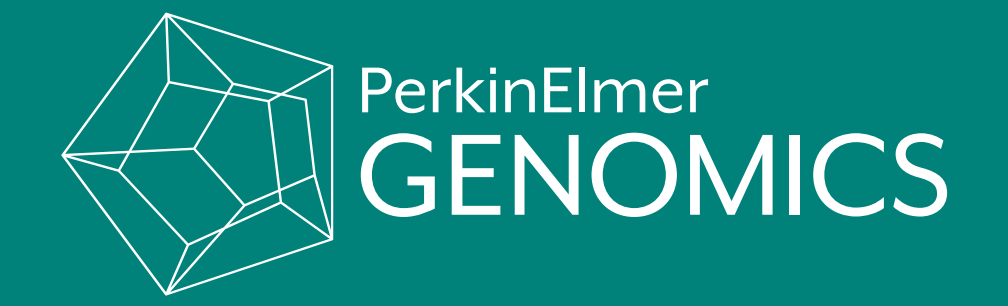


# Importance of parental segregation studies and its role in variant classification

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## INTRODUCTION

Genetic testing has become an integral part of clinical practice for prognostic and diagnostic management of genetic disorders. While the use of advanced next generation sequencing (NGS) technique has provided a convenience for the healthcare system to provide a better diagnostic outcome, it has equally highlighted the need of follow-up parental testing to accurately interpret the results obtained. Here we demonstrate the importance of follow-up parental segregation studies and its impact on the variant classification.

## CASE STUDIES

### Scenario 1: Reclassification of a *De Novo* to Likely Pathogenic

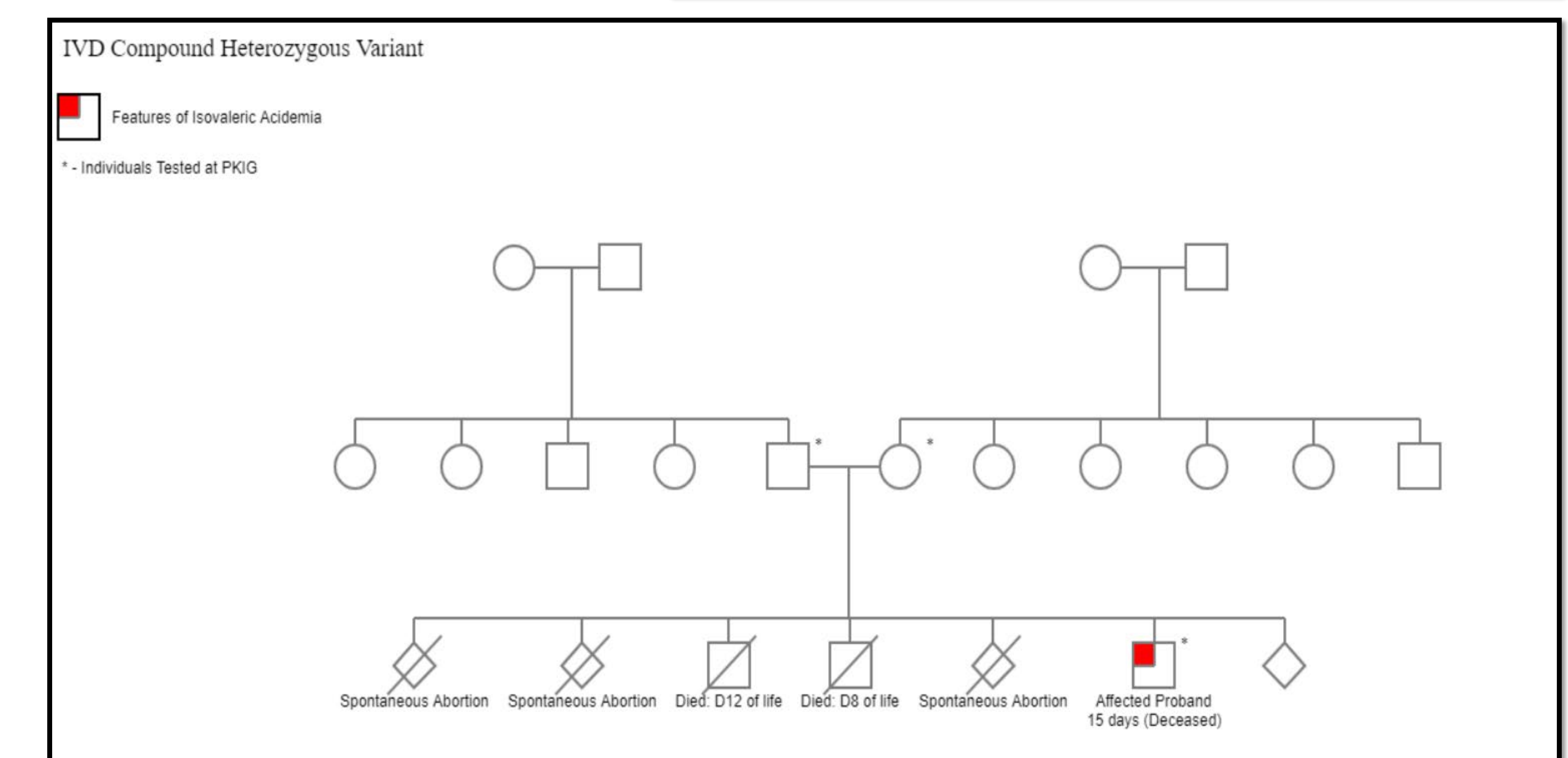
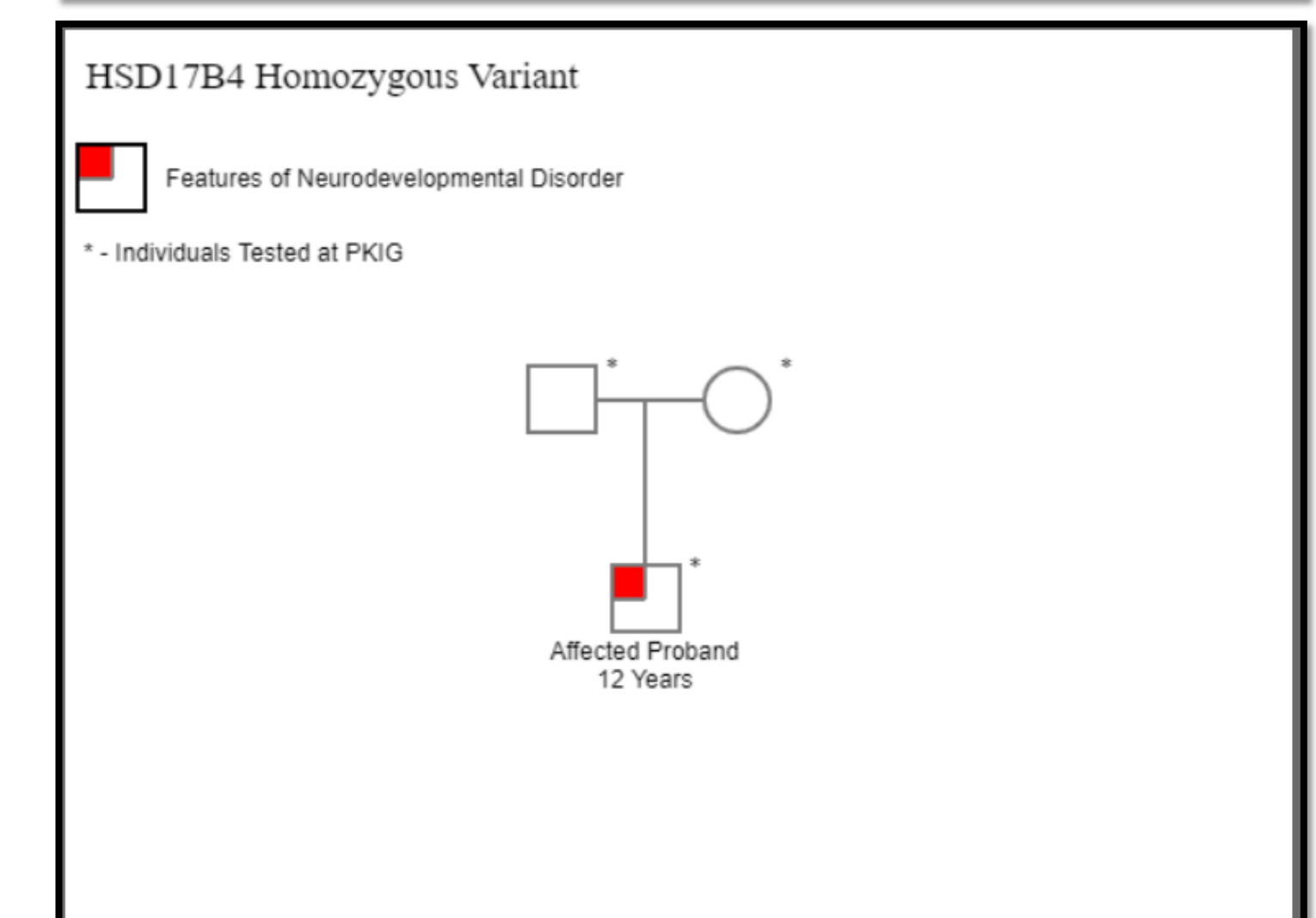
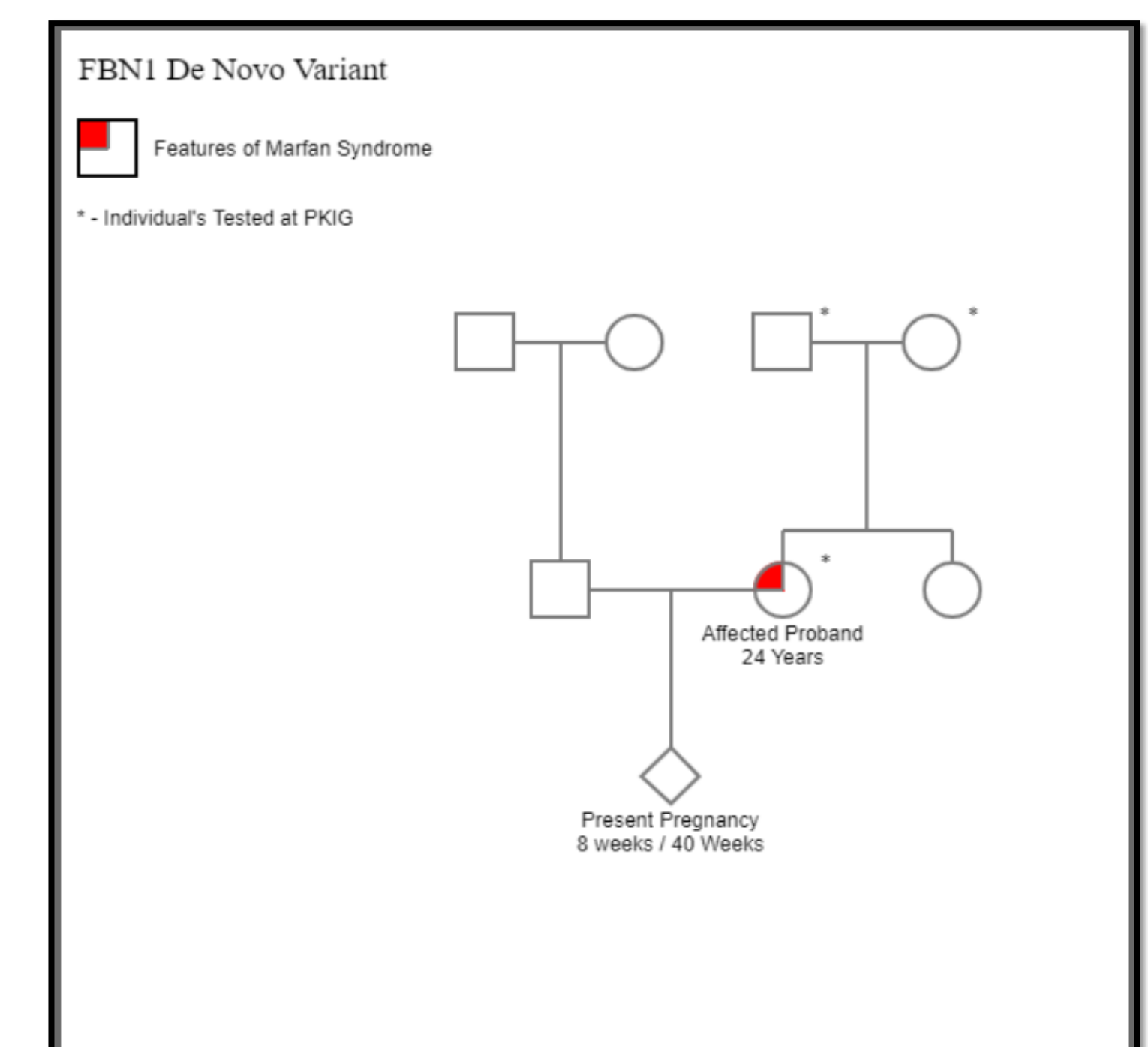
- 25-year-old female with features of high myopia, due to bilateral subluxation of lens, trivial mitral regurgitation, tricuspid regurgitation, mitral valve prolapse, arachnodactyly, positive thumb sign and wrist sign. Clinical suspicion of Marfan syndrome.
- Proband testing via Whole Exome Sequencing: Heterozygous for c.6997+5G>A *FBN1* variant of uncertain significance.
- Parental testing via Sanger Sequencing: Both asymptomatic parents were negative for the variant.
- Additional Testing: STR Analysis

### Scenario 2: Reclassification of a familial variant to Likely Benign

- 12-year-old male with features of neuro regression, long face, hypertonia, spasticity, contracture, café au lait spots, bilateral basal ganglia, few areas of gliosis of bilateral centrum semiovale, genu of corpus callosum, patchy foci of paucity of white matter, subcortical white matter.
- Proband testing via Whole Exome Sequencing: Heterozygous c.958-3\_958-2del *COL6A1* variant of uncertain significance and a homozygous c.625C>G (p.Leu209Val) *HSD17B4* variant of uncertain significance.
- Parental testing via Sanger Sequencing: Asymptomatic mother was homozygous for the *HSD17B4* variant while the asymptomatic father was heterozygous for the *HSD17B4* variant.
- Additional Testing: None

### Scenario 3: Reclassification of compound heterozygous variants to Likely Pathogenic

- 15days-old deceased male with clinical suspicion of isovaleric acidemia and family history of neonatal deaths due IEM in previous pregnancy in parents.
- Proband testing via Single Gene Sequencing: Heterozygous c.263G>A (p.Gly88Glu) and a heterozygous c.131G>A (p.Gly44Glu) *IVD* variants of uncertain significance
- Parental testing via Sanger Sequencing: Asymptomatic mother harbors the c.263G>A (p.Gly88Glu) *IVD* variant and the asymptomatic father harbors the c.131G>A (p.Gly44Glu) *IVD* variant.
- Additional Testing: *IVD* Enzyme Analysis



## RESULTS

- Segregation analysis by sanger sequencing in parents of patient 1 and follow up STR analysis in all three samples confirmed the *de novo* status of the variant in the proband. Taken together, the parental segregation results, the phenotype correlation, and the absence of the variant in the general population, this variant was reclassified from variant of uncertain significance to likely pathogenic.
- Similarly, based on clinical correlation, parental testing by Sanger sequencing for the *HSD17B4* variant of uncertain significance in patient 2, and given that the detection of the variant did not co-segregate with the clinical phenotype in the family, the *HSD17B4* variant was reclassified from uncertain significance to likely benign.
- Testing in parents of patient 3 showed the c.263G>A (p.Gly88Glu) *IVD* variant and the c.131G>A (p.Gly44Glu) *IVD* variant are *in trans* in the affected proband. Additionally, biochemistry analysis showed decreased levels of the enzyme in the proband. The segregation data and biochemical testing helped reclassify both the c.263G>A (p.Gly88Glu) and the c.131G>A (p.Gly44Glu) *IVD* variants from uncertain significance to likely pathogenic.

## CONCLUSION

Follow-up parental segregation analysis helps to verify the phasing of the causative variants as well as to verify the *de novo* occurrences thereby aiding reclassification of the reported variants. We recommend parental analysis post proband testing to better understand complex genetic variations.