Identification of a novel breast cancer-causing mutation in the *BRCA1* gene by targeted next generation sequencing: A case report

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Abstract. Hereditary breast cancer is an autosomal dominant syndrome caused by germ-line mutations in the human breast cancer genes, *BRCA1* and *BRCA2*. Mutations in either *BRCA1* or *BRCA2* are the major causes of familial and early-onset breast cancer. The present study investigated a 33-year-old Chinese female patient with breast cancer using targeted next generation sequencing. A novel heterozygous deletion-insertion was also identified in the *BRCA1* gene, c.311_312delinsAGGTTTGCA, which causes the formation of a truncated BRCA1 protein of 109 amino acids instead of a wild-type BRCA1 protein of 1,863 amino acids. These results could potentially expand the mutational spectra of BRCA1-associated breast cancer. In addition, these findings may be valuable for the mutation-based screening and genetic diagnosis of breast cancer.

Introduction

Hereditary breast cancer (HBC) is an autosomal dominant familial and early-onset breast cancer syndrome manifested by a gradual and exponential risk of developing breast and ovarian cancer. HBC is primarily caused by mutations in the *BRCA1* and *BRCA2* genes. The BRCA1 protein has a major function in the DNA repair system (1). The present study investigated a 33-year-old Chinese female patient with HBC using targeted next generation sequencing (NGS). Through genetic testing, one deletion-insertion mutation (c.311_312delinsAGGTTTGCA) in the *BRCA1* gene was detected, and it was revealed that this was a typical family of inherited tumors following detection of the highest likelihood of mutation in the proband's family members. The proband's uncle and uncle's daughter carry the same mutation as the proband and therefore, it was considered that the proband's father, who had succumbed, and the proband's aunt, who was diagnosed with triple-negative breast cancer (TNBC), may exhibit the same mutation. This deletion-insertion mutation (c.311_312delinsAGGTTTGCA) causes the formation of a truncated BRCA1 protein of 109 amino acids instead of a wild type BRCA1 protein of 1863 amino acids. Hence, this mutation is a loss-of-function mutation.

Case report

The proband is a 33-year-old Chinese female from non-consanguineous parents. The proband was diagnosed with breast cancer and enrolled in the present study. Clinical diagnosis was based on the patient's clinical history. The diagnosis for the patient, TNBC, was supported by her previous clinical information. The present study was performed in the Department of Internal Medicine, The Fourth Hospital of Hebei Medical University (Shijiazhuang, China).

In February 2015, a mass ~2 cm in diameter was identified in the patient's left breast. No other abnormalities were identified during physical examination. The laboratory examination results were within the normal range. Breast-conserving surgery and sentinel lymph node biopsy revealed that the left breast tumor was 1.8 cm in diameter; the breast metaplastic cancer lacked vascular tumor thrombus metastasis of the sentinel lymph nodes with negative margins. H&E staining is presented in Fig. 1A-C, with the following immunohistochemistry: Estrogen receptor (ER), 0; progesterone receptor (PR), 0; human epidermal growth factor 2 (HER2), 0; tumor protein p53 (P53), 60%; Ki-67, 50%; type II topoisomerase (TOMOII), 30%; creatine kinase (CK), +; vimentin (Vim), +; S-100 calcium binding protein (S-100), +; synuclein (Syn), -; and cluster of differentiation 56 (CD56), -/+, belonging to TNBC, as shown in the Fig. 1D-N. The post-operative

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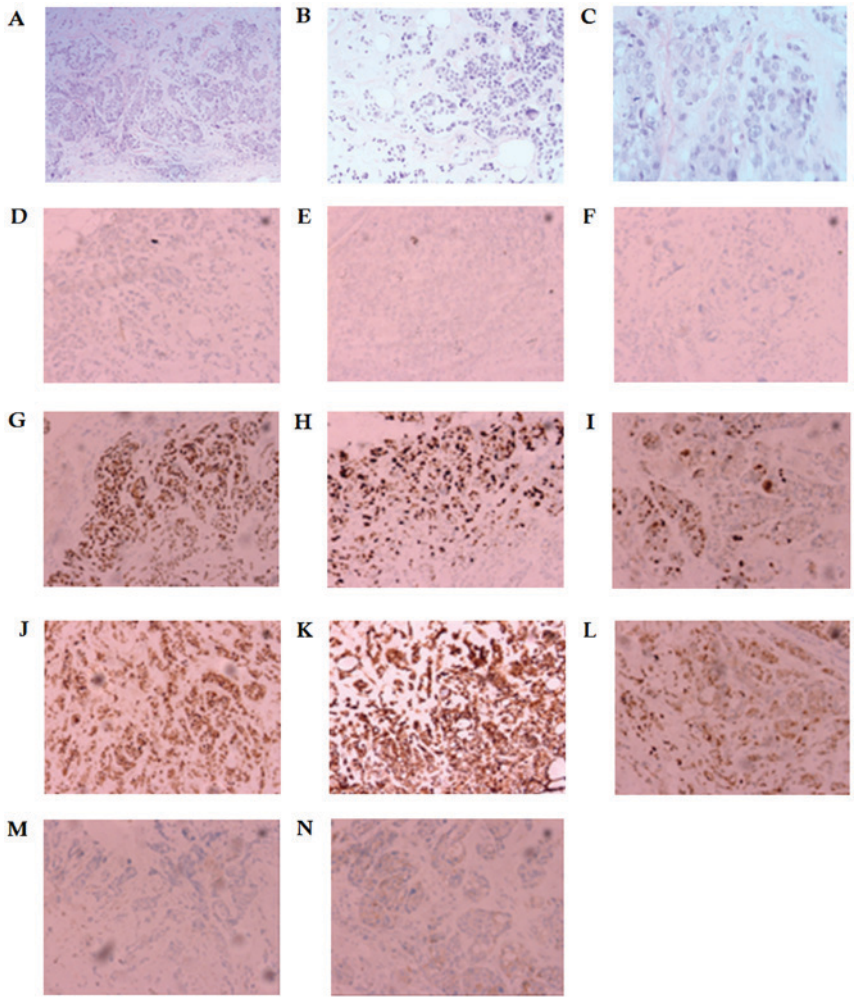

Figure 1. Immunostaining images. H&E staining for tumor tissues. (A) Scale bar=100 μm, (B) scale bar=50 μm and (C) scale bar=25 μm. Immunohistochemistry for (D) ER (negative), (E) PR (negative), (F) HER2 (negative), (G) P53 (60% positive immunoexpression), (H) Ki-67 (50% positive immunoexpression), (I) TOPOII (30% positive immunoexpression), (J) CK(+), (K) Vim(+), (L) S-100(+), (M) Syn(-), and (N) CD56(-/+). Scale bar=50 μm. ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; P53, tumor protein p53; TOPOII, type II topoisomerase; CK, creatine kinase; Vim, vimentin; S-100, S-100 calcium binding protein; Syn, synuclein; CD56, cluster of differentiation 56.

staging was IA (T1cN0M0) (2). Pathology revealed invasive cancer of the left breast with an extensive area of necrosis. The patient was treated with paclitaxel (90-120 mg/m^2) intravenously for 12 weeks, followed by cyclophosphamide treatment (200 mg/m^2) for 12 weeks.

The proband was clinically diagnosed with TNBC at the age of 31 (Fig. 1O, patient III-1) and underwent genetic testing at the age of 33. One mutation in the *BRCA1* gene was detected. As detailed knowledge of the proband's family history was acquired, it was revealed that the proband's father and grandfather were deceased (Fig. 2, I-2 and II-2, respectively); the cause and age of mortality were unknown. The proband's mother was >50 years of age and healthy (Fig. 2, II-1). The proband's aunt was diagnosed with TNBC at the age of 47 and succumbed at the age of 50 (Fig. 2, II-5). The pathological type of the proband was the same as that of her aunt. In addition, the proband has a healthy uncle (Fig. 2, II-4).

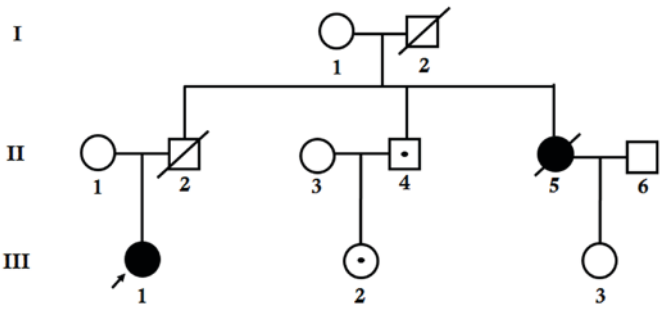

Figure 2. Pedigree structure of the Chinese family with hereditary breast cancer. The proband is indicated with shading and an arrow. Squares and circles denote males and females, respectively. Carriers of this mutation are indicated by a dot within the square or circle. Individuals labeled with a solidus are deceased. Roman numerals indicate generations (I-III).

Prior to performing pedigree verification, it was inferred that there are several hypotheses: i) The proband's aunt and father each carried an identical mutation in the *BRCA1* gene, which was inherited from proband's grandfather or grandmother; in this situation, the proband's uncle has a 50% probability of also carrying this mutation; ii) the mutation exhibited by the proband was inherited from her mother, who was a carrier that did not suffer from cancer; in which case, the proband's aunt who had cancer might have been a sporadic case; or she has other mutations iii) the mutation exhibited

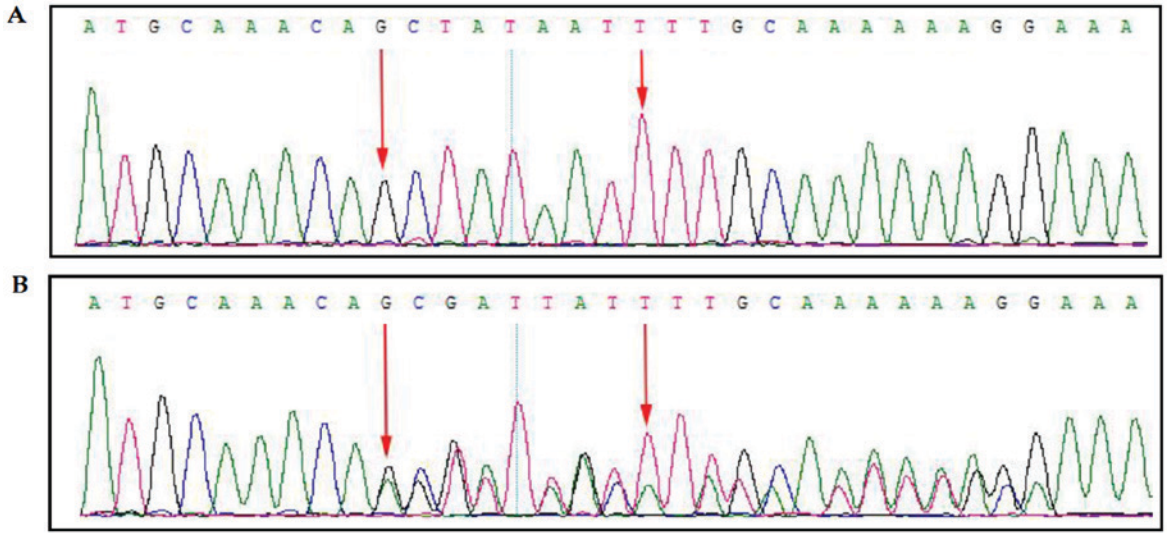

Figure 3. Sanger sequencing of the breast cancer 1 gene identified a germline novel heterozygous deletion-insertion mutation. (GenBank accession no., NM_007294). (A) DNA sequence derived from unaffected family member (II:3). (B) The equivalent region from the proband (III:1). The arrows indicate the novel heterozygous deletion-insertion mutation (c.311_312delinsAGGTTTGCA).

by the proband was not inherited from her parents, but was *de novo*; the proband's aunt's breast cancer is not associated with this mutation.

As the grandmother of the proband (Fig. 2, I-1) refused to undergo genetic testing, and the proband's aunt and father were already deceased, samples from the proband's mother were tested initially. It was revealed that the mother of the proband did not carry any mutations in the *BRCA1* gene and therefore, the first hypothesis was highly suspected. Next, verification was performed with the proband's uncle, the proband's uncle's daughter (Fig. 2, III-2), and the proband's aunt's daughter (Fig. 2, III-3). It was revealed that the proband's uncle and the proband's uncle's daughter carry the same mutation as the proband. This result confirms the first hypothesis. The uncle of the proband was 54 years old, the proband's uncle's daughter was 28 years old, and the two of them were healthy. A risk management scheme was provided for them to facilitate early cancer detection, prevention and risk of developing the disease in following generations.

Diagnosis and treatment

In March 26, 2015, the proband received paclitaxel liposome (180 mg, intravenously, day 1). Due to the presence of proteinuria, chemotherapy was suspended and radiotherapy was administered. At the end-point of the study (July 2017), no tumor recurrence had been observed.

Following written informed consent being obtained, a sample of the proband's peripheral blood was collected and genomic DNA was extracted. Targeted NGS was performed with a panel of 21 genes (*BRCA1, BRCA2, CHEK2, PALB2, BRIP1, TP53, PTEN, STK11, CDH1, ATM, BARD1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, PMS1, PMS2, RAD50, RAD51C*) with a target area of 172,959 bp. The coverage and the depth of the target area was 99.49% and 586.13X respectively.

A novel heterozygous mutation (c.311_312delinsAGG TTTGCA) was identified in the proband. MutationTaster version 2 (http://www.mutationtaster.org/) predicted that this mutation causes the formation of a premature stop codon which leads to a frameshift followed by the formation of a truncated BRCA1 protein (3). This mutation was confirmed by Sanger sequencing (Fig. 3A and B); Fig. 3A presents the wild-type result, while Fig. 3B presents the proband's result, which revealed the mutation. This mutation was not present in the Human Gene Mutation database (www.hgmd.cf.ac.uk) or OMIM (omim.org). The mutation was not identified in the BGI database, which contains ~30,000 Chinese Han samples (www.genomics.cn/en/).

Discussion

The present study investigated a 33-year-old Chinese female with HBC. A heterozygous novel heterozygous deletion-insertion (c.311_312delinsAGGTTTGCA) in the *BRCA1* gene was identified in the proband. This mutation causes formation of a truncated BRCA1 protein with 109 amino acids instead of the wild-type of the BRCA1 protein with 1,863 amino acids.

Genetic screening for *BRCA1* and *BRCA2* genes and identification of novel variants serves a key role for timely diagnosis, proper counseling, successive follow-up and management of disease (4). By contrast, a large number of variants of unknown clinical significance (VUS) in the *BRCA1* and *BRCA2* genes has been identified in the patients and their family members by NGS. Functional characterization of germ-line mutations at transcriptional or translational levels is required to understand the dominant negative effect of the mutation in functional wild-type proteins (5).

BRCA1 gene mutations have a 60-80% probability of causing breast cancer in females; these mutations also increase the risk of developing ovarian cancer in females and prostate cancer in males. Germ-line mutations in the *BRCA2* gene are observed in ~35% of families with early-onset breast cancer in females; these mutations lead to an increased risk of developing ovarian cancer in females and breast cancer in

males (6). Breast cancer caused by a mutation in the *BRCA1* gene has a higher incidence, higher mitotic rate and more lymphatic penetrance than sporadic breast cancer (7). These types of cancer are more likely to lack expression of ER, PR and HER-2nue receptors, and to have a somatic mutation in the *P53* gene (7-10). In addition to having an increased risk of developing breast cancer, *BRCA1* or *BRCA2* gene mutation carriers have an increased risk for other types of cancer, including colon, prostate, pancreatic, melanoma and gastric cancer (11,12). Novel mutations in the *BRCA1* and *BRCA2* genes are very rare, as 2,000 mutations have been discovered in these two genes so far. The most common mutation forms are small insertions, small deletions, nonsense mutations, missense mutations, premature transcription terminations and splicing troubles. Deletion and insertion mutations lead to a frame shift. According to the Breast Information Core, the majority of the breast cancer-causing mutations in the *BRCA1* and *BRCA2* genes lead to the production of truncated protein through nonsense, frame shift and splicing mutations (13).

However, *BRCA1* and *BRCA2* genes associated with human breast and ovarian cancer occur with an autosomal dominant mode of inheritance and late onset of age. Therefore, genetic screening for patients and their family members are the key factors for the proper clinical management, accurate follow-up and understanding of the disease risk for all the family members. Additionally, prenatal genetic screening, as well as prenatal diagnosis for the family members with breast and ovarian cancer, would be a great step in the future in order to reduce the risk of disease occurrence in the successive generations in a family with several patients with BRCA1/2-associated breast/ovarian cancer.

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Availability of data and materials

The datasets used and/or analyzed in the present study are available from the corresponding author upon reasonable request.

Authors' contributions

SB, ZP, DJ designed and coordinated the study. DJ, QZ, XZ, YC, JL and HJ assessed the clinical findings of the cases. YW, HH, JW, KL, WC and JX performed the molecular genetic studies and analyzed the data. YW and WC wrote the draft of the manuscript with input from the other co-authors. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All study participants provided written informed consent and the study design was approved by an Institutional Ethics Review Board of the Department of Internal Medicine, The Fourth Hospital of Hebei Medical University (Shijiazhuang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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