Multiplexed assays of variant effect for all possible missense alterations located in the DNA Binding Domain of BRCA2.

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Many inherited missense variants (>4000) have been detected in *BRCA2* by clinical genetic testing. Few of these have been established as pathogenic variants that inactivate BRCA2 function and confer high risks of breast and ovarian cancer, or as benign missense variants that have no influence on BRCA2 function. Here we report on the functional characterization of 450 clinically observed missense variants in the DNA binding domain (DBD) of BRCA2 using a homology directed DNA repair (HDR) cell-based assay that evaluates the influence of variants on the homologous recombination DNA repair activity of BRCA2. Among these, 137 reduced BRCA2 activity greater than 70%. Combining the results from the HDR assay that provides strong evidence of pathogenicity under the PS3/BS3 rule from the ClinGen/ACMG/AMP variant classification model led to clinical classification of 439 of 450 (97.6%) of these variants. In parallel, high throughput deep mutational scanning of all possible single nucleotide changes in the exons encoding the helical and OB1 regions of the DNA binding domain of BRCA2 was conducted using a cell survival assay in HAP1 haploid human cell line. Of 2296 possible missense variants resulting from these alterations, 90.4% yielded reproducible functional results from triplicate experiments. Sensitivity and specificity based on stop codons and synonymous variants was greater than 90% for all exons. From these studies, over 300 missense variants were identified as deleterious. Comparison with results from individual HDR functional assays showed greater than 95% positive correlation. Combining the functional results with other rules-based data in a ClinGen/ACMG/AMP classification model resulted in classification of greater than 95% of all missense variants. These findings appear to resolve the clinical relevance of the majority of VUS in this region of BRCA2. The results are expected to improve clinical management of many patients diagnosed with these BRCA2 VUS.