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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

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...
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/m²-cyclophosphamide 600 mg/m²) 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 (Figure 1, II-3 and II-9) died of unknown cancers. In F3 generation, her daughter (Figure 1, 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 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).
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. 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. BRCA1 gene mutations causing breast cancer according to family pedigree have been recorded in several studies such as in China, Italy, Greece, Iran, and the United States. 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-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. More locally, Vietnam experienced a higher incidence rate with 20.6% (about 15229 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 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 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. To the best of our knowledge,
p.Tyr1666Terfs mutation has not been known in Vietnamese population but based on our research result, the role of it was clarified more clearly in the heredity of family. Speciality, 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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REFERENCES
1. Narod SA. Early-onset breast cancer: what do we know about the risk factors? A Countercurrents Series. Curr Oncol. 2011;18:204-205.
2. Marchina E, Fontana MG, Speziani M, et al. BRCA1 and BRCA2 genetic test in high risk patients and families: counselling and management. Oncol Rep. 2010;24:1661-1667.
3. Devilee FATP. Tumours of the Breast and Female Genital Organs. Washington, DC: IARC Press; Geneva, Switzerland: WHo; 2003.
4. Apostolou P, Fostira F. Hereditary breast cancer: the era of new susceptibility genes. Biomed Res Int. 2013;2013:747318.
5. Kwong A, Ng EK, Tang EY, et al. A novel de novo BRCA1 mutation in a Chinese woman with early onset breast cancer. Fam Cancer. 2011;10:233-237.
6. Wang Y, Jiang D, Zhao Q, et al. Identification of a novel breast cancer-causing mutation in the BRCA1 gene by targeted next generation sequencing: a case report. Oncol Lett. 2018;16:3913-3916.
7. Antonucci I, Provenzano M, Sorino L, Rodrigues M, Palka G, Struppia L. A new case of “de novo” BRCA1 mutation in a patient with early-onset breast cancer. Clin Case Rep. 2017;5:238-240.
8. Fostira F, Tsoukalas N, Konstantopoulou I, Georgoulas V, Christophyllakis C, Yannoukakos D. A paternally inherited BRCA1 mutation associated with an unusual aggressive clinical phenotype. Case Rep Genet. 2014;2014:875029.
9. Sadr-Nabavi A, Dastpak M, Homaei-Shandiz F, Bahrami AR, Bidkhori HR, Raesoulmohaddeseen M. Analysis of novel mutations in BRCA1 in Iranian families with breast cancer. Hereditas. 2014;151:38-42.
10. Marshall M, Solomon S, Lawrence Wickerham D. Case report: de novo BRCA2 gene mutation in a 35-year-old woman with breast cancer. Clin Genet. 2009;76:427-430.
11. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
12. Ginsburg OM, Dinh NV, To TV, et al. Family history, BRCA mutations and breast cancer in Vietnamese women. Clin Genet. 2011;80:89-92.
13. Kim H, Choi DH. Distribution of BRCA1 and BRCA2 mutations in Asian patients with breast cancer. J Breast Cancer. 2013;16:357-365.
14. Lal A, Ramazzotti D, Weng Z, Liu K, Ford JM, Sidow A. Comprehensive genomic characterization of breast tumors with BRCA1 and BRCA2 mutations. BMC Med Genomics. 2019;12:84.