Vietnam Journal of Biotechnology **24**(2): 185-198, 2026. DOI: 10.15625/vjbt-23548

Research Article

IDENTIFICATION OF GENETIC VARIANTS IN VIETNAMESE PATIENTS WITH BREAST CANCER BY WHOLE EXOME SEQUENCING

Duc Huan Le[1,2], Thi Kim Lien Nguyen[1], Ngoc Lan Nguyen[3], Thi Thanh Huong Tran[4], Thi Huyen Phung[5], Van Chu Nguyen[6], Van Dung Tran[7], Hong Huy Luu[7], Chi Kien Dong[8], Van Manh Dang[5], Minh Long Duong[6], Thi Hien Anh Nguyen[9], Ngoc My Le[10], Hong Hanh Ha[1] and Van Tung Nguyen[1,✉]

[1]*Institute of Biology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam*

[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nghia Do, Hanoi, Vietnam*

[3]*Center for Gene and Protein Research, Hanoi Medical University, 1 Ton That Tung Street, Hanoi 11521, Vietnam*

[4]*Vietnam National Cancer Institute, K Hospital, 43 Quan Su, Cua Nam, Hanoi, Vietnam*

[5]*Department of Medical Oncology 6, K Hospital, 30 Cau Buou, Thanh Liet, Hanoi, Vietnam*

[6]*Quan Su Cytopathology Department, K Hospital, 43 Quan Su, Cua Nam, Hanoi, Vietnam*

[7]*Department of International Collaboration and Research, K Hospital, 30 Cau Buou, Thanh Liet, Hanoi, Vietnam*

[8]*Department of Medical Oncology 5, K Hospital, 30 Cau Buou, Thanh Liet, Hanoi, Vietnam*

[9]*Douglass Hanly Moir Pty. Sonic Health, 333-339 Geoger Street, Sydney, Australia*

[10]*Microbiology Department, Blood Transfusion and Hematology Hospital, 118 Hong Bang street, Cho Lon, Ho Chi Minh city, Vietnam*

✉To whom correspondence should be addressed. Email: tungnv53@gmail.com

Received: 28 October 2025; Accepted: 29 April 2026; Published online: 29 May 2026

ABSTRACT

Breast cancer is becoming the most common type of cancer among women in Vietnam and globally, in terms of both incidence and mortality rates. In addition to factors such as lifestyle habits and environmental exposure, genetic mutations are a significant concern in the development of this disease. Identifying these mutations is essential for understanding hereditary risks and improving early diagnosis. In this study, we utilized whole exome sequencing (WES) to analyze and identify genetic mutations potentially associated with breast cancer in ten affected women. All patients had at least one family member diagnosed with breast cancer, ovarian cancer, or other types of cancer, suggesting a hereditary component. The analysis and screening process focused on mutations in breast cancer-

related genes with a minor allele frequency (MAF) of less than 1%. The screening results indicated that five out of ten patients carried only benign mutations, whereas pathogenic mutations were identified in three patients. Specifically, two heterozygous mutations, c.5314C>T (p.Arg1772Ter) and c.66dupA (p.Glu23ArgfsTer18), both located in the *BRCA1* gene, along with a frameshift variant, c.1350dupT (p.Gly451TrpfsTer3), in the *BARD1* gene, were identified. These mutations caused the introduction of premature stop codons, leading to truncated protein products with a significant impact on gene/protein function. Our findings provide a deeper understanding of the genetic basis of breast cancer and contribute to improving the genetic diagnosis of familial breast cancer in Vietnam.

Keywords: *BARD1*, *BRCA1,* breast cancer, genetic variants, Vietnamese patient, whole exome sequencing (WES).

INTRODUCTION

Breast cancer (OMIM #114480) represents the most commonly diagnosed malignancy in women, with incidence rates continuing to rise globally and in Vietnam (Zhang *et al*., 2025). This disease arises from the uncontrolled proliferation of breast tissue cells, leading to abnormal cellular growth. Over time, these abnormal cell populations may develop into tumors, which can be either benign or malignant. The malignant forms, in particular, pose a serious health threat due to their potential for invasion and metastasis (Khuwaja and Abu-Rezq, 2004). The cancer cells arise from the epithelial lining of the lobules and the ducts, which are responsible for the production of milk. Sarcomas are a rarer type of breast cancer in which the abnormality starts in the stromal components of breast tissues (Lim *et al*., 2016). Cancer cells can spread to different organs, such as bones, liver, or lungs, causing serious damage to the patient's body. According to Globocan, there were over 2 million people suffering from breast cancer in 2022, and Asia accounted for 42.9% of the total number of breast cancer cases worldwide (Bray *et al*., 2024). Sex, age, reproductive and hormonal factors (such as having fewer children, lack of breastfeeding, or hormone replacement therapy), an unhealthy lifestyle (including being overweight or consuming alcohol), family history, and genetic predisposition are some of the risk factors that may increase the chances of developing breast cancer (Obeagu and Obeagu, 2024). In Vietnam, breast cancer is one of the most common cancers. The incidence of the disease in the age group of 30–34 is increasing rapidly. It is worth noting that younger patients tend to have a worse prognosis and lower cure rates compared to older patients (Trieu *et al*., 2015). The early diagnosis of breast cancer is crucial for effective treatment, with up to 95% of cases being curable when identified at an early stage (Pham *et al*., 2019).

Although hereditary factors are only responsible for 5 to 10% of the total cases, family history remains a significant clinical indicator in risk assessment and diagnosis (Castelló *et al*., 2015). According to previous studies, women with a first-degree relative affected by breast cancer may face up to a fourfold increase in risk compared to those without such a history (Balmaña *et al*., 2011). The most common breast cancer-related genes include *BRCA1, BRCA2, MAP3K1, PALB2, TP53, ATM, PTEN, FGFR1*, and *RB1* (Pereira et al., 2016; Stephens et al., 2012). Half of familial or hereditary breast cancer cases are associated with *BRCA1/2* mutations (Hu *et al*., 2021).

Deleterious gene mutations involved in breast cancer have been classified into three groups: high, moderate, and low penetrance (Hong and Xu, 2022). Mutations in high-penetrance genes such as *PALB2, BRCA1, BRCA2,* and *TP53* confer a breast cancer risk exceeding threefold relative to the general population, whereas variant in moderate-penetrance genes, including those in *BARD1, CHEK2, CDH1*, and *STK11*, typically contribute to a two- to threefold elevation in risk (Hong and Xu, 2022). *BARD1* has been identified as a potential susceptibility gene for breast and ovarian cancers. Its encoded protein interacts with *BRCA1* and displays notable structural and functional similarities, suggesting a cooperative role in DNA repair pathways (Westermark *et al*., 2003). These features support the hypothesis that alterations in *BARD1* may contribute to hereditary cancer predisposition (Hashizume *et al*., 2001). The *BARD1* gene is located on chromosome 2q34–35 and comprises 11 exons, encoding a protein of 777 amino acids with multiple functional domains, including a RING-finger motif, BRCT domains, and Ankyrin repeats. Functionally, *BARD1* forms a heterodimeric complex with *BRCA1*, a partnership that plays a critical role in maintaining genomic stability (Tarsounas and Sung, 2020). This interaction has been implicated in key biological processes such as DNA damage repair, cell cycle regulation, and hormone signaling, thereby supporting the classification of *BARD1* as a potential tumor suppressor gene (Zhao *et al*., 2017). In addition, *BARD1* also has *BRCA1*-independent functions, such as interacting with p53 to stabilize it and enhance p53-induced apoptosis activity in response to DNA damage. Low-penetrance genes, such as *ATM, BRIP1, NF1,* and *RAD51C,* contribute to a 1- to 2-fold increased risk (Hu *et al*., 2021). Breast cancer is a highly heterogeneous disease with multiple risk-associated genes (Testa *et al*., 2020). Therefore, with its ability to detect multiple variants across numerous genes simultaneously, next generation sequencing (NGS) represents a suitable method for studying the genetic factors of this disease.

In this study, we performed whole-exome sequencing on ten Vietnamese breast cancer patients to identify genetic variants associated with disease susceptibility. The results are expected to enhance understanding of the molecular basis of breast cancer in the Vietnamese population.

MATERIALS AND METHODS

Ethics

Blood samples were taken after obtaining consent from the patients. The study was approved by the Ethics Committee of the Institute of Genome Research, Vietnam Academy of Science and Technology (No. 01-2021/NCHG-HĐĐĐ).

Subjects

A total of ten female patients with invasive breast cancer were selected for the research. All patients had a family history of cancer, with at least one relative affected by breast, ovarian, or other cancer types (Table 1).

DNA extraction and whole-exome sequencing

Patient blood samples were obtained from the Vietnam National Cancer Hospital (K Hospital), collected in EDTA tubes, and kept at -20°C until use. Genomic DNA was isolated with the QIAamp DNA Mini Kit (QIAGEN, Cat. No. 51304, Germany). DNA libraries were constructed using the

SureSelect XT Library Prep Kit ILM (Agilent, Cat. No. G9956A, USA). Whole-exome sequencing was then carried out on the Illumina system (Illumina, CA, USA).

Table 1. Family history of patients.

Patient ID	Relatives who have breast or ovarian cancer	Relatives with other cancer
BCK1	Aunt (Breast cancer)	Father (Rectal cancer)
BCK2	Aunt (Breast cancer)	None
BCK3	None	Father (Liver cancer)
BCK4	None	Mother (Rectal cancer)
BCK5	Mother (Ovarian cancer)	None
BCK6	None	Sister (Stomach cancer)
BCK7	None	Older and younger brothers (Lung cancer)
BCK8	Mother (Breast cancer)	Aunt (Bone cancer)
BCK9	Sister (Breast cancer)	Daughter (Brain cancer)
BCK10	None	Father (Lung cancer)

Bioinformatics analysis

Variant calling was conducted according to the GATK best practices workflow (Heldenbrand *et al*., 2019; Tung *et al*., 2021). Paired-end sequencing reads were aligned to the GRCh38 reference genome using Burrows-Wheeler Aligner version 0.7.17 (Li, Durbin, 2009). After alignment, duplicate reads were marked and other quality refinements were applied using the Picard toolkit to ensure data accuracy and reduce potential artifacts. Subsequently, genetic variants, including single nucleotide variants and small insertions/deletions (indels), were identified through the Genome Analysis Toolkit (GATK) version 4.1. (Van der Auwera *et al*., 2013). The resulting variants were annotated and assessed using public databases, such as ClinVar (Landrum *et al*., 2018), dbSNP (Sherry *et al*., 2001), the 1000 Genomes Project (Auton *et al*., 2015), and gnomAD (Karczewski *et al*., 2020). Variants were excluded if they: (i) were located in intronic regions, (ii) exhibited a minor allele frequency (MAF) greater than 1% in the gnomAD and 1000 Genomes Project, (iii) were classified as benign/likely benign in ClinVar, (iv) were predicted by SnpEff to have low impact. The clinical significance of the identified variants was interpreted in accordance with the guidelines issued by the American College of Medical Genetics and Genomics (Amendola *et al*., 2016). Accordingly, variant pathogenicity was classified into five categories: benign, likely benign, variant of uncertain significance (VUS), likely pathogenic, and pathogenic.

PCR and Sanger sequencing

Three primer pairs were used to amplify the fragment of genes which contained pathogenic variants (PHUSA Genomics, Vietnam) (Table 2).

Table 2. Sequences of PCR primers.

Primer ID	Sequence	Product length
BARD1_BCK3F	5' - TCAGTTCCTCCGACCTCTTT - 3'	673 bp
BARD1_BCK3R	5' - TGGTCTCAAACTCCTGACCT - 3'	
BRCA1_BCK5F	5'- CCTGATGGGTTGTGTTTGGT - 3'	356 bp
BRCA1_BCK5R	5'- CGAGACGGGAATCCAAATTA - 3'	
BRCA1_BCK9F	5'- GCCCCAGTGCAGAACCAATC - 3'	675 bp
BRCA1_BCK9R	5'- AAGGCTACCACCACCTACCC - 3'	

The PCR thermal cycle was followed as 95ºC/12 min; (95ºC/45 sec; 58ºC/30 sec; 72ºC/45 sec) x 35 cycles and 72ºC/8 min. The candidate pathogenic variants in patients and controls were confirmed through Sanger sequencing. The PCR amplification primers were also used as sequencing primers for Sanger validation.

RESULTS

Variant screening

After excluding benign and likely benign variants, a total of seven remaining variants were identified in five patients, including three pathogenic mutations and four variants of uncertain significance (VUS) (Table 3). Three pathogenic variants consisted of two frameshift variants (c.66dupA in the *BRCA1* gene and c.1350dupT in the *BARD1* gene), and a nonsense variant (c.5314C>T in the *BRCA1* gene). No disease-related mutations were detected in five remaining patients.

Table 3. Variants in coding regions of genes associated with breast cancer.

Patient	Transcript	Gene Name	c.DNA change	Amino acid change	dbSNP151	Pathogenicity classification (ACMG)
BCK2	NM_000251	*MSH2*	c.2197G>A	p.Ala733Thr	rs772662439	VUS
	NM_001304717	*PTEN*	c.182T>C	p.Leu61Ser	-	VUS
BCK3	NM_000465	*BARD1*	c.1350dupT	p.Gly451Trpfs Ter3	rs758278293	Pathogenic
	NM_000465	*BARD1*	c.1348A>C	p.Asn450His	rs776756736	VUS
BCK4	NM_002555	*SLC22A 18*	c.707G>A	p.Arg236Gln	rs148740521	VUS
BCK5	NM_007300	BRCA1	c.5314C>T	p.Arg1772Ter	rs80357123	Pathogenic
BCK9	NM_007294	*BRCA1*	c.66dupA	p.Glu23Argfs Ter18	rs80357783	Pathogenic

In silico prediction tools were applied to evaluate the functional impact of four VUS variants detected in the patients. The c.2197G>A variant in the *MSH2* gene of patient BCK2 was consistently predicted to be pathogenic. In contrast, for the remaining three VUS, the prediction outcomes across different algorithms were not fully concordant. Nevertheless, the majority of tools indicated that these variants are more likely to be benign (Table 4).

Table 4. *In silico* analysis of variants of uncertain significance.

	Variant information			
Patient	BCK2	BCK2	BCK3	BCK4
Gene name	*MSH2*	*PTEN*	*BARD1*	*SLC22A18*
HGVS.c	c.2197G>A	c.182T>C	c.1348A>C	c.707G>A
HGVS.p	p.Ala733Thr	p.Leu61Ser	p.Asn450His	p.Arg236Gln
dbSNP	rs772662439	-	rs776756736	rs148740521
***In silico* prediction**				
Revel	Moderate pathogenic (Score: 0.85)	Benign (Score: 0.16)	Benign (Score: 0.1)	Benign (Score: 0.17)
Varity	Deleterious (Score: 0.87)	Benign (Score: 0.05)	Deleterious (Score: 0.44)	Benign (Score: 0.05)
Mutation Assessor	High deleterious (Score: 3.6)	Low (Score: 1.35)	Neutral (Score: 0.04)	Low (Score: 1.36)
MutationTaster	Deleterious (Score: 1)	Benign (Score: 0)	Deleterious (Score: 0.75)	Benign (Score: 0)
DANN	Deleterious (Score: 1)	Deleterious (Score: 0.98)	Deleterious (Score: 0.99)	Deleterious (Score: 0.97)
MetaLR	Deleterious (Score: 0.81)	Benign (Score: 0.23)	Benign (Score: 0.25)	Benign (Score: 0.25)
BayesDel	Moderate pathogenic (Score: 0.27)	Benign (Score: -0.40)	Benign (Score: -0.41)	Benign (Score: -0.41)
GenoCanyon	Deleterious (Score: 1)	Benign (Score: 0.5)	Deleterious (Score: 0.84)	Benign (Score: 0.5)

Sanger sequencing of pathogenic variants

Sanger sequencing was used to verify the presence of three pathogenic variants in three patients. Sanger sequencing revealed that patient BCK3 carries a heterozygous c.1350dupT (p.Gly451TrpfsTer3) mutation in the *BARD1* gene (Figure 1). This is a frameshift mutation in which a thymine (T) nucleotide is inserted at position 1350 of the c.DNA sequence (Figure 1), altering the reading frame starting from the codon encoding glycine at position 451. This change causes a frameshift that results in the appearance of a premature stop codon after three amino acids beyond the mutation site.

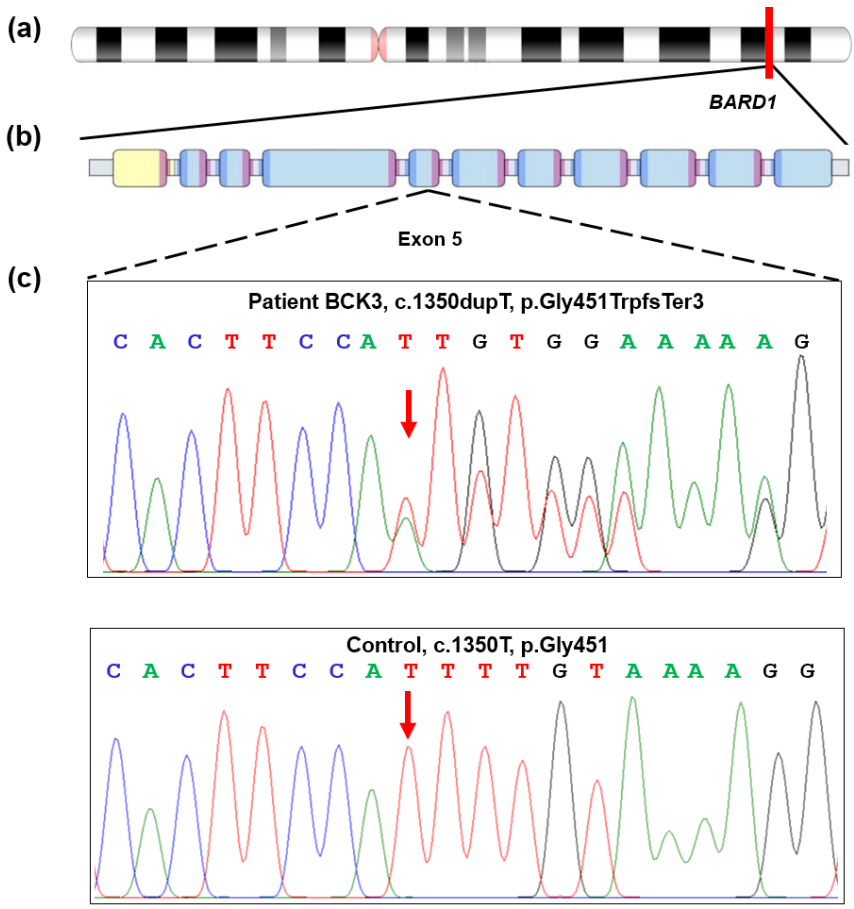

Figure 1. Analysis of pathogenic mutation in patient BCK3 and control. (a) *BARD1* is located on chromosome 2q35. (b) Graphical representation of the *BARD1* exon–intron structure and mutation c.1350dupT occurs in exon 5. (c) Sanger sequencing of the c.1350dupT mutation.

Sanger sequencing was employed to validate the variants initially detected through whole-exome sequencing. The analysis confirmed the presence of a heterozygous nonsense mutation, c.5314C>T (p.Arg1772Ter), in the *BRCA1* gene of patient BCK5. In addition, a heterozygous frameshift mutation, c.66dupA (p.Glu23ArgfsTer18), was identified in the *BRCA1* gene of patient BCK9 (Figure 2).

Patient BCK5 carries a nonsense mutation c.5314C>T on exon 20 of the *BRCA1* gene (Figure 2b), which replaces the amino acid arginine (Arg) with a stop codon at residue 1772, resulting in a truncated protein chain that is 112 amino acids shorter (Figure 3a). The c.66dupA mutation occurs in exon 2 of the *BRCA1* gene of patient BCK9 (Figure 2b), leading to an early stop codon at the 41st amino acid (Figure 3b).

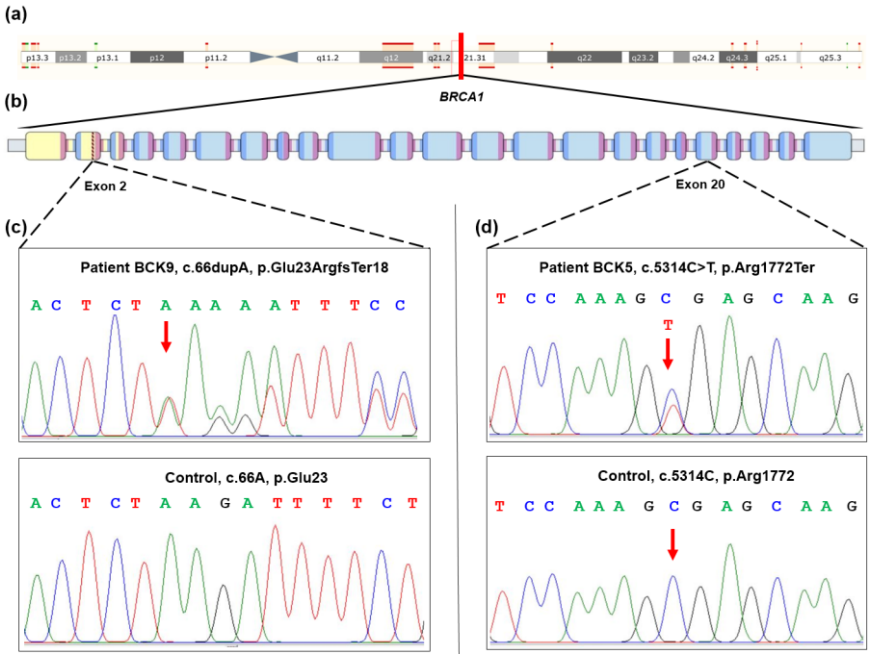

Figure 2. Analysis of pathogenic mutations in the *BRCA1* gene of two patients (BCK9 and BCK5) and a control. (a) *BRCA1* is located on chromosome 17q21.31. (b) Schematic illustration of the *BRCA1* gene exon–intron structure. (c) Sanger sequencing of the c.66dupA mutation. (d) Sanger sequencing of the c.5314C>T mutation.

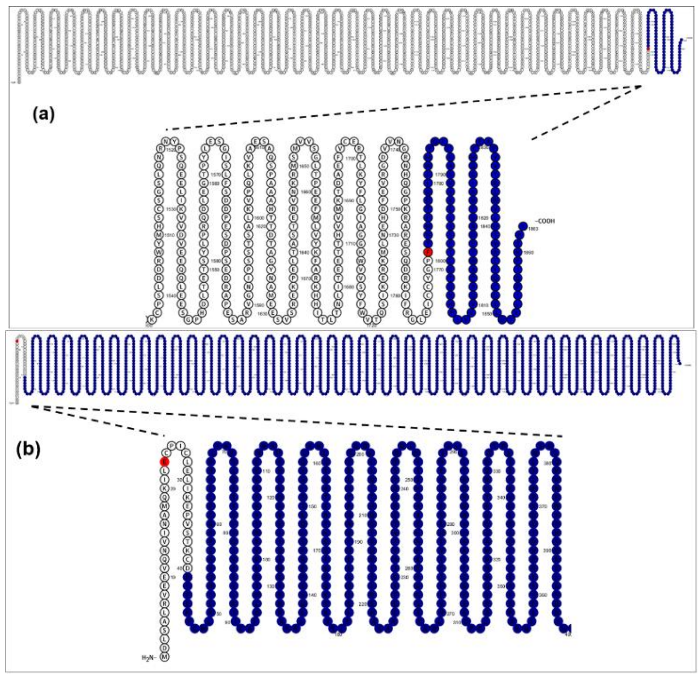

Figure 3. Topology of the BRCA1 protein. (a) The mutation at residue 1772 (red circle) leads to a truncation of 112 amino acids (blue circles). (b) The mutation at residue 23 (red circle) leads to a truncation of amino acids from 41 to the C-terminus of the BRCA1 protein (blue circles).

DISCUSSION

In this study, we identified one pathogenic variant in the *BARD1* gene and two in the *BRCA1* gene among three of the ten Vietnamese breast cancer patients. The BRCA1 protein is essential for preserving genomic stability by facilitating the repair of DNA damage, especially double-strand breaks, through the homologous recombination (HR) pathway (Jiang and Greenberg, 2015). When the *BRCA1* gene is mutated, its ability to repair DNA is impaired, elevating cancer susceptibility, especially breast and ovarian cancer. Therefore, *BRCA1* is considered a key tumor suppressor gene that helps protect cells from abnormal growth and the risk of cancer formation (Gorodetska *et al*., 2019). The BRCA Exchange currently lists approximately 35,598 variants identified in the *BRCA1* and *BRCA2* genes (Abreu *et al*., 2022). Among these, 2,233 variants in the BRCA1 gene have been classified as pathogenic or likely pathogenic. Therefore, it becomes understandable when we identified two *BRCA1* pathogenic variants in two out of ten breast cancer patients in this study.

The *BRCA1* c.66dupA (p.Glu23ArgfsTer18) variant was detected in patient BCK9. This mutation has identified as a germline mutation in a Chinese patient (Zhong et al., 2016). Furthermore, c.66dupA was detected in one out of a total of 259 Vietnamese breast cancer patients (Ginsburg *et al*., 2011). This mutation occurs at residue 23 of the protein, leading to the appearance of a stop codon after 18 amino acids, resulting in a truncated protein that severely affects the structure and function of the BRCA1 protein; therefore, it meets the ACMG criterion PVS1. This variant has been reported in at least 10 individuals affected with breast and ovarian cancers and was also identified in three cases in a breast cancer case–control meta-analysis, supporting an increased prevalence among affected individuals (ACMG criterion PS4) (Liede *et al*., 2002; Thirthagiri *et al*., 2008). The variant has not been observed in population reference databases such as gnomAD or the 1000 Genomes database (ACMG criterion PM2). The *BRCA1* c.66dupA variant fulfils the ACMG criteria PVS1, PS4, and PM2, indicating strong evidence for pathogenicity. Accordingly, the variant was classified as pathogenic.

In patient BCK5, the *BRCA1* c.5314C>T (p.Arg1772Ter) variant was detected in a heterozygous state. The identified variant in *BRCA1* is a nonsense mutation predicted to result in a truncated protein (ACMG criterion PVS1). This variant has been reported in numerous individuals with personal or familial histories consistent with BRCA1-associated breast and ovarian cancer susceptibility, as documented across multiple independent studies (Apessos *et al*., 2018; Janavičius, 2010; S. Zhang *et al*., 2011) supporting enrichment of the variant among affected individuals (ACMG criterion PS4). A study by Le *et al*. (2022) using high-throughput sequencing identified this mutation in four out of 33 Vietnamese patients with hereditary breast and ovarian cancer syndrome. Using Ion Torrent PGM sequencing technology, Ngo *et al*. (2020) identified this mutation in one out of 10 Vietnamese ovarian carcinomas. Additionally, this nonsense mutation has been found in a Vietnamese patient with stage 3 invasive ductal carcinoma type (Tung *et al*., 2023). Functional evidence further supports pathogenicity, as published assays demonstrated that this variant is unable to

support cellular viability in a haploid cell line, indicating a damaging effect on *BRCA1* function (Findlay *et al*., 2018) (ACMG criterion PS3). In addition, the variant is absent or present at an extremely low frequency in large population databases, including gnomAD, consistent with rarity in the general population (ACMG criterion PM2). Collectively, these lines of evidence support classification of this variant as pathogenic according to ACMG/AMP guidelines.

The *BARD1* gene produces a protein that binds to the N-terminal region of *BRCA1*, forming a functional complex. The heterodimeric complex formed by *BRCA1* and *BARD1* plays a crucial role as a tumor suppressor in breast and ovarian cancers. This complex contributes to several vital cellular processes, including DNA damage repair, stabilization of replication forks, regulation of transcription, and the maintenance of tumor-suppressive functions (Tarsounas and Sung, 2020). Genetic variants that impair the stability of this heterodimer may trigger harmful degradation of both *BRCA1* and *BARD1* proteins, thereby promoting cancer development. For reasons that have not been well studied, variants in the *BARD1* gene are rarer than *BRCA1* variants in hereditary breast cancer and are often associated with a lower breast cancer risk (Suszynska et al., 2019). Disruptions in *BARD1* itself may also contribute to the onset of breast and ovarian cancers, indicating its potential as a target for oncogenic mutations (Alenezi *et al*., 2020). The c.1350dupT (rs758278293) mutation introduces an early stop codon (p.Gly451TrpfsTer3) in the *BARD1* gene, likely leading to a truncated or nonfunctional protein (ACMG criterion PVS1). Notably, this variant is absent from population databases, including gnomAD (ACMG criterion PM2). Moreover, similar premature stop codons have been reported in individuals diagnosed with breast cancer (Sun *et al*., 2017) (ACMG criterion PS4). Overall, these findings support classification of the variant as pathogenic according to ACMG/AMP guidelines. In a study on Chinese patients, Kwong *et al*. (2025) identified 8 *BARD1* mutations in 12 individuals. Carriers of these mutations often had family histories of other cancers, such as liver, prostate, and cervical cancers. Although *BARD1* mutations are rare, these findings suggest that individuals carrying mutations in this gene, along with a related family history of cancer, should be monitored more closely.

Although family segregation analysis was not performed in the current study, future co-segregation studies in additional family members would provide further supporting evidence (ACMG criterion PP1) for the hereditary significance of these germline pathogenic variants in *BRCA1* and *BARD1*.

In this study, alongside the three pathogenic variants, four alterations were classified as variants of uncertain significance based on the ACMG/AMP guidelines. Previous reports indicate that approximately 30–50% of *BRCA1* and *BRCA2* variants detected through genetic testing fall into this category (Hofstra *et al*., 2008). The VUS group encompasses both common polymorphisms, present in more than 1% of alleles in the general population, and rare variants whose association with breast cancer susceptibility remains unclear. These variants require further investigation and evaluation to determine whether they are indeed related to pathogenic mechanisms of breast cancer.

CONCLUSION

In this study, ten female patients with breast cancer in Vietnam were investigated using WES. Among them, two patients were identified with pathogenic mutations in the *BRCA1* gene, including c.5314C>T (p.Arg1772Ter) and c.66dupA (p.Glu23 ArgfsTer18). In addition, one patient carried the c.1350dupT (rs758278293) mutation in the *BARD1* gene. These findings provide valuable new insights into genetic mutations in Vietnamese breast cancer patients.

AUTHOR CONTRIBUTIONS

Study concept and design: T.K.L.N., N.L.N. and H.H.H.; data collection: T.T.H.T., T.H.P., V.C.N., V.D.T., H.H.L., C.K.D., V.M.D., M.L.D. and N.M.L.; analysis and interpretation of results: V.T.N., D.H.L., N.N.L., T.H.A.N. and H.H.H.; draft manuscript preparation: T.K.L.N and V.T. N; writing—review and editing: D.H.L., V.T.N and H.H.H. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

This research was supported by the Ministry of Science and Technology, Vietnam (grant no. KC-4.0-37/19-25). The authors thank sample donors for contributing to this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Abreu R. B. V., Gomes T. T., Nepomuceno, T. C., Li X., Fuchshuber-Moraes M., De Gregoriis G., *et al.* (2022). Functional restoration of BRCA1 nonsense mutations by aminoglycoside-induced readthrough. *Fron- tiers in Pharmacology*, *13*, 935995. https://doi.org/10.3389/fphar.2022.935995

Alenezi W. M., Fierheller C. T., Recio N., and Tonin P. N. (2020). Literature review of BARD1 as a cancer predisposing gene with a focus on breast and ovarian cancers. *Genes*, *11*(8), 856. https://doi.org/10.3390/genes110 80856

Amendola L. M., Jarvik G. P., Leo M. C., McLaughlin H. M., Akkari Y., Amaral M. D., *et al.* (2016). Performance of ACMG-AMP Variant-interpretation guidelines among nine laboratories in the clinical sequencing exploratory research consortium. *American Journal of Human Genetics*, *98*(6), 1067–1076. https://doi.org/10.1016/j.ajhg.2016.03.024

Apessos A., Agiannitopoulos K., Pepe G., Tsaousis G. N., Papadopoulou E., Metaxa-Mariatou, V., *et al.* (2018). Comprehensive BRCA mutation analysis in the Greek population. Experience from a single clinical diagnostic center. *Cancer Genetics*, *220*, 1–12. https://doi.org/10.1016/j.cancergen.2017.10.002

Auton A., Abecasis G. R., Altshuler D. M., Durbin R. M., Abecasis G. R., Bentley D. R., *et al* (2015). A global reference for human genetic variation. *Nature*, *526*(7571), Article 7571. https://doi.org/10.1038/nature15393

Balmaña J., Díez O., Rubio I. T., Cardoso F., and ESMO guidelines working group. (2011). BRCA in breast cancer: ESMO clinical practice guidelines. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, *22 Supplementary 6*, vi31-34. https://doi.org/10.1093/annonc/mdr373

Bray F., Laversanne M., Sung H., Ferlay J., Siegel R.L., Soerjomataram I., *et al.* (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians, 74(3): 229-263.* https://doi.org/10.3322/caac.21834

Castelló A., Martín M., Ruiz A., Casas A. M., Baena-Cañada J. M., Lope V., *et al.* (2015).

Lower breast cancer risk among women following the World Cancer Research Fund and American Institute for Cancer Research Lifestyle Recommendations: EpiGEICAM Case-Control Study. *PloS One*, *10*(5), e0126096. https://doi.org/10.1371/journal.pone.0126096

Findlay, G. M., Daza, R. M., Martin, B., Zhang, M. D., Leith, A. P., Gasperini, M., *et al.* (2018). Accurate classification of BRCA1 variants with saturation genome editing. *Nature*, *562*(7726), 217–222. https://doi.org/10.1038/s41586-018-0461-z

Ginsburg O. M., Dinh N. V., To T. V., Quang L. H., Linh N. D., Duong B. T. H., *et al.* (2011). Family history, BRCA mutations and breast cancer in Vietnamese women. *Clinical Genetics*, *80*(1), 89–92. https://doi.org/10.1111/j.1399-0004.2010.01545.x

Gorodetska I., Kozeretska I., and Dubrovska, A. (2019). BRCA Genes: the role in genome stability, cancer stemness and therapy resistance. *Journal of Cancer*, *10*(9), 2109–2127. https://doi.org/10.7150/jca.30410

Hashizume R., Fukuda M., Maeda I., Nishikawa H., Oyake D., Yabuki Y., *et al.* (2001). The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *The Journal of Biological Chemistry*, *276*(18), 14537–14540. https://doi.org/10.1074/ jbc.C000881200

Heldenbrand J. R., Baheti S., Bockol M. A., Drucker T. M., Hart S. N., Hudson M. E., *et al.* (2019). Recommendations for performance optimizations when using GATK3.8 and GATK4. *BMC Bioinformatics*, *20*(1), 557. https://doi.org/10.1186/s12859-019-3169-7

Hofstra R. M. W., Spurdle A. B., Eccles D., Foulkes W. D., de Wind N., Hoogerbrugge N., *et al.* (2008). Tumor characteristics as an analytic tool for classifying genetic variants of uncertain clinical significance. *Human Mutation*, *29*(11), 1292–1303. https://doi.org/10.1002/humu.20894

Hong R., and Xu B. (2022). Breast cancer: An up-to-date review and future perspectives. *Cancer Communications*, *42*(10), 913–936. https://doi.org/10.1002/cac2.12358

Hu C., Hart S. N., Gnanaolivu R., Huang H., Lee K. Y., Na J., *et al.* (2021). A population-based study of genes previously implicated in breast cancer. *The New England Journal of Medicine*, *384*(5), 440–451. https://doi.org/10.1056/NEJMoa2005936

Janavičius R. (2010). Founder BRCA1/2 mutations in the Europe: Implications for hereditary breast-ovarian cancer prevention and control. *The EPMA Journal*, *1*(3), 397–412. https://doi.org/10.1007/s13167-010-0037-y

Jiang Q., and Greenberg, R. A. (2015). Deciphering the BRCA1 tumor suppressor network. *The Journal of Biological Chemistry*, *290*(29), 17724–17732. https://doi.org/10.1074/jbc.R115.667931

Karczewski K. J., Francioli L. C., Tiao G., Cummings B. B., Alföldi J., Wang Q., *et al.* (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, *581*(7809), Article 7809. https://doi.org/10.1038/s41586-020-2308-7

Khuwaja G. A., and Abu-Rezq A. N. (2004). Bimodal breast cancer classification system. *Pattern Analysis and Applications*, *7*(3), 235–242. https://doi.org/10.1007/BF02683990

Kwong A., Ho C. Y. S., Au C. H., and Ma E. S. K. (2025). Germline BARD1 mutation in high-risk chinese breast and ovarian cancer patients. *Cancers*, *17*(15), 2524. https://doi.org/10.3390/cancers17152524

Landrum M. J., Lee J. M., Benson M., Brown G. R., Chao C., Chitipiralla S., *et al.* (2018). ClinVar: Improving access to variant interpretations and supporting evidence. *Nucleic Acids Research*, *46*(D1), D1062–D1067. https://doi.org/10.1093/nar/gkx1153

Le T.N.N., Tran V.L., Nguyen T.T., Vo N.S., Hoang T.H., Vo H.L., *et al.* (2022) *BRCA1/2* mutations in Vietnamese patients with hereditary

Breast and ovarian cancer syndrome. *Genes*, *13*(2), 268. https://doi.org/10.3390/genes13020268

Li H., and Durbin R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Liede A., Malik I. A., Aziz Z., Rios P. de los., Kwan E., and Narod, S. A. (2002). Contribution of BRCA1 and BRCA2 Mutations to Breast and Ovarian Cancer in Pakistan. *American Journal of Human Genetics*, *71*(3), 595–606. https://doi.org/10.1086/342506

Lim S. Z., Ong K. W., Tan B. K. T., Selvarajan S., and Tan P. H. (2016). Sarcoma of the breast: An update on a rare entity. *Journal of Clinical Pathology*, *69*(5), 373–381. https://doi.org/10.1136/jclinpath-2015-203545

Ngo D.P., Tuong N.V., Hoa P.H., Nhu B.T.H., Nhan V.T., Thanh L.Q *et al*. (2020) Preliminary study of BRCA1 and BRCA2 mutations among Vietnamese ovarian carcinomas population by using ion personal genome machine platforms. *Hue University Journal of Science: Natural Science*, *129*(1A), 49-60. https://doi.org/10.26459/hueuni-jns.v129i1A.5471

Obeagu E. I., and Obeagu G. U. (2024). Breast cancer: A review of risk factors and diagnosis. *Medicine*, *103*(3), e36905. https://doi.org/10.1097/MD.0000000000036905

Pereira B., Chin S.-F., Rueda O. M., Vollan H.-K. M., Provenzano E., Bardwell H. A., *et al.* (2016). The somatic mutation profiles of 2,433 breast cancers refine their genomic and transcriptomic landscapes. *Nature Communications*, *7*(1), 11479. https://doi.org/10.1038/ncomms11479

Pham T., Bui L., Kim G., Hoang D., Tran T., and Hoang M. (2019). Cancers in Vietnam—burden and control efforts: a narrative scoping review. *Cancer Control: Journal of the Moffitt Cancer Center*, *26*(1), 1073274819863802. https://doi.org/10.1177/1073274819863802

Sherry S. T., Ward M. H., Kholodov M., Baker J., Phan L., Smigielski E. M., *et al.* (2001). dbSNP: The NCBI database of genetic variation. *Nucleic Acids Research*, *29*(1), 308–311. https://doi.org/10.1093/nar/29.1.308

Stephens P. J., Tarpey P. S., Davies H., Van Loo P., Greenman C., Wedge D. C., *et al.* (2012). The landscape of cancer genes and mutational processes in breast cancer. *Nature*, *486*(7403), 400–404. https://doi.org/10.1038/nature11017

Sun J., Meng H., Yao L., Lv M., Bai J., Zhang, J., *et al* (2017). Germline mutations in cancer susceptibility genes in a large series of unselected breast cancer patients. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *23*(20), 6113–6119. https://doi.org/10.1158/1078-0432.CCR-16-3227

Suszynska M., Kluzniak W., Wokolorczyk D., Jakubowska A., Huzarski T., Gronwald J., *et al* (2019). BARD1 is a low/moderate breast cancer risk gene: evidence based on an association study of the central European p.Q564X recurrent mutation. *Cancers*, *11*(6), 740. https://doi.org/10.3390/cancers11060740

Tarsounas M., and Sung, P. (2020). The antitumorigenic roles of BRCA1–BARD1 in DNA repair and replication. *Nature Reviews Molecular Cell Biology*, *21*(5), 284–299. https://doi.org/10.1038/s41580-020-0218-z

Thirthagiri E., Lee S., Kang P., Lee D., Toh G., Selamat S., *et al* (2008). Evaluation of BRCA1 and BRCA2 mutations and risk-prediction models in a typical Asian country (Malaysia) with a relatively low incidence of breast cancer. *Breast Cancer Research: BCR*, *10*(4), R59. https://doi.org/10.1186/bcr2118

Trieu, P. D. (Yun), Mello-Thoms, C., and Brennan, P. C. (2015). Female breast cancer in Vietnam: A comparison across Asian specific regions. *Cancer Biology and Medicine*, *12*(3), 238–245. https://doi.org/10.7497/j.issn.2095-3941.2015.0034

Tung N. V., Huan L. D., Hien D. M., Mai B. B., Phuong P. C., Lien N. T. K., *et al.* (2023). A nonsense mutation in BRCA1 gene in a Vietnamese patient with breast cancer. *Vietnam Journal of Biotechnology*, *21*(2), Article 2. https://doi.org/10.15625/1811-4989/18036

Tung N. V., Lien N. T. K., and Hoang N. H. (2021). A comparison of three variant calling pipelines using simulated data. *Academia Journal of Biology*, *43*(2), Article 2. https://doi.org/10.15625/2615-9023/16006

Ugo T., Germana C., and Elvira P. (2020). Breast Cancer: A molecularly heterogenous disease needing subtype-specific treatments. *Medical Sciences (Basel, Switzerland)*, *8*(1). https://doi.org/10.3390/medsci8010018

Van der Auwera G. A., Carneiro M. O., Hartl C., Poplin R., del Angel G., Levy-Moonshine A., *et al.* (2013). From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics*, *43*(1), 11.10.1-11.10.33. https://doi.org/10.1002/0471250953.bi1110s43

Westermark U. K., Reyngold M., Olshen A. B., Baer R., Jasin M., and Moynahan M. E. (2003). BARD1 participates with BRCA1 in homology-directed repair of chromosome breaks. *Molecular and Cellular Biology*, *23*(21), 7926–7936. https://doi.org/10.1128/MCB.23.21.7926-7936.2003

Zhang S., Royer R., Li S., McLaughlin J. R., Rosen B., Risch H. A., *et al.* (2011). Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. *Gynecologic Oncology*, *121*(2), 353–357. https://doi.org/10.1016/j.ygyno.2011.01.020

Zhang Y., Ji Y., Liu S., Li J., Wu J., Jin Q., *et al.* (2025). Global burden of female breast cancer: New estimates in 2022, temporal trend and future projections up to 2050 based on the latest release from GLOBOCAN. *Journal of the National Cancer Center*, *5*(3), 287–296. https://doi.org/10.1016/j.jncc.2025.02.002

Zhao W., Steinfeld J. B., Liang F., Chen X., Maranon D. G., Jian Ma C., *et al.* (2017). BRCA1-BARD1 promotes RAD51-mediated homologous DNA pairing. *Nature*, *550*(7676), 360–365. https://doi.org/10.1038/nature24060

Zhong X., Dong Z., Dong H., Li J., Peng Z., Deng L., *et al.* (2016). Prevalence and prognostic role of brca1/2 variants in unselected chinese breast cancer patients. *PLoS ONE*, *11*(6), e0156789. https://doi.org/10.1371/journal.pone .0156789