**Supplemental Material 1: Using the Copy Number Variation (CNV) Scoring Metrics**

**General Instructions**

*Appropriate Use of the Scoring Metrics*

The use of these scoring metrics presumes that a CNV otherwise meets the laboratory’s size and quality thresholds for reporting. It is recommended that any size restriction for inclusion of a CNV in a clinical report be based on the laboratory’s consideration of the performance characteristics and limitations of the methodology used and generation of a reasonable amount of clinical follow-up, rather than assumptions regarding clinical significance based on size alone.

The scoring metrics (Table 1 and Table 2, Main Text) are intended primarily for the evaluation of CNVs associated with genes/genomic regions associated with dominant, Mendelian inheritance patterns. They were not developed with the intent of independently evaluating CNVs exhibiting non-Mendelian inheritance patterns; however, many of the concepts presented here may prove useful in the evaluation of such CNVs.

It should also be noted that many recurrent CNVs exhibiting incomplete penetrance and/or variable expressivity have already been evaluated by resources such as the ClinGen Dosage Sensitivity (DS) curation process, which provides ongoing review of these and other genes and genomic regions (<https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/>).  Those CNVs with well-documented clinical features and significance replicated across multiple independent studies (e.g., 16p11.2 proximal region deletion, 22q11.2 proximal [DiGeorge/Velocardiofacial Syndrome] region duplication) have ClinGen DS scores of 3; such CNVs would be classified as Pathogenic utilizing Category 2A of the scoring metrics (“complete overlap with established dosage sensitive gene/genomic region”).  Conversely, certain recurrent CNVs have been observed across multiple independent studies to lack any consistent phenotypic association, and to have evidence against dosage sensitivity when evaluated in case versus control populations (e.g., 15q11.2 [BP1-BP2] region duplications, 15q13.3 [D-CHRNA7 to BP5, nested] region duplications).  These regions have been given ClinGen Dosage Sensitivity scores of “dosage sensitivity unlikely.” As such, these would be classified as Benign utilizing category 2F in the deletion metric, or categories 2C, 2D, or 2F in the duplication metric (“overlap with established benign gene/genomic region”).  Use of these metrics for recurrent regions other than those with definitive evidence classifications should be performed with caution.

The scoring metrics are intended primarily for the evaluation of the most commonly encountered CNVs (i.e. single copy gains and losses). However, they may also be adapted to evaluate CNVs other than typical losses (i.e., copy number of 1) or gains (i.e. copy number of 3), including the following:

Evaluation of other copy number states

The metrics may be adapted for use in the evaluation of other copy number states, such as homozygous deletions (copy number of 0), or gains beyond a single extra copy (copy number of 4 or more).  When evaluating any case, regardless of copy number state, one must look for evidence identical or similar in scope to the CNV type under evaluation. For example, if one were evaluating a triplication (copy number of 4), one would go through the gain scoring metric (Table 2) focusing on evidence relating to tetrasomy, though overlapping gains with established triplosensitivity may also be appropriate to include.  Another example would be evaluating homozygous deletions. In this scenario, one must evaluate using evidence relating to both haploinsufficiency and nullisomy. With both copies of the allele lost, the patient could be at risk for either a more severe presentation of an autosomal dominant disorder (i.e., if the loss of both copies were not lethal), or an autosomal recessive condition.

Complex Rearrangements

When dealing with complex rearrangements, such as unbalanced translocations, one should evaluate each CNV separately (for example, evaluate the deletion portion of the translocation using the deletion metric, and the duplication portion using the duplication metric).  The overall classification for the event should default to the most deleterious classification (for example, if the deletion portion were classified as “pathogenic” and the duplication portion was classified as “uncertain significance,” the event as a whole should be classified as “pathogenic.”  The mechanism and consequences of complex rearrangements, including recurrence risks, should be discussed in the report.

CNVs on the X Chromosome

The scoring metrics may be used to evaluate losses or gains on the X chromosome, which represent different copy number types in males (copy number of 0 or 2, respectively) and females (copy number of 1 or 3, respectively).  In many circumstances, males are affected by X-linked disorders and females are asymptomatic carriers. However, there are also X-linked disorders in which females are the predominantly affected sex (due to male lethality), or in which carrier females are known to display symptoms of varying degrees of severity (due to skewed X-inactivation and/or other factors).   When evaluating CNVs on the X-chromosome, utilize any appropriate evidence related to affected individuals, regardless of sex. Classification of losses involving X-linked recessive genes in females should be based on the predicted impact in a male (i.e. pathogenic), though overall clinical significance may be uncoupled. See Supplement 4, Example 6, for an example of how to report an X-linked deletion in a female.

*Beginning the Evaluation*

After selecting the appropriate scoring metric (Table 1 for deletions, Table 2 for duplications), work through each evidence category moving from top to bottom. If a section does not apply, skip to the next section. For example, if the observed CNV does not overlap any established dosage sensitive or benign genes or genomic regions (Section 2 in both metrics), move on to Section 3.

When evaluating individual CNVs using case-based evidence from the literature or internal/external databases, the following may be considered relevant: CNVs (of the same copy number state/type) with identical or highly similar in genomic content to the one under evaluation; LOF variants involving individual genes within the deleted region; or whole gene duplications of individual genes within the duplicated region. For many CNVs, particularly those without an established HI or TS gene/genomic region, it is often difficult to identify other CNVs with identical or highly similar genomic content, and evaluating individual genes within the CNV itsel may be the best approach to identifying evidence. It may be most appropriate to focus first on evaluating gene(s) within the interval that have established disease relationships. Resources with well-documented gene-disease annotations, such as OMIM (<https://omim.org>) and ClinGen (<https://search.clinicalgenome.org/kb/gene-validity/>) can a good starting point for evaluating phenotypic overlap, mechanism of pathogenicity, and inheritance patterns of individual genes.

The ClinGen Dosage Sensitivity map specifically annotates and scores genes and genomic regions in relation to dosage sensitivity, and should be used to interrogate most CNVs. When selecting the gene(s) to take through the scoring metric, give precedence to the genes in the region that are associated with dominantly inherited disorders caused by an appropriate mutational mechanism (see below).  If a laboratory chooses to report on carrier status for autosomal recessive carrier status, they should consider only those conditions for which loss of function is an established disease mechanism. Resources useful for evaluating gene inheritance patterns include OMIM and the ClinGen Gene-Disease Validity curations.

When evaluating gene-level evidence, one must evaluate the given CNV in the context of the mutational mechanism observed in that gene. Dominant disorders may result from mechanisms other than dosage sensitivity (for example, gain of function or dominant negative mechanisms), and in some cases, differing dominant phenotypes may be associated with the same gene (e.g., gain of function/activating mutations in *FGFR1* result in skeletal dysplasias, whereas deletions/loss of function variants are associated with Kallmann syndrome1). A full extra copy of a haploinsufficient gene should not be presumed to be disease-causing unless it is specifically known to also be associated with a triplosensitive disorder (e.g., *PMP22*, which is associated with Charcot-Marie-Tooth disease [MIM: 118220] when duplicated and Hereditary Neuropathy with liability to Pressure Palsies [MIM:162500] when deleted). Full-gene duplications are commonly present as benign variation in the human genome2, 3.

*Scoring Ranges*

A range is provided for most scoring categories to allow the user flexibility when considering evidence of different relative strengths. For example, in Section 4, individual probands described in the literature, public databases, and/or internal laboratory data may be more or less convincing than others depending on factors such as the mode of ascertainment, depth of clinical data, phenotypic specificity, inheritance pattern, and/or the presence of additional or alternative genetic or non-genetic etiologies. Users should assign more points for particularly strong evidence (e.g., a well-phenotyped, *de novo* case involving the same genomic content as the CNV being evaluated) and fewer points to weaker evidence (e.g., a case with limited clinical data and no inheritance information). At times, the recommended range will include 0; just because a piece of evidence in a particular category is available does not mean it warrants points. For example, a case-control study involving the CNV under evaluation may be published, but, for whatever reason, the study is deemed to be of poor quality. The user is not obligated to assign points for a poorly-designed study; if a score of “0” is the most appropriate, then that is what should be assigned. Whenever a laboratory opts to deviate from the default recommended points for a particular evidence category, the reasons should be documented in their internal tracking systems and/or in the clinical report in an effort to move toward greater transparency in variant classification.

*Arriving at a Classification*

Once a score of 0.99 or greater (or -0.99 or fewer) has been obtained, the CNV classification has been made (pathogenic or benign, respectively). However, one should consider working through the remaining sections of the metric (particularly section 4, focusing on literature evaluation) to determine if there is additional information of clinical relevance to report. Consider, for example, a deletion involving multiple genes. If one of those genes is an established HI gene, then 1.00 points may be assigned per category 2A (deletion metric), and the variant classification is “Pathogenic.” However, there may be mounting evidence that HI of a different gene in that interval could be involved in a different phenotype. This does not change the classification (the CNV is still pathogenic), but the laboratory may wish to also comment on the potential clinical significance of the loss of the other gene in the clinical report.

It is recommended that laboratories keep track of the CNVs they evaluate and the evidence that supports them to alleviate the need to start the evaluation from the beginning each time a particular CNV is observed. Once a CNV has reached a classification of “pathogenic” via this scoring metric, it is not always necessary to fully reevaluate the evidence each time it is observed, though laboratories should confirm that no new evidence contradicting pathogenicity has emerged since the variant was last evaluated.

The following text describes each section in the scoring metrics in detail, providing examples to illustrate various concepts. Note that the section numbers are the same in both the deletion and the duplication scoring metrics, but the individual categories therein may differ.

**Section 1: Initial assessment of genomic content**

In this first evidence category, genomic content within the CNV is evaluated in a general manner. If the CNV contains or overlaps protein-coding genes or other known functionally important elements4 (1A), further evaluation (using the subsequent evidence categories) of the CNV is required. Examples of functionally important elements include: the *SHOX* enhancer 160 kb downstream of the gene5, the zone of polarizing activity regulatory sequence (ZRS) affecting the *SHH* gene (reviewed here:<https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/clingen_region.cgi?id=ISCA-37467>), etc.

CNVs that are completely void of gene content, including intronic sequence, repetitive elements or pseudogenes, fall into category 1B. The recommended point score for these types of events is -0.60. In these scenarios, there may not be any evidence to interrogate about the genomic region, as no genes are present. With no evidence supporting or refuting pathogenicity, the default classification should be one of uncertainty, which is reflected in the recommended default score. Additional information, however, could move a CNV like this toward either pathogenic (P) or benign (B) as appropriate. Inheritance information from the family being studied (considered in Section 5) could be used to classify this type of CNV as likely benign (LB) or B. However, the possible presence of functionally important elements, such as promoters, enhancers, or other regulatory regions near coding sequence, should be considered with clinical correlation for possible effects on gene expression. In addition, given that CNV breakpoints are not precisely mapped due to gaps in probe or bait coverage, it is important to consider all genes in the maximum CNV interval before presuming a CNV to be clinically benign (even though reporting is often done on the minimal interval only). Further evaluation may be necessary to clarify the genomic content of the CNV for appropriate clinical interpretation. Generally, it is acceptable to adopt a laboratory policy not to report these CNVs or report them in a supplemental list, as there is no relevant literature to interrogate. An exception might be made if the CNV exceeds a size cutoff established by the laboratory.

**Section 2: Overlap with Established (or Predicted) Pathogenic or Benign Dosage Sensitive Genes/Genomic Regions**

In this evidence category, the CNV is evaluated for overlap with established dosage sensitive genes or genomic regions, or any established benign genes or genomic regions (i.e., known to be copy number variable in the general population). To aid the genomics community in identifying those genes and genomic regions that are HI and/or TS, ClinGen has developed the Dosage Sensitivity Map6, a publicly available resource cataloging evidence supporting and/or refuting the role of dosage sensitivity for genes and genomic regions. For the purposes of this framework, “established dosage sensitive” (DS) genes or genomic regions are those that have been evaluated and shown to have sufficient evidence for HI and/or TS (i.e., a score of 3). “Established benign” genes or genomic regions are those that have been evaluated and shown to be copy number variable in the general population (i.e. a score of “dosage sensitivity unlikely”). A list of genes and regions that have been evaluated using these criteria is updated daily and can be accessed at <https://www.ncbi.nlm.nih.gov/projects/dbvar/clingen/>.

Those CNVs completely containing established dosage sensitive genes or genomic regions may be considered “pathogenic” (category 2A; illustrated in Figures S1.1 and S1.2). If the CNV under evaluation partially overlaps an established dosage sensitive genomic region, further evaluation is required to determine whether the causative gene or critical region for the established region is known. If the critical gene/region has not yet been established (category 2B; illustrated in Figure S1.2), additional evidence will be necessary to determine the clinical relevance of the CNV under evaluation. For example, though the clinical significance of the 1p36 deletion (MIM: 607872) has been appreciated for some time, there is still no established causative gene or critical region7, 8; deletions overlapping (but not completely encompassing this region) would fall under category 2B, and would require further evidence to determine their classification and clinical significance. If the CNV partially overlaps a curated HI or TS gene, additional evaluation to determine the potential functional effect is required based on breakpoint location, involvement of coding sequence (for partial gene deletion/duplication) and evidence from the literature (categories 2C-2D in the deletion metric [Table 1], 2J-2K in the duplication metric [Table 2]; illustrated in Figure S1.3).

Similar logic applies when a CNV overlaps an established benign gene or genomic region. For the purposes of this framework, “established benign” genes or genomic regions are those evaluated and determined to be “dosage sensitivity unlikely” per the ClinGen Dosage Sensitivity Map. In general, these are CNVs that occur at high frequency in the general population (1% or higher), are not known to be more frequent in cases compared to controls and are not associated with any consistent phenotype. When a CNV under evaluation is contained completely within one of these genes/regions, it may be considered “benign” (category 2F in the deletion metric [Table 1], and categories 2C, 2D, and 2F in the duplication metric [Table 2]; illustrated in Figure S1.4). If the CNV under evaluation overlaps an established benign gene/region but is larger, it may contain clinically relevant genes or functionally important elements (such as regulatory regions for nearby HI genes) (category 2G; illustrated in Figure S1.4), and additional evaluation is recommended.

In general, if a CNV reaches a 1 or -1 score due to complete overlap with an established dosage sensitive gene or region, users of these metrics may not need to proceed further; however, for some CNVs, particularly those with incomplete penetrance and/or variability expressivity, additional evaluation may be necessary, and caution is recommended before interpreting a CNV based on this information alone. When reviewing ClinGen DS scores, it is important to note the date of last evaluation, as curations reflect a temporally static assessment. New evidence may have emerged since the date of last evaluation, either supporting or refuting the original assessment.

*Deletions partially overlapping established HI genes (categories 2C-2D in the deletion metric)*

Special considerations may apply when a deletion partially overlaps an established HI gene. Note that these evidence categories are only applicable if the testing platform has sufficient resolution to identify such events. If the 5’ end of the gene is involved, one must consider whether the deletion also involves additional coding sequence (category 2C-1), or if it is just restricted to the 5’ untranslated region (UTR) (category 2C-2) (illustrated in Figure S1.3).

If additional coding sequence is involved, scoring should be based on the likelihood that a functional protein could still be produced. Deletions that include 5’ UTR exons, first coding exons, or internal exons are typically deleterious in LOF genes. If the gene has alternative in-frame methionine downstream of the deletion, or if an alternatively spliced isoform is not encompassed by the deletion, consider downgrading from the suggested default number of points. If a significant portion of the gene is deleted, or if a known functional domain is deleted, consider upgrading the suggested default number of points. If the deletion only involves the 5’ UTR, but the promoter is well-characterized and included in the deleted interval, consider upgrading the suggested default number of points.

When only the 5’UTR is involved, the recommended default score is 0. However, there are genes in which there is evidence supporting the role of 5’UTR variants in disease9. In these scenarios, it may be appropriate to upgrade within category 2C-2.

If a deletion partially overlaps the 3’ end of the gene (category 2D; illustrated in Figure S1.1), one must consider whether or not the resulting protein product is expected to undergo nonsense-mediated decay (NMD). Deletions that involve the last exons of a LOF gene are not universally pathogenic if amino acids in the carboxyl terminus of the encoded protein are dispensable. If only the 3’ UTR is involved (category 2D-1), the recommended default score is 0. If the deletion involves only the last exon, the resulting protein product is expected to escape NMD. If, however, there is evidence to suggest that the last exon is critical to the protein’s function (for example, if other established pathogenic variants have been documented in that exon), then such a deletion may be disease-causing (category 2D-2). Use the scoring range to reflect the level of confidence that the last exon is critical to gene function (e.g., increasing points with increasing number of documented pathogenic variants, functional studies showing that loss of the last exon results in disrupted function, the last exon is within an established variation hotspot, etc.). If there is no evidence to suggest that the last exon is critical to the gene function (for example, no other pathogenic variants have been reported in that exon) (category 2D-3), the recommended default score is 0.30. If a deletion overlaps the 3’ end of the gene and includes exons other than the last exon (category 2D-4), NMD is expected to occur, and the recommended default score is 0.90. Consider upgrading from the default score if there is additional evidence to suggest a detrimental effect on the protein (for example, if a significant percentage of the protein is expected to be missing).

*Deletions and duplications within individual genes (category 2E in the deletion metric; 2I in the duplication metric)*

With the increasing resolution of array and NGS-based approaches, it is now possible to identify losses and gains of genomic material within individual genes. As opposed to multigenic CNVs (i.e., those CNVs involving more than one gene), these smaller, intragenic variants may be evaluated similarly to sequence-level variants. Guidelines for the interpretation of sequence variants already exist10, and additional efforts are underway to further clarify how best to utilize these11-13. The ACMG/AMP sequence variant evidence category most directly applicable to intragenic deletions and duplications is PVS1, the rule that evaluates null variants in genes for which LOF is a known disease mechanism. The original publication, however, did not provide specific guidance regarding when PVS1 should be applied, or scenarios in which PVS1 (a “very strong” piece of evidence) should be used at the original strength or downgraded. The ClinGen Sequence Variant Interpretation (SVI) working group recently put forth some additional considerations regarding use of the PVS1 rule14. To promote consistency and transparency across the field, we recommend that intragenic deletions and duplications within established HI genes6 be evaluated according to these criteria. A helpful flow-chart is provided in that manuscript that walks the user through how to determine the relative evidential strength of a given intragenic deletion or duplication. Each PVS1 category as described in that manuscript has been associated with a numerical point score in both the deletion (category 2E) and duplication (category 2I) metrics. Briefly, for intragenic deletions, one must consider: whether or not the deletion disrupts the reading frame; whether or not the deletion is predicted to undergo NMD; if the deleted exons are present in biologically-relevant transcript(s); if the truncated/altered region is critical to protein function; the frequency of LOF variants in the involved exon(s) in the general population; and the amount of protein removed by the deletion.

For intragenic duplications, one must consider: whether or not the duplication is in tandem; if the reading frame is disrupted; and if NMD is predicted to occur. Duplications affecting individual genes are typically expected to be in tandem rather than as insertions or translocations elsewhere in the genome15. Partial-gene duplications including terminal coding exons, i.e., either the first or last exons, of any type of gene are also often not deleterious because functional gene structure may be preserved. Surveys of benign variation have shown a preponderance of such duplications as well in the general population3. In contrast, duplications with breakpoints entirely within a gene and not involving terminal exons can be deleterious because they can disrupt the reading frame or splicing. In specific cases, it is essential to accurately determine the reading frame because it has known clinical implications (e.g., distinguishing between Duchenne and Becker muscular dystrophy).

When intragenic CNVs are being identified by chromosomal microarray (CMA), it is at times not possible to determine with certainty which elements may be involved, or whether or not the variant will disrupt the reading frame, due to array design and/or gaps in probe coverage. If information necessary to work through the PVS1 flow chart14 is not available, always default to the least impactful option. For example, if it is not clear whether a particular intragenic deletion disrupts the reading frame, select the arm of the flow chart that corresponds to deletions that preserve the reading frame. If available/feasible, one may also refer the patient for additional molecular testing to better characterize the event if the particular testing platform has technical limitations.

*Evaluation of HI predictors (Section 2H, deletion metric only)*

Computational (*in silico*) predictors of gene and variant function provide supportive evidence in the evaluation of sequence variants. Similarly, for the evaluation of CNVs, HI predictors may be used as supporting evidence for copy number losses. These types of *in silico* predictions do not need to be used for deletions that involve a gene which has been already established to cause disease due to HI or LOF (categories 2A-2E, deletion metric); use of this evidence is restricted to intervals encompassing genes of uncertain clinical significance.

Two HI predictors currently in common use are the gnomAD probability of loss of function intolerance (pLI) score16 and the DECIPHER HI index17. Note that these tools represent predictions; to our knowledge, the sensitivity and specificity of these tools to accurately predict haploinsufficiency have not been studied. Conservatively, both predictors must have strong HI scores to be included as supportive evidence. A deletion involving at least one gene with a gnomAD pLI score ≥ 0.9 (and upper bound of the observed/expected confidence interval <0.35) and a DECIPHER HI index of ≤10% will qualify for scoring in this category. The points associated with this category should only be given once; do not count this piece of evidence multiple times, even if there is more than one gene in the region predicted to be HI.

**Section 3: Evaluation of Gene Number**

Though CNV size is generally used as a proxy for pathogenicity, exceptions often occur. Some CNVs that are very large, including cytogenetically visible imbalances, can be benign in nature18-21, while very small CNVs, such as focal gene and intragenic deletions and duplications, can be pathogenic due to dosage sensitivity.

In order to understand the impact of gene content on CNV pathogenicity, we evaluated the gene content of over 5000 clinically-classified copy number gains and losses from publicly available datasets (studies nstd37 and nstd101 in dbVar). Autosomal CNVs included in this study were classified using a 5-tier clinical classification system (benign, likely benign, uncertain, likely pathogenic, pathogenic). We controlled for CNV size (200 kb-5 Mb) and reduced the number of duplicate CNVs by only including a single representative CNV if all associated clinical classifications were the same. We excluded duplicate CNVs with multiple clinical classifications, non-protein coding genes, known dosage sensitive genes and known pathogenic or benign regions within dbVar study nstd45. Gene arrays were defined by greater than five genes in a family within a CNV and were reduced to count as one gene, by utilizing a custom script to identify genes with the same three starting characters.

We found that the median gene content of duplications classified as pathogenic (n=732) was 23.5 (average number of genes = 29.39, min=0, max=151), whereas for the median gene content of duplications classified as benign (n=990) was 4 (average number of genes = 5.33, min=0, max=38). Similarly, for deletions classified as pathogenic (n=1367), the median gene content was 18 (average number of genes = 22.35, min=0, max=91), and for deletions classified as benign (n=607), the median gene content was 4 (average number of genes = 4.36, min=0, max=26). We used over-lay of histograms (bin size = 10) of pathogenic and benign CNVs to determine optimal data segments (Figure S1.5).

Given these results, a stratified approach was developed that combined reported standard practice of participating laboratories with the results of gene content analysis. Deletions containing less than 24 genes and duplications containing less than 34 genes (category 3A) are not assigned any points; in general, pathogenicity in this gene range can be quite variable and very dependent upon the actual genes therein (i.e., whether or not one or more is dosage sensitive). Deletions containing between 25 and 34 genes, or duplications containing between 35-49 genes (category 3B) are assigned 0.45 points (a strong level of evidence). Deletions containing 35 or more genes, or duplications containing 50 or more genes (category 3C) are assigned 0.90 points (a very strong level of evidence). Note that, although these gene bins were determined using data from autosomes only, the relationship between gene count and pathogenicity should generally also apply to CNVs on the X-chromosome. Our results demonstrate that, in general, when a CNV contains a very large number of genes, it becomes more likely that loss or gain of this amount of genetic material will result in some demonstrable phenotypic consequence.

In practice, when using Section 3 in the gain and loss scoring metrics, err on the side of caution if a CNV involves clusters of genes or gene families, particularly those that are non-coding or lack any known clinical association. We recommend counting each cluster/family as a single gene. Exceptions include gene families in which individual genes are known to be associated with disease (such as the SCN gene cluster region at 2q24.3); in these cases, the disease genes may be counted individually.

**Section 4: Detailed Evaluation of Genomic Content Using Cases from Published Literature, Public Databases, and/or Internal Laboratory Data**

Many CNVs will not overlap established dosage sensitive genes or genomic regions; for these CNVs, gene content should be scrutinized for documented and relevant clinical association. When evaluating other data sources, such as published literature, public databases, and/or internal laboratory data, one is looking for case-level or case-control data that support or refute the clinical significance of the genomic region under evaluation. The primary source for this type of information is peer-reviewed medical literature, however supportive information may also be obtained from public databases (ClinVar22, DECIPHER23, etc.) or internal laboratory databases. We recommend that laboratories develop processes to document, track, and re-evaluate previously classified CNVs, includingpathogenic CNVs, CNVs of uncertain significance, and CNVs that have been determined to represent benign variation, and share this information with the community through publicly available databases such as ClinVar.

Use judgment when opting to include data from a public or internal laboratory database as evidence. For data available in public databases, consider the amount of supporting evidence available to allow for independent evaluation of the clinical classification provided. For internal laboratory data, consider possible sources of bias, such as platform-specific artifacts and population sampling bias. Consider assigning higher or lower points based on the quantity and quality of the data available. Primary data should involve observations of related variants in humans, while *in vitro* or *in vivo* studies may be viewed as supportive. Use of evidence derived solely from model systems is generally discouraged, particularly for CNVs associated with non-specific conditions (e.g., intellectual disability) and/or limited evidence regarding gene function (e.g., the only information available for a gene is documented neuronal expression).

Individual Case Evidence

Case-level data includes other reported probands with CNVs similar in genomic content to the CNV under evaluation. For copy number losses, one may also consider probands with deletions or LOF variants of individual genes within the loss under evaluation; for copy number gains, one may also consider probands with full gene duplications of individual genes within the gain under evaluation. When evaluating case-level data reported in the literature, one must consider the clinical context in which the variant was observed. In general, probands with similar, highly specific, well-defined phenotypes represent stronger evidence than probands with disparate or non-specific phenotypes. For the purposes of this framework, “highly specific, well-defined” phenotypes are those that are both distinct and have a known genetic etiology with limited genetic heterogeneity. Examples of “highly specific, well-defined” phenotypes include congenital anomalies (e.g., skeletal dysplasias, midline facial defects, holoprosencephaly, etc.) or a constellation of findings (e.g., coloboma, heart defects, choanal atresia, growth delays, genital anomalies, and ear abnormalities associated with CHARGE syndrome (MIM:214800)). “Non-specific” phenotypes are those that may be more common in the general population, have more considerable genetic heterogeneity, and/or can be caused by etiologies other than genetic variation. Examples of “non-specific” phenotypes include autism spectrum disorder and intellectual disability. Inheritance information, when available, can be used to modify the strength of case-level evidence. For example, a higher score should be assigned to cases with *de novo* occurrences or strong segregation amongst similarly affected family members compared to cases with unknown inheritance.

*De novo Occurrences (categories 4A-D)*

The fact that a variant occurred *de novo* in an affected individual with no family history of disease lends credence to that variant’s possible clinical significance; this information alone, however, is not enough to infer that a particular variant is disease-causing. The ClinGen SVI working group has developed parameters for evaluating the relative strength of *de novo* occurrence evidence based upon the phenotypic consistency, total number of *de novo* observations, and whether or not parental relationships have been confirmed (<https://www.clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_0.pdf>).

Briefly, those cases with highly specific and relatively unique phenotypes (e.g., those with pathognomonic features for a specific condition) and confirmed parental relationships (i.e., both maternity and paternity have been confirmed) constitute the strongest level of evidence (category 4A). Cases with highly specific (but not necessarily unique) phenotypes, consistent with what is expected for a given gene/genomic region, represent a moderate level of evidence (category 4B). Examples of this scenario may include phenotypes that are relatively rare in the general population but may also be associated with more than one known genetic cause, such as early infantile epileptic encephalopathy. Phenotypes that are consistent with what is expected for a given gene/genomic region but are not highly specific and are noted to have high genetic heterogeneity (e.g., intellectual disability, autism spectrum disorder, etc.) are considered supporting evidence (category 4C). In all categories, if parental relationships are assumed but not confirmed, relative strength is decreased, and points are subsequently reduced. A range is provided for all categories to allow for flexibility when assessing phenotypes of different relative specificity.

The ClinGen SVI *de novo* criteria recommendations were written for the evaluation of sequence-level variants within genes of known clinical significance and focus on the consistency of the observed phenotype with the phenotype that is known to be associated with variation in a particular gene. However, these concepts can be adapted for the process of CNV evaluation, which often requires the assessment of both genes of known and unknown clinical significance. When the gene(s) being assessed are of known clinical significance, points should be assigned for each reported *de novo* observation according to the appropriate category (4A-4D) up to a maximum total value of 0.90 points. For example, variation in gene X may be commonly associated with disease Y; however, gene X has not previously been evaluated by the ClinGen Dosage Sensitivity process, and it is unclear whether haploinsufficiency is the disease mechanism for disease Y. The CNV under evaluation is a deletion that includes gene X. There have been two reports of probands in the literature with *de novo* LOF variants in gene X and phenotypic features consistent with and highly specific for disease Y (Category 4A). One of these probands has confirmed parental relationships (0.45 points), the other has assumed parental relationships (0.30 points). Based on this information 0.75 total points would be assigned in this section.

When the gene(s) being assessed are of unknown clinical significance, the expected phenotype associated with the gene is unknown. In this scenario, consistency of the observed phenotypes with each other, as well as their relative specificity is critical. Because there is no “expected” phenotype against which to compare, there must be at least two *de novo* cases with similar phenotypes before evidence can be counted. Each additional case observed with a consistent phenotype may be awarded additional points. For example, consider gene X as a gene of uncertain significance. There have been 3 reported *de novo* deletions in the literature, each with developmental delay. Developmental delay is a non-specific and highly heterogeneous phenotype; however, it is consistently observed across all three cases (Category 4C). All 3 cases have confirmed parental relationships. In this scenario, 0.15 points would be assigned to the first two cases, and an additional 0.15 would be assigned to the third, for a total of 0.30 points.

When there is only a single *de novo* case reported in the literature for in a gene of uncertain clinical significance, it may be unclear whether the observed phenotype is due to the observed *de novo* variant or some other cause entirely (genetic or otherwise). In this scenario, if the variant under evaluation by the laboratory is *de novo*, and the phenotype reported to the laboratory is consistent with the phenotype of the single reported *de novo* observation in the literature, these two cases could be used together to meet the consistency requirement. However, if this is the case, the proband under evaluation should not be assigned additional points in Section 5 of either scoring metric.

If the reported phenotype is either NOT consistent with what is expected for a given gene/genomic variant, or simply not consistent in general, clinical judgement should be used to determine if this should result in 0 added points to the evaluation, or negative point values (i.e., evidence *against* a role in pathogenicity). Negative point values could be considered with increasing evidence of inconsistency. For example, consider a *de novo* deletion of a particular gene or genomic region reported twice in the literature - once in a seven-year-old with developmental delay, and once in a newborn with a congenital anomaly. Though the reported phenotypes are not the same, there is not enough information here to constitute evidence against a role in pathogenicity - the newborn may ultimately go on to be diagnosed with developmental delay, and it may be unclear whether the older child was assessed for the congenital anomaly. A scenario like this may warrant 0 points. Next consider a scenario in which *de novo* deletions have been observed in 5 cases in the literature - all in well-phenotyped, older individuals - 1 with developmental delay, 1 with a history of cardiac defect and normal development, 1 with a history of genitourinary anomalies and normal development, and 2 in individuals from the general population. In this scenario, with such disparate phenotypes and reasonable confidence that there are no overlapping phenotypes, negative points may be assigned.

*Unknown Inheritance (category 4E)*

Occasionally, particularly in older literature, there are probands with compelling phenotypes and CNVs similar in genomic content to the CNV under evaluation (or other variants appropriate for inclusion, such as LOF variants in genes included in copy number losses, or whole gene duplications of genes included within copy number gains, etc.) but without inheritance information. Caution should be used when deciding whether to include these cases as evidence, as knowing whether a particular variant is *de novo*, inherited from an affected parent, or inherited from an apparently unaffected parent constitute different levels of evidence in the clinical evaluation of that variant. It is recommended that these types of cases only be included as evidence if the phenotype in the proband is highly specific and consistent with that of other probands for which inheritance is known, and only be assigned a minimal number of points. These types of cases should not be used in the setting of nonspecific phenotypes (e.g., developmental delay or autism spectrum disorder).

*Segregation Among Similarly Affected Family Members (categories 4F-4H)*

Segregation of a variant among similarly affected family members can lend support to the argument that the variant may be disease-causing. It is important to remember that segregation implicates a locus, not necessarily a particular gene or variant. While a given variant may be segregating within a family, it may be in linkage disequilibrium with the true causative variant. At least 3 documented segregations among affected individuals must be documented in order to assign points per this scoring metric; segregations may be added across families. In order to simplify the process of assessing segregation evidence, we recommend an approach consistent with the ClinGen gene-disease validity evaluation process24; only those individuals with both the genotype and the phenotype, or individuals who are obligate carriers by virtue of their position in the pedigree, should be counted as evidence toward segregation. This is a conservative approach. Given the variability in literature reporting, it is often not possible to consistently gather accurate information on other family members’ phenotypes and/or genotypes, and therefore we believe that such a conservative approach is appropriate. The number of segregations and suggested points included in categories 4F-4H serve as a guide; consider assigning fewer than the suggested number of points if the variant was observed via candidate gene sequencing only (as opposed to other genome-wide assessments, such as exome sequencing). Note that phenotypes segregating in a family may exhibit variable expressivity but may still be part of the same phenotypic spectrum. For example, autism spectrum disorder, intellectual disability, seizures, and schizophrenia observed in different individuals may all be caused by the same CNV as part of the developmental brain disorder spectrum25.

*Apparent Non-Segregation (categories 4I-4K)*

Instances in which the variant appears NOT to segregate with affected status must be interpreted with caution. Apparent non-segregations include: 1) instances in which another *affected* individual in the family is found *not* to have the variant in question (category 4I), and 2) instances in which an *unaffected* individual *is* found to have the variant in question (categories 4J and 4K). In scenario 1 (category 4I), before considering this evidence against the pathogenicity of the variant and assigning negative points, one must consider whether there may be a biologically plausible explanation for this non-segregation, such as the presence of phenocopies. This scenario is more likely to occur in the setting of disorders that are more common in the general population (for example, breast cancer) and/or disorders that are known to have both genetic and non-genetic causes (for example, cardiomyopathy). Assign the default number of negative points (-0.45) when the individuals in the family are affected with similar, highly specific phenotypes (with no known phenocopies) and are not found to carry the same variant. Consider downgrading this evidence when phenocopies are a possibility.

In scenario 2 (categories 4J and 4K), one must consider whether the family member found to have the variant is truly unaffected. This can be difficult to determine, particularly in cases ascertained through the literature, public databases, or even internal laboratory databases, as it is often not possible to request additional clarifying information. Truly affected individuals may appear to be unaffected in the context of variable expressivity, reduced or age-dependent penetrance, disorders with subtle clinical manifestations, incomplete clinical evaluation, or novel disorders in which the phenotypic spectrum has not yet been well characterized. Specificity of the phenotype plays a role in how confident one can be in a family member’s “unaffected” status, and the recommended point values vary accordingly (e.g., finding a variant in a person unaffected for a specific, unique phenotype [4J] is stronger evidence against pathogenicity than finding a variant in a person unaffected for a non-specific phenotype [4K]). For example: the phenotype under investigation is specific and well-defined, such as a rare eye phenotype only appreciable with dilated eye examination. If the family member were evaluated by an ophthalmologist with dilated eye exam and found not to have said eye phenotype, assign the default number of negative points for a family member with the variant but unaffected with the specific, well-defined phenotype (-0.30 points, category 4J). If the family member were evaluated by a general practitioner, and it is unclear whether a dilated eye exam was performed, consider downgrading this evidence. In this scenario, 0 points may be appropriate if a dilated eye exam was not performed; there would be no way to know whether this individual was truly unaffected or not without this study.

Now consider a less specific phenotype, such as autism spectrum disorder. If the family member were thoroughly evaluated (for example, by a developmental pediatrician using validated measures of cognitive and social development) and said to be unaffected, consider awarding the default number of negative points for a family member with the variant but unaffected with a non-specific phenotype (-0.15 points, category 4K). Consider downgrading or not awarding any negative points at all if, for example, that family member is simply stated in a publication not to be affected, but no details of their evaluation are provided. Note that, when evaluating literature, public databases, and/or internal laboratory databases, points can be assigned per independent family observed up to the maximum number of points recommended for that particular category.

*Case-Control or General Population Data (categories 4L-4O)*

If the CNV has been studied as part of a well-powered case-control study with adequate numbers of cases and controls, points may be added or deducted based on enrichment (or lack thereof) in the clinical population. Interpretation of case-control data should include evaluation of significance (i.e. p-value), effect size (e.g. likelihood ratio), and clinical information (e.g. phenotypic specificity). Similar to categories defined for sequence variants10, CNVs in this category will be observed at a significantly higher frequency in cases versus controls (p< 0.05), and with a strong effect size (odds ratio or likelihood ratio >5) and relatively narrow associated 95% confidence interval (lower bound >1). CNVs with the highest points values in this category will be observed in association with a consistent, specific, well-defined phenotype (0.45 points, category 4L); those lacking phenotypic specificity, but enriched nonetheless will also be counted (0.30 points, category 4M). Alternatively, CNVs that are not enriched are observed at similar (or higher) frequencies in controls compared to cases, may be deducted points, particularly when such CNVs are also relatively common (e.g. >0.1% frequency, -0.90 points, category 4N).

When evaluating case-control studies, be cognizant of the methods by which controls were ascertained, as well as the methods by which they were tested. Be aware of biases that might exist if alternate testing platforms were used for cases and controls. CNVs that have been observed in both affected and reportedly unaffected individuals, outside of the context of a disorder for which reduced penetrance and variable expressivity are well understood, should not be immediately dismissed as potentially causative variants without careful consideration. Clinical judgment should be used to determine the appropriate classification for these types of CNVs. A points range (to 0, categories 4L-4N) is provided to account for case-control studies that are underpowered, biased, conflicting, and/or indicate weaker statistical associations.

Though a variant may not have been evaluated as part of a formal case control study, it is still possible to gather information about its presence or absence in the general population through resources which catalog copy number variation in reference populations, such as the Database of Genomic Variants (DGV)2 or the gnomAD structural variant (SV) dataset (<https://www.biorxiv.org/content/10.1101/531210v3>) (category 4O). The DGV compiles variation observed across multiple studies - these studies are often conducted on different platforms and at different levels of quality. To aid the user in identifying the most high-confidence, high quality CNVs observed in reference populations, DGV has developed a curated “Gold Standard” dataset. The Gold Standard dataset is a subset of the total DGV data that includes only those CNVs from genome-wide, high resolution assays with the most accurate breakpoint resolution that have passed internal quality control metrics. Using these data, DGV can provide more accurate frequency estimates and population distribution information. This data is available as a standard track on the DGV website, <http://dgv.tcag.ca/dgv/app/home>.   In 2019, the gnomAD database released a set of approximately 500,000 structural variant calls from approximately 15,000 genome sequencing samples with accompanying frequency information.  Of note, population data for copy number variants has historically not been as robust as population data for sequence variants; as resources like DGV and gnomAD continue to add data and new populations, these datasets will become increasingly more valuable in the variant classification process. In general, for the purposes of clinical CNV evaluation, a CNV may be considered “common” in the general population if it is present at a frequency of 1% or greater in the DGV Gold Standard dataset (or other high confidence dataset); variation observed at this frequency in the general population may receive the default number of points for this category, -1 points. Consider assigning less weight if a variant is observed in the general population, but at a frequency lower than 1%. Note that expected carrier frequencies associated with autosomal recessive diseases may be higher than 1%; do not use this criterion to classify a CNV as “benign” if there is a well-known autosomal recessive disease gene (for which loss of function is the established disease mechanism) in the interval.

Consider downgrading if there are reasons to question the reported population frequency of a variant. When evaluating variation in the general population, consider the number of samples the variant was observed in compared to the number of the samples where that particular locus was examined; when the frequency is extremely high (i.e., >99%), it may be because the locus was only examined in a very small proportion of the overall cases, and/or the variant may represent a reference artifact. Additionally, consider the genomic architecture in the area under investigation; common population variants may occur near known disease-causing regions. For example, there are segmental duplications around the promoter and first exon of *KANSL1*, the gene that has been established as causative of Koolen-Devries syndrome26, 27. As a result, variation around the 5’ end of this gene appears frequently in the general population.

Additional considerations when evaluating case-control or general population data include:

* Dosage of copy number imbalance reported in the general population studies. The CNV observed in the general population might exclusively represent a copy number gain. If the CNV in question overlaps the same region, but is a copy number loss, a pathogenic outcome cannot be excluded. The opposite situation also applies. Similarly, a CNV observed commonly as a heterozygous deletion in the general population may have a pathogenic outcome when present in a homozygous state.
* Size of the reported benign CNV relative to the CNV in question. One should ensure that the CNV reported in the general population includes the same gene content as the CNV being interrogated. Different array platforms represented in public databases can lead to differences in reported sizes of identical CNVs.
* Sex of individual in database relative to patient sex. This consideration is particularly important for X-linked CNVs in males, as many of the variants are seen in females who may be non-manifesting carriers of the condition. In addition, consider that contributors to these databases may have excluded the sex chromosomes from analysis; therefore, CNVs mapping to the X and Y chromosomes may be under-represented.
* Validity of the CNV reported in general population databases. The majority of CNVs reported from large population studies have not been experimentally validated; therefore, CNVs reported in a single study or through use of a single platform should be interpreted with caution.
* Clinical characterization of “normal” individuals. One should consider the extent of clinical characterization of individuals represented in the database. Each population series is selected based on defined criteria, usually outlined in the primary reference. Consider how these individuals were selected for inclusion and how likely it is that the clinical phenotype presented in the patient of interest might be present in the “normal” population. Factors such as incomplete penetrance, variable expressivity, age of onset, and parent of origin imprinting effects need to be considered before classification of a CNV as benign in all instances. CNVs occurring with relatively high frequency in the general population and in multiple publications may be interpreted with more confidence as benign in nature. Of note, many publications use the same reference set (e.g., HapMap); therefore, a CNV represented in multiple publications may represent the same individual studied multiple times.

**Section 5: Evaluation of Inheritance Pattern/Family History for the Patient Being Studied**

When available, inheritance and family history information for the patient being studied may provide evidence supporting or refuting the pathogenicity of the observed variant. Although this is a very relevant line of evidence by which to gauge clinical significance, it is important to stress that it is difficult and often imprudent to attribute clinical significance based on the inheritance pattern of a CNV in a single family. It is only through ascertainment of significantly large families with multiple affected and unaffected family members segregating a given CNV or ascertainment of multiple individuals with the same CNV that a true measure of clinical significance can be confidently assessed. For this reason, the ACMG strongly supports efforts to collect and curate de-identified patient data from clinical studies to facilitate rapid and unambiguous assessment of the clinical significance of CNVs23, 28, 29.

Points should be assigned for inheritance information in the patient being studied (i.e., *de novo*, inherited from an unaffected family member, inherited from an affected family member) using the same considerations and with the same caveats as discussed in the section above about scoring inheritance information for subjects obtained from the literature, public databases, or internal laboratory databases. If inheritance information is unknown or uninformative (for example, only one parent is available for follow-up, and the CNV is not found in the parent available for study), and no information is known about the patient phenotype, no points can be awarded (category 5F). As discussed above, when a CNV is found in a parent or other relative, the carrier parent and other relevant family members should have a thorough medical evaluation for the presence or absence of the clinical features present in the proband. When this information is not provided to the laboratory, this should be included in the report with a recommendation for correlation with parental clinical features. Do not add or deduct points for inheritance from a parent if the affected status of that parent is unknown.

When inheritance information is unavailable or uninformative, but the patient phenotype is consistent with what has been described in similar cases (categories 5G and 5H), points may be assigned dependent upon the relative specificity of the phenotype. In general, cases under evaluation by the clinical laboratory should be scored in a similar manner to relevant cases observed in the scientific literature (e.g., *de novo* cases scored similarly to literature *de novo* cases, inherited cases scored similarly to other cases with the same inheritance pattern, etc.). In categories 5G and 5H, the ranges allow the laboratory to upgrade or downgrade evidence from their observed case based upon their presumed ability to obtain more detailed clinical information, including pertinent negatives (e.g., phenotypes reported in the literature that the patient under evaluation has been determined NOT to have), from the referring clinician, something that is typically not possible in literature cases. If this is not possible, or if any questions remain about how well a patient’s phenotype matches with what has been previously described, score as you would an additional case observed in the literature.

When evaluating inheritance in the context of “affected” vs. “unaffected” family members, consider the specificity of the phenotype, as discussed above. Special considerations that preclude confident inference and may only be well defined after ascertainment of multiple families include the following:

* Incomplete penetrance: The CNV may be pathogenic but non-penetrant in the carrier parent30, 31.
* Variable expressivity: The carrier parent may have subclinical features that will later be shown to be in the spectrum of the disorder caused by the CNV32.
* Parent of origin imprinting effects: The CNV region may be imprinted, such that the disorder only manifests when inherited from a particular sex (and the carrier parent is not manifesting the disorder because of chance inheritance)33, 34.
* Sex-limited traits: Expression of the phenotype may be limited to one sex or the other.  For example, the phenotype may only be confined to the prostate, ovaries, oocytes, sperm, etc.
* Second variant not detectable by original testing methodology: The proband may be manifesting a recessive disorder (e.g., a deletion may be inherited from an unaffected carrier parent and an undetectable variant inherited in a gene within the interval from the other parent). Alternatively, the proband may have one or more “modifier” genes/DNA elements not present in the unaffected parent.
* Mosaic CNV in parent: The CNV may not be present in all tissues of the parent, and therefore, the parent may not manifest all clinical features associated with the CNV35, 36. In general, however, points should not be deducted in the context of “non-segregation” if a parent with a CNV in the mosaic state does not exhibit the same features as a proband with the same CNV observed constitutionally.  The same logic applies for hemizygous CNVs inherited from unaffected heterozygous mothers; in general, these should not be considered non-segregations.

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