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A NONSENSE MUTATION IN *BRCA1* GENE IN A VIETNAMESE PATIENT WITH BREAST CANCER

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SUMMARY

Breast cancer is the most frequent female cancer, and it is increasing at an alarming rate. The typical symptom of breast cancer is breast lumps or swellings, though they can also develop close to the breast or under the arm. Breast cancer usually spreads to distant organs such as the bone, liver, lungs, and brain. Approximately 95% of breast cancer patients who arrive with the early-stage disease show no macroscopic signs of metastases. Although it is possible to reduce some breast cancer risks through prevention, the majority of patients in low-income countries are detected at a late stage. Because of this, even though current therapy is improving, early diagnosis is still crucial for improving the quality of treatment and the survival rate. Sex, age, family history, and an unhealthy lifestyle are some of the risk factors that may increase the chances of getting this disease. Familial or hereditary breast cancer accounts for 10% of breast cancers. Mutations in the *BRCA1* and *BRCA2* genes were responsible for 50% of all familial or hereditary breast cancer cases. In this research, mutations in a Vietnamese patient with familial breast cancer were analyzed using whole exome sequencing. As a result, 17 variants have been reported in the ClinVar database as "germline mutations" in familial breast cancer were detected. Of these, a heterozygous nonsense mutation c.5314C>T (p.R1772X) in the *BRCA1* gene was identified as the genetic cause of this case. This mutation has been previously reported as pathogenic in the ClinVar database (ClinVar variation ID: 55480). Our results provide insight into the genetic causes of breast cancer as well as support the genetics diagnosis of familial breast cancer.

Keywords: *BRCA1*, breast cancer, hereditary breast and ovarian cancer syndrome, genetic variants, Vietnamese patient, whole exome sequencing

INTRODUCTION

Breast cancer (OMIM#114480) ranks as the fifth most common cancer mortality cause globally, with 685,000 fatalities in 2020 (Sung *et al*., 2021). According to Globocan 2020, an estimated 2.3 million new cases of female breast cancer, or approximately 11.7% of all cancer cases, were reported. The prevalence of this disease is estimated to be 34.2 incidences per 100,000 people per year. In Vietnam, the 5-year prevalence of all ages is about 124.65 cases per 100,000 people (Sung *et al*., 2021).

Research showed that having breast cancer under the age of 40 was not a common condition. Between the ages of 30 and 39, the breast cancer developing possibility is 0.04% per year, while the probability in those over 80 years increases to >10% (Cardoso *et al*., 2012). The major causes of this disease are assumed to be related to environmental factors and lifestyle, while only 5–10% of all cases are attributed to genetic disorders (Castelló *et al*., 2015). However, previous studies demonstrated that if a woman had a first-degree relative with breast cancer (her sister, mother, or daughter was diagnosed with this disease), her chance of this disorder developing was 1.7–4.0 times higher. Therefore, family history still plays an important role as a risk factor for breast cancer (Edlich *et al*., 2008). At least two persons with breast or ovarian cancer are close relatives; among those at least one case was diagnosed before age 50, commonly known as Hereditary Breast and Ovarian Cancer Syndrome (HBOC).

The first significant gene discovered and isolated as a susceptibility breast cancer gene was *BCRA1* (OMIM#113705), which was found on chromosome 17q21 (Easton *et al*., 1995). This gene has a function of suppressing tumors involved in double-strand DNA breaks and also supporting the stability of the genome and cell survival (O'Donovan, Livingston, 2010). For a female who has a mutation in the *BRCA1* gene, the lifetime risk of developing breast cancer is between 56 and 87% (Edlich *et al*., 2008). Moreover, mutations in the *BRCA1* and *BRCA2* genes account for nearly 50% of familial hereditary breast cancer cases (Kuchenbaecker *et al*., 2017).

Herein, whole exome sequencing was performed on a Vietnamese case with Hereditary Breast and Ovarian Cancer Syndrome in an effort to better understand the genetics of the disease.

MATERIALS AND METHODS

Ethics

A blood sample was taken after obtaining consent from the patient. The study was approved by the Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology (No. 01-2021/NCHG-HĐĐĐ).

Medical report of the proband

To perform the study, patients suspected of having breast cancer without clinical and paraclinical tests, histopathology, a family history of breast cancer, or any other genetic disease will not be selected. A 41-year-old Vietnamese woman was diagnosed with stage 3 of the invasive ductal carcinoma (IDC) type at the age of 38. The tumor appeared in the lower quarter of the right breast. The patient had her first period at the age of 16 and has not reached menopause yet. The patient is childless and is on hormone replacement therapy. The patient, whose aunt was diagnosed with ovarian

cancer at the age of 65. She does not have a history of lobular carcinoma in situ (LCIS) and no previous treatment with radiotherapy in the chest. The patient's tumors were classified as negative ER/PR/HER2 markers (Triple-negative breast cancer, TNBC) and Ki-67 marker (70%) positive. The patient was treated with chemotherapy. The patient was informed of the detection of a pathogenic variant on the *BRCA1* gene and was indicated for treatment with a PARP inhibitor, but the patient died from COVID-19, so we do not have further data on the treatment effect of a PARP inhibitor in this patient.

DNA extraction and whole-exome sequencing

A blood sample from the patient was provided by the Nuclear Medicine and Oncology Center at Bach Mai Hospital, stored in EDTA tubes, and preserved at -20^{o}C before DNA extraction. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) following the manufacturer's guidelines. The DNA library of the patient was prepared using SureSelect XT Library Prep Kit ILM (Agilent, USA), and the whole exome was sequenced using the Illumina NextSeq 500 platform (Illumina, CA, USA).

Variant calling and data analysis

Variants were called by following GATK Best Practice pipeline instructions (Heldenbrand *et al*., 2019; Tung *et al*., 2021). Briefly, the paired-end reads were mapped to the reference human genome GRCh38 using BWA 0.7.17 (Li, Durbin, 2009). The Picard tool (http://broadinstitute.gith-ub.io/picard/) was used to process post-alignment data, including creating indexes, marking, and removing repeated reads from the alignment bam file. Variants were called using HaplotypeCaller in the GATK package version 4.1 (Van der Auwera *et al*., 2013). The discovered variants were assessed using online databases including dbSNP (Sherry *et al*., 2001), the 1000Genome project (Auton *et al*., 2015), ClinVar (Landrum *et al*., 2018), the Human Gene Mutation Database (HGMD, https://www.hgmd.cf.ac.uk/ac/index.php), gnomAD (Karczewski *et al*., 2020), and the Vietnamese Genetic Variation Database (Le *et al*., 2019). The pathogenicity of the mutation was predicted using *in silico* tools: MutationTaster2021 (Steinhaus *et al*., 2021), and CADD (Rentzsch *et al*., 2019).

PCR and Sanger sequencing

A fragment of the *BRCA1* gene was amplified by using a specific primer designed via Primer Blast. Forward primer: 5'- CCTGATGGGTTGTGTTTGGT - 3', reverse primer: 5'-CGAGACGGGAATCCAAATTA - 3' (PHUSA Genomics, Vietnam). The PCR thermal cycle conditions used for the amplification were 95^{o}C/12 min; (95^{o}C/45 sec; 57^{o}C/45 sec; 72^{o}C/45 sec) x 35 cycles and 72^{o}C/8 min. The PCR product (356 bp) was performed by electrophoresis in agarose 1%.

RESULTS

Whole-exome sequencing of the breast cancer patient yielded 10.2 GB of sequence reads. Of these, over 99.8% were mapped to the human genome, and 70.1% were mapped to exome regions (Table 1).

Table 1. Summary of whole exome sequencing data.

Total number of reads	**41,721,094**
Q20 (%)	98.0
Q30 (%)	94.4
GC (%)	49.5
Number of mapped reads to human genome (%)	41,675,619 (99.8%)
Number of reads mapped to exome regions (%)	28,293,866 (70.1%)
Coverage of target region (≥50X)	60.3%

Table 2. Summary of variants found in patient.

Total non-synonymous variants	336
Benign/Likely benign	50
Uncertain significance	2
Pathogenic	1
No Available	283

By following the GATK Best Practices pipeline, a total of 80,651 SNPs were obtained, including 11,939 synonymous variants, 11,519 missense variants, 108 stop-gained variants, 37 stop-lost variants, and 10,570 indel variants. By focusing on genes associated with breast cancer, 336 non-synonymous variants were detected in exome regions (Table 2).

There were 17 variants found in familial breast cancer or hereditary cancer predisposing syndrome, the majority of which were classified as benign in the ClinVar database (Table 3). Among those, only one heterozygous nonsense mutation (c.5314C>T) in exon 20 of the *BRCA1* gene (transcript ID: ENST00000471181) was identified that may be the cause of disease in the patient. Therefore, it was selected for further Sanger sequencing validation. This mutation changed from Arginine (Arg) to stop codon at amino acid residue 1772 and led to the premature termination of protein (R1772X).

Results from the Sanger sequencing confirmed that the patient had a heterozygous c.5314C>T in exon 20 of the *BRCA1* gene, and the control sample had a normal genotype (Figure 1). CADD evaluated *BRCA1* c.5314C>T with a score of 38, which indicates that the mutation was predicted to be the ~0.1% most deleterious substitution in the human genome. Besides, MutationTaster2021 predicted the mutation as “Deleterious” with the complex_aae model and tree vote 197|3. For mutations leading to early stop codons, MutationTaster2021 uses a model called complex_aae, which uses 200 Random Forest trees for predictions. Tree vote 197|3 means that 197 decision trees in the Random Forest have indicated deleteriousness and only 3 decision trees have indicated benign alteration.

Table 3. Variants related to familial breast cancer or hereditary cancer predisposing syndrome in the patient.

Gene Name	Zygosity	HGVS.c	HGVS.p	ClinVar Clinical Significance	Variant disease name in ClinVar
BARD1	HET	c.1519G>A	V507M	Benign	Hereditary cancer predisposing syndrome
BARD1	HET	c.1134G>C	R378S	Benign	Hereditary cancer predisposing syndrome
PALLD	HOM	c.406A>G	S136G	Benign	Hereditary cancer predisposing syndrome
PMS2	HOM	c.1621A>G	K541E	Benign	Lynch syndrome
PMS2	HET	c.1454C>A	T485K	Benign	Lynch syndrome Hereditary cancer predisposing syndrome
PMS2	HET	c.1408C>T	P470S	Benign	Lynch syndrome Hereditary cancer predisposing syndrome
NBN	HET	c.553G>C	E185Q	Benign	Hereditary cancer predisposing syndrome
ATM	HOM	c.5948A>G	N1983S	Benign	Hereditary cancer predisposing syndrome
BRCA2	HET	c.1114A>C	N372H	Benign	Breast ovarian cancer familial, susceptibility to, 2 Hereditary cancer predisposing syndrome
BRCA2	HOM	c.7397T>C	V2466A	Benign	Familial cancer of breast Breast ovarian cancer familial, susceptibility to, 2
PALB2	HET	c.1676A>G	Q559R	Benign	Familial cancer of breast Hereditary cancer predisposing syndrome
CDH1	HET	c.1409C>T	T470I	Likely benign	Hereditary cancer predisposing syndrome Hereditary diffuse gastric cancer
BRCA1	HET	c.5314C>T	R1772X	Pathogenic	Breast ovarian cancer familial, susceptibility to, 1 Hereditary cancer predisposing syndrome

BRCA1	HOM	c.4900A>G	S1634G	Benign	Breast ovarian cancer familial, susceptibility to, 1 Hereditary cancer predisposing syndrome
BRCA1	HOM	c.3548A>G	K1183R	Benign	Breast ovarian cancer familial, susceptibility to, 1 Hereditary cancer predisposing syndrome
BRCA1	HOM	c.3113A>G	E1038G	Benign	Breast ovarian cancer familial, susceptibility to, 1 Hereditary cancer predisposing syndrome
BRCA1	HOM	c.2612C>T	P871L	Benign	Breast ovarian cancer familial, susceptibility to, 1 Hereditary cancer predisposing syndrome

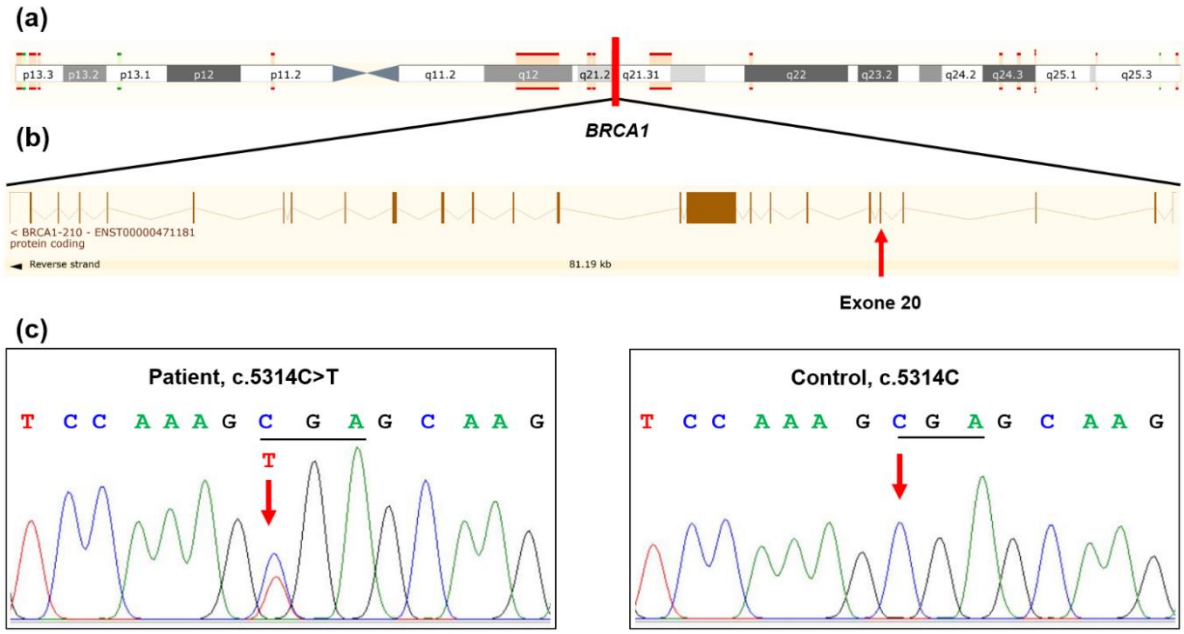

Figure 1. Analysis of the c.5314C>T mutation in the patient and the control. (A) *BRCA1* is located on chromosome 17q21.31. (B) Exon-intron graph of the *BRCA1* gene. (C) Sanger sequencing of the *BRCA1* c.5314C>T mutation in the patient and the control.

The *BRCA1* c.5314C>T mutation was classified as "pathogenic" following ACMG Standards (Richards *et al*., 2015), with criteria for classification shown in Table 4. This mutation has been previously reported in dbSNP (ID: rs80357123), as pathogenic in the ClinVar database (ClinVar Variation ID: 55480; ClinVar RCV ID: RCV000048882), and in the COSMIC database (ID: COSM51256).

Besides, we identified a heterozygous missense mutation c.1409C>T (T470I) in exon 10 of the *CDH1* gene (transcript ID: NM_004360) with a dbSNP ID as rs370864592. Although this mutation was evaluated as "Damaging" by several *in silico* tools (Sift score: 0.025; Polyphen_2 score: 0.999), the rs370864592 has been previously reported as "Benign" in the Clinvar database (ClinVar Variation Id: 127913; ClinVar RCV ID: RCV000123236).

Table 4. Criteria for classifying *BRCA1* c.5314C>T mutation following ACMG Standard.

Evidence of pathogenicity	Annotation
PVS1 (Very Strong)	Null variant (nonsense) in gene *BRCA1*, predicted to cause nonsense-mediated decay. Loss-of-function is a known mechanism of disease (gene has 3,253 reported pathogenic LOF variants). The exon contains 78 pathogenic variants. The truncated region contains 269 pathogenic variants.
PS3 (Strong)	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product, confirmed by a functional study, mentioned in PMID: 12142080 (Ladopoulou *et al*., 2002).
PM2 (Moderate)	GnomAD genomes homozygous allele count = 0 is less than 2 for AD/AR gene BRCA1, good gnomAD genomes coverage = 30.4. GnomAD exomes homozygous allele count = 0 is less than 2 for AD/AR gene BRCA1, gnomAD exomes coverage is unavailable.
PP5 (Supporting)	ClinVar classifies this variant as Pathogenic, 3 stars (expert panel, 34 submissions), citing 11 articles.

DISCUSSION

BRCA1 protein functions in DNA repair, cell division, and growth, which is related to an increased risk of breast and ovarian cancer in both men and women, depending on the types and locations of the mutations. Three breast cancer cluster areas (BCCRs) were detected, which are located at c.179 to c.505, c.4328 to c.4945, and c.5261 to c.5563, as well as an ovarian cancer cluster area (OCCR) from c.1380 to c.4062 (Rebbeck *et al*., 2015). To date, more than 2529 mutations in the *BRCA1* gene have been reported in the HGMD database. Almost all reported variants were involved in breast and/or ovarian cancer. The c.5314C>T mutation led to the truncation of 112 amino acids downstream, which may affect the DNA repair function of the BRCA1 protein. Deficient BRCA1 proteins are unable to help repair DNA damage due to their function in homologous recombination repair. Additionally, rs80357123 affects the BRCA1-C-Terminal domain of the *BRCA1* gene (BRCT), where BRCA1 and BACH1

along with other functional proteins interact. Mutation of the BRCT domain inhibits the interaction of BRCA1 with BACH1, leading to an uncontrolled cell cycle and abnormal homeostasis, which may promote cancer.

Up to now, approximately 34.000 variants of the *BRCA1* gene have been recorded in the BRCA Exchange database (Abreu *et al*., 2022). Of these, nonsense mutations account for 20% of variants classified as "pathogenic" in the Clinvar database. The rs80357123 mutation has been reported in heterozygosity in at least 50 individuals with hereditary breast and ovarian cancer (Findlay *et al*., 2018). It has also been observed in 0.005% (1/19954) of East Asian chromosomes by the gnomAD database. This mutation is the ninth most frequently observed mutations of the *BRCA1* gene in African Americans (2%) and the fifth most common *BRCA1* mutation in Greece and Hungary (Rebbeck *et al*., 2018). The c.5314C>T mutation has been found in 3 out of 4 Vietnamese patients in a study about 200 Asian American patients (Kurian *et al*., 2008). In Vietnam, this mutation has been described in a family with three patients with hereditary breast ovarian cancer syndrome (a female patient, her mother, and maternal grandmother) (Hoang *et al*., 2010). In a study of Vietnamese patients with HBOC using the NGS approach, Le *et al*. (2022) found this mutation in four out of 33 cases.

CONCLUSION

In summary, by performing whole exome sequencing, we detected 17 mutations that were described as "germline mutations" in the ClinVar database in a Vietnamese patient with Hereditary breast and ovarian cancer syndrome. Of these, the nonsense mutation R1772X in the *BRCA1* gene may be the cause of the phenotype in the patient. The results provide useful information on the genetic pathogenesis of hereditary breast and ovarian cancer syndrome in Vietnam.

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