Breast cancer risk associated with missense variants in *BRCA2*

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Characters: 2,538 (limit:2,600)

Purpose
Greater than 90% of missense variants in *BRCA2* are classified as variants of uncertain significance (VUS). In addition, among the few known missense pathogenic variants (PVs) in *BRCA2*, breast cancer risk has been reported to be lower compared to pathogenic truncating variants (PTVs) (Li et al., 2022). Herein, we aim to reclassify several missense variants in *BRCA2* and investigate the associated breast cancer risk.

Methods
A total of 450 *BRCA2* missense variants in the C-terminal DNA binding domain (DBD) were evaluated for functional effect utilizing a previously validated homology-directed DNA double-strand repair (HDR) assay. The results of the functional evaluation along with clinical, structural, and in-silico data were applied to the rules-based ACMG/AMP criteria for variant classification to identify pathogenic/likely pathogenic (P/LP) variants and benign/likely benign (B/LB) variants. A pooled case-control analysis was performed comparing the frequency of reclassified P/LP missense variants among 82,372 women with breast cancer within the Ambry Genetics database as cases and 174,167 unaffected women within gnomAD, CARRIERS, and BRIDGES datasets as controls to evaluate associated breast cancer risk. Separate case-control analysis and comparisons with *BRCA2* DBD PTVs, exon 11 PTVs, and known missense PV standards in ENIGMA were also performed.

Results
From the 450 missense variants in the DBD, 137 (30.4%) were noted to be functionally deleterious and 313 (69.6%) were functionally neutral by the HDR assay. Incorporation of the functional and other data into an ACMG/AMP model led to the reclassification of 439 (97.6%) variants, with 131 as P/LP and 308 as B/LB variants. In a pooled case-control analysis, reclassified *BRCA2* missense P/LP variants were associated with clinically significant increased risks of breast cancer (Odds Ratio (OR):5.6, 95%CI:3.9–8.0), whereas B/LB missense variants were not (OR:1.1, 95%CI:1.1–1.2). Similarly elevated risks of breast cancer were observed for *BRCA2* PTVs in the DBD (OR:6.8, 95%CI:5.1–9.0) and missense PV standards in ENIGMA (OR:7.8, 95%CI:4.1–14.7). However, PTVs in exon 11 of *BRCA2* were associated with a lower risk of breast cancer (OR:4.4, 95%CI:3.8–4.9) compared to PTVs in DBD, confirming the ovarian cancer cluster region effect in *BRCA2*.

Conclusions
The study demonstrates that the HDR functional assay in conjunction with other clinical and genomic data can be a powerful tool that can aid in the reclassification of >95% of missense variants in *BRCA2*. In addition, missense PVs in DBD were noted to have a similarly elevated risk of breast cancer as PTVs. The reclassification of the *BRCA2* missense VUS and identification of associated breast cancer risk in this study will significantly influence breast cancer risk assessment and management strategies among carriers of these missense variants.