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

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Purpose
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>4000) 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 (SGE)-based functional characterization of 97% of all possible single nucleotide variants (SNVs) in the BRCA2 DNA Binding Domain hotspot encoded by exons 15 to 26 for pathogenic missense variants.

Methods
SGE of exons 15 to 26 of *BRCA2* (MANE transcript ENST00000380152.8) was performed in the haploid human HAP1 cell line. Each exon along with 10bp flanking intronic sequence was studied as an independent target region. The most efficient sgRNA for each target region was selected and cloned into a Cas9 expressing plasmid. Site-saturation mutagenesis (SSM) libraries for each of the 14 target regions were designed to target every nucleotide and were generated by site directed mutagenesis using NNN-tailed PCR primers. HAP1 cells were transfected and harvested at Day5 and 14. Cell survival in response to each variant was assessed based on deep sequence analysis of the surviving endogenously targeted HAP1 cells at Day 5 and Day 14.

Results
A total of 7013 SNVs were characterized as functionally abnormal (n=955), intermediate/uncertain, or functionally normal (n=5224) based on 95% precision for ClinVar known pathogenic and benign standards. Results were validated relative to batches of nonsense and synonymous variants and variants evaluated using a homology directed repair (HDR) functional assay. Breast cancer case-control association studies showed that pooled SNVs encoding functionally abnormal missense variants were associated with increased risk of breast cancer (odds ratio (OR) 3.89, 95%CI: 2.77-5.51). The functional data were added to

other sources of information in a ClinGen/ACMG/AMP-like model and 700 functionally abnormal SNVs, including 220 missense SNVs, were classified as pathogenic or likely pathogenic, while 4862 functionally normal SNVs, including 3084 missense SNVs, were classified as benign or likely benign.

Conclusions

The production of functional maps for 97% of all SNVs in the BRCA2 DBD allowed for separation of nucleotide/protein-level functional aberrations. When coupled with genetic and clinical sources of evidence this study led to clinical classification of 5500 individual variants. These data will prove useful in the future, through integration with other datasets, for characterization and clinical classification of all variants in this region in individuals from all racial and ethnic backgrounds and for all BRCA2 associated forms of cancer.