Functional validation and clinical classification of all missense variants characterized by saturation genome editing of RAD51C

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Currently approximately 1000 missense variants of uncertain significance (VUS) have been reported in the RAD51C tumor suppressor gene. A high-throughput CRISPR/cas9-based cell survival assay in haploid HAP1 cells was used to evaluate all possible SNVs (n=885) in exons 2/3 encoding the ATP-binding pocket of RAD51C (Exon 2/3 MAVE), which is a hotspot for inactivating missense variants. Using a two-component mixture model, all functionally evaluated variants were attributed posterior probabilities of pathogenicity and were assigned to seven functional categories: Benign Strong (n=467), Benign Moderate (n=41), Benign Supporting (n=22), VUS (45), Pathogenic Strong (P_Strong) (n=265), Pathogenic Moderate (n=35), and Pathogenic Supporting (n=10). The model achieved high accuracy (96.6% and 97.1%) in classifying silent and nonsense SNVs. A homology-directed repair (HDR) assay was used to validate the findings and showed 94.4% sensitivity for 199 predicted pathogenic SNVs and 91.6% specificity for 96 predicted benign variants. A comparison with results from a recent CRISPR/cas9 based analysis of the entire RAD51C gene found >90% concordance for the fast depleted variants and the Exon2/3 P_Strong variants from the assays. However, the slow-depleted category from the all-RAD51C MAVE showed poor corrections with the Exon 2/3 MAVE and the HDR assays. In case-control analysis the P_Strong missense SNVs from the Exon 2/3 MAVE were associated with an increased risk for ovarian cancer (OR=10.72, 95%CI:5.31-22.83) and breast cancer (OR=3.04, 95%CI:1.6-6). Similar associations with ovarian cancer (OR=8.08, $p=3.74X10^{-18}$) and breast cancer (OR=2.39, $p=5.97X10^{-5}$) were observed for the fast-depleted variants from the All-RAD51C MAVE. Integration of the functional data from the Exon 2/3 MAVE into an ACMG/AMP variant classification framework classified 121 missense variants as pathogenic/likely pathogenic (P/LP) and 454 as benign/likely benign. In addition, only 82 of 1579 missense variants outside exons 2/3 were classified as P/LP. This study will improve precise clinical management of individuals carrying these variants.