>Previously reported with substantial evidence that the variant causes the disease in question OR novel variants in a gene where loss of function is a known mechanism of disease, including:</p> <ul style="list-style-type: none"> • Nonsense or frameshift changes including interruption of the normal start or stop codon • Alteration of a splice donor or acceptor site 	<p>Novel or previously reported variants, with some evidence suggesting that they cause disease, including:</p> <ul style="list-style-type: none"> • Changes in a gene known to cause specific features seen in the patient • Changes in a highly-conserved and/or well-characterized functional domain of the gene product • Functional studies showing a damaging effect on the gene product • Changes that segregate with disease in a family 	<p>Novel variants that may or may not cause disease, including:</p> <ul style="list-style-type: none"> • Missense changes • In-frame insertions or deletions <p>OR variants that have been previously reported but have weak, incomplete or conflicting evidence about whether or not it is disease-causing.</p>	<p>Novel or previously reported variants, with some evidence suggesting that they are neutral, including:</p> <ul style="list-style-type: none"> • Intronic changes that are unlikely to affect splicing • Changes in a highly variable region of a gene without a known function • Functional studies showing no damaging effect on the gene product • Changes that do not segregate with disease in a family 	<p>Previously reported variants that are present at a higher frequency in the general population than expected for that disease.</p>

CLINICAL CONSIDERATIONS AND RECOMMENDATIONS

- Discuss the possibility of finding VUSs with your patients before ordering testing, including the accuracy, limitations and implications of these results.
- Information such as clinical findings, family history, ethnic background, and other laboratory data must be effectively communicated to the lab to guide the most thorough investigation of variant interpretation.
- Follow-up studies looking for the presence of a VUS in a patient’s family members may be useful to draw a correlation between the variant and the patient’s condition. Results of family studies should be interpreted in the context of the likely inheritance pattern expected for the disease (i.e. autosomal recessive, autosomal dominant, X-linked).
- As databases and literature change, the classification of variants may change as well. Therefore, results should be reinterpreted or repeated in light of new and/or relevant discoveries.

For more information, contact the Genome Diagnostics Laboratory at the SickKids at 416-813-7200 x1 or www.sickkids.ca/genome-diagnostics

To locate a genetics center near you, visit the Canadian Association of Genetic Counsellors website at www.caqc-accg.ca or the National Society of Genetic Counsellors website at www.nsgc.org.