BRCA1 frameshift variants leading to extended incorrect protein termini

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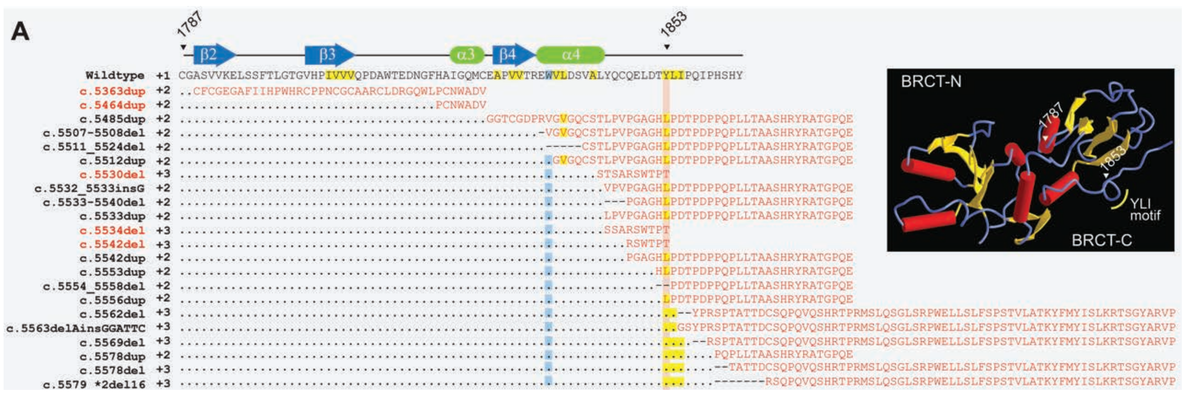
ABSTRACT

Carriers of BRCA1 germline pathogenic variants are at significantly higher risk of developing breast and ovarian cancer than the general population. The accurate identification of at-risk individuals is crucial for risk stratification and the targeted implementation of risk reducing and therapeutic interventions. Despite significant progress in classification efforts, a significant number of reported BRCA1 variants are variants of uncertain clinical significance (VUS). Variants leading to premature protein termination and loss of essential functional domains are typically classified as pathogenic. However, the impact of frameshift variants that result in an extended incorrect terminus (EIT) is unclear. We combined functional assessment, structural modeling, clinical and family data to systematically examine 17 naturally-occurring EIT variants previously reported. Consistent with previous reports, our data show that the loss of more than seven wild-type amino acid residues at the C-terminal portion of BRCA1 results in a striking reduction of the protein activity regardless of the EIT produced. Moreover, steady-state protein levels are markedly reduced for most EITs, suggesting that their loss of activity is due to protein instability. Only one variant, c.5578dup (p.His1860ProfsTer20), displayed transcriptional activation (TA) activity in a validated assay and expression levels similar to the wild-type protein. We also show that p.His1860ProfsTer20 interacts with CtIP at levels comparable to the wild-type protein, suggesting that it may constitute a likely benign/benign or a reduced penetrance variant. These results indicate that most, but not all, BRCA1 variants leading to incorrect extended termini are likely to be pathogenic and highlight the need for functional assays of individual variants.

RESULTS



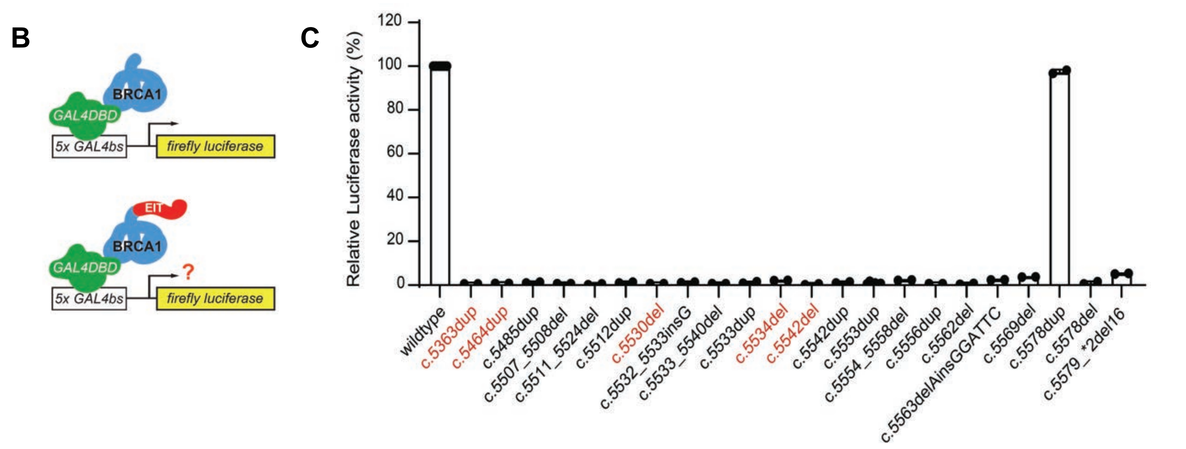


Figure 1. BRCA1 EIT dataset and transctiptional activation assay. (**A**) Protein amino acid sequence alignment of human BRCA1 C-terminal wildtype sequence (aa 1787 to 1863; NP_009225.1) and frameshift variants assessed in this study. Vertical red highlight line indicates position of codon 1853. Residues in red represent incorrect protein sequences. Highlighted positions indicate hydrophobic residues (yellow) and tryptophan 1837 (blue) conserved in BRCT domains. Premature protein termination (PPT) variants are shown in red font. Inset, PDB image 1JNX of BRCA1 tandem BRCT highlighting features in the amino acid sequence. (**B**) Schematic representation of the mammalian transcriptional activation assay (**C**) Transcription activity of extended incorrect termini variants (EITs) and premature protein termination (PPT) controls (c.5363dup, c.5464dup, c.5530del, c.5534del, and c.5542del) relative to wildtype BRCA1 in HEK293FT cells.

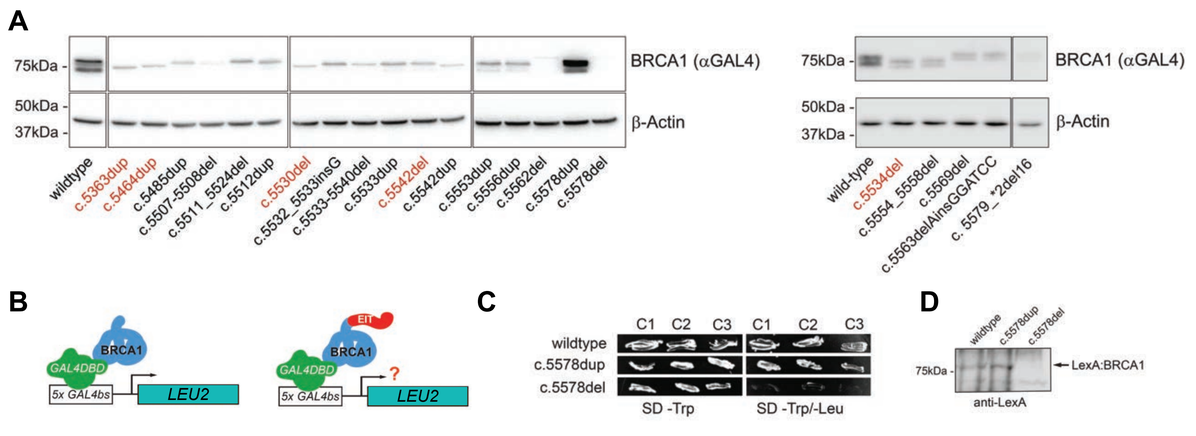


Figure 2. Steady state Protein levels and Yeast Transctiptional activation assay. (**A**) BRCA1 protein levels in whole cell extracts from transfected HEK293FT cells by immunoblotting using anti-GAL4 DNA binding domain (DBD) and anti-β-actin. (**B**) Schematic representation of the yeast-based transcriptional activation assay (**C**) Growth assay of EGY48 cells expressing c.5578dup and c.5578del. Reporter activity was assessed by the ability of three independent clones (C1, C2, and C3) to transactivate the genomic LEU2 auxotrophic reporter driven by LexA operators. (**D**) BRCA1 protein levels of c.5578dup and c.5578del in whole cell extracts from transformed EGY48 cells by immunoblotting using anti-LexA DBD.

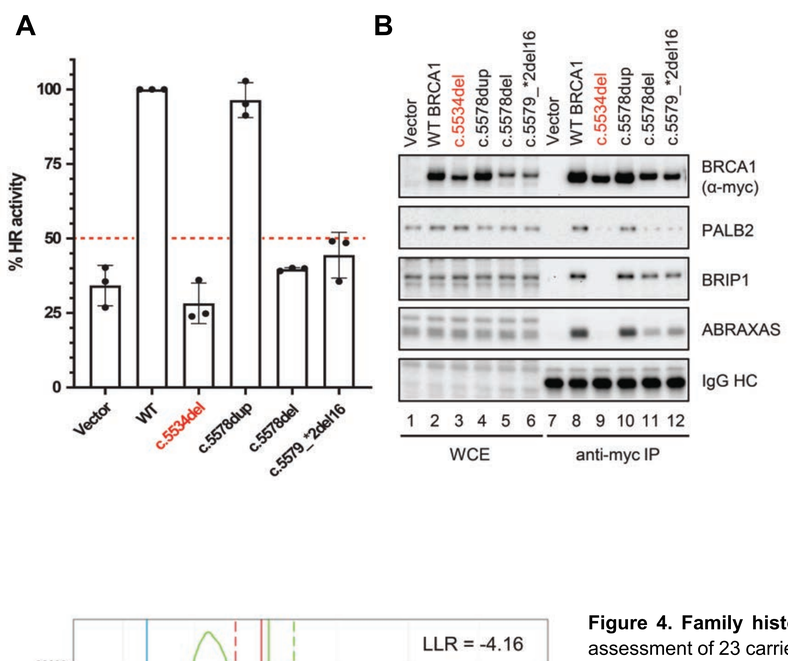


Figure 3. Homologous Recombination (HR) and protein-protein interaction assays. (**A**) Relative HR activities of the BRCA1 variants studied. Variants were introduced into full-length BRCA1 with 3XMyc tag at the N-terminus and their activities assessed in U2OS/DR-GFP cells depleted of the endogenous BRCA1. Activity values were normalized against that of the WT BRCA1. Error bars represent standard deviations from 3 independent experiments. (**B**) Expression levels of the variants studied and their binding capacities to known BRCA1 interacting partners in 293T cells. Cells were transfected with an empty vector (EV) or 3XMyc-tagged BRCA1 constructs. Whole cell extracts (WCEs) were subjected to direct western or immunoprecipitation (IP) with anti-Myc antibody followed by western blotting analysis as indicated.

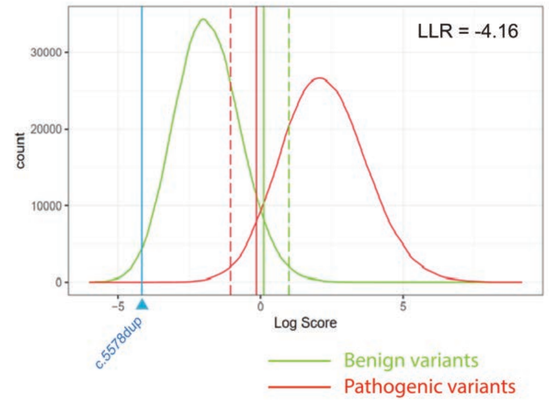


Figure 4. Family history analysis. Family history likelihood model assessment of 23 carriers of c.5578dup plotted against the distribution of benign (green line) and pathogenic (red line) variants. Family history data from 154,698 individuals tested on multigene panels was used to train a logistic regression model. The model predicts carrier status based on personal and family history of cancer. Application of the model to a new patient yields a likelihood ratio which is the probability that the variant is pathogenic divided by the probability the variant is neutral. When multiple patients carry the same variant, the product of the patient level LRs are taken to yield the variant level LR. This LR is then plotted against simulated pathogenic and neutral variants with the same number of families to generate ascertain significance. Note that these likelihood ratios are an updated version of Li et al 2020

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