Illumina Clinical Services Laboratory Variant Classification for Rare and Undiagnosed Genetic Disease

Summary

Classification of single nucleotide variants and small insertion and deletion variants in nuclear genes for rare and undiagnosed genetic disease (RUGD) in the Illumina Clinical Services Laboratory (ICSL) is performed in accordance with the recommendations of the American College of Medical Genetics and Genomics (ACMG) (Richards et al. 2015), and evaluation of the curated gene-disease relationship (GDR), relevant evidence gleaned from the literature and other resources, and variant frequency information obtained from population databases.

Gene Curation and Classification

- Prior to curation of a variant of interest, the strength and classification of the GDR is established through gene curation in accordance with the ClinGen framework for evaluating the clinical validity of a GDR (Strande et al. 2017). Using this framework, GDRs may be classified as Definitive, Strong, Moderate, Limited, No Known Disease Relationship, or Conflicting Evidence Reported (Disputed or Refuted).
- If a GDR has been previously curated by ClinGen, by Ceyhan-Birsoy et al. (2017), or by ICSL, and classified as Strong or Definitive, further curation is not required, and these data are used to support variant classification and reporting.
- If gene curation data are unavailable or if the curated GDR is classified as Moderate or below, gene curation is performed following the current gene-disease validity standard operating procedure from ClinGen.
- Where applicable, professional judgement and expertise are utilized in determining the evidential strength and the final clinical validity classification.

Variant Curation and Classification

- Variants of interest are subjected to a manual curation. Evidence is collected to support the application of appropriate criteria as described by Richards et al. (2015).
- Population frequency information is gathered from the Genome Aggregation Database (gnomAD). Frequency information also may be interrogated from our internal database of whole genome sequences.
- Literature searches are performed in Google Scholar, Google, and ClinVar for each variant using the gene name, cDNA change, amino acid change (where applicable), and rsID, utilizing alternative nomenclature as available.
- Additional resources are consulted as required depending on the variant. For example, the effect of potentially truncating or elongating variants is confirmed using Mutalyzer.
- Variants are classified based on assessment and concordance of the available evidence. Each variant classification is subject to professional review.

- ICSL's classification system has been developed from the recommendations of the ACMG for variant classification and reporting (Richards et al. 2015), with additional internal guidelines to allow application of the classification criteria at different strengths depending on the weight of the available evidence and to ensure consistent application of criteria.
- Variants may be classified as Pathogenic, Likely Pathogenic, Variant of Unknown Significance, Likely Benign, or Benign, depending on the available evidence, application of the classification criteria, and the strength of the GDR.

Sources Used in Variant and Gene Curation

Frequency information:
Genome Aggregation Database (gnomAD): http://gnomad.broadinstitute.org/about

Literature searches:
PubMed: https://www.ncbi.nlm.nih.gov/pubmed
PubMed Central (PMC): https://www.ncbi.nlm.nih.gov/pmc
Google Scholar: https://scholar.google.com/
Google: https://www.google.com/

Gene and disease information:
GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1116/
Genetics Home Reference: https://ghr.nlm.nih.gov/
Orphanet: http://www.orpha.net/consor/cgi-bin/index.php?lng=EN
Online Mendelian Inheritance in Man (OMIM): https://www.ncbi.nlm.nih.gov/omim

Additional resources:
ClinGen: https://clinicalgenome.org/
ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
dbSNP: https://www.ncbi.nlm.nih.gov/SNP/
DECIPHER: https://decipher.sanger.ac.uk/
Franklin: https://franklin.genoox.com/home
HUGO Genome Nomenclature Committee (HGNC): http://www.genenames.org/
Human Genome Variation Society: http://www.hgvs.org
Human Phenotype Ontology (HPO) Browser: https://www.human-phenotype-ontology.github.io/
The Human Protein Atlas: http://www.proteinatlas.org/
International Mouse Phenotyping Consortium (IMPC): https://www.mousephenotype.org/
Monarch Disease Ontology (MonDO): https://ebi.ac.uk/ols/ontologies/mondo
Mouse Genome Informatics: https://www.jax.org/jax-mice-and-services
Mutalyzer: https://mutalyzer.nl/
NCBI Gene: http://www.ncbi.nlm.nih.gov/gene
NCBI Protein database: https://www.ncbi.nlm.nih.gov/protein
UCSC Genome Browser: http://genome.ucsc.edu
Uniprot: https://uniprot.org
Varsome: https://varsome.com/

References

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Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. 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. Genet Med. 2015 May; 17(5):405-24.

Strande NT, Riggs ER, Buchanan AH, Ceyhan-Birsoy O, DiStefano M, Dwight SS, Goldstein J, Ghosh R, Seifert BA, Sneddon TP, Wright MW, Milko LV, Cherry JM, Giovanni MA, Murray MF, O'Daniel JM, Ramos EM, Santani AB, Scott AF, Plon SE, Rehm HL, Martin CL, Berg JS. Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. Am J Hum Genet. 2017 Jun 1; 100(6):895-906.