Rare *BRCA1* c.3418_3419insTGACTACT:p.S1140Mfs*18 germline mutation in a family with breast and ovarian cancer

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Abstract. *BRCA1* is among one of the most frequently mutated tumor suppressor genes in breast and ovarian cancer. In the present retrospective study, a novel heterozygous germline mutation, *BRCA1* c.3418_3419insTGACTACT:p.S1140Mfs*18 (C>CAGTAGTCA), was identified in a family with breast and ovarian cancer via next-generation and Sanger sequencing analysis and the health status of 18 individuals (three with breast cancer, one with ovarian cancer, one with gastric cancer, one with lung cancer, one with colorectal cancer, three with non-cancer disease and eight healthy individuals) recruited from the 3201 Hospital of Xi'an Jiaotong University (Hanzhong, China) between September 2023 and January 2025 was examined. The aforementioned mutation occurred in four female patients with breast and ovarian and one healthy individual between September 2023 and January 2025, accounting for 27.78% (5/18) of the enrolled family members and 57.14% (4/7) of the patients with cancer. As of March 2025, the treatment of these patients with *BRCA1* c.3418_3419insTGACTACT mutations have demonstrated notable therapeutic effects. In particular, in two patients who underwent neoadjuvant chemotherapy, surgical resection and targeted therapy, no recurrence or metastasis as of March 2025. Age risk was analyzed in the present cohort; the area under the curve was 0.9107 and the P-value was 0.0078 between patients with cancer and healthy individuals. Age may also be a key factor that affects tumorigenesis in breast and ovarian cancer. In conclusion, *BRCA1* c.3418_3419insTGACTACT:p.S1140Mfs*18 (C>CAGTAGTCA) heterozygous germline mutation may affect the occurrence and development of cancer.

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Introduction

Breast cancer (BC) is the leading cause of morbidity and mortality among females globally; in 2023 there were an estimated 2.3 million BC incident cases (1), and multitarget detection improves the accuracy of diagnosis (2). *BRCA1* is among the most frequently mutated tumor suppressor genes in ovarian cancer and BC; 20-30% of ovarian or patients with BC carry BRCA1 mutations. Loss of *BRCA1* triggers homologous recombination repair deficiency, leading to genomic instability and PARP inhibitor-associated synthetic lethality (3). Germline pathogenic variants in the *BRCA1* and *BRCA2* genes confer elevated risk of breast and ovarian cancer (4). Compared with non-carriers, patients with cancer who are carriers of the *BRCA1/2* pathogenic variant have a higher histological grade, triple-negative disease rate, Ki-67 proliferation index and rate of no special type of carcinoma (5). In patients with a *BRCA1* or *BRCA2* pathogenic variant, risk-reducing mastectomy (RRM) decreases the risk of BC (6,7). Mosaic *BRCA1* promoter methylation (*BRCA1*meth) increases the risk of early-onset and triple-negative BC and ovarian cancer (8).

There are several types of *BRCA1* and *BRCA2* mutation. Among these, the pathogenicity of the *BRCA1* c.5017_5019del (p. His1673del) has been confirmed (9). In accordance with American College of Medical Genetics and Genomics guidelines, *BRCA1* c.4358-2A>G and *BRCA2* c.475+5G>C are classified as pathogenic variants (10). Founder alleles such as *BRCA1* c.3629_3630delAG in Chechens, *BRCA2* c.6341delC in North Ossetians, *BRCA2* c.5351 dupA in Ingush and *BRCA1* c.2907_2910delTAAA in Karachays have been identified in North Caucasus regions (11). Compared with that in patients with cancer, the mutational spectrum of *BRCA* in healthy Chinese Han individuals is distinct, with a prevalence of pathogenic/likely pathogenic variants comprising 0.53% (1/189) of mutations. The prevalence of *BRCA1* c.5470_5477del is high (0.44%) in North Han Chinese individuals (12).

In Italy, a novel *BRCA2* pathogenic variant, c.7094_7100del (p.His2365LeufsTer9), was identified in a family with a history of hereditary BC (13). In Brazil, 15 germline mutations (13 in *BRCA1* and two in *BRCA2*) have been identified, of which c.5266dupC and c.2215 A > T are the most frequent variants. Furthermore, c.7645dupT and c.921dupT mutations have been reported (14). Another novel germline mutation (Phe1695Val)

in *BRCA1* has been identified using whole-exome sequencing in a Chinese family with multiple types of cancer (15).

The present retrospective study investigated the rare BRCA1 gene mutation in BC and ovarian cancer, and its potential impact on treatment.

Patients and methods

Samples. In the present retrospective study, 18 individuals [seven with cancer, three with non-cancer disease (emphysema or coronary heart disease) and eight healthy individuals (Table I)] were included, with seven males and 11 females. Among the patients with cancer, the cancer types were lung, gastric, ovarian, breast and colorectal.

All subjects were enrolled from The 3201 Hospital of Xi'an Jiaotong University (Hanzhong, China) between September 2023 and January 2025. The present study was approved by the 3201 Hospital Medical Ethics Committee (approval no. LLSC-KYLW-2025-009; Hanzhong, China) and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) (16). All patients provided written informed consent. The treatment strategies for cancer patients were based on guidelines of the American Society of Clinical Oncology (CSCO) and National Comprehensive Cancer Network (NCCN) (17,18). Magnetic resonance imaging (MRI) was conducted with GE Signa HDxt 3.0T MRI Machine. Subject 13 received neoadjuvant chemotherapy and targeted therapy starting in January 2024. The regimen comprised four cycles of epirubicin 150 mg plus cyclophosphamide 900 mg, followed by four cycles of docetaxel 150 mg combined with trastuzumab (initial 440 mg, subsequent 330 mg per cycle), totaling eight cycles. Post-treatment tumor size regressed to 0.6x0.5 cm. Modified left radical mastectomy was performed in March 2024. Postoperatively, trastuzumab 330 mg (6 mg/kg) was administered intravenously every 3 weeks for another 14 cycles, and oral tamoxifen 20 mg once daily was prescribed for 5-year endocrine therapy. Quarterly follow-up showed no recurrence or metastasis as of March 2025. Subject 4 initiated four cycles of neoadjuvant chemo-targeted therapy in June 2024 with albumin paclitaxel (PTX) 300 mg, carboplatin 400 mg, and bevacizumab 500 mg intravenously. The residual tumor size was 4.2x4.0x3.9 cm after treatment. Radical resection of ovarian cancer and sigmoid lesion was performed in October 2024. Two additional cycles of adjuvant chemo-targeted therapy with the original (albumin PTX, 300 mg; carboplatin, 400 mg) regimen plus bevacizumab (500 mg) were completed. Oral olaparib 300 mg twice daily was given for 2 years. No recurrence or metastasis was detected as of March 2025. Subject 6 underwent modified radical mastectomy in August 2016. Adjuvant chemotherapy consisted of four cycles of epirubicin 90 mg/m^2 plus cyclophosphamide 600 mg/m^2, followed by four cycles of docetaxel 90 mg/m^2. Conventional segmented radiotherapy at a total dose of 50 Gy in 25 fractions was delivered to the chest wall and axillary region. The patient received 5-year endocrine therapy with oral tamoxifen 20 mg once daily. No disease recurrence or metastasis was observed as of January 2025.

Inclusion criteria for patients with BC or ovarian cancer were as follows: i) BC or ovarian cancer confirmed following WHO Tumor Classification (19), 5th Edition; ii) no other cancer; and iii) no treatment and surgery.

The exclusion criteria for the present study were as follows: i) Patients with BC or ovarian cancer combined with other malignant cancer; ii) no samples; and iii) voluntary withdrawal.

BRCA1 c.3418_3419insTGACTACT mutation analysis. Peripheral blood samples from subjects 13 and 4 underwent next-generation sequencing (NGS) for exon detection. Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen GmbH; cat. No. 51104). The quantity and purity of the extracted genomic DNA were evaluated using a Qubit® 3.0 fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.) and a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc.). DNA was fragmented using an E220 focused ultrasonicator at 500 kHz for 95 sec at 7˚C and the target fragment length was 200 bp. Library preparation was performed using the Agilent SureSelectXT Low Input Target Enrichment System, with hybridization probes provided by Diaying Biotechnology. Following quality control and quantification using an Agilent 2100 Bioanalyzer (Agilent Technologies), quantitative analysis was conducted using a Qubit® 3.0 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.). Libraries were subjected to paired-end sequencing (2x75 bp) on an Illumina NextSeq CN500 platform (Illumina Inc.) at a final loading concentration of 1.8 pM (calculated from Agilent 2100 data). The sequencing kit used was the NextSeq CN500 Mid Output v2 kit 150 cycles (cat. No. R0151, Illumina). FastQC (version 0.11.8; github.com/s-andrews/FastQC/releases/tag/v0.11.8) was used to assess the quality of raw sequencing data. Subsequently, fastp (version 0.23.2; github.com/OpenGene/fastp/releases/tag/v0.23.2) with default parameters was used to trim adapter sequences, filter low-quality reads, remove short sequences, and eliminate reads with excessive N bases, resulting in high-quality clean data. The clean reads were aligned to the human reference genome hg19 using BWA (version 0.7.17; github.com/lh3/bwa/releases/tag/v0.7.17). The generated BAM files were processed by GATK4 (version 4.2.6.1; github.com/broadinstitute/gatk/releases/tag/4.2.6.1) for sequence sorting, PCR repeat marking and recalibration of base quality values. Single nucleotide variations (SNVs) and insertion-deletion variations (InDels) were detected using VarDict (version 1.8.0; github.com/AstraZeneca-NGS/VarDictJava/releases/tag/v1.8.0). Only variations in exon regions and splice site regions were retained and synonymous mutations were excluded; variations with a sequencing depth of less than 10 and an allele frequency of less than 15% were filtered; common polymorphic sites with the minor allele frequency (MAF) ≥0.05 based on the 1000 Genomes Project dataset (accession no. 1000g2015aug_all), National Heart, Lung, and Blood Institute Exome Sequencing Project and Exome Aggregation Consortium databases were excluded; variations predicted as benign by PolyPhen-2_HDIV (version 2.2.3; genetics.bwh.harvard.edu/pph2/) and marked as benign or possibly benign in the ClinVar database (ncbi.nlm.nih.gov/clinvar/) and meeting the criteria of the American College of Medical Genetics and Genomics were excluded (20).

Table I. Characteristics of the present study cohort.

Subject	Age, years	Sex	Disease
1	73	Male	Lung cancer
2	72	Female	Gastric cancer
3	67	Male	Emphysema
4	61	Female	Ovarian cancer
5	71	Male	Coronary heart disease
6	68	Female	Breast cancer
7	58	Male	Healthy
8	50	Female	Breast cancer
9	38	Male	Heart disease
10	51	Female	Healthy
11	35	Female	Healthy
12	27	Female	Healthy
13	42	Female	Breast cancer
14	57	Male	Colorectal cancer
15	27	Female	Healthy
16	13	Female	Healthy
17	30	Male	Healthy
18	15	Female	Healthy

Peripheral blood samples from subjects 6, 8, 11 and 12 underwent Sanger sequencing detection. The *BRCA1* c.3418_3419insTGACTACT mutation sequence was verified by Sanger sequencing.

HER2 immunohistochemistry (IHC) and evaluation. Tissue specimens were fixed in 10% neutral buffered formalin at room temperature for 8 h. Routine dehydration in graded ethanol, clearing in xylene and paraffin embedding were performed. Tumor paraffin blocks were automatically sectioned and stained using the BenchMark GX (Roche Diagnostics). Representative tumor paraffin blocks were cut into 4 μm sections, which were baked at 60˚C for 1 h. Dewaxing was performed with EZ Prep solution (Roche Diagnostics), followed by gradient rehydration in a descending alcohol series and final washing with Reaction Buffer (Roche Diagnostics). Antigen retrieval was performed with CC1 buffer containing EDTA (pH 8.0; cat. No. 06414575001, Roche Diagnostics, USA) at 100˚C for 30 min. Endogenous peroxidase activity was blocked by incubating sections in 3% hydrogen peroxide at 37˚C for 5 min and slides were then washed with Reaction Buffer. Slides were incubated with VENTANA anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody (cat. no. 790-4493, Roche Diagnostics, USA) at 37˚C for 32 min. HRP-conjugated goat anti-rabbit/mouse polymer (ready-to-use, cat. No. 760-500, Roche Diagnostics) was applied at 37˚C for 8 min. Visualization was performed using DAB at 37˚C for 8 min. The reaction was terminated by washing with Reaction Buffer. Counterstaining was performed using Mayer's hematoxylin at room temperature for 12 min, followed by bluing with Bluing Reagent at room temperature for 4 min. Sections were dehydrated in 70, 95, and 100% ethanol sequentially for 2 min each, cleared in xylene twice for 3 min each, and mounted with neutral balsam. Evaluation was conducted under a bright-field light microscope. HER2 IHC results were evaluated following the American Society of Clinical Oncology/College of American Pathologists 2018 guidelines (21,22). The HER2 IHC scoring system was as follows: 0+, no staining or incomplete and faint/barely perceptible membrane staining in ≤10% of tumor cells; 1+, incomplete and faint/barely permeable membrane staining in >10% of tumor cells; 2+, weak/moderate complete membrane staining in >10% of tumor cells or complete and intense membrane staining in ≤10% of tumor cells; and 3+, complete and intense membrane staining in >10% of tumor cells (Fig. S1).

Fluorescence in situ hybridization (FISH). FISH was performed on formalin-fixed paraffin-embedded tumor tissue sections using the HER2 Gene Detection kit (cat. number YZY-ISH-P012A, YZY Medical) in accordance with the manufacturer's instructions. The probes were ~100 base pairs in length and consisted of an orange-labeled HER2 probe and a green-labeled CEP17 probe. Initially, 4 μm tissue sections were baked at 80˚C for 30 min. Dewaxing was performed in dewaxing agent at 68˚C for 15 min. Washing was performed at 25˚C in 100% ethanol for 5, 85% ethanol for 5 min, 75% ethanol for 5 min, and deionized water for 1 min. Permeabilization was conducted in deionized water at 95˚C for 30 min. The slides were then left at room temperature for 1 min. Digestion was performed by incubation in enzyme working solution at 37˚C for 20 min. Dehydration was performed in 75, 85, and 100% ethanol solutions for 3 min each. The slides were air-dried at room temperature. For hybridization, 5 μl HER2 FISH probe was applied to each tissue section; the sections were denatured at 85˚C for 5 min and hybridized at 42˚C for 2 h. Washing was performed in washing solution A at 37˚C for 1 min, washing solution B at 68˚C for 2 min, and deionized water at 37˚C for 1 min, followed by air-drying at room temperature. Counterstaining was performed with 5 μl DAPI at room temperature for 10 min. Positive amplification was defined as a HER2/CEP17 ratio ≥2.0 and an average HER2 copy number/cell ≥4.0, whereas negative amplification was defined as a HER2/CEP17 ratio <2.0 and an average HER2 copy number/cell <4.0.

Statistical analysis. All data are presented as the mean ± standard deviation. Area under the receiver operating characteristic curve (AUC) was analyzed using GraphPad Prism (version 10.0; Dotmatics). $P<0.05$ was considered to indicate a statistically significant difference.

Results

*Rare BRCA1 c.3418_3419insTGACTACT:p.S1140Mfs*18 germline mutation analysis*. There were 18 subjects in the present study cohort, including seven patients with cancer, three with non-cancer diseases (emphysema or heart disease) and eight healthy individuals (Table I; Fig. 1A). The cancer types included BC and lung, gastric, ovarian and colorectal cancer.

To formulate an individualized treatment strategy, the proband (subject 13) underwent NGS for polygenic detection using peripheral blood sample, which

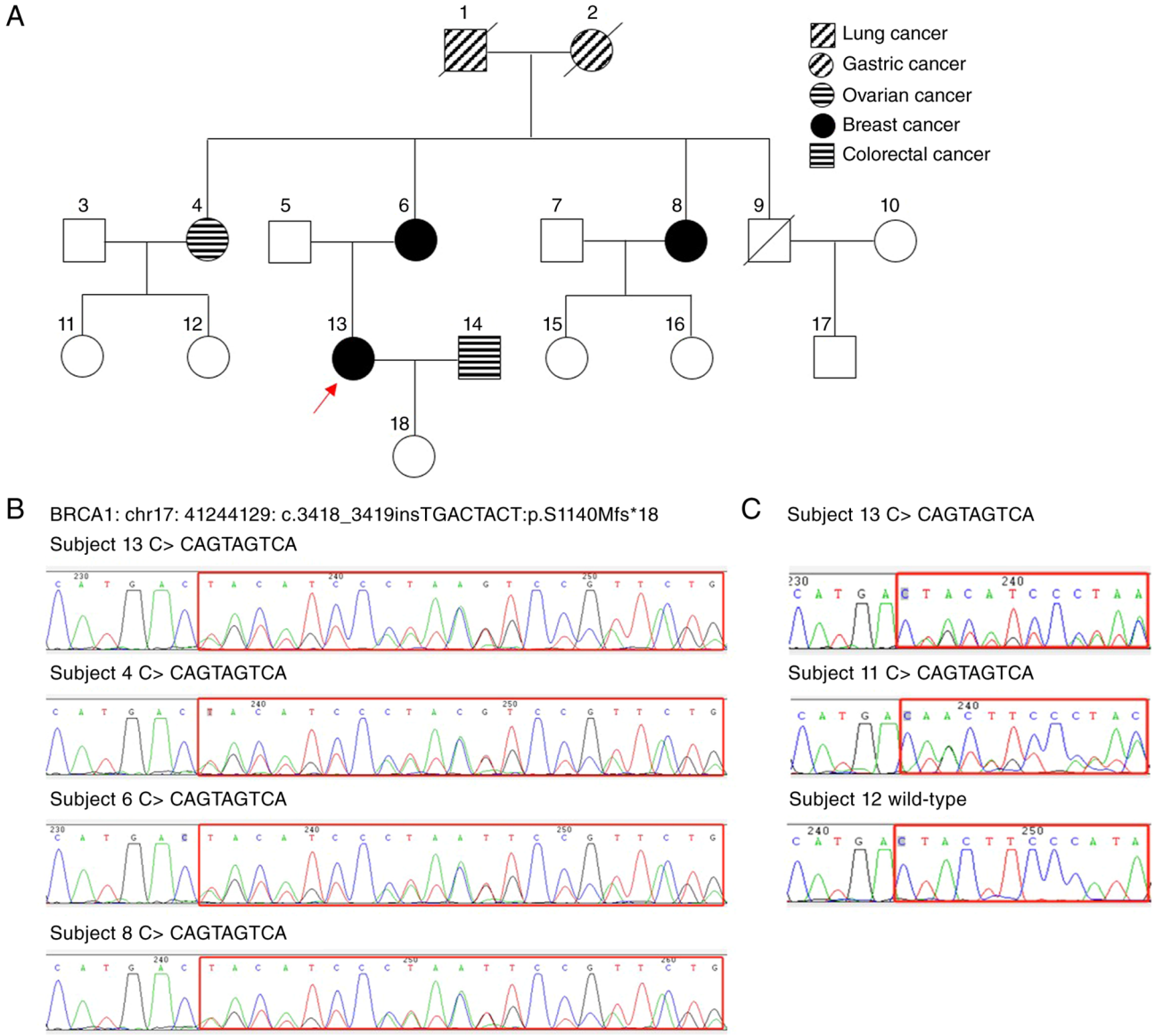

Figure 1. Pedigree of the multicancer family. (A) Pedigree of the 18 family members. Arrow, proband. (B) Analysis of *BRCA1* insertion mutations in subjects 4, 6, 8 and 13. (C) Analysis of *BRCA1* insertion mutations in subjects 11, 12 and 13.

revealed a heterozygous germline mutation in *BRCA1*: chr17:41244129: c.3418_3419insTGACTACT:p.S1140Mfs*18 (C>CAGTAGTCA). This mutant genotype was confirmed by sequence analysis (Fig. 1B). The other three patients with cancer had the same *BRCA1* c.3418_3419insTGACTACT germline mutant genotype (Fig. 1B).

To explore the *BRCA1* c.3418_3419insTGACTACT mutation, this gene was retrospectively studied in two adult daughters of subject 4. Subject11 was a 35-year-old female and subject 12 was a 27-year-old female. Subject 11 had the same *BRCA1* c.3418_3419insTGACTACT mutation as subject 4, whereas subject 12 had wild-type *BRCA1* (Fig. 1C).

Patient information and treatment follow-up. Subject 13 (Fig. 1), a 42-year-old female, was diagnosed with BC in September 2023 in 3201 Hospital of Xi'an Jiaotong University. Pretreatment pathological examination revealed invasive ductal carcinoma of the left breast, grade II (2 points for ductal formation, 3 points for nuclear atypia, 2 points for nuclear division; total score of 7 points) and high-grade ductal carcinoma *in situ*, according to CSCO and NCCN. The tumor size was 4.30x3.70x1.80 cm (Fig. 2A). Tumor stage was classified as cT2N0M0, II A, according to the AJCC (19). IHC revealed the following: E-cadherin (EC+), p120 (+), estrogen receptor (ER+; 70%), progesterone receptor (PR+; 60%), androgen receptor (AR+; 90%), HER2 (2+), CK5/6 (+), Ki-67 (+; 30%), CD8 (+), doublecortin-like kinase 1 (DCLK1+), forkhead box C1 and p53 (-) (Fig. S2). IHC revealed HER2 expression as 2+ and fluorescence *in situ* hybridization confirmed HER2 positivity (Fig. S3). Neoadjuvant chemotherapy and targeted therapy were performed in January 2024 [epirubicin (150 mg) and cyclophosphamide (900 mg) four cycles, docetaxel, 150 mg; trastuzumab, first dose 440 mg and subsequently 330 mg four cycles, for a total of eight cycles. Following treatment, the tumor size was 0.6x0.5 cm (Fig. 2B). Modified radical mastectomy of the left breast was performed in March 2024. The postoperative pathological report was as follows: Complete resection specimen of the left breast,

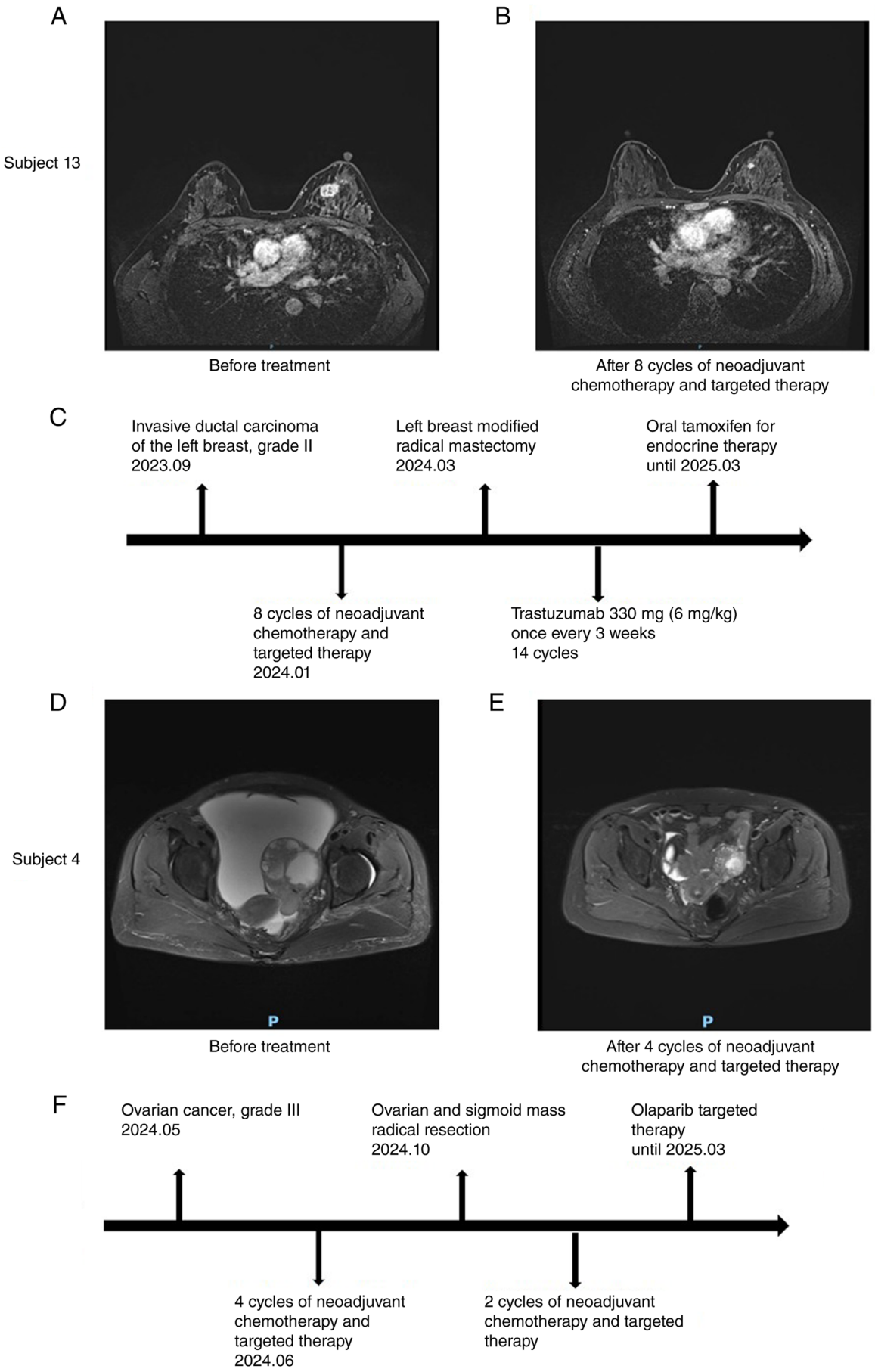

Figure 2. Patient treatment and follow-up. MRI imaging (A) before and (B) following eight cycles of neoadjuvant chemotherapy and targeted therapy for subject 13. (C) Treatment plan for subject 13. Molybdenum imaging (D) before and (E) following four cycles of neoadjuvant chemotherapy and targeted therapy for subject 4. (F) Treatment plan for subject 4.

containing a solitary tumor lesion measuring 0.60x0.50 cm in the upper quadrant of the left breast, 20 mm from the nipple. Histologically, the tumor was diagnosed as invasive ductal carcinoma. It was assigned 1 point for glandular differentiation, 3 points for nuclear grade and 2 points for mitotic count, resulting in a total score of 6 points consistent with histological grade II. A total of 22 lymph nodes was examined, none of which revealed cancer metastasis. Pathology of the BC lesion following neoadjuvant therapy revealed the following: Miller-Payne grade, 3 (23); Residual Cancer Burden grade, II (24); and Staloff, T-B (25). IHC revealed the following: ER (+; 60%), PR (+; 10%), AR (+; 60%), HER2 (1+), Ki-67+ (1%) and p53 (-, mutant type; Fig. S4). Following the surgery, trastuzumab (330 mg; 6 mg/kg) intravenous infusion was continued for targeted therapy, which was administered every 3 weeks for a total of 14 cycles. Oral tamoxifen (20 mg once daily) was also administered as endocrine therapy for 5 years. Re-examination were conducted every 3 months and no signs of metastasis or recurrence were observed as of March 2025 (Fig. 2C).

The second aunt of the proband (Fig. 1; subject 4), a 61-year-old female, was diagnosed with ovarian cancer. The subject was admitted in 3201 Hospital of Xi'an Jiaotong University for examination in May 2024. IHC revealed the following: CK7 (+), CK20 (-), villin (-), paired box-8 (+), Wilms' tumor-1 (+), thyroid transcription factor-1 (-), napsin A(-), GATA-binding protein 3 (-), human bone marrow endothelial cell-1 (+), CK5/6 (+), podoplanin (D2-40) (-) and Ki-67 (+; 30%; Fig. S5) and the tumor was stage III according to the WHO Tumor Classification (19), 5th Edition. The tumor size was 7.20x5.80x6.20 cm (Fig. 2D). Neoadjuvant chemotherapy and targeted therapy were performed in June 2024, for a total of four cycles using albumin paclitaxel (PTX), 300 mg; carboplatin, 400 mg combined with bevacizumab (500 mg) intravenous infusion. Following treatment, the size of the tumor was: 4.2x4.0x3.9 cm (Fig. 2E). In October 2024, the subject underwent radical resection of the ovarian cancer and sigmoid mass (total hysterectomy, bilateral adnexectomy, omentectomy, ovariectomy, pelvic lymph node dissection and sigmoid mass resection). Postoperative pathological examination revealed a diagnosis of high-grade serous papillary carcinoma of the left and right ovaries following the WHO 2020 Classification (19), with multiple cancerous emboli identified in the ovarian tunica, chronic inflammation of the cervical mucosa and atrophic endometrium present. A total of 10 lymph nodes was negative for metastasis, however, nodules on the sigmoid surface revealed metastatic cancer, with carcinoma nodules visible in the omentum. Following surgery in October 2024, the original treatment plan (albumin PTX, 300 mg; carboplatin, 400 mg) combined with bevacizumab (500 mg) was continued for two cycles of adjuvant chemotherapy and targeted therapy. Due to postoperative genetic test indicating BRCA1 mutation, olaparib was administered orally at a dose of 300 mg twice daily for 2 years. Re-examination every 3 months revealed no tumor recurrence or metastasis as of March 2025 (Fig. 2F).

The mother of the proband (Fig. 1; subject 6), a 68-year-old female, was diagnosed with BC in 1994, underwent modified radical mastectomy in 1994 and received postsurgical radiotherapy. As of January 2025, the subject was free of recurrence and metastasis.

The third aunt of the proband (Fig. 1; subject 8), a 50-year-old female, was diagnosed with BC. The disease was diagnosed as grade II invasive ductal carcinoma of the right breast following WHO Tumor Classification (19), 5th Edition and the tumor size was 4x5 cm. There were 20 axillary lymph nodes, four of which were positive for metastasis. IHC revealed EC (+), p120 (+), ER (+; 90%), PR (+; 60%), AR (+; 90%), HER2 (1+), DCLK1 (+), CK5/6 (+), Ki-67 (+; 40%) and p53 (-; Fig. S6). The subject underwent modified radical mastectomy in August 2016. Epirubicin (90 mg/m^2) combined with cyclophosphamide (600 mg/m^2) was administered for four cycles, followed by docetaxel (90 mg/m^2) treatment for four cycles intravenous infusion, and conventional segmental radiotherapy of the chest wall and axilla was given with 50 Gy, 25 times. The subject underwent tamoxifen (20 mg once daily, by mouth) endocrine therapy for 5 years. As of January 2025, neither recurrence nor metastasis was observed.

The grandfather of the proband (subject 1) was diagnosed with lung cancer and died at 73 years of age; the grandmother of the proband (subject 2) was diagnosed with gastric cancer and died at 72 years of age; the husband of the proband (subject 14), a 57-year-old male, was diagnosed with colorectal cancer; the daughter of subject 4 (subject 11), a 35-year-old female, had the same *BRCA1* c.3418_3419insTGACTACT mutation (C>CAGTAGTCA) as subject 4; and another daughter of patient 4 (subject 12), a 27-year-old female, had wild-type *BRCA1* (Fig. 1).

Age. All four female patients with cancer had *BRCA1* c.3418_3419insTGACTACT:p.S1140Mfs*18 (C>CAGTAGTCA) mutations and all were aged >40 years. One subject had the *BRCA1* c.3418_3419insTGACTACT mutation, but the subject was <40 years of age and a healthy individual. The mean age of the patients with cancer was 60 years, 59 years for patients without cancer and 32 years for healthy individuals (Fig. 3A). There was a significant age gap between patients and healthy individuals. When the cancer (n=7) and healthy group (n=8) were compared, the AUC was 0.9107, with a P-value of 0.0078 (Fig. 3B). When the cancer (n=7) and non-cancer group (n=11) were analyzed, the AUC was 0.8082 and the P-value was 0.0265 (Fig. 3C). Overall, age was a risk factor for cancer development.

Discussion

BRCA1 is among the most frequently mutated tumor suppressor genes in cancer (3). *BRCA1*meth increases the risk of early-onset and triple-negative BC and ovarian cancer (8). *BRCA1* is affected by multiple mutations such as *BRCA1* c.4358-2A>G (10), *BRCA1* c.3629_3630delAG, *BRCA1* c.2907_2910delTAAA (11) and *BRCA1* Phe1695Val (15). Germline *BRCA1/2* mutations have been identified in 13-15% of ovarian cancer cases (26). In 3,220 patients in Japan with solid tumors, *BRCA1* was more commonly associated with BC and ovarian cancer, while *BRCA2* was more extensively detected in prostate and pancreatic cancer and cholangiocarcinoma (27). In the present study, a novel heterozygous germline mutation *BRCA1* c.3418_3419insTGACTACT:p.S1140Mfs*18

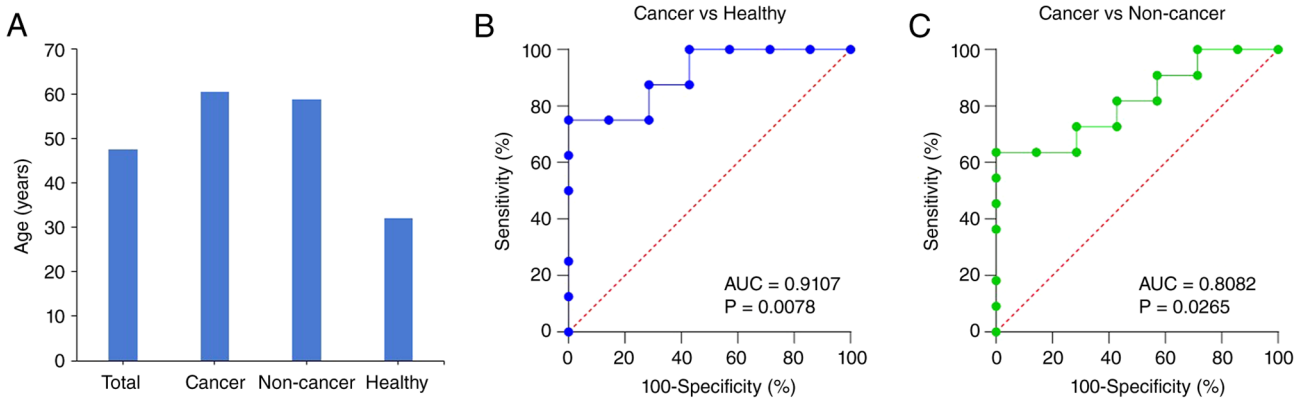

Figure 3. Cancer risk analysis by age. (A) Mean age analysis. (B) AUC between patients with cancer (n=7) and (B) healthy individuals (n=8) and (C) non-cancer diseases (n=11). AUC, area under the curve.

(C>CAGTAGTCA) was identified in exon 10 of the *BRCA1* gene in both BC and ovarian cancer. Notably, the *BRCA1* c.3418_3419insTGACTACT insertion mutations in the present four female patients with cancer were of the same type.

Olaparib + trastuzumab may be effective and safe in pre-treated patients with HER2-positive germinal *BRCA* mutations advanced BC (28). Poly (ADP-ribose) polymerase inhibitors were approved by U.S Food and Drug Administration for patients with *BRCA1/2* mutations (29). Patients with advanced-stage *BRCA*-mutant high-grade ovarian cancer demonstrate progression-free survival benefit from maintenance of olaparib and bevacizumab, regardless of mutation location (30). In the present retrospective study, reasonable treatment plans were formulated in accordance with CSCO and NCCN clinical treatment guidelines on the basis of the tumor type of each patient, expression of tumor markers, gene types and other clinical diagnostic results. Tumor progression in four patients (subjects 4, 6, 8 and 13) was also effectively controlled.

In the present patient cohort, the grandfather of the proband (subject 1) had lung cancer and the grandmother of the proband (subject 2) had gastric cancer. Since both subjects had passed away, the *BRCA1* mutation types of subjects 1 and 2 could not be determined. In addition, the uncle of the proband (subject 9) had coronary heart disease and had died; therefore, the *BRCA1* mutation and its potential association with heart disease could not be determined. These missing data had a bias impact on the present retrospective statistical study. To the best of our knowledge however, no other subjects with the same mutations have been identified thus far.

Furthermore, more subjects with the same mutation need to be recruited to obtain more accurate results. *BRCA1* c.3418_3419insTGACTACT mutations were detected in the two daughters of subject 4. Subject 11 had the same *BRCA1* c.3418_3419insTGACTACT mutation as subject 4; subject 12 had the wild-type gene.

Patients with *BRCA1* double heterozygosity (DH) are more likely to have a family history of BC compared with patients with a single *BRCA1* mutation, and patients with *BRCA1* DH are more likely to have TNBC (31). However, in the present retrospective study, subject 8 was ER (+; 90%), PR (+; 60%) and HER2 (1+) and subject 13 was ER (+; 70%), PR (+; 60%) and HER2 (2+), this indicated that the function of the *BRCA1* c.3418_3419insTGACTACT mutation required more exploration. For patients with *BRCA1* or *BRCA2* pathogenic variant, RRM may decrease the risk of BC (6). Further retrospective studies with larger sample sizes are required to confirm whether patients with the *BRCA1* c.3418_3419insTGACTACT heterozygous germline mutation type identified in the present study benefit from RRM.

To the best of our knowledge, no studies have investigated the biological effects of the *BRCA1* c.3418_3419insTGACTACT mutation and whether there are improved treatment options for patients with cancer with these mutations. Future studies should establish a *BRCA1* c.3418_3419insTGACTACT mutation BC cell model to detect the effects of treatment drugs on this type of cell (32). BC mouse or rat models carrying the *BRCA1* c.3418_3419insTGACTACT mutation should be used to investigate tumor progression and drug treatment efficacy (33,34). It is recommended that healthy individuals who carry this mutant gene undergo regular health check-ups (35,36).

Age is a key factor in carcinogenesis. BC is rare among young female patients, affecting 4-6% of female patients aged <40 years (37). The risk of developing BC in patients aged up to age 80 for female patients with *BRCA1/2* mutations is 69-72% (38). The cumulative risk of invasive BC among patients aged 60-80 years is 20.1% for those with a *BRCA1* mutation (39). In the present study, the mean patient age was 60 years and the mean age of healthy individuals was 32 years. The AUC between the cancer (n=7) and the healthy group (n=8) was 0.9107, and the P-value was 0.0078; thus, age was also a high-risk factor for cancer. In addition to BC, *BRCA1* mutation increases the risk of colorectal cancer by 5-fold among *BRCA1* mutation carriers aged <50 years (40). In the present retrospective study, subject 11, a 35-year-old female subject, had a *BRCA1* c.3418_3419insTGACTACT mutation and did not receive risk-reducing intervention; therefore, this individual should undergo follow-up to confirm the health status in future. For carriers of *BRCA1* mutations, guidance is provided in accordance with the regulations stipulated in the Chinese Expert Consensus on BRCA in patients with BC (41). During the screening process, family members of patients with cancer carrying *BRCA1* mutations should undergo examination as soon as possible. It is recommended to undergo relevant examinations for the breast and ovaries, such as breast

and gynecological ultrasound once/year, mammography and MRI every 2 years and tumor marker tests (carcinoembryonic antigen and carbohydrate antigen 125) once/year.

In summary, the *BRCA1* heterozygous germline mutation, c.3418_3419insTGACTACT:p.S1140Mfs*18 (C>CAGTAGTCA) may have affect the occurrence and development of BC and ovarian cancer and age is a key risk factor for cancer.

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Availability of data and materials

The data generated in the present study may be found in The National Center for Biotechnology Information Sequence Read Archive under accession number PRJNA1347611 or at the following URL: ncbi.nlm.nih.gov/bioproject/PRJNA1347611.

Authors' contributions

CZ and DX conceived and designed the study and interpreted data. CZ and LJ analyzed data. CW performed the experiments. LC recruited and followed up patients. TH provided clinical information and performed the experiments. DX obtained funding. All authors have read and approved the final manuscript. CZ and DX confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was performed in line with the principles of the Declaration of Helsinki. The present study was approved by the Ethics Committees of the 3201 Hospital of Xi'an Jiaotong University (approval no. LLSC-KYLW-2025-009; Hanzhong, China). All participants provided written informed consent.

Patient consent for publication

Written patient consent was obtained for the publication of the present study, including potentially identifiable patient data and images.

Competing interests

The authors declare that they have no competing interests.

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