**Methods**

- Mosaicism was challenging to detect using conventional sequencing methods and therefore may be an under-reported cause of genetic disease.
- NGS with high read-depth provides a more sensitive and semi-quantitative approach for the identification of mosaicism.
- This is a retrospective study to identify the frequency of mosaicism observed by gene and disease category.
- Data was generated by NGS using mosaic-aware variant calling and optimized bioinformatics.
- Genes were assigned to phenotypic categories based on the major disease association for each gene.
- Frequency of mosaicism was calculated by gene and disease category.

**Results**

- 1.4% (111/7859) of PV/LPV in 188 high sensitivity disease genes were classified as mosaic.
- Mosaic variants were identified in 59/188 genes and accounted for 4.8% of all PV/LPVs in these genes.
- Genes associated with brain malformation syndromes and epilepsy/intellectual disabilities had the highest frequency of mosaic PV/LPV. This is in contrast to genes associated with cardiomyopathy/arrhythmia and neuromuscular disorders. This finding may be explained by the nature of these disorders (Figure 1).
- NGS allows for low-level mosaic detection (<10% of reads) in parents of a proband with a known PV/LPV. Each dot represents results from a single parent, with the percent reads of the mosaic PV/LPV observed for that parent (y-axis). The bioinformatics detection cutoff for proband testing is indicated by a solid line (~10% threshold). Detection of low-level mosaicism in parents is critical to assess recurrence risk (Figure 2).

**Conclusions**

- Mosaic PV/LPV are relatively common in genes associated with NDD, specifically, brain malformation syndromes, epilepsy-related disorders, and intellectual disabilities.
- Diagnostic testing for patients with NDD should include NGS with high read-depth and mosaic-aware variant calling.
- NGS could be considered in probands who received a negative Sanger sequencing result for a gene with a high mosaic rate.
- Parents of probands with apparently de novo variants in genes with high mosaic rates should be tested by NGS or a comparable method to detect low-level mosaicism.

**Reference**