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**Closing the gap: systematic clinical integration of multiplexed functional data to resolve variants of uncertain significance**

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**Abstract:**
Clinical interpretation of missense variants identified by genetic testing is challenging because the majority are rare and their functional effects are unknown. Despite intense focus on the interpretation of variants in the cancer predisposition genes *BRCA1*, *TP53* and *PTEN*, the majority of missense variants in these genes are reported in ClinVar as variants of uncertain significance (VUS) (*BRCA1*: 78%, n=2,142; *TP53*: 61%, n=537; *PTEN*: 71%, n=369). Multiplexed assays of variant effect (MAVEs), where the consequences of thousands of single nucleotide variants are simultaneously measured, provide rich functional data that can help resolve VUS. However, a rigorous assessment of the clinical value of these multiplexed functional data is lacking. To assess the utility of multiplexed functional data in reinterpreting VUS, we systematically combined previously published *BRCA1*, *TP53*, and *PTEN* multiplexed functional data with patient phenotype and family history data from Ambry Genetics' clinical variant interpretations. First, we curated 49,281 variant functional scores from MAVEs across the three genes according to the Brotman Baty Institute Mutational Scanning Working Group guidelines. For *BRCA1* and *PTEN* we used the wild type-like and damaging functional classifications from the original papers. For *TP53*, we developed a naïve Bayes classifier to integrate functional scores from four different MAVEs into a single functional prediction for each variant. We then determined the strength of evidence provided by each multiplexed functional dataset based on their ability to predict established pathogenic and benign variants. Following the American College for Medical Genetics and Genomics Association for Molecular Pathology guidelines, we reevaluated 324 VUS classifications (*BRCA1*=110, *TP53*=166, *PTEN*=48). We found that multiplexed functional data was effective in driving variant reclassification when combined with existing lines of evidence, moving 42% of VUS for *BRCA1* (VUS -> likely benign (LB)=32, VUS -> likely pathogenic (LP)=14), 60% for *TP53* (VUS -> LB=82, VUS -> LP=19), and 15% for *PTEN* (VUS -> LB=0, VUS -> LP=7). Thus, multiplexed functional data, which are becoming available for numerous genes, are poised to have a positive impact on clinical variant interpretation. However, we identified two major factors limiting the utility of multiplexed functional data: the modest predictive value of some assays and the scarcity of established pathogenic or benign variants for some genes. To overcome these challenges MAVEs should be designed and piloted with clinical integration in mind, prioritizing genes with established pathogenic and benign variants.