**BACKGROUND**

- The identification of pathogenic mutations in the *BRCA1*, *BRCA2* and Lynch syndrome genes (MLH1, MSH2, MSH6 and PMS2) significantly impacts patient management, but the identification of a variant of unknown significance (VUS) makes clinical management less certain. Thus, there is a significant need to reclassify such variants to a more definitive category.

- Homozygosity or compound heterozygosity (Figure 1) for pathogenic mutations within these genes has been demonstrated to result in embryonic lethality (*BRCA1*) or severe early onset clinical phenotypes, such as Fanconi Anemia (FA) (*BRCA2*) or Constitutional Mismatch Repair Deficiency syndrome (CMMR-D) (Lynch genes).3

- The identification of individuals homozygous for a particular variant, or with a variant co-occurring in *trans* with a pathogenic or likely pathogenic mutation, provides very strong evidence that the variant is benign.

- We have validated the use of *in trans/homozygous* findings to reclassify a variant to benign or likely benign.

**METHODS**

- Unique variants were selected for analysis based on proposed criteria for usage of this reclassification methodology in the diagnostic setting. These criteria included patient age at testing (if unaffected), personal cancer history, and age at diagnosis, if applicable.

- Figure 2 outlines the process of variant classification by *in trans/homozygous* analysis and validation by independent classification methods.

**RESULTS**

- 334 variants with ≥1 *in trans* observation with a known pathogenic mutation were selected for validation (Figure 3A).

- *In trans* analysis showed 99.7% (333/334) concordance with other reclassification methodologies.

- 274 variants with ≥1 homozygous observation were selected for validation (Figure 3B).

- Analysis of homozygous variants demonstrated 99.6% (275/274) concordance with other reclassification methodologies.

- One variant *in trans* to a deleterious mutation and one homozygous variant were not downgraded due to conflicting data at the time of the observation, highlighting the importance of evaluating multiple lines of evidence in variant classification.

**CONCLUSIONS**

- This study demonstrates that *in trans* and homozygosity analyses can be used as methods to downgrade variants in the *BRCA1*, *BRCA2*, and Lynch syndrome genes. However, caution must be taken to ensure that clinical phenotypes are carefully considered for each variant.

**REFERENCES**