ACMG TECHNICAL STANDARDS | Genetics inMedicine

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)

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Purpose: Copy-number analysis to detect disease-causing losses and gains across the genome is recommended for the evaluation of individuals with neurodevelopmental disorders and/or multiple congenital anomalies, as well as for fetuses with ultrasound abnormalities. In the decade that this analysis has been in widespread clinical use, tremendous strides have been made in understanding the effects of copy-number variants (CNVs) in both affected individuals and the general population. However, continued broad implementation of array and next-generation sequencing–based technologies will expand the types of CNVs encountered in the clinical setting, as well as our understanding of their impact on human health.

Methods: To assist clinical laboratories in the classification and reporting of CNVs, irrespective of the technology used to identify them, the American College of Medical Genetics and Genomics has developed the following professional standards in collaboration with the National Institutes of Health (NIH)–funded Clinical Genome Resource (ClinGen) project.

Results: This update introduces a quantitative, evidence-based scoring framework; encourages the implementation of the five-tier classification system widely used in sequence variant classification; and recommends "uncoupling" the evidence-based classification of a variant from its potential implications for a particular individual.

Conclusion: These professional standards will guide the evaluation of constitutional CNVs and encourage consistency and transparency across clinical laboratories.

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INTRODUCTION

Genome-wide assessment of copy-number variants (CNVs), including losses (deletions) and gains (duplications and triplications), is recommended as a first-tier approach for the postnatal evaluation of individuals with intellectual disability, developmental delay, autism spectrum disorder, and/or multiple

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congenital anomalies, as well as for prenatal evaluation of fetuses with structural anomalies observed by ultrasound.[1–3] For over a decade, CNV analysis by chromosomal microarray (CMA) has been broadly implemented in the clinical setting for detection of genomic imbalances at a much higher resolution than conventional cytogenetic methods (e.g., G-banded karyotype). In some cases, exon-focused array designs have also been used for detecting CNVs involving individual genes associated with monogenic disorders. More recently, next-generation sequencing (NGS)–based CNV analysis is increasingly used in clinical testing through genome, exome, or gene panel sequencing. Together, these methods have enabled genome-wide detection of CNVs, ranging in size from single exons to whole chromosomes in clinically affected individuals, as well as in the general population.

Though many recurrent CNVs (such as those flanked by segmental duplications) have been well characterized, most CNVs are unique, requiring further investigation to determine their potential clinical significance. This can be challenging for several reasons, including absent, limited, or conflicting associations with clinical phenotypes described in published literature and genomics databases. Accurate clinical interpretation of CNVs requires consistent methods of evaluating the genomic content of a CNV region and correlating clinical findings with those reported in the medical literature, with the ultimate goal of producing consistent, evidence-based clinical classification across laboratories.[4] Inconsistency among laboratories can create confusion for clinicians and their patients, leaving them unable to confidently use genetic information to manage health-care decisions.[5] Standards that are widely available, up-to-date, and flexible enough to incorporate lessons learned from the ever-evolving genomics knowledge base should help to reduce discordance in clinical classifications.

METHODS

To assist in the evaluation of CNVs and promote consistency and transparency in classification and reporting across clinical laboratories, the American College of Medical Genetics and Genomics (ACMG) and the National Institutes of Health (NIH)–funded Clinical Genome Resource (ClinGen) project[6] formed a collaborative working group with the goal of updating the existing ACMG professional clinical laboratory practice standards for evaluating CNVs.[7] The working group held an in-person meeting in the fall of 2015 to review the existing version of the interpretation standards[7] and discuss how laboratories had incorporated them (and any modifications) into their clinical practice, as well as new resources, tools, and technologies that became available in the intervening years. Through group consensus, evidence categories most relevant to CNV classification were determined (including genomic content, dosage sensitivity predictions and curations, predicted functional effect, clinical overlap with patients in the medical literature, evidence from case and control databases, and inheritance patterns for individual CNVs), and a relative weight was assigned to each. In this manner, a semiquantitative point-based scoring system was developed (described in detail in Supplemental Material 1).

Development of the new framework was an iterative process; working group members tested the analysis metrics using cases observed in their clinical laboratories and provided feedback for refinement that ensured objective and rigorous assessment of the available evidence. In 2017, after the framework had been developed and assessed by the working group, we identified a group of 11 additional board-certified clinical cytogeneticists to further evaluate both the performance of the analysis metrics and their usability in the clinical setting. Using both the outside reviewers and the committee members, we evaluated a total of 114 CNVs (58 deletions, 56 duplications); most CNVs ($n = 111$) were each evaluated by two independent reviewers. A full description of the validation process is provided in Supplemental Material 2. Feedback from this process led to the current version of the scoring metrics.

Proposed criteria for the evaluation of constitutional copy-number variants

These standards build upon the previous version[7] by introducing a semiquantitative point-based scoring metric for CNV classification. Owing to the distinct properties and inherent differences between copy-number losses and copy-number gains, separate scoring metrics were developed for each (Tables **1** and **2**, respectively); each scored evidence category is labeled (1A, 1B, etc.) for easy referencing. Full descriptions of each evidence category, including caveats to consider while scoring and illustrative examples, are provided in Supplemental Material 1. We strongly recommend the user to carefully review the explanatory material provided in the Supplement before utilizing these scoring metrics in clinical practice. Example cases scored using the metrics are provided in Supplemental Material 3.

As clinical laboratories incorporate more NGS-based techniques for CNV detection and integrate results from multiple technologies (some capable of identifying both copy-number and sequence variants), consistency across interpretation processes and reporting is critical. Thus, where possible, evidence categories and concepts presented in this CNV scoring system were developed to align with terminology and processes currently utilized for clinical sequence variant classification and interpretation.[8]

The point values assigned to each piece of evidence roughly correspond to the categorical strengths of evidence present in the sequence variant interpretation guidelines[8] as well as recommendations put forth by the ClinGen Sequence Variant Interpretation (SVI) Working Group to model the ACMG/Association for Molecular Pathology (AMP) sequence variant interpretation guidelines into a more quantitative Bayesian framework;[9] however, it is important to note that these numbers have not been statistically derived. In general, evidence receiving 0.90 points or higher is considered “very strong"; 0.45 points is considered “strong"; 0.30 points is considered “moderate"; and 0.15 points or lower is considered “supporting” evidence. Scores

Table 1 CNV interpretation scoring metric: copy-number loss

Section 1: Initial assessment of genomic content			
Evidence type	**Evidence**	**Suggested points/case**	**Max score**
Copy-number loss content	**1A**. Contains protein-coding or other known functionally important elements.	0 (Continue evaluation)	0
	1B. Does NOT contain protein-coding or any known functionally important elements.	−0.60	−0.60
Section 2: Overlap with established/predicted haploinsufficiency (HI) or established benign genes/genomic regions *(Skip to section 3 if your copy-number loss DOES NOT overlap these types of genes/regions)*			
Overlap with ESTABLISHED HI genes or genomic regions and consideration of reason for referral	**2A**. Complete overlap of an established HI gene/genomic region.	1.00	1.00
	2B. Partial overlap of an established HI genomic region • The observed CNV does NOT contain the known causative gene or critical region for this established HI genomic region OR • Unclear if known causative gene or critical region is affected OR • No specific causative gene or critical region has been established for this HI genomic region	0 (Continue evaluation)	0
	2C. Partial overlap with the 5′ end of an established HI gene (3′ end of the gene not involved)…	See categories below	
	2C-1. …and coding sequence is involved	0.90 *(range: 0.45 to 1.00)*	1.00
	2C-2. …and only the 5′ UTR is involved	0 *(range: 0 to 0.45)*	0.45
	2D. Partial overlap with the 3′ end of an established HI gene (5′ end of the gene not involved)…	See categories below	
	2D-1. …and only the 3′ untranslated region is involved.	0 (Continue evaluation)	0
	2D-2. …and only the last exon is involved. Other established pathogenic variants have been reported in this exon.	0.90 *(range: 0.45 to 0.90)*	0.90
	2D-3. …and only the last exon is involved. No other established pathogenic variants have been reported in this exon.	0.30 *(range: 0 to 0.45)*	0.45
	2D-4. …and it includes other exons in addition to the last exon. Nonsense-mediated decay is expected to occur.	0.90 *(range: 0.45 to 1.00)*	1.00
	2E. Both breakpoints are within the same gene (intragenic CNV; gene-level sequence variant).	See ClinGen SVI working group PVS1 specifications • PVS1 = 0.90 *(Range: 0.45 to 0.90)* • PVS1_Strong = 0.45 *(Range: 0.30 to 0.90)* • PVS1_Moderate *or* PM4 (in-frame indels) = 0.30 *(Range: 0.15 to 0.45)* • PVS1_Supporting = 0.15 *(Range: 0 to 0.30)*	See categories at left

Table 1 continued

		• N/A = No points, but continue evaluation	
Overlap with ESTABLISHED benign genes or genomic regions	**2F**. Completely contained within an established benign CNV region.	−1	−1
	2G. Overlaps an established benign CNV, but includes additional genomic material.	0 (Continue evaluation)	0
Haploinsufficiency predictors	**2H**. Two or more HI predictors suggest that AT LEAST ONE gene in the interval is HI.	0.15	0.15
Section 3: Evaluation of gene number			
Number of protein-coding RefSeq genes wholly or partially included in the copy-number loss	**3A**. 0–24 genes	0	0
	3B. 25–34 genes	0.45	0.45
	3C. 35+ genes	0.90	0.90
Section 4: Detailed evaluation of genomic content using cases from published literature, public databases, and/or internal lab data *(Skip to section 5 if either your CNV overlapped with an established HI gene/region in section 2, OR there have been no reports associating either the CNV or any genes within the CNV with human phenotypes caused by loss of function [LOF] or copy-number loss)*			
Individual case evidence—de novo occurrences	Reported proband (from literature, public databases, or internal lab data) has either: • A complete deletion of or a LOF variant within gene encompassed by the observed copy-number loss OR • An overlapping copy-number loss similar in genomic content to the observed copy-number loss AND…	See categories below	
	4A. …the reported phenotype is highly specific and relatively unique to the gene or genomic region,	Confirmed de novo: 0.45 points each Assumed de novo: 0.30 points each *(range: 0.15 to 0.45)*	0.90 (total)
	4B. …the reported phenotype is consistent with the gene/genomic region, is highly specific, but not necessarily unique to the gene/genomic region.	Confirmed de novo: 0.30 points each Assumed de novo: 0.15 point each *(range: 0 to 0.45)*	
	4C. …the reported phenotype is consistent with the gene/genomic region, but not highly specific and/or with high genetic heterogeneity.	Confirmed de novo: 0.15 point each Assumed de novo: 0.10 point each *(range: 0 to 0.30)*	
Individual case evidence—inconsistent phenotype	**4D**. …the reported phenotype is NOT consistent with what is expected for the gene/genomic region or not consistent in general.	0 points each *(range: 0 to −0.30)*	−0.30 (total)
Individual case evidence—unknown inheritance	**4E**. Reported proband has a highly specific phenotype consistent with the gene/genomic region, but the inheritance of the variant is unknown.	0.10 points each *(range: 0 to 0.15)*	0.30 (total)
Individual case evidence—segregation among similarly affected family members	**4F**. 3–4 observed segregations	0.15	0.45
	4G. 5–6 observed segregations	0.30	
	4H. 7 or more observed segregations	0.45	
Individual case evidence—nonsegregations	**4I**. Variant is NOT found in another individual in the proband's family AFFECTED with a consistent, specific, well-defined phenotype (no known phenocopies).	−0.45 points per family *(range: 0 to −0.45)*	−0.90 (total)
	4J. Variant IS found in another individual in the proband's family UNAFFECTED with the specific, well-defined phenotype observed in the proband.	−0.30 points per family *(range: 0 to −0.30)*	−0.90 (total)

Table 1 continued

	4K. Variant IS found in another individual in the proband's family UNAFFECTED with the nonspecific phenotype observed in the proband.	−0.15 points per family *(range: 0 to −0.15)*	−0.30 (total)
Case–control and population evidence	**4L**. Statistically significant increase amongst observations in cases (**with a consistent, specific, well-defined phenotype**) compared with controls.	0.45 per study *(range: 0 to 0.45 per study)*	0.45 (total)
	4M. Statistically significant increase amongst observations in cases (**without a consistent, nonspecific phenotype OR unknown phenotype**) compared with controls.	0.30 per study *(range: 0 to 0.30 per study)*	0.45 (total)
	4N. No statistically significant difference between observations in cases and controls.	−0.90 (per study) *(range: 0 to −0.90 per study)*	−0.90 (total)
	4O. Overlap with common population variation.	−1 *(range: 0 to −1)*	−1
Section 5: Evaluation of inheritance pattern/family history for patient being studied			
Observed copy-number loss is de novo	**5A**. *Use appropriate category from de novo scoring section in section 4.*	*Use de novo scoring categories from section 4 (4A–4D) to determine score*	0.45
Observed copy-number loss is inherited	**5B**. Patient with **specific, well-defined** phenotype and no family history. CNV is inherited from an apparently unaffected parent.	−0.30 *(range: 0 to −0.45)*	−0.45
	5C. Patient with **nonspecific** phenotype and no family history. CNV is inherited from an apparently unaffected parent.	−0.15 *(range: 0 to −0.30)*	−0.30
	5D. CNV segregates with a consistent phenotype observed in the patient's family.	*Use segregation scoring categories from section 4 (4F–4H) to determine score*	0.45
Observed copy-number loss—nonsegregations	**5E**. *Use appropriate category from nonsegregation section in section 4.*	*Use nonsegregation scoring categories from section 4 (4I–4K) to determine score*	−0.45
Other	**5F**. Inheritance information is unavailable or uninformative.	0	0
	5G. Inheritance information is unavailable or uninformative. The patient phenotype is nonspecific, but is consistent with what has been described in similar cases.	0.10 *(range: 0 to 0.15)*	0.15
	5H. Inheritance information is unavailable or uninformative. The patient phenotype is highly specific and consistent with what has been described in similar cases.	0.30 *(range: 0 to 0.30)*	0.30

Only those CNVs otherwise meeting the reporting thresholds determined by your laboratory should be evaluated using this metric. See Supplemental Material 1 for a detailed description of each evidence category. Scoring: *pathogenic* 0.99 or more points, *likely pathogenic* 0.90 to 0.98 points, *variant of uncertain significance* 0.89 to −0.89 points, *likely benign* −0.90 to −0.98 points, *benign* −0.99 or fewer points.
CNV copy-number variant, *SVI* sequence variant interpretation, *UTR* untranslated region.

Table 2 CNV interpretation scoring metric: copy-number gain

Evidence type	Evidence	Suggested points/case	Max score
Section 1: Initial assessment of genomic content			
Copy-number gain content	**1A**. Contains protein-coding or other known functionally important elements.	0 (Continue evaluation)	0
	1B. Does NOT contain protein-coding or any known functionally important elements.	−0.60	−0.60
Section 2: Overlap with established triplosensitive (TS), haploinsufficient (HI), or benign genes or genomic regions ***(Skip to section 3 if the copy-number gain DOES NOT overlap these types of genes/regions)***			
Overlap with ESTABLISHED TS genes or genomic regions	**2A**. Complete overlap; the TS gene or minimal critical region is fully contained within the observed copy-number gain.	1	1
	2B. Partial overlap of an established TS region • The observed CNV does NOT contain the known causative gene or critical region for this established TS genomic region OR • Unclear if the known causative gene or critical region is affected OR • No specific causative gene or critical region has been established for this TS genomic region.	0 (Continue evaluation)	0
Overlap with ESTABLISHED benign copy-number gain genes or genomic regions	**2C**. Identical in gene content to the established benign copy-number gain.	−1	−1
	2D. Smaller than established benign copy-number gain, breakpoint(s) does not interrupt protein-coding genes.	−1	−1
	2E. Smaller than established benign copy-number gain, breakpoint(s) potentially interrupts protein-coding gene.	0 (Continue evaluation)	0
	2F. Larger than known benign copy-number gain, does not include additional protein-coding genes.	−1 *(range: 0 to −1.00)*	−1
	2G. Overlaps a benign copy-number gain but includes additional genomic material.	0 (Continue evaluation)	0
Overlap with ESTABLISHED HI gene(s)	**2H**. HI gene fully contained within observed copy-number gain.	0 (Continue evaluation)	0
Breakpoint(s) within ESTABLISHED HI genes	**2I**. Both breakpoints are within the same gene (gene-level sequence variant, possibly resulting in loss of function [LOF]).	See ClinGen SVI working group PVS1 specifications • PVS1 = 0.90 *(Range: 0.45 to 0.90)* • PVS1_Strong = 0.45 *(Range: 0.30 to 0.90)* • N/A = 0 (Continue evaluation)	
	2J. One breakpoint is within an established HI gene, patient's phenotype is either inconsistent with what is expected for LOF of that gene OR unknown.	0 (Continue evaluation)	0
	2K. One breakpoint is within an established HI gene, patient's phenotype is highly specific and consistent with what is expected for LOF of that gene.	0.45	0.45
Breakpoints within other gene(s)	**2L**. One or both breakpoints are within gene(s) of no established clinical significance.	0 (Continue evaluation)	0
Section 3: Evaluation of gene number			
Number of protein-coding RefSeq genes wholly or partially included in the copy-number gain	**3A**. 0–34 genes	0	0
	3B. 35–49 genes	0.45	0.45
	3C. 50 or more genes	0.90	0.90
Section 4: Detailed evaluation of genomic content using cases from published literature, public databases, and/or internal lab data ***(Note: If there have been no reports associating either the copy-number gain or any of the genes therein with human phenotypes caused by triplosensitivity, skip to section 5)***			
Individual case evidence—de novo occurrences	Reported proband (from literature, public databases, or internal lab data) has either: • complete duplication of one or more genes within the observed copy-number gain OR • an overlapping copy-number gain similar in genomic content to the observed copy-number gain AND…	See categories below	
	4A. …the reported phenotype is highly specific and relatively unique to the gene or genomic region.	Confirmed de novo: 0.45 points each Assumed de novo: 0.30 points each *(range: 0.15 to 0.45)*	0.90 (total)
	4B. …the reported phenotype is consistent with the gene/genomic region, is highly specific, but is not necessarily unique to the gene/genomic region.	Confirmed de novo: 0.30 points each Assumed de novo: 0.15 point each *(range: 0 to 0.45)*	
	4C. …the reported phenotype is consistent with the gene/genomic region, but not highly specific and/or with high genetic heterogeneity.	Confirmed de novo: 0.15 point each Assumed de novo: 0.10 point each *(range: 0 to 0.30)*	
Individual case evidence—inconsistent phenotype	**4D**. …the reported phenotype is NOT consistent with the gene/genomic region or not consistent in general.	0 points each *(range: 0 to −0.30)*	−0.30 (total)
Individual case evidence—unknown inheritance	**4E**. Reported proband has a highly specific phenotype consistent with the gene/genomic region, but the inheritance of the variant is unknown.	0.10 points each *(range: 0 to 0.15)*	0.30 (total)

Table 2 continued

Individual case evidence—segregation among similarly affected family members	**4F**. 3–4 observed segregations	0.15	0.45
	4G. 5–6 observed segregations	0.30	
	4H. 7 or more observed segregations	0.45	
Individual case evidence—nonsegregations	**4I**. Variant is NOT found in another individual in the proband's family AFFECTED with a consistent, specific, well-defined phenotype (no known phenocopies).	−0.45 points per family *(range: 0 to −0.45)*	−0.90 (total)
	4J. Variant IS found in another individual in the proband's family UNAFFECTED with the specific, well-defined phenotype observed in the proband.	−0.30 points per family *(range: 0 to −0.30)*	−0.90 (total)
	4K. Variant IS found in another individual in the proband's family UNAFFECTED with the nonspecific phenotype observed in the proband.	−0.15 points per family *(range: 0 to −0.15)*	−0.30 (total)
Case–control and population evidence	**4L**. Statistically significant increase among observations in cases (with a consistent, **specific, well-defined phenotype**) compared with controls.	0.45 per study *(range: 0 to 0.45 per study)*	0.45 (total)
	4M. Statistically significant increase among observations in cases (with a consistent, **nonspecific phenotype or unknown phenotype**) compared with controls.	0.30 per study *(range: 0 to 0.30 per study)*	0.45 (total)
	4N. No statistically significant difference between observations in cases and controls.	−0.90 per study *(range: 0 to −0.90 per study)*	−0.90 (total)
	4O. Overlap with common population variation.	−1 *(range: 0 to −1)*	−1
Section 5: Evaluation of inheritance patterns/family history for patient being studied			
Observed copy-number gain is de novo	**5A**. *Use appropriate category from de novo scoring section in section 4.*	*Use de novo scoring categories from section 4 (4A–4D) to determine score*	0.45
Observed copy-number gain is inherited	**5B**. Patient with a **specific, well-defined phenotype** and no family history. Copy-number gain is inherited from an apparently unaffected parent.	−0.30 *(range: 0 to −0.45)*	−0.45
	5C. Patient with **nonspecific phenotype** and no family history. Copy-number gain is inherited from an apparently unaffected parent.	−0.15 *(range: 0 to −0.30)*	−0.30
	5D. CNV segregates with consistent phenotype observed in the patient's family.	*Use segregation scoring categories from in section 4 (4F–4H) to determine score*	0.45
Observed copy-number gain—nonsegregations	**5E**. *Use appropriate category from nonsegregation section in section 4.*	*Use nonsegregation scoring categories from section 4 (4I–4K) to determine score*	−0.45
	5F. Inheritance information is unavailable or uninformative.	0	0
	5G. Inheritance information is unavailable or uninformative. The patient phenotype is nonspecific, but is consistent with what has been described in similar cases.	0.10 *(range: 0 to 0.15)*	0.15
	5H. Inheritance information is unavailable or uninformative. The patient phenotype is highly specific and consistent with what has been described in similar cases.	0.15 *(range: 0 to 0.30)*	0.30

Only those CNVs otherwise meeting the reporting thresholds determined by your laboratory should be evaluated using this metric. See Supplemental Material 1 for full description of each evidence category. Scoring: *pathogenic* 0.99 or more points, *likely pathogenic* 0.90 to 0.98 points, *variant of uncertain significance* 0.89 to −0.89 points, *likely benign* −0.90 to −0.98 points, *benign* −0.99 or fewer points.
CNV copy-number variant, *SVI* sequence variant interpretation.

for each observed piece of evidence, both in support of (positive values) and refuting (negative values) pathogenicity, are summed to arrive at a CNV classification. CNVs with a final point value ≥0.99 are considered pathogenic, while point values between 0.90 and 0.98 are considered likely pathogenic; this approach aligns with the sequence variant interpretation guidelines[8] (i.e., variants interpreted as pathogenic should have a 99% level of confidence and variants interpreted as likely pathogenic should have a 90% level of confidence). The variant of uncertain significance (VUS) category is the broadest, corresponding to points between −0.89 and 0.89, while refuting evidence arriving at scores between −0.90 and −0.98, or ≤−0.99 are considered likely benign and benign, respectively.

To facilitate use of this semiquantitative system, a web-based CNV classification calculator based on these scoring metrics is publicly available (http://cnvcalc.clinicalgenome.org/cnvcalc/). This tool allows users to apply points for individual evidence categories for a given CNV and will automatically calculate the final point value and corresponding CNV classification. This tool will be continually supported and updated, allowing timely integration of new information as it emerges.

These standards were developed for evaluating evidence in the context of constitutional CNVs, including those detected during postnatal or prenatal testing. Laboratories may choose to use specific reporting practices based on factors such as CNV classification and clinical context, and these may vary across different test types and clinical settings (e.g., choosing to only report likely pathogenic or pathogenic variants associated with dominantly inherited conditions in a prenatal setting). These specific reporting practices should be documented in the laboratory's interpretation and reporting protocol.

These standards do not apply to acquired CNVs in neoplasia. In addition, this document does not address analytical validation of CNV detection methods, which have been addressed elsewhere, and assumes that any laboratory using the provided standards is confident that a reported CNV represents a true biological event.[10] These standards serve as a reference for clinicians to enable them to understand the complexity of CNV interpretation and to appropriately communicate test results to patients and families. Although these standards attempt to comprehensively incorporate commonly available resources and processes used in CNV classification and interpretation, it is important to recognize that no singular algorithm will be applicable in all potential scenarios. The semiquantitative scoring framework is meant to serve as a guide. Professional judgment should always be used when evaluating the evidence surrounding a particular genomic variant and assigning a classification.

Recommended variant classification categories

Using the scoring metrics described in Supplemental Material 1, a laboratory geneticist should assign any CNV reported in a patient to one of five main classification categories. It is strongly recommended that consistent terminology for these categories be used in clinical reporting to facilitate unambiguous communication of clinical significance throughout the medical community.

The classification categories represent a significant update from the previous version of these guidelines.[7] To align closely with recommendations in the ACMG/AMP sequence variant interpretation guidelines[8] and with the manner in which these terms are now commonly used, we have updated the existing three-tiered system of clinical significance (in which the term "variant of uncertain significance" had the optional qualifiers of "likely pathogenic" or "likely benign") to the five-tiered system described below.

Pathogenic

Pathogenic (P) CNVs are those that score 0.99 points or higher using the evidence scoring metric (Supplemental Material 1). Although the full clinical effect of a CNV on a patient's phenotype may not be known (due to zygosity or other reasons), the pathogenic nature of the CNV should not be in question.

Examples of P CNVs may include (1) CNVs reported in association with consistent clinical phenotypes across multiple peer-reviewed publications, with well-documented penetrance and expressivity, even if reduced and/or variable; (2) unique CNVs that overlap completely with an established dosage-sensitive region; and (3) multigenic CNVs in which at least one gene is known to be dosage sensitive,[11] even if the other genes are of uncertain significance.

Except for well-established cytogenetic heteromorphisms, this category will include most cytogenetically visible alterations (generally >5 Mb). In the absence of loci clearly associated with defined genetic syndromes within the interval, cytogenetically visible alterations should still be cautiously evaluated, taking the gene content into consideration.

Likely pathogenic

Likely pathogenic (LP) CNVs are those that score between 0.90 and 0.98 points using the evidence scoring metric. In general, these variants have strong evidence to suggest that they will ultimately be determined to be disease-causing, but not enough yet to definitively assert pathogenicity. Several evidence types outlined within the scoring metrics could be combined to reach the LP point threshold. However, some particularly strong pieces of evidence may result in the CNV being classified as LP without the need for additional evidence (although additional information could be added to bring the classification to P). Examples of this type of evidence may include (1) deletions involving the 5' end (plus additional coding sequence) of established haploinsufficient (HI) genes (in scenarios where there are no known alternative start sites) (category 2C-1, deletion metric); (2) deletions involving multiple exons (through the 3' end of the gene) in an established HI gene (category 2D-4); and (3) deletions or duplications involving genes with multiple case reports reported in consistent, highly specific phenotypes.

Uncertain significance

Variants of uncertain significance (VUS) are those that score between −0.89 and 0.89 points using the evidence scoring metric. This represents a broad category and may include findings that are later demonstrated with additional evidence to be either pathogenic or benign. Some CNVs in this category may have more evidence than others to indicate involvement in disease and the likelihood of additional evidence surfacing through published literature may be higher. However, at the time of reporting, if insufficient evidence is available for confident determination of definitive clinical significance and the CNV meets the reporting criteria established by the laboratory, the CNV should be described as a variant of uncertain significance.

Examples of VUS may include (1) a CNV that exceeds a laboratory's size threshold for reporting but has no genes in the affected genomic interval (category 1B); (2) a CNV described in a small number of cases in the general population, but not at a high enough frequency to be considered a polymorphism (>1%) (category 4O, with a downgraded score due to frequency); (3) a CNV that contains a small number of genes, but it is not known whether the genes in the interval are dosage sensitive (category 3A); (4) a CNV described in multiple contradictory publications and/or databases, without firm conclusions regarding clinical significance (multiple categories); (5) a CNV within an individual gene (category 2E, deletion metric, and 2I, duplication metric) with an unclear effect on the transcript reading frame.

Likely benign

Likely benign (LB) CNVs are those that score between −0.90 and −0.98 points using the evidence scoring metric. In general, these variants have strong evidence to suggest that they are likely not involved in Mendelian disease, but do not yet have enough evidence to state this definitively.

Examples of LB CNVs may include (1) variants with no statistically significant difference between observations in cases and controls (category 4N), and (2) variants observed frequently in the general population (although at a lower frequency than 1%, a conventionally accepted threshold for a common polymorphism [category 4O]).

Benign

Benign CNVs are those that score −0.99 or fewer points using the evidence scoring metric. These CNVs have typically been reported in multiple peer-reviewed publications or annotated in curated databases as benign variants, particularly if the nature of the copy-number variation has been well characterized (e.g., copy-number variation of the salivary amylase gene[12]) and/or the CNV represents a common polymorphism. To qualify as a benign polymorphism, the CNV should be documented in >1% of the population. It is important to carefully consider dosage of the CNV documented as a benign variant, given, for example, that duplications of some regions may be benign, whereas deletions of the same interval may have clinical relevance.

Reporting guidelines for copy-number variants in the constitutional setting

In recent years, innovations in microarray and NGS technologies have expanded the diagnostic application of clinical CNV analysis and interpretation from chromosomal microarrays to single- and multigene sequencing panels, and exome or genome sequencing. Each of these tests may have distinct clinical reporting specifications. The following recommendations describe elements of a clinical laboratory report that are necessary to precisely describe the nature of a CNV and clearly communicate the evidence related to its classification and clinical significance. Other required elements of a clinical report (e.g., methodology and relevant disclaimers) are outlined in detail in the ACMG Technical Standards and Guidelines.

Reporting criteria

The laboratory report should include a description of the criteria used for both inclusion of a CNV in the report (e.g., classification type, CNV size) and classification of the CNV (e.g., the scoring metrics included in this document). Laboratories may or may not choose to disclose benign or likely benign CNVs, and this should be indicated in the report and their laboratory reporting protocol.

Description of each CNV detected

Each CNV should be described with the elements below. Appropriate nomenclature from the International System for Human Cytogenomic Nomenclature (ISCN) or the Human Genome Variation Society (HGVS) should be included in the report, but should not serve as a substitute for a clear description of the genomic imbalance for clinical professionals unfamiliar with these conventions.

- Cytogenetic location (chromosome number and cytogenetic band designation).
- CNV size and linear coordinates with the genome build specified. Genomic coordinates for the minimum predicted interval should be specified. When applicable, particularly when gene content of the CNV is unclear, the maximal genomic coordinates may also be provided.
- Copy-number state (e.g., single-copy gain or loss) with CNV mechanism specified when understood (e.g., tandem duplication). Assessment of mechanism may require additional testing methods.
- For intragenic CNVs: Appropriate naming conventions in this scenario may be dependent on the platform used to detect these variants. If the variant is identified using NGS-based technologies, HGVS nomenclature may be preferable, including gene name (using valid Human Genome Organisation Gene Nomenclature Committee [HGNC] nomenclature), transcript, and exons involved. If the variant is identified using CMA, ISCN nomenclature

is generally recommended. The naming convention selected should recognize a location, genomic content, and certainty or uncertainty of precise breakpoints.

Designation of genes in CNV interval

To the extent feasible, genes involved in a CNV should be specified in the laboratory report. For large imbalances, particularly those with well-established clinical significance, it is acceptable to provide only the name of the corresponding syndrome and/or the most clinically relevant genes in the interval. For CNVs of uncertain significance, it is suggested that all validated/curated (i.e., not predicted or hypothetical) genes in the interval be included, when possible, to facilitate periodic reviews of relevant medical literature. The incorporation of links to websites that list the genes in an interval is not recommended because the links may not faithfully direct the clinician to the appropriate gene content in the future. If all genes in the interval are not listed on the report, it is suggested that at least the total number of genes in the CNV interval be provided to highlight the extent of genomic imbalance; other potentially clinically relevant elements may also be noted.

Clear statement of variant classification and clinical significance

Regardless of the type of variant being assessed (CNV, sequence variant, etc.), determining a variant's classification should be performed independently from determining how it contributes to the diagnosis of the individual in whom it is discovered. Uncoupling variant classification (P, LP, etc.) from clinical significance in the context of an individual patient's diagnosis is key to objective and consistent interpretation of genomic variants. While the phenotype of the proband should be taken into account when assessing evidence supporting the pathogenicity of a CNV, classification should not be solely driven by the presentation of the patient under investigation (without consideration of other available evidence). For example, there is compelling evidence in the literature that deletion of a particular gene results in disease X; a laboratory evaluating a deletion of this gene is able to reach 0.99 points using the scoring metric, suggesting a classification of pathogenic. The laboratory should not then disregard all previously collected evidence and classify the variant as "uncertain significance" solely because their patient did not display features of disease X.

The classification of a particular variant should be based upon the evidence available to support or refute its pathogenicity at a given point in time; that body of evidence is ostensibly the same for every patient found to have that variant at that same point in time. As such, the variant should receive the same variant classification (P, LP, VUS, etc.), regardless of the clinical significance it has for each patient (which may differ). For example, there is substantial evidence demonstrating that a particular gene on the X chromosome causes disease via a loss-of-function mechanism. Given the body of evidence, deletions involving this gene should receive the classification of pathogenic each time they are observed, regardless of whether they are observed in hemizygous males or heterozygous females. Within the report, the laboratory should explain the potential consequences of such a deletion for the patient under study—in a male this variant could represent a diagnostic finding; in a female this variant could represent carrier status. Therefore, each description of a CNV should include a clear statement of its classification and the evidence supporting it, as outlined in these recommendations, as well as the clinical significance of that variant for the patient being tested. See Supplemental Material 4 for examples of how these concepts may be conveyed during reporting.

Special considerations regarding reporting: clinically significant findings unrelated to the reason for referral

Occasionally, a CNV may be identified that, although unrelated to the patient's reason for referral, may indicate presymptomatic status for a late-onset disorder or may reveal an ongoing clinically unrecognized condition (i.e., an incidental finding[13]). Some examples of these include deletions involving known tumor suppressor genes,[14] male infertility due to deletions involving the AZF region on the Y chromosome,[15] a deletion disrupting a gene for hereditary spastic paraplegia in a child referred for autism,[16] etc. It is often not possible to specifically avoid interrogation of the types of loci mentioned in the aforementioned cases, because such findings may occur as part of a large CNV involving multiple genes. It is impractical to provide a predefined list of all possible diagnoses to allow a patient to consent specifically to the interrogation of and reporting for each disorder. Therefore, referring clinicians must have a clear understanding of the potential for these discoveries, and patients/families should be duly informed before test ordering. An informed consent process is strongly recommended.

It is recommended that P or LP CNVs indicative of presymptomatic status be reported to facilitate appropriate and timely access to medical care. Individual laboratories may adopt nondisclosure policies for specific conditions and state them as such in their clinical reports.

The ACMG Secondary Findings Working Group has been established to identify genes "associated with highly penetrant genetic disorders and established interventions aimed at preventing or significantly reducing morbidity and mortality."[13] When evaluating CNVs involving these genes, it is important to remember the mechanism of disease associated with each. If haploinsufficiency or triplosensitivity is not an established mutational mechanism for a specific gene, a deletion or duplication is not likely to be clinically relevant. If the mechanism of disease is consistent with haploinsufficiency or triplosensitivity, these CNVs should be reported. Dosage sensitivity evaluations of the genes currently on the ACMG secondary findings list are available at the following link: https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/acmg.shtml.

Clinically significant findings seemingly unrelated to the reason for referral represent another situation where it is important to separate the variant classification from clinical correlation. Historically, the reason for referral has influenced the CNV interpretation process; anecdotally, variants with clear evidence for pathogenicity have been classified as VUS because they did not "explain" the patient's stated reason for referral. The reasons for referral provided to laboratories may not always represent a complete picture of the patient's phenotypic features, and assumptions that a patient does or does not have a particular feature are not prudent without appropriate consultation with the referring clinician. Open channels of communication between the laboratory and the ordering physician are critical to guide clinical correlation.[17]

It is certainly appropriate to consider available phenotype information about a given patient as evidence in variant evaluation; if the patient undergoing testing has a phenotype that is consistent with the described phenotype for an observed CNV, this may be considered evidence supporting pathogenicity. It is not appropriate, however, to provide a different classification for the same CNV simply because it was identified in an individual with a different reason for referral. For example, there is substantial evidence demonstrating that loss-of-function variants in gene X result in hearing loss. If a laboratory observes a deletion of this gene in an individual referred for hearing loss, and the exact same deletion in another individual referred for speech delay, they should not interpret that variant as P in the former case and VUS in the latter. The variant should be classified as P in both instances. The variant is directly relevant to the reason for referral in the individual with hearing loss, but may represent an incidental finding or an explanation for an unobserved/unreported phenotype in the second. The pathogenicity of the variant, however, should not be in question given the depth of the supporting evidence. The reason for referral alone should not be used to justify varying classifications for the same CNV in different individuals.

Special considerations regarding reporting: carrier status

Detection of some CNVs, particularly deletions, will indicate carrier status for autosomal recessive or X-linked disorders mapping within the CNV interval. Although exhaustive reporting of carrier status may be considered difficult to standardize and beyond the intended scope of genome-wide microarrays (particularly for very large multigenic events), improvements in informatics could support reporting of such information in the future. Individual laboratories may choose to adopt specific disclosure policies for recessive conditions. If a laboratory chooses to include a list of carrier alleles, its reports should clearly separate the primary CNV results related to the reason for referral from a secondary list of carrier status alleles. If carrier status is not routinely assessed, reports should clearly state that carrier status may not be disclosed, and that any clinical concern for recessive disorders should be communicated to the reporting laboratory for appropriate consideration. There are some situations when disclosure of carrier status is recommended:

1. Well-characterized disorders where loss of function is the established disease mechanism. In such cases, there may be justification for reporting carrier status to provide opportunity for reproductive counseling and additional testing in the proband or relevant family members, particularly when the carrier frequency is reasonably high, and/or screening is commonly available (e.g., cystic fibrosis). It should be recognized that these disclosures will represent serendipitous findings, and no claim should be made to the ordering clinician or patient that this test will routinely detect carrier status for any condition.
2. Disorders with clinical features consistent with the patient's reason for referral. In such cases, a laboratory may have identified a CNV that represents one allele of an expected pair consistent with the referral diagnosis. The laboratory may then recommend ancillary molecular testing for this disorder in an effort to identify the other disease-causing allele. This should be restricted to well-described disorders with clear clinical consequence. The report should clearly state the recessive nature of the condition, and that the CNV is not diagnostic of affected status without confirmation of a second pathogenic variant.
3. CNVs involving dosage-sensitive genes on the X chromosome in females. Given the significant reproductive risk to female carriers of X-linked conditions, we recommend reporting these variants because it provides the opportunity for the patient and relevant family members to pursue additional testing/counseling as needed. Additionally, females may manifest symptoms in many X-linked disorders; these variants may ultimately have an impact on their medical management.

To make these nuances clearer to users of the laboratory report, we recommend dividing the report into sections describing primary variants considered relevant to the stated reason for referral separately from any variants that represent secondary or incidental findings or carrier status. Laboratories may decide at their discretion if additional subcategories are necessary.

Recommendation for appropriate clinical follow-up

The laboratory report should include recommendations for any necessary further cytogenetic characterization of the CNV, genetic counseling, and evaluation of relevant family members as appropriate. In addition, when a CNV is of uncertain significance, the report may include a recommendation for continued surveillance of the medical literature for new information that may alter the classification of the CNV and provide clarification on its clinical significance. The responsibility for monitoring the medical literature for a specific patient lies primarily with the physician with an ongoing patient relationship,[18] but laboratories may choose to offer amended reports when reclassifications occur.

CONCLUDING REMARKS

Understanding the clinical relevance of CNVs is a complex, continually evolving process that constitutes the practice of medicine. As evident from the numerous considerations outlined in this document, no one formula or algorithm for CNV interpretation will substitute for adequate training in genetics and sound clinical judgment. We recommend that clinical reporting of constitutional CNVs be performed by individuals with appropriate professional training and certification (those individuals certified by the American Board of Medical Genetics and Genomics [ABMGG] in clinical cytogenetics, molecular genetics, and/or laboratory genetics and genomics). In addition, given the complexity of CNV interpretation, the different laboratory methodologies utilized for CNV characterization, and the evaluation of additional family members, an ideal laboratory setting for CNV analysis should include both cytogenetic and molecular genetic expertise.

This document for the first time lays out explicit guidance for interpreting CNVs that occur within individual genes. As detecting CNVs from sequencing-based platforms becomes more commonplace, it is important that CNV and single-nucleotide variant (SNV) analyses are appropriately aligned in their approaches to variant classification. Ideally, a CNV should receive the same classification whether it was detected on a CMA or an NGS platform, and whether or not it was interpreted by someone board-certified in cytogenetics or molecular genetics. The recommendations presented here (and in Supplemental Material 1) represent an initial effort to move toward more consistent CNV interpretation between laboratories and across technologies.

Systematic approaches to variant interpretation (such as this one) will evolve over time, particularly as knowledge regarding the relationships between genomic variation and human health improve. Groups are encouraged to use this framework as a guide, always using professional judgment when opting to incorporate emerging knowledge, methods, and resources, and documenting the process by which this evidence is used to arrive at a variant classification.

To summarize, major updates from the previous document[7] include:

- CNV classification categories will change to the five-tier classification system recommended in the ACMG/AMP sequence variant interpretation guidelines.[8]
- Variants should be classified consistently between patients; while patient presentation and/or reason for referral may be used as evidence to support a particular classification, this information should not be used to justify disparate classifications of the same variant. Variant classifications should be based on evidence; at a given point in time, evidence supporting/refuting a given variant's pathogenicity should be the same. Therefore, the classification of that variant should be the same regardless of patient-specific factors such as reason for referral, sex, age, etc.
- Laboratories should consider utilizing headers or subsections in the clinical report to clearly communicate primary versus incidental or secondary findings, such as carrier status for autosomal recessive conditions, pathogenic variants unrelated to the stated reason for referral, etc. (examples may be found in Supplemental Material 4).
- Explicit new guidance for interpreting CNVs occurring within individual genes (intragenic deletions and duplications) (described in detail in Supplemental Material 1).
- Points-based scoring rubrics (Tables **1** and **2**) to guide laboratories toward more consistent CNV interpretations. We anticipate that updates to these metrics will be required as laboratories gain experience using them, and as evidence and technologies change.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-019-0686-8) contains supplementary material, which is available to authorized users.

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