Original Article

BRCA1/2 mutation spectrum in Chinese early-onset breast cancer

Mengjia Shen[1,2], Libo Yang[1,2], Ting Lei[1,2], Lin Xiao[1], Li Li[1,3], Peichuan Zhang[1,3], Weiyi Feng[4], Feng Ye[1,3], Hong Bu[1,2,3]

[1]Laboratory of Pathology, Clinical Research Center for Breast, [2]Department of Pathology, [3]Key Lab of Transplant Engineering and Immunology, Ministry of Health, West China Hospital, Sichuan University, Chengdu 610041, China; [4]Big Data Research Center, School of Computer Science and Engineering, University of Electronic Science and Technology of China, Chengdu 610054, China

Contributions: (I) Conception and design: M Shen, F Ye; (II) Administrative support: H Bu; (III) Provision of study materials or patients: L Xiao, L Li; (IV) Collection and assembly of data: L Yang, T Lei; (V) Data analysis and interpretation: P Zhang, W Feng; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dr. Feng Ye. Laboratory of Pathology, West China Hospital, Sichuan University, Chengdu 610041, China. Email: fengye@scu.edu.cn.

Background: Breast cancer is the most commonly diagnosed cancer among women. Although many studies have reported the *BRCA* mutations among breast cancer patients, few studies have focused among Chinese early-onset breast cancer patients. The purpose of this study is to identify *BRCA1* and *BRCA2* mutation features and their clinical significance of early-onset Chinese breast cancer patients.

Methods: A total of 54 female patients diagnosed with breast cancer were enrolled in this study, of which 27 were younger than 40 (study group, mean age 32 years, range, 23–40 years) and 27 were older than 40 (control group, mean age 52 years, range, 41–68 years). Tumor FFPE samples were collected for somatic mutation test, while blood samples or normal tissue were used for germline mutation by both PGM and Miseq platform. All codon exons and functional introns for *BRCA1/2* were covered. The clinical significance of mutation types was cross analyzed in several available database. The novel mutations were confirmed by sanger sequencing.

Results: In study group, 14.8% (4/27) and 3.7% (1/27) patients had deleterious *BRCA1/2* germline and somatic mutations respectively. While in control group, only 3.7% (1/27) and 7.4% (2/27) had deleterious *BRCA1/2* germline and somatic mutations respectively. *BRCA1* germline mutation c.2623C>T and *BRCA2* germline mutation c.5852G>A were found to be novel mutation sites and confirmed by sanger sequencing.

Conclusions: Our study found two novel *BRCA1/2* mutation sites in early-onset breast cancer, and also showed that early-onset breast cancer patients are more likely to harbor germline mutations with deleterious and uncertain significance.

Keywords: *BRCA1*; *BRCA2*; early-onset breast cancer; next-generation sequencing (NGS)

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Introduction

Breast cancer is the most commonly diagnosed cancer among women and is the second leading cause of cancer death for women in US (1). Early-onset breast cancer, though only accounting for 7% of all breast cancers, is the most common cancer among young females (2), and has been described to be more biologically aggressive than in older women, which has been associated with a worse prognosis (3). Nowadays, with the development of new techniques, an increasing number of susceptibility gene mutations related to early-onset breast cancer has been detected to improve diagnosis and therapy of early-onset breast cancer and predict outcome. Among all these detected mutations, *BRCA1/2* are still figured out to play an important role in early-onset breast cancer (4-7).

Among the multitude of markers involved into the breast cancer tumorigenesis as *EGFR*, *RANK* (8), *BRCA1*

Table 1 The characteristics of patients according to the age of diagnosis

Characteristics	Study group (n=27)	Control group (n=27)	P value
Mean age (years)	32	52	<0.001
Estrogen-receptor (ER) status			0.704
Positive	24	22	
Negative	3	5	
Progesterone-receptor (PR) status			0.750
Positive	21	20	
Negative	6	7	
Human epidermal growth factor-2 (HER-2)			0.669
Positive	23	25	
Negative	4	2	
Molecular phenotypes			0.525
Luminal A	1	1	
Luminal B ($HER2^+$)	22	22	
Luminal B ($HER2^-$)	2	0	
$HER2^+$	2	3	
Triple negative	0	1	
TNM stage			
T stage (1 missing)			0.551
Tis	1	0	
T1	8	8	
T2	15	17	
T3	3	1	
T4	0	0	
N stage (1 missing)			0.273
N0	14	14	
N1	9	7	
N2	0	3	
N3	4	2	

and *BRCA2* are the first two genes found directly related to hereditary breast cancer. *BRCA1* is located on 17q21.31, and the exon count is 24. *BRCA2* is located on 13q13.1, and the exon count is 27. Both *BRCA1* and *BRCA2* are considered tumor suppressor gene which involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair. Inherited mutations in *BRCA1* and *BRCA2*, confer increased lifetime risk of developing breast cancer (9). Due to the length of *BRCA1/2*, and the randomness of mutation sites, using the traditional sanger sequencing, qPCR, or MLPA to detect the whole gene is not ideal. In contrast, the high throughput next-generation sequencing (NGS) could be more efficient, that can detect all the exons and their adjacent regions of *BRCA1/2* at a time.

As is mentioned in several published research (10-13), compared with other countries, Chinese women carry different *BRCA* mutations rate and types. In this study, we tried to figure out a detailed *BRCA1/2* germline and somatic mutation spectrum in young Chinese breast cancer patients.

Methods

Cases and samples

A total of 54 female patients diagnosed with breast cancer were enrolled in this study, of which 27 patients (mean age 32 years, range, 23–40 years) diagnosed at the age younger than 40 and the rest 27 (mean age 52 years, range, 41–68 years) diagnosed at the age older than 40 in West China Hospital from January 2010 to December 2016 consecutively, belonging to study group and control group, respectively. DNA of 54 FFPE samples of cancer tissue were collected to test the somatic *BRCA1/2* mutations, while DNA of 31 blood samples and 23 FFPE samples of normal tissue were used to exclude the germline *BRCA1/2* mutations by two NGS platforms PGM and Miseq. Clinicopathological characteristics were reviewed including age, estrogen-receptor (ER) status, progesterone-receptor (PR) status, human epidermal growth factor-2 (HER-2) status, Ki-67, molecular phenotypes, TNM staging, etc. Patients' clinical information is showed in *Table 1*. Approval for the study was granted by the Ethics Committee of West China Hospital (number: 2013-191).

Next generation sequencing on PGM platform and Miseq platform

DNA was extracted using QIAamp DNA FFPE Tissue Kit. For library construction, 30 ng of gDNA (measured using the Qubit fluorometer in combination with the Qubit dsDNA HS assay kit) was amplified using BRCAimPLUS DNA panel (SINGLERA Genomics Inc.) and the Ion Ampliseq™ HiFi Master Mix (Ion Ampliseq™ Library kit 2.0). The amplicons were then digested, barcoded and amplified using the Ion Ampliseq™ Library kit 2.0, Ion Xpress™ barcode adapters kit (Life technologies), and

Ion-to-Miseq primers according to the manufacturer's instructions. The library size was checked using the Agilent High Sensitivity DNA Kit by the Bioanalyzer 2100 instrument (Agilent Technologies), and library concentration was evaluated using the Qubit fluorometer and the Qubit dsDNA HS assay kit (Life technologies).

For PGM platform, 50 pM of each library was multiplexed and clonally amplified on the Chef instrument with the Ion PGM™ Hi-Q™ Chef Solutions Cartridge, Ion PGM™ Hi-Q™ Chef Reagents Cartridge, Ion PGM™ Hi-Q™ Chef Supplies and Ion 318™ Chip v2 breast cancer (Life technologies) according to the manufacturer's instructions. Finally, the Ion 318™ chips loaded with enriched template ISP were sequenced on a PGM™ sequencer with the Ion PGM™ sequencing 200 kit v2 according to the manufacturer's instructions.

For Miseq platform, all purified libraries were quantified by real-time PCR using the SYBR Fast Illumina Library Quantification Kit (Kapa Biosystems) and pooled to give equal genome coverage from each library. Each multiplexed library pool was sequenced an Illumina MiSeq for 151 cycles from each end read according to the manufacturer's instructions.

We then analyzed the sequencing data from both Miseq and PGM platforms using the BRCAimPLUS DNA pipeline-a customized bioinformatic analysis workflow for cancer panel. As other bioinformatic pipelines, it involves processing a series of data transformation steps: alignment, variant calling, annotation, filtering and reporting (14-17).

Variants confirmation

DNA was extracted using QIAamp DNA FFPE Tissue Kit. PCR reactions were run in final volumes of 25 μL containing 200 ng DNA, 0.25 mM dNTPs, 10pmol of each primer and 1.25 unit of Taq polymerase [TIANGEN BIOTECH (BEIJING) CO., LTD.]. PCR was performed in an T100 thermal cycler (Bio-Rad, Hercules, CA, USA) with initial denaturation at 95 ℃ for 3 min, followed by 35 cycles of 95 ℃ for 30 s, 60 ℃ for 30 s, 72 ℃ for 30 s. The purified PCR products were sequenced by Sanger's sequencing according to manufacturer's instruction.

Variants evaluation

Variants both detected by two platforms were then to be evaluated. According to the classification system of International Agency for Research on Cancer (IARC), American College of Medical Genetics and Genomics (ACMG), and Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA), the mutations of *BRCA* gene were divided into five categories: pathogenic (Class 5, the rate of pathogenicity is higher than 0.99), likely pathogenic (Class 4, the rate of pathogenicity is between 0.95 and 0.99), uncertain significance (Class 3, the rate of pathogenicity is between 0.05 and 0.949), likely benign (Class 2, the rate of pathogenicity is between 0.001 and 0.049), benign (Class 1, the rate of pathogenicity is lower than 0.001).

Also, the *BRCA* gene variants identified were checked for pathogenicity in 4 databases: Breast Cancer Information Core (BIC) (18), Leiden Open Variation Database (LOVD) (19), the Catalogue of Somatic Mutations in Cancer database (COSMIC) (20) and ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

Statistical analysis

All the statistical analyses were performed using the statistical program for social sciences (SPSS) software package version 19.0 (Chicago, IL, USA). Two independent sample t tests were applied in comparison between groups. Enumeration data was expressed as cases or percentage. Chi-square test was used in comparison between groups, with $P<0.05$ represented for the difference was statistically significant.

Results

NGS test performance

In the cohort of 54 patients, we obtained an average of 4.1 million reads per sample, with a mean coverage of 90% at a mean X coverage of 2031X on the PGM platform and 5.1 million reads per sample with a mean coverage of 92% at a mean X coverage of 2543X on the Miseq platform. In the early-onset breast cancer patients, 2 had no mutations in *BRCA1/2* genes. In the rest 25 patients, a total of 12 mutations of *BRCA1* were detected by both PGM and Miseq platform in 19 patients (*Table 2*). Eleven mutations of *BRCA2* gene were detected in 22 patients (*Table 3*). In control group, two patients had no *BRCA* mutations, while 7 *BRCA1* mutations were detected in 22 patients (*Table 2*), and 9 *BRCA2* mutations were detected in 22 patients (*Table 3*).

Germline BRCA1/2 mutations detected in young breast cancer patients

In study group, the 11 *BRCA1* germline mutations detected

Table 2 *BRCA1* gene mutations detected by two platforms

Groups	Exon	Intron	Type	Consequence	cDNA_change	Pro_change	Germline mutation (n)	Somatic mutation (n)
Study group								
BRCA1	–	4/23	SNP	Splice_acceptor_variant	c.213-1G>A	–	0	1
BRCA1	10/24	–	SNP	Missense_variant	c.988G>A	p.Asp330Asn	1	0
BRCA1	10/24	–	SNP	Missense_variant	c.1036C>T	p.Pro346Ser	1	0
BRCA1	10/24	–	Indel	Frameshift_variant	c.1299dupC	p.Ser434GlnfsTer2	1	0
BRCA1	10/24	–	SNP	Stop_gained	c.2059C>T	p.Gln687Ter	1	0
BRCA1	10/24	–	SNP	Missense_variant	c.2566T>C	p.Tyr856His	4	0
BRCA1	10/24	–	SNP	Missense_variant	c.2612C>T	p.Pro871Leu	14	1
BRCA1	10/24	–	SNP	Missense_variant	c.2623C>T	p.Pro875Ser	1	0
BRCA1	10/24	–	SNP	Missense_variant	c.3113A>G	p.Glu1038Gly	14	1
BRCA1	10/24	–	SNP	Missense_variant	c.3548A>G	p.Lys1183Arg	14	1
BRCA1	15/24	–	SNP	Splice_region_ variant&synonymous_variant	c.4674A>G	c.4674A>G (p.Leu1558=)	1	0
BRCA1	16/24	–	SNP	Missense_variant	c.4837A>G	p.Ser1613Gly	15	0
Control group								
BRCA1	7/24	–	SNP	Missense_variant	c.446A>C	p.Glu149Ala	1	0
BRCA1	10/24	–	Indel	Frameshift_variant	c.2398_2401delAAAT	p.Lys800ValfsTer2	0	1
BRCA1	10/24	–	SNP	Missense_variant	c.2566T>C	10	1	0
BRCA1	10/24	–	SNP	Missense_variant	c.2612C>T	p.Pro871Leu	16	2
BRCA1	10/24	–	SNP	Missense_variant	c.3113A>G	p.Glu1038Gly	17	4
BRCA1	10/24	–	SNP	Missense_variant	c.3548A>G	p.Lys1183Arg	17	2
BRCA1	16/24	–	SNP	Missense_variant	c.4837A>G	p.Ser1613Gly	17	3

were classified as following: 2 5-pathogenic, 4 3-uncertain, 5 1-benign, in accordance with the data found in ClinVar (*Tables S1*,*S2*). c.2623C>T was identified as novel germline mutation site found in a patient diagnosed as breast cancer at the age of 27 (*Figure S1A*). Among all, 4 mutations were found to be pending, while the rest were not found in BIC. According to the Leiden Open Variation Database, 2 mutations were definitely indicated to affect function. In COSMIC database, 4 mutations were found to be neutral.

As for *BRCA2*, 10 germline mutations were detected involving 2 5-pathogenic, 3 3-uncertain, 5 1-benign in accordance with the data found in ClinVar (*Tables S1*,*S2*). c.5852G>A identified as a novel site found in a patient diagnosed as breast cancer at the age of 28 (*Figure S1B*). Among all, 1 mutation c.10234A>G was found to be class 1 in BIC database, 2 mutations were found to be pending, while the rest were not found in BIC. According to the Leiden Open Variation Database, 1 mutation was definitely indicated to affect function. In COSMIC database, 4 mutations were found to be neutral, and 1 referring to be pathogenic.

In control group, there were only 6 *BRCA1* germline in total 27 patients. c.446A>C was a mutation with uncertain significance. c.2566T>C, c.2612C>T, c.3113A>G, c.3548A>G, c.4837A>G were benign mutations. When considered *BRCA2*, 1 pathogenic mutation c.9294C>G, 2 uncertain mutations c.10150C>G, c.3445A>G were identified as well as 5 benign mutations.

Table 3 *BRCA2* gene mutations detected by two platforms

Groups	Exon	Type	Consequence	cDNA_change	Pro_change	Germline mutation (n)	Somatic mutation (n)
Study group							
BRCA2	27/27	SNP	Missense_variant	c.10234A>G	p.Ile3412Val	0	1
BRCA2	25/27	Indel	Frameshift_variant	c.9401delG	p.Gly3134AlafsTer29	1	0
BRCA2	18/27	SNP	Missense_variant	c.8187G>T	p.Lys2729Asn	1	0
BRCA2	11/27	SNP	Missense_variant	c.5852G>A	p.Ser1951Asn	1	0
BRCA2	11/27	SNP	Missense_variant	c.5785A>G	p.Ile1929Val	1	0
BRCA2	11/27	SNP	Missense_variant	c.2971A>G	p.Asn991Asp	6	0
BRCA2	10/27	SNP	Missense_variant	c.1462A>G	p.Ile488Val	1	0
BRCA2	10/27	SNP	Stop_gained	c.1399A>T	p.Lys467Ter	1	0
BRCA2	10/27	SNP	Missense_variant	c.1114A>C	p.Asn372His	13	1
BRCA2	10/27	SNP	Missense_variant	c.865A>C	p.Asn289His	6	0
BRCA2	5/27	SNP	Missense_variant	c.461A>G	p.Gln154Arg	1	0
Control group							
BRCA2	27/27	SNP	Missense_variant	c.10234A>G	p.Ile3412Val	3	0
BRCA2	27/27	SNP	Missense_variant	c.10150C>G	p.Arg3384Gly	1	0
BRCA2	25/27	SNP	Stop_gained	c.9294C>G	p.Tyr3098Ter	1	0
BRCA2	14/27	Indel	Frameshift_variant	c.7414_7415delAA	p.Lys2472ValfsTer2	0	1
BRCA2	11/27	SNP	Missense_variant	c.5785A>G	p.Ile1929Val	1	0
BRCA2	11/27	SNP	Missense_variant	c.3445A>G	p.Met1149Val	1	0
BRCA2	11/27	SNP	Missense_variant	c.2971A>G	p.Asn991Asp	7	2
BRCA2	10/27	SNP	Missense_variant	c.1114A>C	p.Asn372His	11	6
BRCA2	10/27	SNP	Missense_variant	c.865A>C	p.Asn289His	7	2

Somatic BRCA1/2 mutations detected in young breast cancer patients

In study group, there were 4 *BRCA1* somatic mutations detected in total, including pathogenic mutation c.213-1G>A and three benign mutation c.2612C>T, c.3113A>G, c.3548A>G only detected in one patient respectively. Also, two *BRCA2* somatic benign mutations c.10234A>G and c.1114A>C were detected.

In control group, 5 *BRCA1* somatic mutations involving pathogenic mutation c .2398_2401delAAAT detected in one patient, and four benign mutations c.2612C>T, c.3548A>G, c.4837A>G, c.3113A>G. Also, 4 *BRCA2* somatic mutations were detected, of which c.7414_7415delAA was pathogenic with the rest c.2971A>G, c.865A>C, and c.1114A>C turned out to be benign.

BRCA1/2 mutations of pathogenic and uncertain significance in young breast cancer patients

Mutations defined as pathogenic/likely pathogenic and uncertain were selected for further analysis, as is shown in *Table 4*. In total, 11 germline (7 3-uncertain, 4 5-pathogenic) and 1 somatic (5-pathogenic) of *BRCA1/2* mutations were detected in study group, while 4 germline (3 3-uncertain and 1 5-pathogenic) and 2 somatic (2 5-pathogenic) of *BRCA1/2* mutations were detected in control group.

In study group, 14.8% (4/27) and 3.7% (1/27) patients had deleterious *BRCA1/2* germline and somatic mutations respectively. While in control group, only 3.7% (1/27) and 7.4% (2/27) had deleterious *BRCA1/2* germline and somatic mutations, respectively.

Table 4 The distribution of 3-uncertaion and deleterious *BRCA1/2* mutations in two groups

Groups	Variations	Clinical significance	Germline mutations (n)	Somatic mutations (n)
Study group				
BRCA1	c.988G>A	3-uncertain	1	0
BRCA1	c.4674A>G	3-uncertain	1	0
BRCA1	c.1036C>T	3-uncertain	1	0
BRCA1	c.2623C>T	3-uncertain	1	0
BRCA1	c.2059C>T	5-pathogenic	1	0
BRCA1	c.1299dupC	5-pathogenic	1	0
BRCA1	c.213-1G>A	5-pathogenic	0	1
BRCA2	c.1462A>G	3-uncertain	1	0
BRCA2	c.461A>G	3-uncertain	1	0
BRCA2	c.5852G>A	3-uncertain	1	0
BRCA2	c.9401delG	5-pathogenic	1	0
BRCA2	c.1399A>T	5-pathogenic	1	0
Control group				
BRCA1	c.446A>C	3-uncertain	1	0
BRCA1	c.2398_2401delAAAT	5-pathogenic	0	1
BRCA2	c.10150C>G	3-uncertain	1	0
BRCA2	c.3445A>G	3-uncertain	1	0
BRCA2	c.9294C>G	5-pathogenic	1	0
BRCA2	c.7414_7415delAA	5-pathogenic	0	1

The 4 pathogenic germline mutations were c.2059C>T, c.1299dupC, c.9401delG, c.1399A>T found in study group at the age of 40, 28, 36, 40. And 7 uncertain germline mutations existed in study group were c.988G>A, c.4674A>G, c.1036C>T, c.2623C>T, c.1462A>G, c.461A>G, c.5852G>A. When it turns to control group, there was only one pathogenic germline mutation c.9294C>G found in patient at the age of 57 and 3 uncertain germline mutations c.446A>C, c.10150C>G, c.3445A>G.

Discussion

BRCA status is not only important for the identification of familial cancer predisposition but also to therapeutic choices for breast cancer patients, e.g., the PARP inhibitor therapy (21-23). *BRCA* gene mutation is closely related to the early-onset breast cancer occurrence. In most national and international guidelines, testing criteria of *BRCA* includes patients with breast cancer aged less than 35 or 40 years (24). *BRCA1/2* mutation carriers diagnosed with breast cancer before age 50 are prone to a worse survival (25). In our study, the mean age of 27 early-age onset breast cancer patients was 32, with the minimum hospitalized age 23. Only 2 had no *BRCA1/2* mutation. Twenty patients had *BRCA1* mutations and 22 had *BRCA2* mutations, with 3 (11.1%) patients had pathogenic mutations involving c.1299dupC, c.2059C>T, c.213-1G>A in *BRCA1/2* (7.4%) had pathogenic mutations involving c.9401delG, c.1399A>T in *BRCA2*, which were not found in control group. The mutation frequency of the deleterious germline mutation in our study is a little bit higher than in other research (24,26,27) may attribute to insufficient number of analyzed cases.

BRCA1 mutation c.2623C>T was identified as a germline mutation for the first time in this study, which was a SNP detected in EXON10 leading to the protein change p.Pro875Ser. The patient who had c.2623C>T mutation as the only *BRCA* mutation diagnosed as breast cancer at the age of 27. As for *BRCA2*, c.5852G>A was also identified as a germline mutation for the first time in this study which was a SNP detected in EXON11 leading to the protein change

p.Ser1951Asn. The patient who had c.5852G>A mutation as the only *BRCA* mutation other than benign mutations diagnosed as breast cancer at the age of 28. Since these two new mutation sites were detected in patients at such young age without other suspicious *BRCA* mutation sites, we have reason to suspect that these two mutation sites may play a role in the pathogenesis of the early-onset breast cancer which need to be further confirmed.

A large number of literatures show strong correlation between *BRCA* mutation and familial early-onset breast cancer (28-30). In our study, in total, 11 germline (7 3-uncertain, 4 5-pathogenic) and 1 somatic (5-pathogenic) of *BRCA1/2* mutations were detected in study group, while 4 germline (3 3-uncertain and 1 5-pathogenic) and 2 somatic (2 5-pathogenic) of *BRCA1/2* mutations were detected in control group. In study group, 14.8% (4/27) patients had deleterious *BRCA*1/2 germline mutations, and 3.7% (1/27) had deleterious *BRCA1/2* somatic mutations, while in control group, 3.7% (1/27) had deleterious *BRCA1/2* germline mutations, and 7.4% (2/27) had deleterious *BRCA*1/2 somatic mutations, displaying a trend that early-onset group is more likely to have germline mutations than elderly counterparts. Therefore, there is a strong recommendation for the early-onset breast cancer patients despite of the family history to get *BRCA* test for potential benefit for their family members as well as benefit for the patient of PARP inhibitor therapy.

One of the limitations of the study is the absence of triple negative samples in the study groups which is among the breast cancer histotypes the more aggressive with the poor prognosis caused by insufficient cases, but we tried to figure out a detailed spectrum of *BRCA1/2* germline and somatic mutations of early-onset breast cancer patients in West China Hospital using NGS. Several deleterious and uncertain mutations were observed in this cohort and it is recommended that a more thorough and functional examination of these mutations should be conducted in the future.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.03.02). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Approval for the study was granted by the Ethics Committee of West China Hospital (number: 2013-191). And the study was done with all patients' informed consent.

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Supplementary

Table S1 *BRCA1* variations found and their evaluations in BRCA databases

Variations	Clinical significance	BIC database clinically importance/clinical classification	LOVD	COSMIC	ClinVar
Study group					
c.213-1G>A	5-pathogenic	Pending	Affects function	Not found	Pathogenic
c.988G>A	3-uncertain	Not found	Not found	Not found	Uncertain significance
c.4674A>G	3-uncertain	Not found	Effect unknown	Not found	Uncertain significance
c.1299dupC	5-pathogenic	Not found	Not found	Not found	Pathogenic
c.2623C>T	3-uncertain	Not found	Not found	Not found	Not found
c.1036C>T	3-uncertain	Pending	Effect unknown; affects function	Not found	Conflicting interpretations of pathogenicity
c.2059C>T	5-pathogenic	Not found	Affects function	Not found	Pathogenic
c.2566T>C	1-benign	Pending	Does not affect function	Not found	Benign
c.2612C>T	1-benign	Not found	Does not affect function	Neutral	Benign
c.3113A>G	1-benign	Not found	Does not affect function	Neutral	Benign
c.3548A>G	1-benign	Pending	Does not affect function	Neutral	Benign
c.4837A>G	1-benign	Pending	Does not affect function	Neutral	Benign
Control group					
c.446A>C	3-uncertain	Not found	Not found	Not found	Uncertain significance
c.2398_2401delAAAT	5-pathogenic	Not found	Not found	Not found	Pathogenic
c.2566T>C	1-benign	Pending	Does not affect function	Not found	Benign
c.2612C>T	1-benign	Not found	Does not affect function	Neutral	Benign
c.3113A>G	1-benign	Not found	Does not affect function	Neutral	Benign
c.3548A>G	1-benign	Pending	Does not affect function	Neutral	Benign
c.4837A>G	1-benign	Pending	Does not affect function	Neutral	Benign

BIC, Breast Cancer Information Core; LOVD, Leiden Open Variation Database; COSMIC, Catalogue of Somatic Mutations in Cancer database.

Table S2 *BRCA2* variations found and their evaluations in BRCA databases

Variations	Clinical significance	BIC database clinically importance/ clinical classification	LOVD	COSMIC	ClinVar
Study group					
c.9401delG	5-pathogenic	Not found	Not found	Not found	Pathogenic
c.8187G>T	1-benign	Pending	Does not affect function	Pathogenic	Benign
c.5852G>A	3-uncertain	Not found	Not found	Not found	Not found
c.1462A>G	3-uncertain	Not found	Not found	Not found	Conflicting interpretations of pathogenicity
c.1399A>T	5-pathogenic	Not found	Affects function	Not found	Pathogenic
c.461A>G	3-uncertain	Not found	Not found	Not found	Uncertain significance
c.10234A>G	1-benign	Class 1	Does not affect function	Neutral	Benign
c.5785A>G	1-benign	Pending	Does not affect function; Effect unknown	Not found	Benign
c.2971A>G	1-benign	Not found	Does not affect function	Neutral	Benign
c.1114A>C	1-benign	Not found	Does not affect function	Neutral	Benign
c.865A>C	1-benign	Not found	Does not affect function	Neutral	Benign
Control group					
c.10150C>G	3-uncertain	Not found	Not found	Not found	Uncertain significance
c.9294C>G	5-pathogenic	Class 5	Affects function	Not found	Pathogenic
c.7414_7415delAA	5-pathogenic	Not found	Not found	Not found	Pathogenic
c.3445A>G	3-uncertain	Pending	Effect unknown	Not found	Conflicting interpretations of pathogenicity
c.10234A>G	1-benign	Class 1	Does not affect function	Neutral	Benign
c.5785A>G	1-benign	Pending	Does not affect function; Effect unknown	Not found	Benign
c.2971A>G	1-benign	Not found	Does not affect function	Neutral	Benign
c.1114A>C	1-benign	Not found	Does not affect function	Neutral	Benign
c.865A>C	1-benign	Not found	Does not affect function	Neutral	Benign

BIC, Breast Cancer Information Core; LOVD, Leiden Open Variation Database; COSMIC, Catalogue of Somatic Mutations in Cancer database.

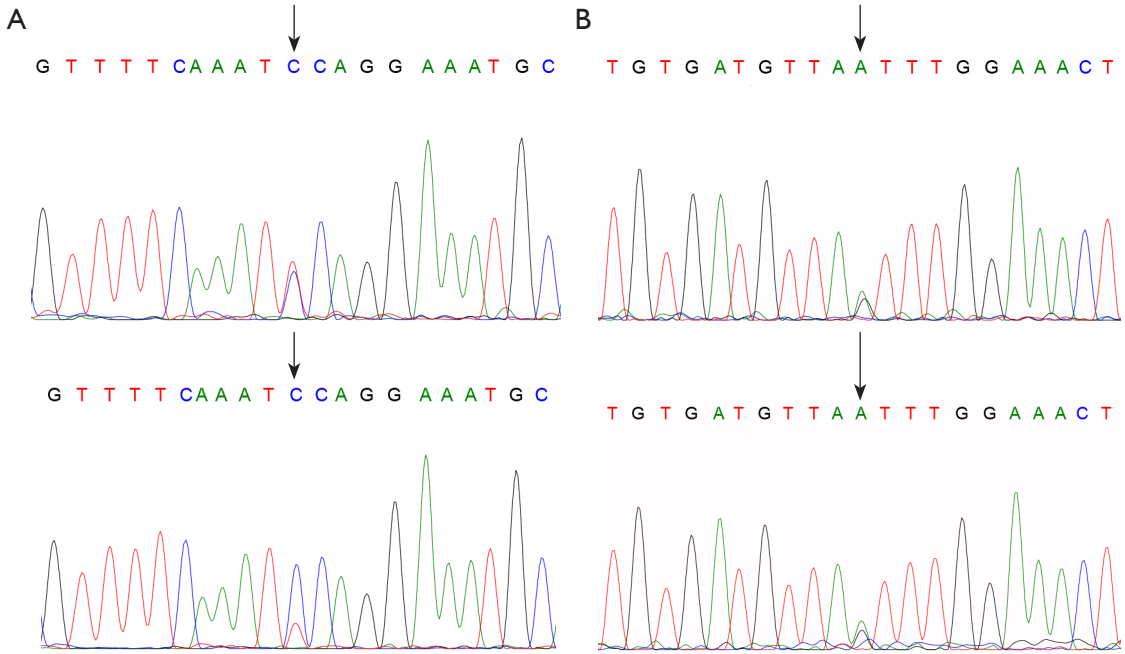

Figure S1 Sanger sequence of two novel mutation sites. (A) The position indicated by the arrows show the mutation site c.2623C>T. The figure above shows the sequencing result of tumor tissue, while the lower figure shows the result of normal tissue. (B) Arrows indicate the mutation site c.5852G>A. The figure above shows the sequencing result of tumor tissue, while the lower figure shows the result of normal tissue.