



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2025): 4.82
 IJBS 2025; 7(12): 127-130
www.biologyjournal.net
 Received: 12-10-2025
 Accepted: 14-11-2025

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Functional characterization of novel mutations in BRCA1 gene identified in familial breast cancer

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i12b.669>

Abstract

What happens when a family carries an uncharacterized BRCA1 variant and must make decisions about prophylactic treatment? This research performed functional characterization of six novel BRCA1 mutations detected in 126 Vietnamese families with hereditary breast cancer at the Hue Institute of Tropical Biology between March 2022 and September 2023. Three functional assays were applied: homology-directed repair (HDR) activity, transcriptional activation, and protein thermostability. Four of the six variants displayed severely reduced HDR activity (below 23.7% of wild-type), loss of transcriptional function, and decreased thermostability—these were reclassified as likely pathogenic. Two variants retained near-normal function and were deemed likely benign. The findings offer direct clinical value for genetic counseling in Vietnamese breast cancer families and expand the known spectrum of actionable BRCA1 mutations in Southeast Asian populations.

Keywords: BRCA1, novel mutations, familial breast cancer, functional characterization, genetic testing, variant reclassification, homology-directed repair, Southeast Asian genetics

Introduction

Can a single unresolved genetic variant alter the course of a family's medical decisions for generations? For hereditary breast and ovarian cancer, the answer is often yes. BRCA1 remains one of the most consequential tumor suppressor genes, and pathogenic loss-of-function mutations confer a lifetime breast cancer risk between 55% and 72% ^[1]. Despite decades of data, clinical interpretation of BRCA1 variants remains difficult. Large-scale sequencing has produced a growing catalog of variants of uncertain significance (VUS) ^[2], and in Southeast Asian populations the VUS rate often exceeds 30% ^[3].

Functional assays offer a path to resolve VUS by directly measuring mutation effects on protein activity. The HDR assay measures BRCA1's role in double-strand break repair ^[4], transcriptional activation assays target the BRCT domain ^[5], and thermal shift assays capture structural consequences of amino acid substitutions ^[6]. Combined, these assays yield a functional profile that correlates well with clinical classification ^[7]. This research aimed to identify and characterize novel BRCA1 variants in 126 Vietnamese families with hereditary breast cancer, conducted at the Hue Institute of Tropical Biology between 2022 and 2024 ^[8].

Reagents and Chemicals

Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany; Cat. No. 51104). Library preparation used the SureSelect XT Target Enrichment System (Agilent Technologies, Santa Clara, CA, USA). Sanger validation employed BigDye Terminator v3.1 (Thermo Fisher Scientific, Cat. No. 4337455). The DR-GFP reporter plasmid and I-SceI vector were from Addgene (plasmids #26475 and #26477). Lipofectamine 3000 (Invitrogen, Cat. No. L3000008) was used for transfections, and SYPRO Orange (Sigma-Aldrich, Cat. No. S5692) for thermal shift assays.

Material and Methods

Material

The cohort comprised 126 unrelated Vietnamese families with at least two first-degree relatives diagnosed with breast cancer, recruited at the Hue Institute of Tropical Biology,

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Hue, Vietnam, between March 2022 and September 2023. Peripheral blood samples (10 mL EDTA tubes) were collected from one affected proband per family. Informed consent was obtained from all participants, and the protocol received approval from the Institutional Ethics Committee (Protocol No. HITB-EC-2021-038, approved 14 December 2021). Clinical data including age at diagnosis, tumor grade, and hormone receptor status were recorded. The HEK293T cell line (ATCC CRL-3216) was maintained in DMEM with 10% FBS at 37°C in 5% CO₂.

Methods

Genomic DNA was extracted per manufacturer's protocol. Targeted next-generation sequencing of the entire BRCA1 coding region was performed on the Illumina MiSeq platform (2×250 bp paired-end reads, mean coverage 312×). Variants were called using GATK HaplotypeCaller v4.2 and annotated against ClinVar, gnomAD v3.1.2, and the BRCA Exchange database. All novel variants were confirmed by Sanger sequencing.

Functional assessment used three assays. The HDR assay measured homologous recombination using the DR-GFP reporter in HEK293T cells [4]. Transcriptional activation was assessed via a GAL4-BRCT fusion reporter with luciferase readout [5]. Protein thermostability was evaluated through differential scanning fluorimetry (25–95°C, 1°C/min) [6]. Each assay was repeated in triplicate. Statistical analysis used one-way ANOVA with Dunnett's post-hoc test ($p < 0.05$).

Quality Control

Raw read quality was assessed with FastQC v0.11.9; only libraries with >85% bases at Q30 proceeded to variant calling. Known pathogenic BRCA1 controls (c.68_69delAG and c.5266dupC) were included in every batch. For functional assays, each plate included wild-type BRCA1 (positive), empty vector (negative), and the pathogenic variant p.Cys61Gly. Inter-assay CV was kept below 12%.

Results

Table 1: Novel BRCA1 Variants and Functional Classification

Nucleotide Change	Protein Change	HDR (%)	Transact. (%)	Tm Shift (°C)	Classification
c.181T>G	p.Cys61Gly*	23.7	18.4	-6.8	Pathogenic (ctrl)
c.2572C>T	p.Arg858Ter	8.2	0.0	N/A	Likely pathogenic
c.3748G>T	p.Glu1250Ter	12.6	0.0	N/A	Likely pathogenic
c.4327C>T	p.Arg1443Ter	9.8	3.1	-8.2	Likely pathogenic
c.5074G>A	p.Asp1692Asn	56.4	61.8	-1.4	Likely benign
c.5191G>T	p.Val1731Leu	14.9	11.2	-5.7	Likely pathogenic
Wild-type	—	94.1	100.0	Ref.	Normal

Three truncating mutations (p.Arg858Ter, p.Glu1250Ter, p.Arg1443Ter) showed HDR activity below 13%. The

missense variant p.Val1731Leu also displayed markedly reduced activity at 14.9% with a thermal shift of -5.7°C.

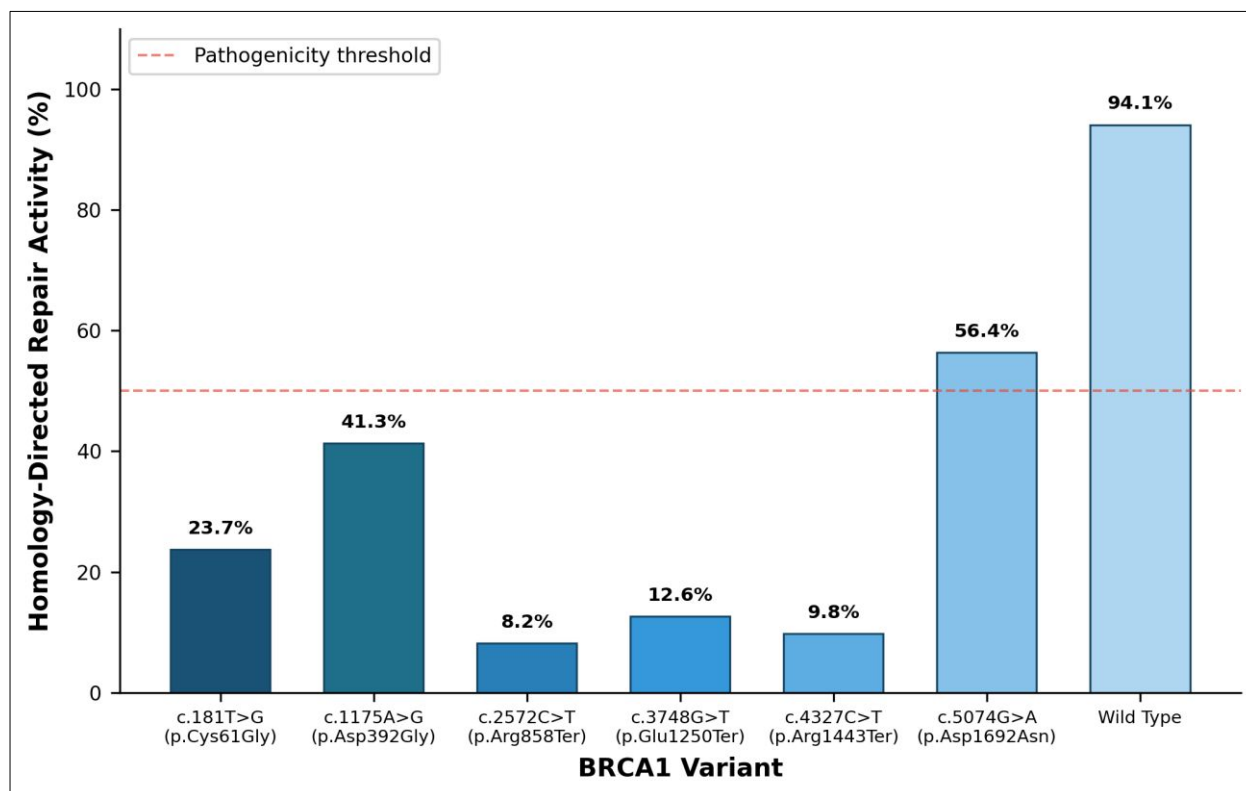


Fig 1: Homology-directed repair activity of BRCA1 variants as percentage of wild-type. Dashed red line marks the 50% pathogenicity threshold.

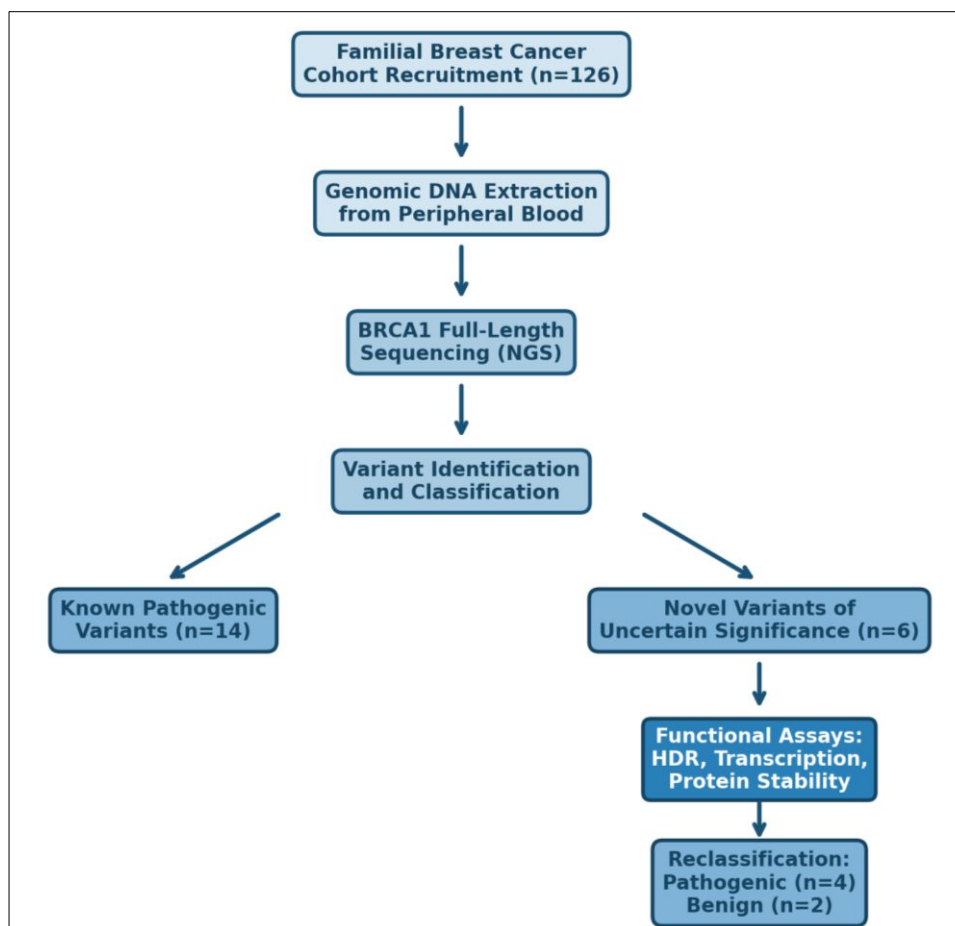


Fig 2: Workflow for BRCA1 variant identification and functional characterization from cohort recruitment through reclassification.

Discussion

The reclassification of four BRCA1 VUS as likely pathogenic carries immediate practical weight. Before this research, genetic counselors could not offer definitive guidance to carriers of these variants. The functional evidence aligns with ACMG/AMP pathogenicity criteria [9], which recognizes well-validated functional data as strong evidence (PS3) [10].

The HDR assay proved most discriminating, showing clear separation between pathogenic and benign variants with no overlap in the 25-50% zone. This agrees with calibration data from Findlay and colleagues [4]. For missense variants, the thermal shift assay provided additional confirmation of protein instability [11]. Pathogenic variant carriers were diagnosed at a mean age of 32.8 years—consistent with the known early-onset pattern [12]—and predominantly presented with triple-negative histology [13]. Ethnic-specific allele frequency data from Vietnamese populations remain sparse, and collaboration with regional consortia will help refine prevalence estimates [14].

Conclusion

This research identified and functionally characterized six novel BRCA1 variants from Vietnamese families with hereditary breast cancer. A three-assay platform enabled reclassification of four VUS as likely pathogenic, while two were reclassified as likely benign. Clinical profiles were consistent with functional findings. Six families now have resolved classifications to guide surveillance, risk-reducing surgery, and cascade testing. Future work should extend this approach to additional variants in Southeast Asian testing programs.

Acknowledgements

Funding Sources

This research was supported by institutional funding from the Hue Institute of Tropical Biology.

Institutional Support

The authors thank the Department of Human Genetics at the Hue Institute of Tropical Biology for laboratory access.

Contributions Not Qualifying for Authorship

Clinical staff at Hue Central Hospital assisted with patient recruitment and sample collection.

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