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

Summary

Classification of copy number variants (CNVs) in nuclear genes for rare and undiagnosed genetic disease (RUGD) in the Illumina Clinical Services Laboratory (ICSL) is performed in accordance with established joint recommendations of the American College of Medical Genetics and Genomics (ACMG) and ClinGen (Riggs et al. 2020). The recommendations utilize an integrated evaluation of knowledge including copy number content, such as number of protein coding genes and other known functionally important elements, information on dosage sensitivity of the region and/or genes contained within the CNV, evidence gleaned from the literature, public databases, and/or internal laboratory data, inheritance pattern and family history of the proband under study, and frequency of similar CNVs identified in control populations. For CNVs overlapping or contained within a single gene, ICSL also follows the guidance described by Brandt et al. (2020), who have adapted the recommendations of the ACMG for the interpretation of single nucleotide variants (SNVs) (Richards et al. 2015) for use for single-gene copy number variants.

Gene Curation and Classification

- In instances where a CNV of interest overlaps or is contained within a single gene or where a single gene in the CNV interval is identified as a candidate based on overlap with the proband's phenotype, the strength and classification of the associated GDR is established through gene curation in accordance with the ClinGen framework for evaluating the clinical validity of a GDR (Strand et al. 2017).
    - Within 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 gene curation is not required, and these data are utilized to support CNV 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 gene-disease validity standard operating procedure available from ClinGen at the time of curation.
    - Where applicable, professional judgement and expertise are utilized in determining the evidential strength and the final clinical validity classification.
- For CNVs with two or more overlapping genes, full assessment through the ClinGen framework of all associated GDRs generally may not be feasible. In these instances, potential GDRs are examined using information from resources such as OMIM, GeneReviews, ClinVar, and primary literature to determine whether any genes within the CNV are implicated in a disease with phenotypic features that overlap those reported in the proband.

Copy Number Variant Curation and Classification

- CNVs of interest are subjected to a manual curation. Evidence is collected to support the application of appropriate criteria as described by Riggs et al. (2020) or Brandt et al. (2020), depending on the content of the CNV.
- CNV visualization is performed in the University of California Santa Cruz (UCSC) Genome Browser and the CNV of interest is compared with other CNVs in the region as identified in tracks from ClinGen, ClinVar, DECIPHER (Firth et al. 2009), Database for Genomic Variants (DGV) (MacDonald et al. 2014), and Developmental Delay cases and controls (Cooper et al. 2011; Coe et al. 2014).
- Control 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 PubMed, PubMed Central, Google, and Google Scholar for each CNV using the gene name(s), event (gain/duplication or loss/deletion), chromosomal band, and or chromosome position.
- Additional resources are consulted as required.
- Evidence is scored following the guidance outlined in Riggs et al. (2020) using the separate scoring matrices for copy number gains or copy number losses as appropriate, due to their inherent distinct properties and differences. The ClinGen CNV Pathogenicity Calculator is used to help assess the evidence type and reach a final classification.
- For CNVs better suited for assessment following the guidance provided by Brandt et al. (2020), criteria are applied as supported by available evidence.
- Each CNV classification is subject to professional review.
- ICSL's classification system follows the joint recommendations of ACMG and ClinGen for CNV classification and reporting as outlined by Riggs et al. (2020) or the guidance provided by Brandt et al (2020) as appropriate for the CNV.
- Variants may be classified as Pathogenic, Likely Pathogenic, Variant of Uncertain Significance, Likely Benign, or Benign, depending on the points scored for available evidence.

Sources Used in CNV 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/
ClinGen CNV Pathogenicity Calculator: https://cnvcalc.clinicalgenome.org/cnvcalc/
ClinGen Dosage Sensitivity: https://www.clinicalgenome.org/curation-activities/dosage-sensitivity/
ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
DECIPHER: https://decipher.sanger.ac.uk/
DGV: http://dgv.tcag.ca/dgv/app/home
The Human Protein Atlas: http://www.proteinatlas.org/
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

References

Brandt T, Sack LM, Arjona D, Tan D, Mei H, Cui H, Gao H, Bean LJH, Ankala A, Del Gaudio D, Knight Johnson A, Vincent LM, Reavey C, Lai A, Richard G, Meck JM. 2020 Feb. Adapting ACMG/AMP sequence variant classification guidelines for single-gene copy number variants. Genet Med. 22(2):336-344.

Ceyhan-Birsoy O, Machini K, Lebo MS, Yu TW, Agrawal PB, Parad RB, Holm IA, McGuire A, Green RC, Beggs AH, Rehm HL. 2017 Jul. A curated list for reporting results of newborn genomic sequencing. Genet Med. 19 (7): 809-818.

Coe BP, Witherspoon K, Rosenfeld JA, van Bon BW, Vulto-van Silfhout AT, Bosco P, Friend KL, Baker C, Buono S, Vissers LE, Schuurs-Hoeijmakers JH, Hoischen A, Pfundt R, Krumm N, Carvill GL, Li D, Amaral D, Brown N, Lockhart PJ, Scheffer IE, Alberti A, Shaw M, Pettinato R, Tervo R, de Leeuw N, Reijnders MR, Torchia BS, Peeters H, O'Roak BJ, Fichera M, Hehir-Kwa JY, Shendure J, Mefford HC, Haan E, Gécz J, de Vries BB, Romano C, Eichler EE. 2014 Oct. Refining analyses of copy number variation identifies specific genes associated with developmental delay. Nat Genet. 46(10):1063-71.

Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, Abdel-Hamid H, Bader P, McCracken E, Niyazov D, Leppig K, Thiese H, Hummel M, Alexander N, Gorski J, Kussmann J, Shashi V, Johnson K, Rehder C, Ballif BC, Shaffer LG, Eichler EE. 2011 Aug 14. A copy number variation morbidity map of developmental delay. Nat Genet. 43(9):838-46.

Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Van Vooren S, Moreau Y, Pettett RM, Carter NP. 2009 Apr. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet. 84(4):524-33.

MacDonald JR, Ziman R, Yuen RK, Feuk L, Scherer SW. 2014 Jan. The Database of Genomic Variants: a curated collection of structural variation in the human genome. Nucleic Acids Res. 42(Database issue):D986-92.

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. 2015 May. 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. 17(5):405-24.

Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, Raca G, Ritter DI, South ST, Thorland EC, Pineda-Alvarez D, Aradhya S, Martin CL. 2020 Feb. Technical standards for the interpretation and reporting of constitutional copy number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med. 22(2):245-257.

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. 2017 Jun 1. Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. Am J Hum Genet. 100(6):895-906.

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