A Novel *BRCA1* Gene Mutation Detected With Breast Cancer in a Vietnamese Family by Targeted Next-Generation Sequencing: A Case Report

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ABSTRACT: Hereditary breast cancer is an inherited genetic condition, mainly caused by *BRCA1* and *BRCA2* gene mutations. These genetic changes can increase the risks of breast and ovarian cancers in women, while prostate and breast cancers in men. Especially, mutations in either *BRCA1* or *BRCA2* genes take important roles in early-onset breast cancer. The present study focused on a 47-year-old Vietnamese woman with breast cancer by applying targeted next-generation sequencing technique. A novel *BRCA1* gene mutation, namely NM_007294.3 (BRCA1): c.4998insA (p. Tyr1666Terfs), was identified both in this patient and in some of the members in her family proved the fact that the mutated genes passed down through generations. This change may exponentially initiate breast cancer risks and become a valuable marker for exact clinical prognosis and treatment.

KEYWORDS: *BRCA1* gene mutation, breast cancer, early—onset period, family pedigree

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Introduction

BRCA1 and *BRCA2* genes encode for tumor-suppressor proteins which take responsibility for DNA correction and genetic material stability. Mutations in *BRCA1* gene can boost the risks of breast, ovarian, and prostate cancers. There are many causes of breast cancer but the heredity makes up for about 3% to 10% of cases and 30% of early-onset period.[1] In the family having the genetic hereditary history of cancer, *BRCA1* and *BRCA2* gene mutations constitute about 5% to 10% of breast cancer and 10% to 15% of ovarian cancer causes.[2] Modern molecular techniques, such as next-generation sequencing, allow us to determine the exact mutation sites leading to cancers. These results will help the clinical prognosis and treatment process more effective. In this study, we discovered a novel mutation in the *BRCA1* gene of a woman patient, which might be a cause leading to breast carcinoma.

Method

Peripheral blood samples were collected from the patient and her family members. Genomic DNA was extracted from blood with QuiAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and sequenced by targeted next-generation sequencing. In the library preparation step, we used NEBNext Ultra II DNA Library Preparation Kit from New England BioLabs (Ipswich, MA, USA) for DNA fragmentation and library preparation. The breast cancer susceptibility gene panel containing 17 genes (*APC, MLH1, MSH2, MSH6, BRCA1, BRCA2, PALB2, PTEN, TP53, CDH1, PMS2, EPCAM, MUTYH, STK11, VHL, RB1, RET*) chosen to examine mutation. Predesigned probes were used to capture exons of the genes of interest and a small flanking sequence of introns for those genes from IDTDNA (Coralville, IA, USA). Captured products were amplified with KAPA HiFi HotStart ReadyMix from KAPA Biosystems (Wilmington, MA, USA). Samples were sequenced on Illumina NextSeq platform (Illumina, San Diego, CA, USA). Raw sequences from each sample were aligned to the reference human genome from University of California, Santa Cruz (UCSC) Genome Browser (NCBI build GRCh38) using Burrows Wheeler Aligner (BWA). The aligned output was used to compute depth and breadth of coverage in the target region, and SNP/INDEL calling with GATK standard pipeline. Variants were classified using ClinVar database (National Institutes of Health) and then confirmed by Sanger sequencing technique.

Case Report

A 47-year-old Vietnamese woman was selected as the main proband for our research (Figure 1, II-13, red arrow). The proband was diagnosed with left breast cancer in November 2018 at Vietnam National Cancer Hospital (Hanoi, Vietnam) given by x-ray analysis, pathology interpretation, and breast ultrasound results.

The patient detected a lump in her left breast by herself without nipple fluid discharge and skin abnormality. On the ultrasound, her left breast had scattered cysts with the biggest one measured approximately 4×4 mm. In the site of 2 pm, there was a heterologous, fluid-like and demarcated lesion (14×11 mm). No mammography was taken. Then she

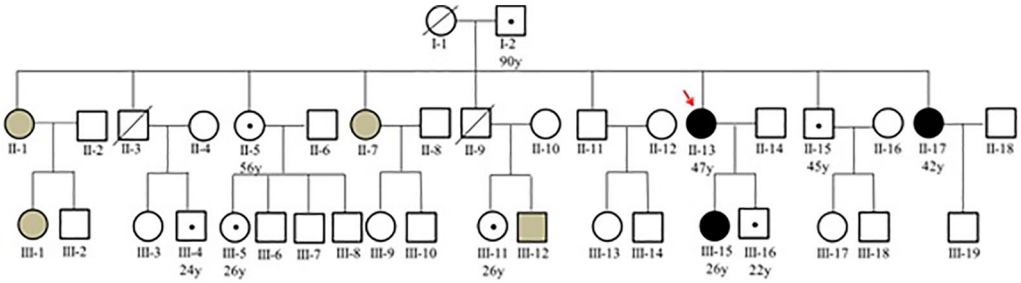

Figure 1. The pedigree of a Vietnamese patient's family in 3 generations with hereditary breast cancer. Squares and circles denote males and females, respectively. The red arrow indicates the main proband. Members have breast cancer clinically and carry gene mutation being denoted by black circles. People have a central dot seen as gene mutation carriers without disease. Brown symbols illustrate individuals who do not carry any mutant. Circles or squares crossed by a line represent member deceased.

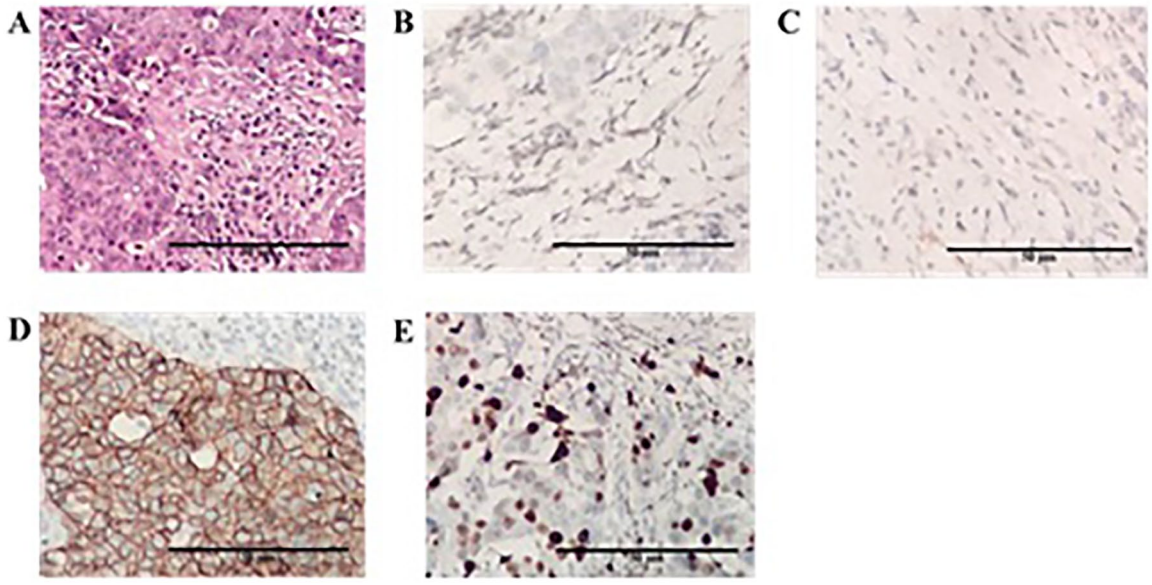

Figure 2. Immunostaining images (magnification 40×, scale bar 50μm). Hematoxylin and Eosin staining for tumor tissues (A). Immunohistochemistry staining for estrogen receptor (B); negative, progesterone receptor (C); negative, Her-2 (D); positive (+++) and Ki67 index 75% (E).

had her lumpectomy in a private clinic because of a benign origin suspicion. On the histological images, tumor cells aggregated in foci and sheets. They were large with irregular, hyperchromatic nuclei and conspicuous nucleoli, abundant cytoplasm. Stroma was sclerotic and infiltrated by numerous lymphocytes and plasmocytes. Immunohistochemical stains revealed estrogen receptor (ER) and progesterone receptor (PR) negative, Her2/neu positive 3 plus. In addition, Ki67 index was 75% (Figure 2). Therefore, the tumor was classified as invasive carcinoma of no special type according to WHO classification (4th edition, 2012) and the molecular type was HER2.[3]

Then the patient was operated to remove her total cancer breast with revision PATEY protocol. No tumor remained on the postsurgery microscopic pictures and 20 lymph nodes were devoid of malignancy, too. In aspect of pathological features, the postoperative stage was pT1cN0Mx. After that, she continued being treated with adjuvant chemotherapy of 4AC (4 doxorubicin 60 mg/m^2-cyclophosphamide 600 mg/m^2) and 4T (paclitaxel 175 mg/m2) in 3 weeks. Then trastuzumab was added right after chemotherapy. Efficiency was evaluated after 4 cycles and cardiac function was assessed after 3 months.

Her family's history was investigated. Surprisingly, several of the patient's family members had been affected by cancer. In F1 generation, her mother (Figure 1, I-1) died of unknown cause, while her father (Figure 1, I-2) remained healthy. Among 9 siblings (F2 generation), there was a younger sister (Figure 1, II-17) who was diagnosed to have left breast cancer in March 2015. Besides, 2 older brothers (Figure1, II-3 and II-9) died of unknown cancers. In F3 generation, her daughter (Figure1, III-15) had left breast cancer at the age of 22 years also. Therefore, we hypothesized that there was a genetic factor related to breast cancer flowing through generations in her family.

To clarify this hypothesis, we decided to perform a screening mutation test with her blood by applying targeted Next-Generation Sequencing method (Illumina, NextSeq, United

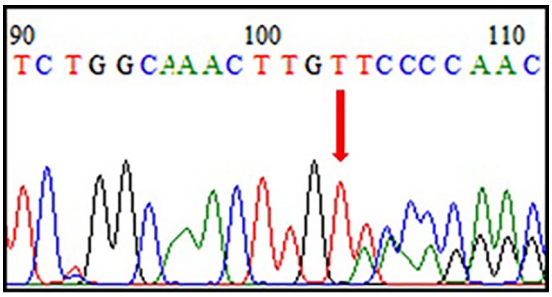

Figure 3. A germline novel heterozygous insertion mutation location was identified by Sanger sequencing denoted by a red arrow (namely NM_007294.3 (BRCA1): c.4998insA (p.Tyr1666Terfs)).

States). The sequencing detected a novel mutation of *BRCA1* gene, while *BRCA2* gene was not mutated. The coverage and the depth of target regions were 99.6% and 271X, respectively. To elucidate the genetic predisposition of the *BRCA1* gene mutation derived from either her mother or father, genomic DNA extracted from blood of other members was sequenced by the same technique. Notably, her father's blood carried the same mutant allele as the patient, which proved the fact that the patient directly inherited mutant allele from him. This is quietly surprising because he is still alive and healthy while her mother died many years ago. Examination results of both patient's children (Figure 1, III-15 and III-16) showed the mutated copy of *BRCA1* gene being similar to their mother's gene status. Among survival siblings, 2 sisters (Figure 1, II-5 and II-17) also had this mutation, while 2 others (Figure 1, II-1 and II-7) did not. In particular, both the proband and the older sister (Figure 1, II-5) passed down this mutation to their daughter (Figure 1, III-5 and III-15, respectively). In addition, another younger brother (Figure 1, II-15) carried this *BRCA1* mutation. In the family of 2 deceased brothers (Figure 1, II-3 and II-9), 1 of their children was detected to carry the *BRCA1* mutated allele (Figure 1, III-4 and III-11, respectively), suggesting that these brothers died of cancer might be related to the *BRCA1* gene mutation. By comparison with the reference human genome from UCSC Genome Browser, the *BRCA1* gene mutation of the proband's family was identified as a novel heterogenous gene mutation, named NM_007294.3 (BRCA1): c.4998insA (p. Tyr1666Terfs), specifically 1 base pair insertion results in the generation of a premature stop codon leading to a truncated protein resulted in hereditary cancer-predisposing syndrome as the mutation of NM_007294.3 (BRCA1): c.4998C>A (p.Tyr1666Ter) effect, according to ClinVar Database of the US National Institutes of Health. To verify the sequencing output, Sanger sequencing was applied to confirm and gave the same result (Figure 3).

Discussion

Currently, breast cancer becomes popular in women, with about 5% to 10% of familial cancers.[4] *BRCA1* and *BRCA2* genes play a role in the repair of double-stranded DNA by homologous recombination, which interacts with RAD51, thereby inhibiting tumor formation and development.[4] *BRCA1* gene mutations causing breast cancer according to family pedigree have been recorded in several studies such as in China,[5,6] Italy,[7] Greece,[8] Iran,[9] and the United States.[10] However, no detailed research has been conducted in Vietnam. Therefore, we performed a survey, collected blood samples and sequenced breast cancer-related genes on a Vietnamese patient whose family also has many members diagnosed with cancer. The investigated patient was a 47-year-old female with hereditary breast cancer. The pathology results showed that this woman has a tumor in the left breast, in the stage of IA (according to AJCC 8th edition). A novel heterozygous germline mutation (NM_007294.3 (BRCA1): c.4998insA (p.Tyr1666Terfs)) in *BRCA1* gene was detected in the patient's blood sample. This genetic alteration led to premature stop codon formation, following by truncated amino acid chains and therefore might affect protein expression. Both next-generation sequencing and Sanger sequencing results emphasized the fact that 8 out of 14 tested members simultaneously carried p.Tyr1666Terfs mutation. Outstandingly, both her sister (Figure 1, II-17) and her daughter (Figure 1, III-15) developed breast cancer at the early stage of life, 38 and 22 years old, respectively. This study alarms us to keep following her 22-year-old son (Figure 1, III-16) who is carrying gene mutation. To be concluded, the *BRCA1* gene mutation has been passed down through generations from fathers (Figure 1, I-2) to subsequent descendants. It is worth noting that this mutation has a genetic potential and increases the risk of early-onset breast cancer.

Accounting for 22.9% of the total invasive cancer portions in women, breast cancer became the most popular cancer disease over the world according to GLOBOCAN 2008. In addition, the incidence rate of the breast cancer was highest among 10 most common cancer types (11.6%), as lung cancer in both sexes.[11] More locally, Vietnam experienced a higher incidence rate with 20.6% (about 15 229 new cases) according to GLOBOCAN 2018. Among these cases, the frequency of *BRCA1/2* mutated gene was just only 0.8% in sporadic breast cancer patients[12,13] but limited research reported detailed statistical data about this frequency in familial breast cancer. *BRCA1* mutation accounted for 72% probability of breast cancer development before 70 years old which is higher than the percentage of cases caused by *BRCA2* mutation (about 60%). Patients with harmful *BRCA1/BRCA2* mutation have a higher risk to develop cancer in opposite breast in the near future. Lal et al[14] suggested that BRCA—mutated tumors were more aggressive than sporadic breast cancer as BRCA pathway changes can affect multiple important signaling networks including mutagenesis and gene dysregulation. There are about 2000 mutations in *BRCA1* and *BRCA2* genes but novel mutation occurs rarely.[6] To the best of our knowledge,

p.Tyr1666Terfs mutation has not known in Vietnamese population but based on our research result, the role of it was clarified more clearly in the heredity of family. Specialty, this mutation has possibility to initiate tumor formation at very early of the age.

In summary, our study would like to emphasize the role of molecular testing, especially the *BRCA1* gene in the breast cancer patients and early genetic screening for other family members if in doubt. The exact mutation identification will help for individualized treatment, prognosis, and follow-up more effective, especially for Poly ADP ribose polymerase enzyme inhibitors therapy.

Author Contributions

T.V.T, N.V.C, D.V.T, and N.T.Q.T equally contributed to this work.

Informed Consent

Written informed consent was applied to the patient before enrolling them to the study. Patient could withdraw from the study at any time without any threats or disadvantages and for no stated reasons.

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