

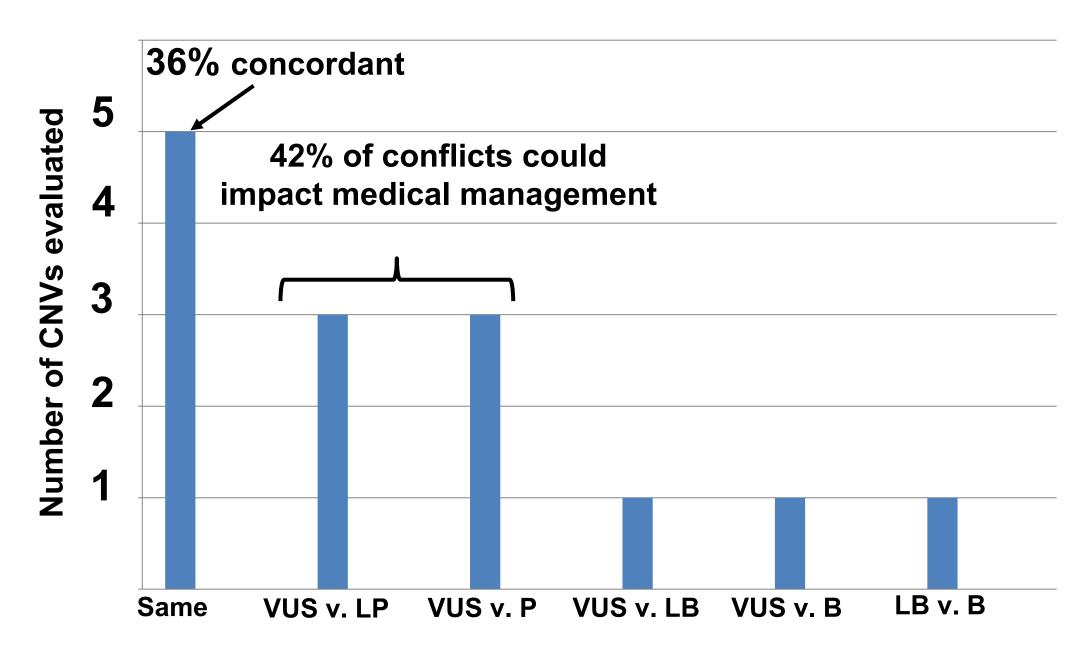
New systematic rubric for clinical interpretation of copy number variants (CNVs) improves interpretation consistency across laboratories

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Introduction

Analysis of CNVs by chromosomal microarray analysis (CMA) is the first-tier genetic test in patients with neurodevelopmental disorders and/or multiple congenital anomalies. In addition, due to advancements in microarray and sequencing technologies, CNVs are now being analyzed at higher resolutions extending down to single-exon CNVs, extending their clinical scope to gene panels and whole exome sequencing.



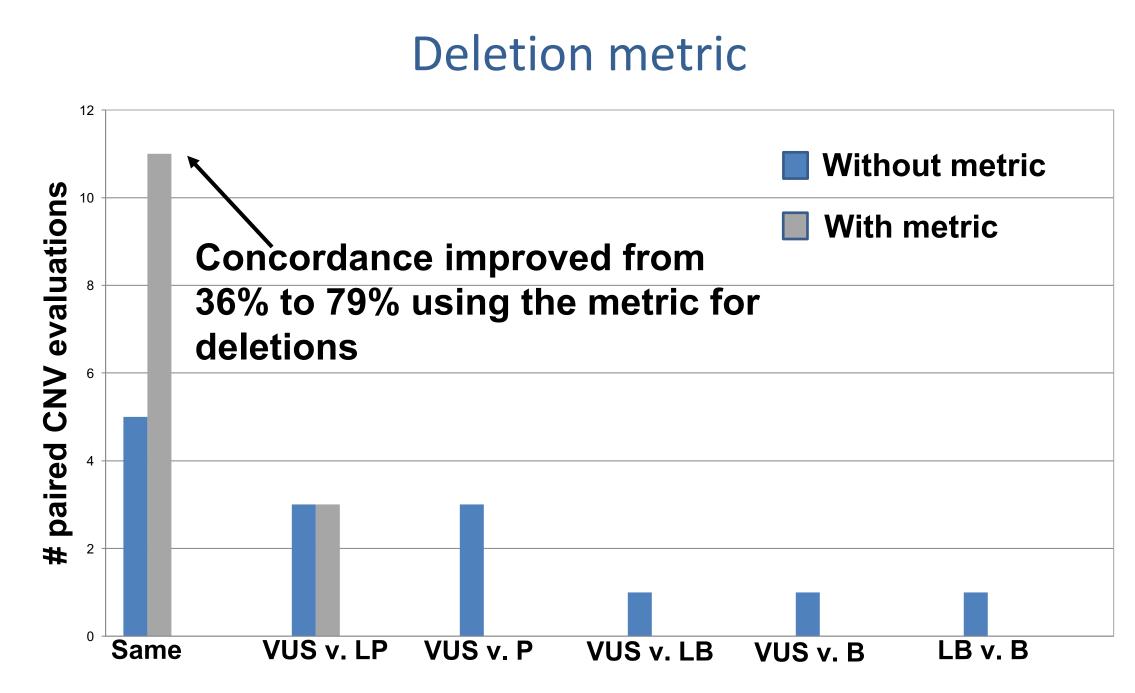
Despite the existence of CNV interpretation standards from ACMG and mounting experience from laboratories analyzing CNVs, inconsistencies in clinical interpretation persist due to differences weighing evidence used for classification.

In an effort to improve consistency, the ACMG and the Clinical Genome Resource (ClinGen) established a collaboration to update the existent CNV classification guidelines with a more standardized clinical classification framework.

CNV clinical interpretation rubric Point-based, hierarchical scoring system Benign Pathogenic Genomic content **Towards Pathogenic Towards Benign** Overlap with established haploinsufficiency/triplosensitive Overlap with benign region region/gene/regulatory region No genes in region Protein truncation (Frame-shift) Disruption of a protein coding gene Published literature and databases **Towards Pathogenic Towards Benign** Strong segregation Statistically significant association with disease (Case v. Controls) Reported in the general population, not statistically associated with disease (Case v. Moderate segregation, presence in case with specific phenotype Weak segregation, reported in case with unspecific phenotype, Controls) variant reported de novo Number of genes involved * In development **Towards Pathogenic** 34-49 genes 15-34 genes Inheritance and family history **Towards Benign Towards Pathogenic** Variant is de novo Variant is inherited from an affected parent Variant is inherited from an unaffected parent

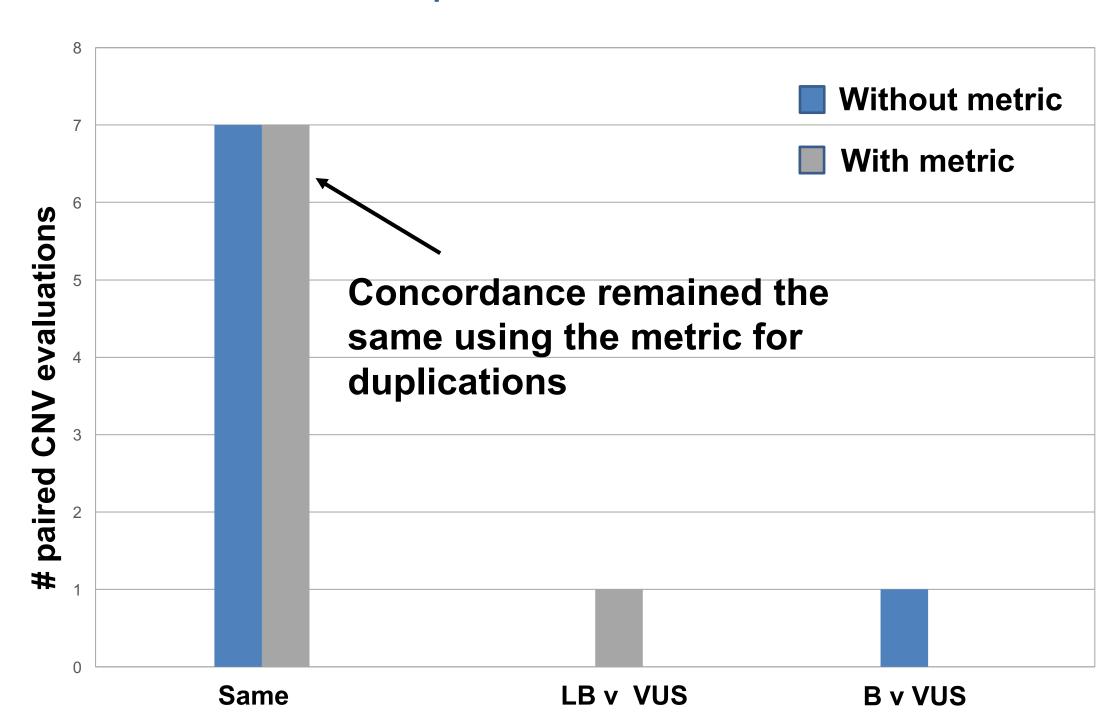
Testing of the metric

Thirty three (33) deletions and 28 duplications with defined clinical classifications from clinical laboratories were used to test the performance of the metric. All CNVs were evaluated independently by 2 geneticist. Fourteen (14) deletions and 8 duplications of the total of CNVs evaluated were also evaluated with the existing guidelines. Concordance between both rubrics was calculated.



When the new rubric was used for evaluation of loss CNV, the concordance among reviewers significantly improved. Of the total number of evaluations (n=66), in 80%, the calculated clinical interpretation was deemed appropriate by an expert panel, 11% differed by a single-step classification difference (LP vs VUS or VUS vs LB), and 4% were confidence differences (P vs LP, LB vs B and vice versa).

Duplication metric



Testing of the duplication metric is in progress. Preliminary data show that when the new rubric was used for evaluation of gain CNV, the concordance among reviewers was high, but did not improve. Of the total number of evaluations (n=62), in 90%, the calculated classification was deemed appropriate by an expert panel, and 8% differed by a single-step classification.

Conclusion and future direction

- We devised a systematic framework for clinical interpretation of discrete CNV events, which is expected to have broad impact by providing a robust system to support the consistent interpretation across clinical laboratories.
- This rubric will be tested with a broader group of clinical laboratory geneticists to identify nuances and refine its guidance.

Disclaimers and funding

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- This work is in progress, and has not yet been reviewed or approved by the American College of Medical Genetics and Genomics' (ACMG) Board; ACMG has no formal or established position on the conclusions of this work at this time.