Saturation genome editing-based functional evaluation and clinical classification of BRCA2 single nucleotide variants

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Germline *BRCA2* loss-of function (LOF) variants identified by clinical genetic testing predispose to breast, ovarian, prostate and pancreatic cancer. However, variants of uncertain significance (VUS) (n>5000) limit the clinical use of testing results. Thus, there is an urgent need for functional characterization and clinical classification of all *BRCA2* variants. Here we report on comprehensive saturation genome editing-based functional characterization of 99% of all possible single nucleotide variants (SNVs) in the BRCA2 DNA Binding Domain hotspot for pathogenic missense variants that is encoded by exons 15 to 26. The assay was based on deep sequence analysis of HAP1 haploid cells endogenously targeted using a CRISPR/cas9 knockin approach. A total of 6959 SNVs were characterized for effects on cell survival. The assay was validated relative to nonsense and synonymous variants, ClinVar pathogenic/likely pathogenic and benign/likely benign variants and homology directed repair cell based DNA repair assay results, all of which showed >94% sensitivity and specificity. Variants were assigned posterior probabilities of pathogenicity using a VarCall two component Bayesian mixture model and were further grouped according to ACMG strength of evidence under the PS3/BS3 rule resulting in Benign Strong (n=5430), Benign Moderate (n=190), Benign Supporting (n=61), VUS (122), Pathogenic Strong (n=1021), Pathogenic Moderate (n=88), and Pathogenic Supporting (n=47). Breast cancer case-control association studies showed that pooled SNVs encoding functionally pathogenic missense variants were associated with increased risks of breast (odds ratio (OR)3.81, 95%CI: 2.88-5.07) and ovarian cancer (OR 5.93, 95%CI: 4.12-8.52). The functional data were also combined with other sources of information in the ClinGen BRCA1/2 VCEP ACMG/AMP-classification model. A total of 785 SNVs, including 261 missense SNVs, were classified as pathogenic or likely pathogenic, while 5566 SNVs, including 3786 missense SNVs, were classified as benign or likely benign. These classified variants can now be used for risk assessment and clinical care of variant carriers.