Partially functional (hypomorphic) missense variants in *BRCA2* are reduced penetrance pathogenic variants

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Abstract

BRCA2 variants with partially aberrant RNA splicing have been associated with a relatively lower breast cancer risk (reduced penetrance) than canonical pathogenic variants. However, the existence of partial loss of function (hypomorphic) missense variants, conferring reduced penetrance, is less certain. Identification and assessment of the clinical relevance of such reduced penetrance pathogenic variants (RPPVs) is needed to provide accurate risk estimates. We describe comprehensive functional characterization of 70 hypomorphic missense variants in the BRCA2 DNA-binding domain. The variants reduced BRCA2 homologous recombination DNA repair activity and endogenous cell survival, decreased RAD51 foci formation, and increased sensitivity to Poly(ADP-ribose) polymerase inhibitors. The variants were associated with moderate breast cancer risks, later ages of breast cancer diagnosis than canonical pathogenic variants, and were identified as "likely pathogenic" by a ClinGen/ACMG/AMP variant classification model. These RPPVs represent a new class of *BRCA2* variants that have implications for the clinical management of carriers.

Introduction

- *BRCA2* (OMIM: 600185) is a well-established tumor suppressor and predisposition gene for breast, ovarian, prostate, and pancreatic cancer[1,2,3].
- A small number of BRCA2 PVs, termed reduced penetrance pathogenic variants (RPPVs), that confer a reduced risk of cancer relative to canonical BRCA2 PVs have been identified[4].
- Recently, several BRCA2 missense variants have been identified in Fanconi anemia patients[5].
- More precise functional and clinical studies are needed to determine whether other clinically relevant RPPVs exist
- NCCN management guidelines for females with BRCA2 PVs include options for risk-reducing mastectomy, and carriers of BRCA2 PVs may benefit from targeted treatment such as PARP inhibitors.
- However, as RPPVs may be associated with moderate risks of cancer when compared to canonical BRCA2 PVs, there is a clinical need to establish the risks of cancers associated with partially functional hypomorphic variants and develop specific management and treatment guidelines for carriers.

Methods and Materials

- A homology directed DNA repair (HDR) cell-based assay was used to evaluate BRCA2 DNA binding domain (DBD) missense variants (amino acids 2479–3186). The HDR assay was calibrated using established pathogenic and benign non-splicing missense variants[6].
- To confirm the partial function of the 70 BRCA2 variants at the endogenous level, the effects of variants on viability and sensitivity to PARP inhibitor (PARPi) olaparib of HAP1 human haploid cells after CRISPR/Cas9-based knock-in were assessed.
- The risks of breast cancer for individuals carrying BRCA2 hypomorphic, pathogenic or benign variants were estimated in a consecutive cohort of women undergoing hereditary cancer testing at Myriad Genetics, and in breast cancer patients referred for genetic testing at Ambry Genetics compared to unaffected All of Us[7] reference populations.
- An American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) variant classification model was used for clinical classification of *BRCA2* hypomorphic variants.

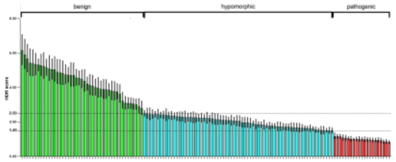

Figure 1. HDR assay evaluation of 70 BRCA2 DBD candidate hypomorphic variants

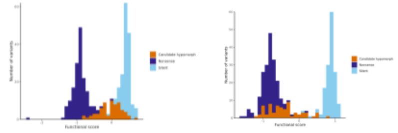

Figure 2. Endogenous effects of BRCA2 hypomorphic variants by CRISPR/cas9 knock-in viability assay.

Results

- The variants consistently demonstrated hypomorphic HDR activity and endogenous survival in HAP1 targeted cells, decreased RAD51 foci formation, and increased sensitivity to PARP inhibitors.
- These variants were associated with a **moderate** increase in breast cancer risk, as shown in two independent studies: one using multivariable logistic analysis adjusted for age, ancestry and family history (OR=2.41, 95%CI=1.51-3.85) and another comparing frequencies of variants in breast cancer cases to public reference controls adjusted for ancestry (OR=2.09, 95% CI=1.16-4.08).
- The mean age of breast cancer diagnosis for carriers of hypomorphic variants was 57 years, which was significantly older than the mean age of diagnosis of 51 years for carriers of protein truncating variants.
- Functional data were combined with other evidence sources in a ClinGen/ACMG/AMP model to classify 14 variants as "likely pathogenic".

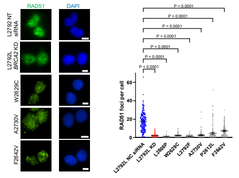

Figure 4. Representative micrographs and quantification of RAD51 focus formation for BRCA2 hypomorphic variants.

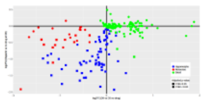

Figure 3. Olaparib PARPi response of BRCA2 hypomorphic variants.

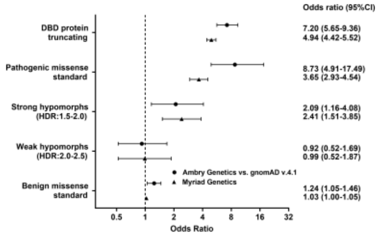
Odds ratio (95%CI)
DBD protein truncating
7.20 (5.65-9.36)
4.94 (4.42-5.52)
Pathogenic missense standard
8.73 (4.91-17.49)
3.65 (2.93-4.54)
Strong hypomorphs (HDR:1.5-2.0)
2.09 (1.16-4.08)
2.41 (1.51-3.85)
Weak hypomorphs (HDR:2.0-2.5)
0.92 (0.52-1.69)
0.99 (0.52-1.87)
Ambry Genetics vs. gnomAD v.4.1
Myriad Genetics
Benign missense standard
1.24 (1.05-1.46)
1.03 (1.00-1.05)
0.5
1
2
4
8
16
32
Odds Ratio

Figure 5. Associations between variants in the BRCA2 DBD and risk of breast cancer.

Discussion and Conclusions

- This is the first study to identify large numbers of partially functional, hypomorphic missense variants associated with reduced penetrance for breast cancer in *BRCA2*.
- Nevertheless, these hypomorphs may not fully represent the behavior of the likely larger number of hypomorphs *BRCA2*. In an ongoing study, additional candidate hypomorphs have been identified through a functional screen of BRCA2 DBD.
- High-throughput multiplexed assays of variant effect (MAVE) for BRCA2 are unable to discriminate hypomorphs from known pathogenic missense in the P-Strong category because MAVE seems to identify all variants with partial to full pathogenic effects.
- Cancer risk management guidelines for this new category of variants should be revisited given that the associated breast cancer risks warrant high risk screening but do not meet the threshold at which risk reducing mastectomy is offered.
- This study supports a shift in paradigm to considering variant-based risks on a continuum, with implications for risk-appropriate cancer risk managements (enhanced risk screening, risk-reducing surgery, and potentially PARPi treatment of cancer.).

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References

1. Couch, F. J., Shimelis, H., Hu, C., Hart, S. N., Polley, E. C., Na, J., ... & Dolinsky, J. S. (2017). Associations between cancer predisposition testing panel genes and breast cancer. *JAMA oncology*, 3(9), 1190-1196.
2. LaDuca, H., Polley, E. C., Yussuf, A., Hoang, L., Gutierrez, S., Hart, S. N., ... & Dolinsky, J. S. (2020). A clinical guide to hereditary cancer panel testing: evaluation of gene-specific cancer associations and sensitivity of genetic testing criteria in a cohort of 165,000 high-risk patients. *Genetics in Medicine*, 22(2), 407-415.
3. Hu, C., Hart, S. N., Polley, E. C., Gnanaolivu, R., Shimelis, H., Lee, K. Y., ... & Couch, F. J. (2018). Association between inherited germline mutations in cancer predisposition genes and risk of pancreatic cancer. *JAMA*, 319(23), 2401-2409.
4. Pal T, Mundt E, Richardson ME, et al. Reduced penetrance BRCA1 and BRCA2 pathogenic variants in clinical germline genetic testing. *NPJ Precis Oncol*. 2024;8(1):247.
5. Johnatty SE, Tudini E, Parsons MT, et al. BRCA1-, BRCA2-, and PALB2-related Fanconi anemia: Scope to expand disease phenotypic features and predict breast cancer risk in heterozygotes. *Am J Hum Genet*. Published online October 30, 2025. doi:10.1016/j.ajhg.2025.10.007
6. Hu, C., Susswein, L. R., Roberts, M. E., Yang, H., Marshall, M. L., Hiraki, S., ... & Couch, F. J. (2022). Classification of BRCA2 Variants of Uncertain Significance (VUS) Using an ACMG/AMP Model Incorporating a Homology-Directed Repair (HDR) Functional Assay. *Clinical Cancer Research*, 28(17), 3742-3751.
7. All of Us Research Program Genomics, I. Genomic data in the All of Us Research Program. *Nature* 627, 340-346 (2024).