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 preventive and therapeutic interventions. Despite significant progress in classification efforts, a sizeable portion of reported BRCA1 variants remains as 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, structure modeling, clinical and family data to systematically probe 17 naturally-occurring EIT variants previously reported in the population. 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 resulted in a striking reduction of the protein activity regardless of the EIT produced. Moreover, steady-state protein levels were 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 interacted 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.

Keywords: BRCA1, Frameshift, Functional assay, VUS