

Novel loss-of-function mutation in *BRCA2* gene identified in a Chinese female with a family history of ovarian cancer: A case report

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Abstract. Inherited loss-of-function mutations in the tumor suppressor *BRCA2* gene are associated with a high risk of ovarian cancer in the Chinese population. The current case report discusses a novel heterozygous insertion in *BRCA2* gene, c.3195_3196insA, in a 54-year-old Chinese female with hereditary ovarian cancer. This frameshift mutation generates a premature stop codon at amino acid 1,076, which leads to a truncated *BRCA2* protein instead of a wild-type *BRCA2* protein with 3,418 amino acids. According to the Breast Cancer Information Core database, this mutation has not been previously reported. However, germline mutations of *BRCA2* are a more prevalent cause of ovarian cancer in Chinese females compared with females in Western populations. The present study expands the mutational spectra of *BRCA2* that is associated with ovarian cancer.

Introduction

Among females, ovarian cancer is a frequently diagnosed cancer type and a leading cause of mortality (1). In

China, the incidence rate of ovarian cancer has increased exponentially over the last decade (2). Inherited germline mutations in *BRCA2* predisposes individuals to a high risk of ovarian cancer. Therefore, genetic screening of *BRCA2* mutations may be a significant component of clinical practice for individuals with a family history of ovarian cancer. In females, *BRCA2* mutations result in a lifetime risk of 11-18% of developing ovarian cancer (3,4). Hereditary ovarian cancer is an autosomal dominant syndrome with incomplete penetrance caused by germline mutations of *BRCA2*. Germline mutations in both *BRCA1* and *BRCA2* are major causes of hereditary ovarian cancer (5,6). *BRCA1* and *BRCA2* serve key roles in the double-strand break repair system (7). Based on the Breast Cancer Information Core (BIC) database (<http://research.nhgri.nih.gov/bic/>), approximately 3,700 *BRCA1/BRCA2* germline mutations have been associated with ovarian cancer. The present study reports a novel heterozygous germline insertion mutation in the *BRCA2* gene, c.3195_3196insA. This single nucleotide insertion causes a frameshift mutation, which results in the formation of a truncated *BRCA2* protein with 1,076 amino acids instead of the wild-type *BRCA2* protein with 3,418 amino acids.

Case report

The proband investigated in the current study is a 54-year-old Chinese female clinically diagnosed with ovarian cancer who belongs to a non-consanguineous Chinese family. The proband's elder sister passed away at the age of 46 years as a result of ovarian cancer. The age and cause of mortality for the proband's parents are unknown (Fig. 1). The proband had been diagnosed with stage III right ovarian cancer, along with liver metastasis. The top edge of the liver exhibited a nodule with mild leukoariosis. The magnetic resonance imaging (MRI) scan demonstrated no metastasis around the brain.

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The biopsy sections (thickness, 3-4 μm) were paraffin embedded, fixed with 4% neutral buffer formaldehyde solution, at room temperature (25°C) for 6-48 h. Pathological biopsy was performed at the Fourth Hospital of Hebei Medical University (Shijiazhuang, China). Hematoxylin and eosin staining of tumor tissue was performed by optical microscopy (magnification, x460) and microscopy images were obtained (Fig. 2A-C). Paraffin-embedded slices were stained with IHC markers (Ventana Benchmark Ultra automatic immunohistochemical staining platform, primary antibodies: ER: Anti-estrogen receptor (SP1) rabbit monoclonal primary antibody, Roche, catalog number: 790-4325; PR: Anti-progesterone receptor (1E2) rabbit monoclonal primary antibody, Roche, catalog number: 790-4296; P53: Anti-P53 mouse monoclonal primary antibody, Roche, catalog number: 800-2912; Ki-67: Anti-Ki-67 (30-9) rabbit monoclonal primary antibody, Roche, catalog number: 790-4286; CEA: Anti-CEA (COL-1) mouse monoclonal primary antibody, From: MXB Biotechnologies, catalog number: Kit-0008; CA125: Anti-CA125 (TA347) mouse monoclonal primary antibody, MXB Biotechnologies, catalog number: MAB-0007; WT1: Anti-WT1 (MX012) mouse monoclonal primary antibody, MXB Biotechnologies, catalog number: MAB-0678; PAX-8: Anti-PAX-8 rabbit polyclonal primary antibody, MXB Biotechnologies, catalog number: RAB-0657; PAX-2: MXB Biotechnologies, catalog number: RAB-0648. Secondary antibodies: MaxVision DAB, From: MXB Biotechnologies, catalog number: KIT-5220. All antibodies were purchased in their ready-to-use format so dilutions weren't required. IHC experimental procedure according to the manufacturer's protocols, the percentage of positive staining was calculated by eye. Immunohistochemistry (IHC) for estrogen receptors demonstrated a high (70%) expression level in cancer cells (Fig. 2D). IHC for progesterone receptors revealed a moderate (30%) expression level in cancer cells (Fig. 2E). IHC for p53 demonstrated zero expression level of p53 in cancer cells (Fig. 2F). IHC for Ki67 revealed a moderate (30%) expression level in cancer cells (Fig. 2G). Staining for cancer antigen 125 demonstrated a high expression level (Fig. 2H). IHC for Wilms tumor protein (WT1) revealed a moderate expression level in cancer cells (Fig. 2I). IHC for paired box gene PAX-2, PAX-8 and CEA was also performed, revealing zero, positive and positive expression, respectively (Fig. 2J-L). An MRI scan was performed, which demonstrated enhancement of the pelvic tumor (Fig. 3A), enhancement of hepatic metastasis (Fig. 3B,C), and thickening of the omentum majus and tumor tissue (Fig. 3D).

The patient's treatment regimen consisted of TC scheme (paclitaxel and carboplatin: Paclitaxel liposome, 240 mg/day 1+ carboplatin, 0.5 g/day 1, intravenous injection) chemotherapy for two cycles. Following treatment, the patient showed an improvement, and a gynecological surgeon contacted the patient's family.

Prior to the experiment, written informed consent was obtained from each patient. Peripheral blood samples of the patients were collected and genomic DNA was extracted. Targeted high-throughput sequencing (DNA polymerase is T4 DNA POLYMERASE, supplier is ENZYMATICS; Qiagen, Inc., Valencia, CA, USA) was performed with a panel of a 21 genes (*BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *BRIPI*, *TP53*, *PTEN*, *STK11*, *CDH1*, *ATM*, *BARD1*, *MLH1*, *MRE11A*, *MSH2*,

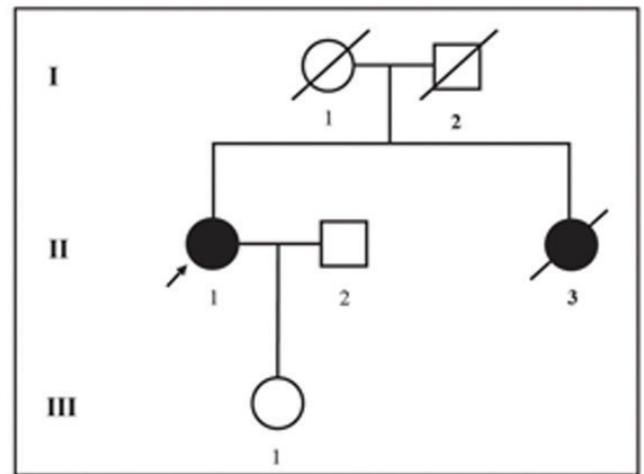


Figure 1. Pedigree structure of the family of the Chinese patient with hereditary breast cancer. Black shapes indicate individuals with hereditary ovarian cancer. Squares and circles denote males and females, respectively. Individuals labeled with a diagonal line are deceased. Roman numerals indicate generations. The arrow indicates the proband.

MSH6, *MUTYH*, *NBN*, *PMS1*, *PMS2*, *RAD50* and *RAD51C*). Roche NimbleGen's (Roche Applied Science, Madison, MI, USA) custom Sequence Capture Human array was used to designed to capture targeted sequence, according to the manufacturer's protocols. A target area of 172,959 bp, a target area coverage of 99.49% and a target area average depth of 586.13x. Samples were sequenced simultaneously on Illumina HiSeq 2500 Analyzers (Illumina Inc., San Diego, CA, USA) for 90 cycles according to the manufacturer's protocols. Targeted high-throughput sequencing identified a novel heterozygous mutation, c.3195_3196insA, in *BRCA2*, which causes a frame-shift mutation that generates a premature stop codon and results in the formation of a truncated *BRCA2* protein. This mutation was confirmed by Sanger sequencing (Fig. 1R). The mutation was absent in the Human Gene Mutation database (www.hgmd.cf.ac.uk/) and the Online Mendelian Inheritance in Man database (www.omim.org). In addition, the mutation could not be identified in our in-house databases, which contains ~30,000 samples from Chinese Han individuals.

Since the proband's elder sister passed away at an age of 46 years due to ovarian cancer, it can be hypothesized that she carried the same mutation in *BRCA2*. In addition, the proband's father or mother may carry the same mutation. However, as the proband's elder sister and parents had passed away, it was not possible to investigate this. The proband's children did not volunteer genetic testing. Therefore, only the following suggestions can be made. Firstly, it could be hypothesized that the proband and her sister carried the same mutation on the *BRCA2* gene, which was inherited from their father or mother. By contrast, it could be hypothesized that the mutation carried by the proband is a *de novo* mutation. In this case, her parents and sister would not have carried the same mutation. With the knowledge that the proband's sister passed away due to ovarian cancer, we speculate that the first hypothesis is more likely. The Ethical Committee of The Fourth Hospital of Hebei Medical University (Shijiazhuang, China) approved the current study protocol, which was in compliance with the Declaration of Helsinki.

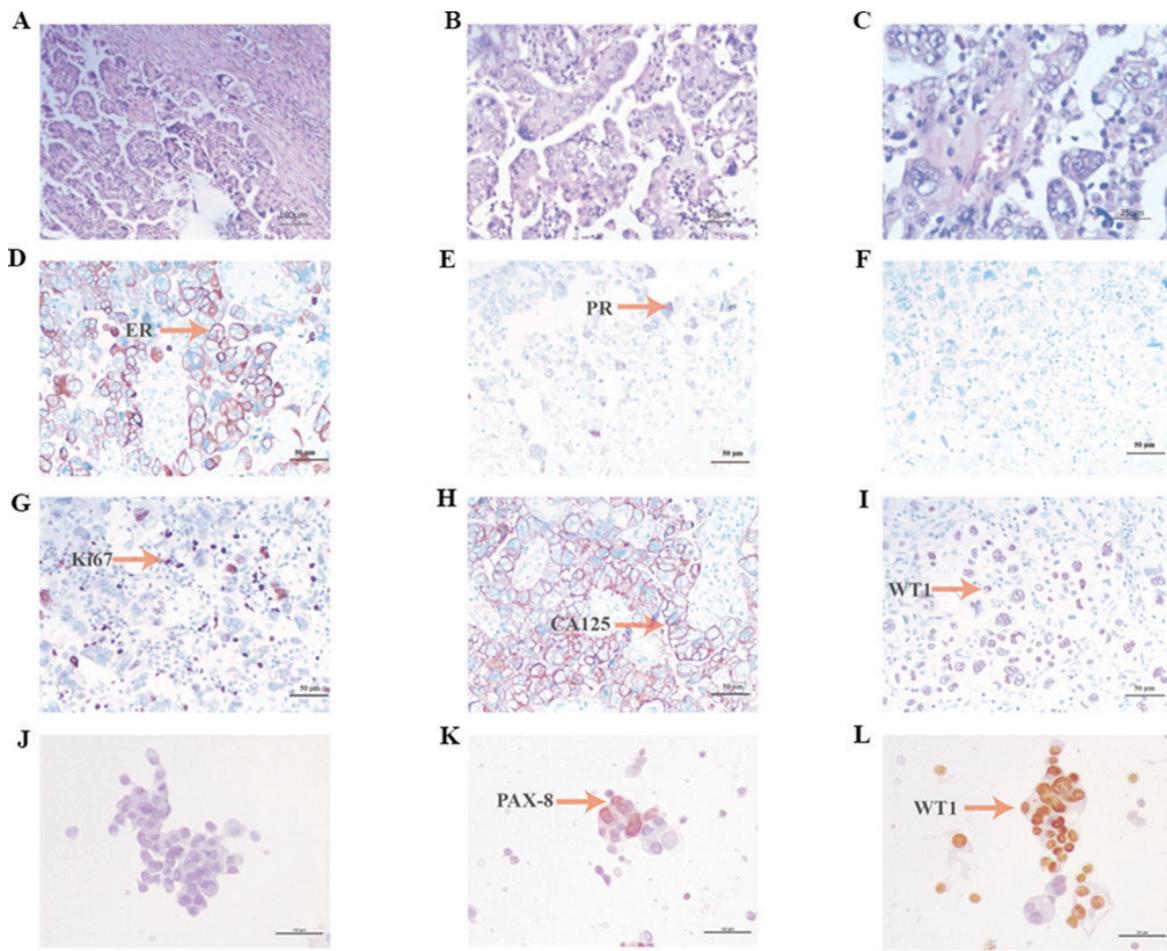


Figure 2. Hematoxylin and eosin staining of tumor tissue was performed, and images were obtained with a (A) scale bar=100 μm, (B) scale bar=50 μm and (C) scale bar=25 μm. (D) Immunohistochemistry for ER (red arrow) indicated a high (70%) expression level in cancer cells. Scale bar=50 μm. (E) Immunohistochemistry for PR (red arrow) demonstrated a moderate (30%) expression level in cancer cells. Scale bar=50 μm. (F) Immunohistochemistry for p53 indicated no expression in cancer cells. Scale bar=50 μm. (G) Immunohistochemistry for Ki67 (red arrow) revealed a moderate (30%) expression level in cancer cells. Scale bar=50 μm. (H) Immunohistochemistry for CA125 (red arrow) demonstrated a high expression level in cancer cells. Scale bar=50 μm. (I) Immunohistochemistry for WT1 (red arrow) indicated a moderate expression level in cancer cells. Scale bar=50 μm. Positive expression was represented by a brownish color in the cytoplasm and membrane. (J) Immunocytochemistry for PAX-2. Scale bar=100 μm. (K) Immunocytochemistry for PAX-8 (red arrow). Scale bar=100 μm. (L) Immunocytochemistry for CEA (red arrow). Magnification, x460. ER, estrogen receptor; PR, progesterone receptor; CA125, cancer antigen 125; WT1, Wilms tumor protein; MRI, magnetic resonance imaging; PAX, paired box gene.

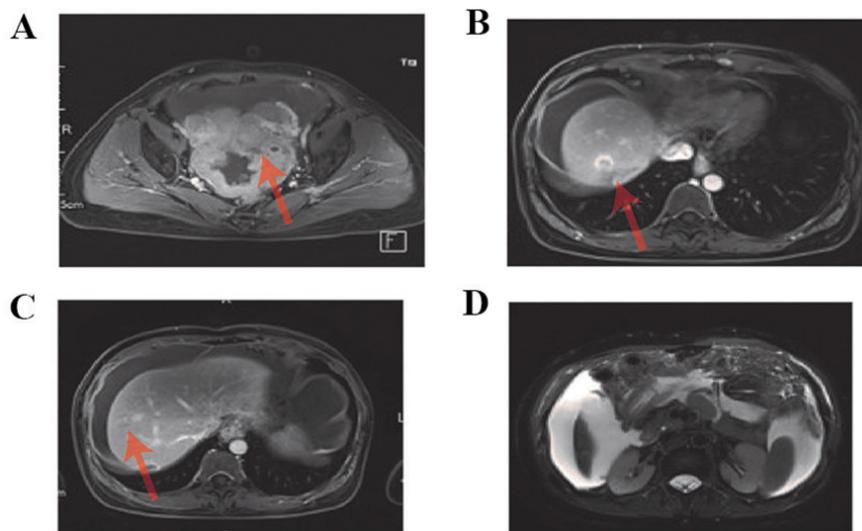


Figure 3. MRI of patient. (A) An MRI scan demonstrated enhancement of the pelvic tumor (red arrow). (B) An MRI scan indicated enhancement of hepatic metastasis (red arrow). (C) An MRI scan revealed enhancement of hepatic metastasis (red arrow). (D) An MRI scan identified thickening of the omentum majus and tumor tissue.

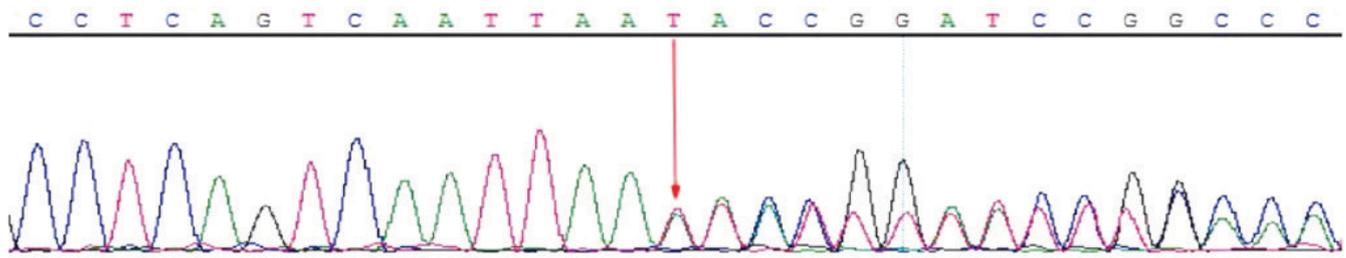


Figure 4. Sanger sequencing of the *BRCA2* gene identified a germline novel heterozygous insertion mutation. The arrow indicates the novel heterozygous insertion mutation (c.3195_3196insA).

Discussion

A c.3195_3196insA frameshift mutation in exon 10 of the *BRCA2* gene, which has not previously been reported in the BIC database, was detected in a 54 year-old female Chinese patient diagnosed with ovarian cancer. Not all genetic variants are harmful; *BRCA1/2* variations can be classified into the five following categories: ‘Pathogenic’, ‘likely pathogenic’, ‘uncertain significance’, ‘likely benign’ and ‘benign’, according to American College of Medical Genetics’ guidelines (8). ‘Pathogenic’ or ‘likely pathogenic’ indicates that a mutation was identified in a specific gene, which increases the lifetime risk of developing certain cancer types, but does not indicate that cancer will definitely develop. Germline *BRCA1/2* deleterious mutations lead to an increased risk of early onset hereditary breast and ovarian cancer (HBOC)-associated tumors, including breast, ovarian and pancreatic cancer in females, and to a lesser extent, breast and prostate cancer in males (9,10).

Genetic sequencing techniques have developed radically during the past few decades; therefore, novel testing techniques have been widely used in the medical sector (11-15). For example, the enrichment step, a preparation technique used prior to next-generation sequencing (NGS), can significantly improve the accuracy and efficiency of medical screening (16). This technique was used in the current study to identify the largest possible number of mutations that may be associated with HBOC. Due to technology limitations, there are still many challenges in detecting copy-number variants by NGS (17), certain patients with a negative NGS testing result may also require multiplex ligation-dependent probe application, particularly patients with a family history of cancer.

BRCA1 and *BRCA2* were not the only genes analyzed in the current study; multiple gene analysis was performed. Numerous genes should be involved in sequencing tests as the mutational spectra of these genes can be accurately detected with NGS technology, which provides additional data that can support clinical diagnoses. However, involving multiple genes in sequencing tests may lead to the detection of a higher number of variants of uncertain significance (VUS) (18). Clinical recommendations may not be standardized for the presence of VUS mutations in certain cancer susceptibility genes, which may complicate results. These complex results are why suggestions from medical geneticists or certified genetic counselors are important. Since a number of NGS-based techniques are used in genetic testing and different genetic

companies perform different techniques, customers should have access to the sensitivity and specificity of any results, as well as comparisons of various techniques (19). In our opinion, positive results should be validated using standard Sanger sequencing, which should also be available to relatives who are concerned about the risk of 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, DJ, ZP designed and coordinated the study. DJ, YC, NZ, JH, FL, SW, QD, QZ and HJ assessed the clinical findings of the cases. YW, HH, JW, KL, WC, WL and JX performed the molecular genetic studies and analyzed the data. YW 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

A written consent was obtained from the patient for the publications of the present study.

Competing interests

The authors declare that they have no competing interests.

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