A novel germline BRCA1 mutation identified in a family with hereditary breast and ovarian cancer syndrome

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ABSTRACT: Pathogenic germline mutations occurring in the BRCA1 (MIM:113705) and BRCA2 (MIM: 600185), which always result in truncated protein or nonsense-mediated mRNA decay, have been identified to increase the risk of hereditary breast, ovarian, pancreatic, prostate, and melanoma cancers. Recent studies show that BRCA1/2 germline mutations also contribute to half of all hereditary breast and ovarian cancer (HBOC).

In this case series, we reported a novel frameshift mutation of the BRCA1 gene. This novel frameshift mutation occurs in exon10 of BRCA1 and may result in a lack of the serine cluster domain and BRCA1 C-terminus domain, which mediates the function of BRCA1 in DNA repair and are responsible for activation function of BRCA1. The mutation was present in a Chinese hereditary male/female breast and ovarian cancer family characterized by a high incidence of breast cancer and/or ovarian cancer among the relatives and by a high incidence of triple negative breast cancer (TNBC).

Our findings speculate that BRCA1 E1148Rfs*7 mutation may be related to the occurrence of HBOC and even TNBC. Interestingly, three cases of TNBC with this novel BRCA1 mutation in this case series showed a good disease-free survival, one of them has a disease-free survival up to 7 years. Therefore, further study is required to confirm that whether this mutation is associated with good prognosis of HBOC.

KEYWORDS: BRCA1 mutation, susceptibility, family history, hereditary breast, ovarian and triple negative breast cancer, case series

Introduction

Breast cancer and ovarian cancer are the most common malignancies in women and account for 15% and 2.9% morbidity, and 6.9% and 2.2% mortality in China per year, respectively.1 About 5%–10% of breast cancer and 10% of ovarian cancer show a hereditary origin.2,3 Hereditary breast and ovarian cancer (HBOC) is a cancer syndrome that defined as a diagnosis of multiple cases of breast cancer and/or ovarian cancer on the same side of the family. The National Comprehensive Cancer Network guideline recommends that women with HBOC family history should perform genetic counseling and testing. To date, over 400 DNA alterations have been identified, including single nucleotide insertions, deletions, or substitutions (http://www.nfdht.nl). Pathogenic germline mutations occurring in the BRCA1 (MIM:113705) and BRCA2 (MIM: 600185), which always result in truncated protein or nonsense-mediated mRNA decay, have been identified to increase the risk of hereditary breast, ovarian, pancreatic, prostate, and melanoma cancers. BRCA1 and BRCA2 pathogenic mutations are mainly frameshift mutations, nonsense mutations, and splice site mutations, few are missense mutations, which mostly focus on BRCA1 C-terminus (BRCT) domain (>90%)4,5 and lead to loss of function of protein.

As the tumor suppressors, BRCA1 and BRCA2 play an essential role in repairing DNA double-strand break by homologous recombination.6 Germline BRCA mutation leads to dys-function of BRCA proteins, which could result in repairing error of DNA and further contribute to genetic aberrations.7 It has been revealed that BRCA1 and BRCA2 mutation is positively linked to increased risk of HBOC.8 In general, the risk of breast cancer is increase from 10%–15% to 45%–65% and the risk of tubo-ovarian cancer is increased from 1%–2% to 20%–50% in BRCA1 and BRCA2 mutation carriers.9 The number of cancer-bearing individuals within a family is generally considered a strong clinical predictor for HBOC, as described in the current guidelines.10 In this case series, we report a novel frameshift mutation of the BRCA1 gene. This novel frameshift mutation occurs in exon10 of BRCA1 in six members belonging to a Chinese hereditary male/female breast and ovarian cancer family.

Case Presentation

Samples collection

This study was approved by the Ethics Committee of Changhai Hospital and was conducted according to the principles of the
Declaration of Helsinki. The ethics approval ID is B2020-011A and the approval date was December 29, 2020. All participants have signed informed consent for sample collection and data publication in this case series. The breast tissue sample from the proband was stained with hematoxylin and eosin (H&E) and immunohistochemistry. Meanwhile, both her tumor tissue (at least 25 mg with 60% tumor purity) and peripheral blood (10 ml) samples were collected and performed for next-generation sequencing (NGS). Subsequently, peripheral blood samples from her relatives were conducted for Sanger sequencing to assay the BRCA1 mutation. The study was a retrospective cohort of our hospital between July 2013 and September 2018.

**Next-generation sequencing and Sanger sequencing**

Genomic DNA and peripheral blood DNA were extracted (DNA Rapid Extraction Kit, TIANGEN) from the tumor tissue and peripheral blood samples of the proband, respectively. This was followed by genomic DNA library construction (HTP/LTP Library Preparation Kits, KAPA), and hybridization capture-based targeted NGS (Illumina HiSeq X Ten) with a panel of 578 cancer-relevant-gene was performed. Genomic alterations including single nucleotide variant (SNV), copy number variant (CNV), Indels, and gene rearrangement were analyzed. For Sanger sequencing analysis, DNA extracted from the patients' peripheral blood sample, then polymerase chain reaction (PCR) amplification (Q5 High-Fidelity DNA Polymerase, NEB) was conducted (Forward primer sequence: TGGGAAGTAGTCATGCATCTCA; Reverse primer sequence: TCGGTAACCCTGAGCCAAAT) and PCR products were purified, followed by Sanger sequencing assay. BRCA1 variants were viewed with the Integrative Genomics Viewer software and Codon Code Aligner, respectively.

**Patient information**

The proband (III-1) is a 34-year-old woman diagnosed with triple-negative breast cancer (TNBC) (infiltrating ductal carcinoma, right breast, no lymph node metastasis, Ki-67 (marker of proliferation) [80%], topoisomerase (Topo) II [40%], E-cadherin (ECAD) [+], estrogen receptor (ER) [−], progesterone receptor (PR) [−], tumor protein (EC :2.7. 1.37) (P53) [−], human epidermal growth factor receptor 2 (HER2) [−]). She was admitted to the ward of our department in 2016. She underwent a right breast modified radical mastectomy followed by adjuvant EC-T chemotherapy regimen: epirubicin (90 mg/m2) and cyclophosphamide 500 mg/m2 every 3 weeks for four cycles; followed by docetaxel 90 mg/m2 every 3 weeks for four cycles. In accordance with the requirements of family members and patients, and after the hospital ethics record, contralateral prophylactic mastectomy was performed. We conducted genetic counseling for this patient and found there was a strong family history of cancer, with five known cases of breast cancer and four known cases of ovarian cancer. Her mother (II-2) was diagnosed with breast cancer at 52 years (infiltrating ductal carcinoma, left breast, no lymph node metastasis, Ki-67 (60%), Topo II [++] , ECAD [+], ER [−],
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PR [−], P53 [−], HER2 [++], FISH−) and also ovarian cancer. She also has three female cousins diagnosed, one of them (III-20) as breast cancer at 52 years (infiltrating ductal carcinoma, left breast, left axillary lymph node metastasis [4/20], Ki-67 [25%], Topo II [30%], ECAD [+], ER [−], PR [−], P53 [80%], HER2 [−]). One of the other (III-13) suffered from breast cancer and another (III-15) was diagnosed with ovarian cancer. The paternal grandmother (I-2) and two maternal aunts (II-9,11) were diagnosed with ovarian cancer. One of two maternal aunts (II-11) was also diagnosed with esophageal cancer. Moreover, one of her niece (IV-1) developed breast cancer when she was 47 (infiltrating ductal carcinoma, left breast, no lymph node metastasis, Ki-67 (30%), Topo II [5%], ECAD [+], ER [30%], PR [70%], P53 [-], HER2 [-]). Four of these patients were treated in our hospital (II-2, III-1, III-20, IV-1) with the standard EC-T regimen. The patient IV-1 continued adjuvant endocrine therapy with Tamoxifen 20 mg/d (for 5 years) after chemotherapy. The patient III-20 underwent conventional radiotherapy after adjuvant chemotherapy due to left axillary lymph node metastasis (4/20). The pedigree of the affected family was shown in Figure 2.

Frequent diagnoses of breast and ovarian cancer among the members of her family diagnosed with cancer as well as a high proportion of TNBC diagnoses (60%) among patients diagnosed with breast cancer, prompted us to perform a comprehensive genetic analysis to identify the potential cause of frequent diagnoses of breast and/or ovarian cancer in this family.

NGS

Results from the targeted panel of NGS revealed a novel pathogenic germline mutation of *BRCA1* in the proband’s peripheral blood sample, which was a kind of frameshift mutation—the *BRCA1* E1148Rfs*7* (NM_007294.3 c.3442delG) mutation. This mutation occurred in exon10 of *BRCA1* located in BRCT domain, resulting in a frameshift change, and may result in a lack of the serine cluster domain (SCD) and BRCT domain, which mediate the function of *BRCA1* in DNA repair, including the binding of BACH-1 and CtIP, and are responsible for activation function of *BRCA1*. Thus, this mutation may cause non-sense mediated mRNA decay, which leads to loss of function of *BRCA1* protein and potential increased sensitivity to targeted therapies such as poly (ADP-ribose) polymerase (PARP) inhibitors. The presence of the *BRCA1* E1148Rfs*7* mutation was also identified in the peripheral blood samples of proband’s five other family members (Figure 3). We also noticed that three of five women who harbor with this mutation suffered from BC and/or OC (II-2, III-1, III-20). Interestingly, all breast cancer patients with *BRCA1* mutation in this family (III-1, II-2, and III-20) were diagnosed with TNBC. Among them, patient IV-1 was ER+ (30%), PR+ (70%), and HER2 - breast cancer and NGS results did not show this *BRCA* mutation. Up to now, none of the patients had recurrence or metastasis, and the patient (II-2) with the longest disease-free survival (DFS) after surgery had been up to 7 years.

Taken together, the *BRCA1* E1148Rfs*7* mutation we present here, to the best of our knowledge, has not been reported in previous database or publications. *BRCA1* E1148Rfs*7* mutation is another novel mutation found in the HBOC family and this variant should be classified as likely pathogenic, according the classification in American College of Medical Genetics and Genomics (ACMG) guideline. Thus, we speculate this mutation is the main cause of cancer susceptibility in the patient and her family. In the future, in vitro functional study will be needed to verify the protein function of *BRCA1* E1148Rfs*7*.

Discussion

In this work, we described a novel frameshift mutation of the *BRCA1* gene in a large family characterized by a high incidence of BC and/or OC among the relatives and by a high incidence of TNBC. This novel frameshift mutation occurs in exon10 of *BRCA1*, and may result in a lack of the SCD and BRCT domain, which mediate the function of *BRCA1* in DNA repair, including the binding of BACH-1 and CtIP, and are responsible for activation function of *BRCA1*. Therefore, we speculate this mutation may be pathogenic.
BRCA1 and BRCA2 are essential tumor-suppressor proteins for cell division, DNA replication error control, DNA repair, and cell apoptosis. Pathogenic of BRCA 1/2 mutations attribute to the susceptibility of multiple cancers, including breast, ovarian, pancreatic, prostate, and melanoma cancer. Previous study indicated that BRCA1 mutation were strongly associated with earlier age at diagnosis and ER negative in breast cancer. 

The risk of breast cancer in women is 71% for those with BRCA1 mutation and 49% for those with BRCA2 mutation, respectively. The lifetime risk for men is 1%–2% and 6% in BRCA1 with BRCA1 gene mutation carriers, respectively. Thus, the incidence of breast cancer is much higher in women than men with BRCA1 mutation. In this case series, five female and one male person are BRCA1 mutation carriers. Among them, all the three breast cancer patients are female. In accordance with previous findings, cancer caused by BRCA1 mutation is more prone to be inherited in women than in men, which might indicate the inherited correlation between genetic mutations and gender.

In fact, approximately, TNBC accounts for 70% of breast cancers with BRCA1 germline mutations. About 20% of TNBC patients harbor a BRCA1 mutation. Simultaneously, BRCA1 mutant phenotype and TNBC share some features, which include the morphological features and immunohistochemical profile. Besides, TNBC is characterized as aggressive clinical behavior and the treatment for TNBC is limited due to lack of recognized molecular targets for therapy. These features resulted in a poorer prognosis of TNBC than other breast cancer subtypes. Nevertheless, clinical studies and meta-analyses of breast cancer have demonstrated that BRCA1/2 mutations are not always a negative prognostic indicator in TNBC and that TNBC patients with BRCA mutants may be more sensitive to chemotherapy.
It is known that BRCA1 mutation is associated with the increased risk of HBOC. The risk of contralateral breast cancer has been found to be significantly increased in BRCA1 mutations carriers with an estimated 10-year risk ranging from 20% to 40% by Bordeleau et al. However, the correlation between prognosis and BRCA1 mutation is in consistent in different studies. Several clinical studies and meta-analyses of breast cancer cases suggest that BRCA1 mutations is also correlated to a poor prognostic significance. On the contrary, two publications indicate that BRCA1 mutation carriers are more sensitive to neoadjuvant anthracycline-based regimens and have similar survival rates to non-carriers when treated with alkylating chemotherapy among TNBC patients. The reason for the prognostic variation with BRCA1 mutation is still unclear. In the present case series, the members of this HBOC family showed that the TNBC patient with BRCA1 E1148Rfs*7 mutation had probable correlation with long-term OS or RFS. Therefore, the prognosis of BRCA1 mutation carriers may be associated with the mutant point.

The discovery of PARP, the prognosis has greatly improved in breast and ovarian cancer patients with BRCA1/2 mutation. BRCA1/2 mutation should be identified before prescribing PARP inhibitors. Therefore, as recommended in various guidelines, genetic testing may be considered for cancer screening and prevention, especially in those with a family history of cancer. Since genetic counseling has become popular within only few years, it is difficult to offer the genetic testing to all the patient if this testing is not covered by medical insurance. That is why the incidence of the BRCA1 E1148Rfs*7 is unknown because limited patients can afford the genetic testing. Fortunately, the patients in this case series showed a relative good DFS.

There are some limitations of this study: (1) There is only one family cohort in the case series. The sample size is small; (2) the incidence of this mutation is not known in the overall BRCA carriers. A larger cohort study should be conducted to figure out the incidence in future; (3) so far, none of the patients had recurrence or metastasis. Whether this phenomenon indicated a good prognosis is unclear. The response to therapy, especially PARP inhibitors, should be investigated in future.

Taken together, the BRCA1 E1148Rfs*7 mutation we present here, to the best of our knowledge, has not been reported in previous database or publications. BRCA1 E1148Rfs*7 mutation is another novel mutation found in the HBOC family and this variant should be classified as likely pathogenic, according the classification in ACMG guideline. Thus, we speculate this mutation is the main cause of cancer susceptibility in the patient and her family. In the future, in vitro functional study will be needed to verify the protein function of BRCA1 E1148Rfs*7. The identification of BRCA germline mutation can provide clinicians with reference of treatment options and improve the clinical management of patients. Mutation screening of BRCA will become a routine test in women at age of 50 or older as a way to conduct disease treatment or prevention. Moreover, it is important to emphasize that people who has family history should consider taking a genetic counseling and risk assessment.

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Author’s contributions
Yanmei Wu, Xiaodong Pan, Juan Dou, Quan Zhang, and Yuantong Li contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. Yuan Sheng and Xishui Liu are corresponding authors who have contributed the most. Yuan Sheng is responsible for confirming the authenticity of the authors’ contributions.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Changhai Hospital and was conducted according to the principles of the Declaration of Helsinki. Clinical samples collection of all patients with the patients’ informed consent.

Patient consent for publication
All patients have signed patients’ informed consent.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

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