Functional Assays Identify Clinically Actionable Variants in Hereditary Breast and Ovarian Cancer Predisposition Genes

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INTRODUCTION: Genomic testing for hereditary breast and ovarian cancer (HBOC) is becoming widespread; however, the interpretation of variants of unknown significance (VUS) in HBOC genes remains a challenge to geneticists and patients. Here we demonstrate an integrated DNA, RNA, and protein functional approach for the assessment of VUS that improves the identification of clinically actionable alterations in HBOC genes.
METHODS: We analyzed sequence data obtained from a cohort of ~23,000 individuals who underwent a HBOC DNA Multi-Gene Panel Test in a clinical laboratory. We then used a combination of clinical data, protein structure, and *in silico* analyses, in order to select VUS for specific functional analysis, depending on the type of alteration. For *BRCA1* and *BRCA2* missense VUS, protein function was determined by measuring homology directed recombination (HDR) efficiency and quantitative infrared western blot analysis. For splicing variants, we performed targeted RNA Studies. Finally, for gross duplications, a novel DNA Breakpoint Assay (DBA) was employed to detect tandem duplications in *BRCA1*, *BRCA2*, *ATM*, *CDH1*, *PALB2* and *CHEK2*.
RESULTS: Most of the VUS in HBOC genes were missense (91.6%), splicing (3.2%), or gross duplications (1%). To date, 22% of the missense alterations tested using the combination of structure and functional assays (HDR assay and quantitative infrared western blot) were reclassified from VUS to likely pathogenic. For splicing alterations, targeted RNA Studies resulted in ~78% reclassifications. Finally, DBA allowed us to ascertain breakpoints for 44 unique gross duplications from 156 consenting probands. We determined that the duplications occurred in-tandem in 123 (79%) individuals from this cohort. Among the in-tandem gross duplications that were eligible for reclassification, 95% of them were upgraded to pathogenic mutations.
CONCLUSION: The use of DNA, RNA, and protein functional assays significantly improved the overall assessment of germline variants in HBOC genes, resulting in a reduction in VUS classifications.