Variant Classification Specifications

Variant classification at Laboratory Corporation of America® Holdings follows the guidelines set forth by the American College of Medical Genetics[1] (ACMG) in conjunction with our in-house developed method of assessment to assure a systematic, traceable and thorough review of all available evidence supporting a classification outcome.

Core elements of our classification include, but are not limited to: **(1)** locus, disease specific, commercial and publicly available databases, **(2)** peer-reviewed literature, **(3)** prediction algorithms, and **(4)** information derived from our internal testing experience. All ascertained evidences are incorporated as specific components of a standardized algorithmically-weighted workflow, which assigns a score that drives the final reportable variant classification.

Components of Variant Classification:

The specific components driving the final variant classification are:

1. Predicted functional impact on the gene or gene product
2. Evidence of actual deleterious impact on the gene or gene product
3. Prevalence of the variant in the unaffected (general) population
4. Genotype-phenotype assessment based upon occurrence in affected individuals

A brief summary of each component is provided below.

1. **Predicted functional impact:**
   What are the predicted effect(s) of the variant on synthesis and/or function of the encoded protein? The variant type is reviewed in the light of the established molecular mechanism(s) of disease attributed to the gene where the variant is located. Variants that are predicted to cause a truncation of the gene product (nonsense mutation or a frameshift mutation), and those affecting the canonical splice sites, are weighted in favor of pathogenicity. Splice prediction algorithms are used as supportive evidence for synonymous variants and intronic variants outside the canonical splice sites. Missense variants are evaluated using a variety of computational tools (eg, SIFT, PolyPhen, MutationTaster, BLOSUM matrix) to predict an impact on protein function. All prediction models are used with caution since most programs have not been clinically validated. Additional computational tools are evaluated and utilized as new sources of information become available.

2. **Evidence of actual deleterious impact:**
   What effect does the variant have in a controlled experimental system? A significant effect of a variant on the synthesis, cellular location, and/or the function of the encoded protein in an experimental system are suggestive of pathogenicity. While in-vitro experimental systems can provide powerful information, the results must be interpreted with caution as they may not reflect the complexities of the actual in-vivo environment. Data for variant effect in an experimental system as ascertained from peer-reviewed literature are critically reviewed and weighted based upon the strength of the reported evidence.

3. **Prevalence of the variant in the unaffected (general) population:**
   Has the variant been observed in the general population? If a variant is observed more frequently in the general population than is compatible with the prevalence and mode of inheritance of the disease, then this variant is weighted in favor of non-pathogenicity. Data sources for variant frequency in the general population are derived from public databases. Additional population databases are evaluated and utilized as new sources of information become available. Some of these include but are not limited to: **(1)** Exome Aggregation Consortium[2] **(2)** dbSNP[3] and **(3)** Exome Sequencing Project.[4] Additionally, control data from publications, and/or information derived from our internal testing experience, are used to supplement our ascertainment.

4. **Genotype-phenotype assessment based upon occurrence in affected individuals:**
   Has the variant been observed in individuals and families affected with disease? If a variant is observed only in diseased individuals and not in the general (healthy) population, it is weighted in favor of pathogenicity. The probability of association depends on such parameters as the number of diseased individuals with the variant and the transmission patterns for co-segregation of variant with disease within families. All data derived from peer-reviewed published literature, as well as available information derived from in-house family testing, are considered when weighing a variant in favor of pathogenicity.

Additional considerations for Variant Classification:

Additional considerations include, but are not limited to: **(1)** co-occurrence of a variant with known pathogenic variants, **(2)** the presence of alternative isoforms and reference sequences used, **(3)** occurrence of a variant in mutually exclusive disease phenotypes, and **(4)** certain gene-specific and/or disease-specific properties.

We use caution when ascertaining the frequency of variants located in complex regions of genome (homologous regions, repetitive sequences, pseudogenes, segmental duplications, gene families) to ensure accuracy of classification.

Variant Classification outcomes:

All reported variants are classified into 5 major reportable categories: "pathogenic", "likely pathogenic", "variant of uncertain clinical significance (VUS)", "likely benign" and "benign". Variants within the VUS category are further sub-classified during reporting, as appropriate, to assist clinicians further with the interpretation: "VUS-possibly benign", "VUS" and "VUS-possibly pathogenic". Family member testing may be suggested to assist in the interpretation of variants classified in the VUS category, following internally-established policies.

The final classification does not reflect severity of disease, but only a probability of association of the variant with a monogenically-inherited disease based upon the strength of supporting evidence, i.e., the confidence that the classification is accurate.

Quality Assurance of Variant Classification:

To ensure the most clinically relevant outcome, all variant classifications are reviewed by a team of PhD-level scientists with broad expertise in human genetics. This is followed by a tiered review of all available evidence and supporting rationale by a team of ABMG-certified Clinical Molecular Geneticists and ABGC licensed Genetic Counselors. The final reports are reviewed and approved in context of the clinical indications of testing by the laboratory director. Extensive interactions between the variant classification group, geneticists, and genetic counselors ensure continuous quality improvements that facilitate an accuracy of classification outcomes. All variant classifications are re-evaluated at defined intervals for relevant updates that could impact the final report interpretation.

Our information technology platform is developed to streamline the variant classification process and serves as a repository of all variants identified and classified at LabCorp. It provides traceability and guarantees variants are systematically revised as new knowledge emerges. It also interfaces with connected reporting applications to generate result reports, concordant with most recent variant classification.

References:
1. Richards, S, Aziz, N et al. 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. *Genetics in Medicine.* 2015;17: 405-423.
2. http://exac.broadinstitute.org/
3. http://www.ncbi.nlm.nih.gov/SNP/
4. http://evs.gs.washington.edu/EVS/

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