VALIDATION OF UTILIZING *IN TRANS* CO-OCCURRENCE OR HOMOZYGOSITY TO DOWNGRADE THE CLASSIFICATION OF GENETIC VARIANTS IN THE *BRCA1*, *BRCA2* AND LYNCH SYNDROME GENES

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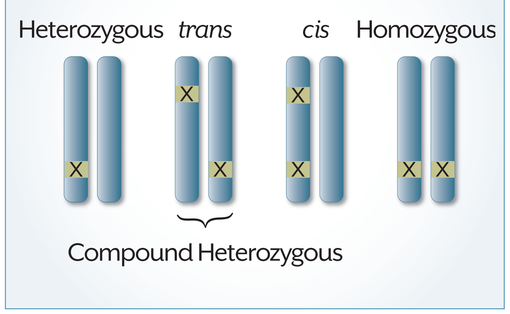
BACKGROUND

- 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*)[1] or severe early onset clinical phenotypes, such as Fanconi Anemia (FA) (*BRCA2*)[2] or Constitutional Mismatch Repair Deficiency syndrome (CMMR-D) (Lynch genes).[3]

Figure 1. Illustration of cis/trans configurations



- 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.

Figure 2. Variant classification by in trans/homozygous analysis

in trans / Homozygous Analysis

A novel variant is classified as benign/likely benign if one of the following is true:

- ≥ 1 *in trans* co-occurrence with a pathogenic mutation as determined by family analysis, haplotype analysis, and/or sequence trace analysis (frameshifts only) *OR*
- ≥ 1 homozygous observation for the variant. Additional molecular and/or family analyses are required to confirm homozygosity.

In all cases, patient phenotype is strongly considered in order to rule out the presence of CMMR-D or FA, where appropriate, as determined from the family history information on the test request form. Additional follow-up and family testing was also pursued in some cases.

Independent Classification

Variants used in this validation were independently scored using one or more independent classification methodologies, which included:

- Statistical family history weighting algorithm (Bowles, poster # 130), mutation co-occurrence algorithm (Coffee, poster # 150), *in silico* mRNA splicing analysis, literature review, and control population frequency.

Variants with insufficient data for independent classification were excluded.

It is important to note that *in silico* splicing analysis, literature review, and control populations frequencies may be insufficient on their own to classify these variants in the clinical setting.

in trans / Homozygous Validation

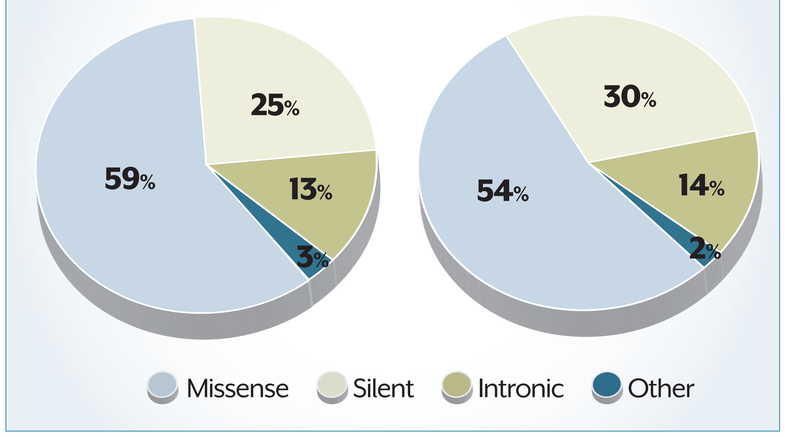
Variants were independently scored as having Concordant or Discordant *in trans*/homozygous analysis results:

- **Concordant**: independent classification methods provide significant data indicating that a given variant is likely benign, in agreement with *in trans* or homozygous analysis.
- **Discordant**: independent classification methods provide significant data indicating that the variant was likely pathogenic.

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% (273/274) 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.

Figure 3. Type of variants assessed by (A) in trans (n=334) and (B) homozygous (n=274) observation



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

1. Evers B, et al. *Oncogene*. 2006; 25:5885–5897.
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3. Wimmer K, et al. *Haematologica*. 2010 May; 95(5): 699–701.

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