Prevalence and Spectrum of BRCA1/2 Germline Mutations in Women with Breast Cancer in China Based on Next-Generation Sequencing

Yi Liang*
Xuexi Yang*
Hong Li*
Anna Zhu
Zhiwei Guo
Ming Li

* These authors contributed equally

Corresponding Author: Ming Li, e-mail: mingli2006_2006@126.com

Source of support: This work was supported by the Special Program for Public Welfare Research and Capacity Construction Project of Guangdong province (Grant number 2015A030401040) and the National Natural Science Foundation for Youth of China (Grant number 81302327)

Background: BRCA1 and BRCA2 (BRCA1/2) play important roles in the development of breast cancer, but information regarding BRCA1/2 mutations in Chinese females remains limited. The aim of this study was to investigate the prevalence and spectrum of BRCA1/2 mutations in China.

Material/Methods: In total, 595 breast cancer patients in China were screened with an amplicon-based panel for the detection of BRCA1/2 mutations in coding regions using next-generation sequencing (NGS) with a Personal Genome Machine. Every pathogenic mutation detected was confirmed by Sanger sequencing. The disease-causing potential of variants of uncertain significance (VUS) was predicted using PolyPhen-2, SIFT, PhyloP, and Grantham.

Results: The prevalence of BRCA1/2 mutations was 8.07% in the Chinese population. Forty-two pathogenic mutations were identified in 48 cases (17 BRCA1 cases and 31 BRCA2 cases), including 19 novel mutations. Nine VUS were predicted to be deleterious by PolyPhen-2 and SIFT and subsequently predicted by PhyloP and Grantham for the evolutionary conservation.

Conclusions: These results suggest that NGS is useful as a rapid, high-throughput, and cost-effective screening tool for the analysis of BRCA1/2 mutations. Based on this panel, we found that BRCA1/2 germline mutations in China exhibit distinct characteristics compared to those in Western populations.

MeSH Keywords: Breast Neoplasms • China • Genes, BRCA1 • Genes, BRCA2 • Germ-Line Mutation • High-Throughput Nucleotide Sequencing

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/905812

1 School of Laboratory Medicine and Biotechnology, Southern Medical University, Guangzhou, Guangdong, P.R. China
2 Guangzhou Darui Biotechnology Co. Ltd., Guangzhou, Guangdong, P.R. China

Authors’ Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

© Med Sci Monit, 2018; 24: 2465-2475
DOI: 10.12659/MSM.905812

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)
Background

The incidence of breast cancer in China has been progressively increasing with the development of the Chinese economy. Indeed, the incidence was estimated to be 272,400 in 2015, with a mortality of 70,700 [1]. Breast cancer is especially prevalent in developed cities, such as Shanghai and Guangzhou, and has become the most common malignancy among women in these cities [2].

BRCA1 (MIM#113705) and BRCA2 (MIM#600185) (BRCA1/2) are 2 high-penetration breast cancer susceptibility genes [3,4]. These 2 genes contribute to 5–10% of all breast cancer cases, and carriers of germline mutations in these genes have an 80% increased risk of developing breast cancer by the age of 70 years [5,6]. Patients with BRCA pathogenic mutations respond better to a recently approved poly (ADP-ribose) polymerase inhibitor (Olaparib) [7], and it has been reported that carriers of BRCA mutations who received prophylactic mastectomies could reduce their risk of breast cancer by approximately 90% [8]. Thus, genetic diagnosis of BRCA-associated breast cancer is essential for the provision of genetic counseling and to establish preventive interventions and therapeutic strategies. However, studies of BRCA-associated breast cancer in China remain limited, and comprehensive BRCA1/2 mutation screening is rarely reported [9,10]. Therefore, investigating the prevalence and spectrum of BRCA1/2 germline mutations in Chinese populations is necessary for developing genetic cancer risk assessments and genetic testing in China.

To date, although Sanger sequencing is still the criterion standard for analysis of BRCA1/2 mutations, the large sizes of the genes (5592 bp and 10257 bp, respectively) and lack of mutation hotspots make this procedure time-consuming and costly. Recent progress in NGS has solved these problems with its high-throughput technique and efficiency [11,12]. Moreover, NGS performs well in detecting a broad spectrum of mutations. Therefore, in this study we used an NGS-based panel to screen entire coding sequences of BRCA1/2 genes in 595 breast cancer patients in China.

Material and Methods

Patients

In total, 595 breast cancer patients were recruited from Hospitals of Guangdong, Chongqing, and Shandong Province from 2014 to 2016, and 2 ml of peripheral blood was obtained from each patient. Among them, 203 were high-risk breast cancer cases who met 1 of the following criteria: 1) at least 1 first- and/or second-degree relative had breast and/or ovarian cancer; 2) younger than 35 years of age at breast cancer onset; 3) bilateral breast cancer; and 4) triple-negative breast cancer (TNBC; estrogen receptor-negative, progesterone receptor-negative, and HER2-negative) [13–15]. The remaining patients were low-risk in terms of family history, age at onset, bilateral breast cancer, and TNBC. Informed consent was obtained from all participants, and approval was granted by the Ethics Committee of Southern Medical University.

DNA extraction

Genomic DNA was extracted from peripheral blood using the QiAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. DNA was quantified using Qubit (Invitrogen, Life Technologies, Carlsbad, CA, USA).

Multiplex PCR target amplification, NGS library construction, and sequencing

Entire BRCA1/2 coding regions were amplified by multiplex PCR using Ion AmpliSeq™ Custom Primer Pools (Invitrogen, Life Technologies) composed of 3 multiplex PCR primer pools with 55, 56, and 56 amplicons, respectively. Each sample used 30 ng of genomic DNA, with 10 ng per primer pool. Thermal cycling was as follows: 99°C for 2 minutes, followed by 22 cycles of annealing and extension at 99°C for 15 seconds, 60°C for 4 minutes, and a final hold at 4°C. Specific barcodes were ligated to each sample after mixing the above 3 PCR products for identification using the Ion AmpliSeq™ Library Kit 2.0 (Life Technologies) following the manufacturer’s instructions. After amplifying the libraries with a second PCR, quantification was performed with Qubit, followed by analysis of the size distribution of the DNA fragments on a 2100 Bioanalyzer using the High Sensitivity Kit (Agilent Technologies, Santa Clara, CA, USA). Equivalent amounts of the patient libraries were pooled to implement Template Preparation using the Ion PGM™ Hi-Q™ OT2 Kit (Life Technologies), followed by quantitative PCR (qPCR) on an ABI 7500 Real-Time PCR System using the SYBR FAST Universal qPCR Kit (Kapa Biosystems, Wilmington, MA, USA). Sequencing of the libraries was performed on a Personal Genome Machine (PGM; Life Technologies) with the Ion 318™ Chip v2 (16 samples per run) using the Ion PGM™ Hi-Q™ Sequencing Kit (Life Technologies) according to the instructions provided.

Sanger sequencing

To verify the panel, each amplicon and pathogenic mutation of BRCA1/2 was confirmed by Sanger sequencing. Sequencing primers were designed using Primer 5.0 software. The details of the primer sequences for the pathogenic mutations are shown in Table 1. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and labeled using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA). Sequencing was performed using the ABI 3730 XL Genetic Analyzer (Applied Biosystems), and the sequencing data was analyzed by the ChromasPro software.
**Table 1.** Primer sequences for *BRCA1/2* mutation identification with Sanger sequencing.

| Mutation | Forward primer (5’- 3’) | Reverse primer (5’- 3’) | Annealing temperature (°C) |
|----------|--------------------------|--------------------------|---------------------------|
| c.1504_1508delTTAAA | GAGGCCCAGATAAATAAGAACGGCTG | GCAGATCTTTTTGAGTAGTATTATGGGG | 60 |
| c.1561_1565delG | TGGTGGCTGAAGAAAAGGAGG | GTGTGGATGGAAAAGGAGG | 60 |
| c.5164_5165delAG | CAG CTA CGG GGA AAA AAG TTA | TGTGCTCACTGTTGAAAATAGG | 60 |
| c.182_182delT | GAGCCACAGATAATACAAGAGCGTG | GCTCACCATTTTTTCTGGAGTGATTCTATTGGG | 60 |
| c.306_2806delAAAC | ATGGAAAAGAATCAAGATGTAT | CTATTGCTTCCTCCCGATGC | 60 |
| c.900_9400delG | CAG CTA CGG GGA AAA AAG TTA | TGTGCTCACTGTTGAAAATAGG | 60 |
| c.1299_1300insC | GAGCCACAGATAATACAAGAGCGTG | GCTCACCATTTTTTCTGGAGTGATTCTATTGGG | 60 |
| c.469_473delAAGTC | TGTGCTCACTGTTGAAAATAGG | GCAGATCTTTTTGAGTAGTATTATGGGG | 60 |
| c.1934_1934delC | AGG CTG AGG AGG AAG TCT TCT ACC | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.304_304delA | GAGCCACAGATAATACAAGAGCGTG | GCTCACCATTTTTTCTGGAGTGATTCTATTGGG | 60 |
| c.5510G>A | ATGGAAAAGAATCAAGATGTAT | CTATTGCTTCCTCCCGATGC | 60 |
| c.1961_1961delT | AGG CTG AGG AGG AAG TCT TCT ACC | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.3353CG | GAGCCACAGATAATACAAGAGCGTG | GCTCACCATTTTTTCTGGAGTGATTCTATTGGG | 60 |
| c.895_8956insA | ATGGAAAAGAATCAAGATGTAT | CTATTGCTTCCTCCCGATGC | 60 |
| c.1961_1961delT | AGG CTG AGG AGG AAG TCT TCT ACC | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.304_304delA | GAGCCACAGATAATACAAGAGCGTG | GCTCACCATTTTTTCTGGAGTGATTCTATTGGG | 60 |
| c.3214_3214delC | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.5718_5719delCT | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.5574_5577delAATT | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.3472G>T | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.1012A>T | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.4222C>T | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.1439_1440insA | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.283_286delCTTG | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.5521_5521delE | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.9317G>A | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.1301_1304delAAAG | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.6952C>T | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |
| c.7562_7563delTTC | TCAATG TCA CCT GAA AGA GAA ATGG | CAG CTA CGG GGA AAA AAG TTA | 60 |

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)
Foster City, CA, USA). Alcohol was used to purify the labeled DNA fragments, and a 3500Dx Genetic Analyzer (Applied Biosystems) was used for sequence analysis.

Bioinformatics analysis

Data from the PGM were analyzed using Torrent Suite Software v4.4 (Life Technologies). Alignment to the BRCA1 NG_005905.2 and BRCA2 NG_012772.1 reference sequences and variant calling were executed after sequence quality filtering and sample identification by barcodes. To discard false-positives or somatic mutations, 3 filters were used in the panel as follows: 1) variants that were not homozygous or heterozygous were discarded; 2) variants located in non-coding regions were filtered; and 3) variant types consisting of single-nucleotide variants (SNVs), multi-nucleotide variants (MNVs), and insertion-deletions (InDels). Variant annotation was performed after variant filtering using Ion Reporter Software 4.4 (Life Technologies) based on the Breast Cancer Information Core (BIC, http://research.nhgri.nih.gov/projects/bic/), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), and ClinVar databases (https://www.ncbi.nlm.nih.gov/clinvar/). The base probability distribution of all missense mutation sites was called from the sequencing data of 18 000 normal Chinese females according to our existing database, which was delved from data of Non-Invasive Prenatal Testing (NIPT) in 18 000 pregnant women for BRCA1/2 genes.

Prediction of functional impact for variant of uncertain significance (VUS) using PolyPhen-2, SIFT software, PhyloP, and Grantham.

The disease-causing potential of BRCA1/2 VUS was predicted using the online tools Polymorphism Phenotyping 2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2) and Sorting Intolerant From Tolerant (SIFT; http://sift.jcvi.org/index.html). The evolutionary conservation of VUS was predicted by phylogenetic p-values (PhyloP) and Grantham.PolyPhen-2 was used to estimate the possible effect of an amino acid substitution on the structure and function of BRCA1 and BRCA2 proteins. In this program, mutations are classified as probably damaging (probability score >0.85), possibly damaging (0.15< probability score <0.85), or benign (probability score <0.15). SIFT evaluates the functional impacts of variants based on the degree of conservation of each amino acid residue in the investigated sequence. SIFT scores ≤0.05 were considered deleterious, and those >0.05 were predicted as tolerated. PhyloP evaluates nucleotide conservation in various species while Grantham quantifies the biochemical difference between 2 amino acids. A site is more conserved than neutral when the PhyloP score is >0.

Statistical analysis

Chi-square analysis or Fisher’s exact test was used to compare the differences between high-risk and low-risk patients according to age, TNBC, family history, and bilaterality. Differences were considered significant when the p-value was <0.05 (two-sided). IBM SPSS 20 (SPSS Inc., Chicago, IL, USA) was used in this study.

Results

Patient characteristics

In our study, the mean age at diagnosis of all patients was 48 years (range 22 to 80 years). Of the 595 cases, 286 were from Guangdong province, 212 were from Shandong Province, and 97 were from Chongqing Province. In total, 76 (13.29%) had early-onset breast cancer (onset age ≤35 years); 52 (10.10%) had a family history of cancer, including breast cancer (24 cases) or ovarian cancer; 90 (16.33%) had TNBC; and 8 (1.44%) had bilateral breast cancer. In brief, 203 (34.12%) patients were defined as high-risk breast cancer patients.

Performance of the BRCA1/2 panel

This panel contained 167 pairs of primers in 3 primer pair pools for 100% amplicon coverage of all targeted exons. The mean depth in our experiment reached 1500× (ranging from 246× to 3628×). The total reads differed for each run, with an average of 4 407 829. With the 3 filters, numerous unsatisfactory variants were discarded. No false-positives were called in this study, resulting in a specificity of 100%.

Prevalence of BRCA1/2 mutations

Forty-two deleterious mutations of the BRCA1/2 genes were identified in 48 cases; the mutation rate of BRCA1/2 for all patients in this study was 8.07% (48/595). All mutations were either frameshift or nonsense mutations. There was no significant difference in the prevalence of BRCA1/2 germline mutations among individuals from Guangdong, Chongqing, and Shandong provinces (p>0.05). Among the early-onset patients, 9 (11.84%) pathogenic mutations were identified: 2 in BRCA1 and 7 in BRCA2 (Table 2). Eight (15.38%) pathogenic BRCA mutations were found in patients with a family history of cancer, 6 of which were BRCA2 mutations. In the TNBC patients, 13 (14.44%) mutations were detected, most of which were BRCA1 mutations. In the bilateral breast cancer cases, 1 (12.5%) mutation was found, which was a BRCA2 mutation. Ten patients had both early-onset breast cancer and a family history, 4 of whom (40%) had a BRCA1/2 mutation. A BRCA2 mutation was also detected in 1 patient who had early-onset bilateral breast cancer.
### Table 2. Prevalence of BRCA mutations according to different risk factors.

| Type        | N     | Mutation |        |        |        | p      |
|-------------|-------|----------|--------|--------|--------|--------|
|             |       | BRCA1    | BRCA2  | Total  |        |        |
| Total number| 595   | 17       | 31     | 48     |        |        |
| High risk   | 203   | 10       | 13     | 23     | 11.33% | P=0.001|
| Low risk    | 369   | 3        | 12     | 15     | 4.07%  |        |
| Early-onset |       |          |        |        |        |        |
| ≤35         | 76    | 2        | 7      | 9      | 11.84% | P=0.01 |
| >35         | 496   | 11       | 10     | 21     | 4.23%  |        |
| Family history |       |          |        |        |        |        |
| Yes         | 52    | 2        | 6      | 8      | 15.38% | P=0.014|
| No          | 463   | 9        | 16     | 25     | 5.40%  |        |
| TNBC        |       |          |        |        |        |        |
| Yes         | 90    | 10       | 2      | 12     | 14.44% | P=0.001|
| No          | 461   | 3        | 20     | 23     | 4.99%  |        |
| Bilateral   |       |          |        |        |        |        |
| Yes         | 8     | 1        | 1      | 1      | 12.50% | P=0.408|
| No          | 547   | 12       | 22     | 34     | 6.22%  |        |

### Table 3. Spectrum of BRCA1 deleterious mutations.

| Sample    | Mutation    | Onset age | Exon | Type* | AA change | BIC record | Note*   |
|-----------|-------------|-----------|------|-------|-----------|------------|---------|
| H1N       | c.1504-1508delTTAAA | 29        | 11   | FS    | p.Leu502Alafs | Y         | TNBC+E  |
| LHY049    | c.3333_3333del A     | 47        | 11   | FS    | p.Glu1112fs  | Y         | TNBC    |
| MBC24     | c.981_982delAT       | 53        | 11   | FS    | p.Cys328fs   | Y         | TNBC    |
| CQ62      | c.1299_1300insC      | 48        | 11   | FS    | p.Ser434fs   | N         | TNBC    |
| CQ98      | c.1934_1934delC      | 36        | 11   | FS    | p.Ser434fs   | N         | TNBC    |
| NF48      | c.3214_3214delC      | 52        | 11   | FS    | p.Leu1072fs  | Y         | TNBC    |
| SD33      | c.5510G>A           | 46        | 24   | NS    | p.Trp1837Ter | Y         | TNBC    |
| GZ54      | c.1961_1961delA      | 61        | 11   | FS    | p.Lys654fs   | Y         | TNBC    |
| GZ65      | c.3353C>T           | 49        | 11   | NS    | p.Lys1841fs  | N         | TNBC    |
| SD136     | c.376C>T            | 32        | 7    | NS    | p.Gln126Ter  | N         | FH+TNBC |
| SD257     | c.5353G>T           | 38        | 22   | NS    | p.Gln1785Ter | Y         | TNBC    |
| NF93      | c.3472G>T           | 43        | 11   | NS    | p.Glu1158Ter | N         | TNBC    |
| NF113     | c.1012A>T           | 55        | 11   | NS    | p.Lys333Ter  | N         | TNBC    |
| ZI116     | c.4222C>T           | –         | 13   | NS    | p.Gln1408Ter | Y         | TNBC    |
| ZI123     | c.1439_1440insA     | –         | 11   | FS    | p.Asn480fs   | Y         | TNBC    |
| ZI1760    | c.283_286delCTTG     | –         | 6    | FS    | p.Leu95fs    | N         |         |
| ZI10040   | c.5521_5521delA     | –         | 24   | FS    | p.Ser1841fs  | Y         |         |

* FS – frameshift; NS – nonsense; TNBC – triple negative breast cancer; FH – family history; BI – bilateral breast cancer; E – early-onset breast cancer.
Table 4. Spectrum of *BRCA2* deleterious mutations.

| Sample  | Mutation                  | Onset age | Exon | Type*  | AA change            | BIC record | Note*  |
|---------|---------------------------|-----------|------|--------|----------------------|------------|--------|
| NFBC2   | c.5164_5165delAG          | 29        | 11   | FS     | p.Ser1722Tyrfs       | Y          | FH+E   |
| NFBC70  | c.182_182delT             | 29        | 3    | FS     | p.Leu61fs            | N          | BI+E   |
| M6      |                           |           |      |        |                      |            |        |
| MBC27   |                           |           |      |        |                      |            | FH     |
| CQ66    | c.3109C>T                 | 11        |      | NS     | p.Gln1037Ter         | Y          |        |
| NF23    |                           |           | 43   |        |                      |            | FH     |
| NF54    |                           |           | 44   |        |                      |            |        |
| NF98    |                           |           | 55   |        |                      |            |        |
| LHY027  | c.2806_2809delAAAC        | 43        | 11   | FS     | p.Lys936_Gln937Tfs   | Y          |        |
| MBC054  | c.5718_5719delCT          | 53        | 11   | FS     | p.Leu1908fs          | Y          |        |
| CQ7     | c.5959C>T                 | 53        | 11   | NS     | p.Gln1987Ter         | Y          | FH     |
| CQ22    | c.9400_9400delTg         | 66        | 25   | FS     | p.Gly3134fs          | N          | TNBC   |
| CQ145   |                           |           | 32   |        |                      |            |        |
| CQ69    | c.469_473delAAAGT         | 46        | 5    | FS     | p.Val159fs           | N          |        |
| CQ146   | c.304_304delA             | 58        | 3    | FS     | p.Leu103fs           | N          |        |
| SD49    | c.7480C>T                 | 50        | 15   | NS     | p.Arg2494Ter         | Y          | TNBC   |
| SD61    | c.3559G>T                 | 54        | 11   | NS     | p.Glu1187Ter         | N          | TNBC   |
| SD208   | c.8955_8956delA           | 32        | 23   | FS     | p.Ile2986fs          | N          | E      |
| GZ174   | c.8827C>T                 | 35        | 22   | NS     | p.Gln2943Ter         | E          |        |
| SD47    | c.464_468delGAGAT         | 25        | 5    | FS     | p.Arg155fs           | N          | FH+E   |
| SD68    | c.8517C>A                 | 31        | 20   | NS     | p.Tyr2839Ter         | N          | FH+E   |
| SD99    | c.5574_5577delAATT        | 59        | 11   | FS     | p.Ile1859fs          | N          |        |
| SD221   | c.3163_3166delAATT        | 36        | 11   | FS     | p.Glu2281fs          | N          |        |
| SD303   | c.5900_5901insG           | 52        | 11   | FS     | p.Ser1968fs          | N          |        |
| NF118   | c.8576_8576delA           | 27        | 20   | FS     | p.Lys2860fs          | N          | E      |
| ZJ30    | c.9317G>A                 | –         | 25   | NS     | p.Trp3106Ter         | Y          |        |
| ZI1212  | c.8951C>G                 | –         | 22   | NS     | p.Ser2984Ter         | Y          |        |
| ZI1776  | c.1301_1304delAAAG        | –         | 10   | FS     | p.Lys437fs           | Y          |        |
| ZJ4611  | c.6952C>T                 | –         | 13   | NS     | p.Arg2318Ter         | Y          |        |
| ZI7730  | c.5718_5721delCTCT        | –         | 11   | FS     | p.Ser1907fs          | Y          |        |
| ZI10024 | c.7562_7563delTc          | –         | 15   | FS     | p.Leu2523fs          | N          |        |

* FS – frameshift; NS – nonsense; * TNBC – triple negative breast cancer; FH – family history; BI – bilateral breast cancer; E – early-onset breast cancer.
cancer. The prevalence of the high-risk group was significantly higher than that of the low-risk group \( (p=0.001) \). Moreover, \( \text{BRCA1/2} \) mutations were determined to be significantly associated with early-onset breast cancer \( (p=0.014) \), and TNBC \( (p=0.001) \).

**\( \text{BRCA1} \) deleterious mutations**

Seventeen deleterious \( \text{BRCA1} \) mutations were detected in our cohort, including 10 frameshift mutations (Table 3). Six novel pathogenic mutations \( (\text{c.1299}_1\text{300insC, c.1934}_1\text{934delC, c.3352C>T, c.376C>T, c.3472G>T, and c.1012A>T}) \) were found, accounting for 35.29\% \( (6/17) \) of all mutations in the \( \text{BRCA1} \) gene. Eleven mutations were located in exon 11, and the majority \( (58.82\%) \) of the samples with mutations were from TNBC patients.

**\( \text{BRCA2} \) deleterious mutations**

Twenty-five deleterious \( \text{BRCA2} \) mutations were detected in our cohort (Table 4). Of these 25 mutations, 16 were frameshift mutations and the rest were nonsense mutations. Thirteen novel pathogenic mutations \( (\text{c.182}_1\text{82delT, c.9400}_1\text{9400delG, c.304}_1\text{304delA, c.3559G>T, c.8955}_1\text{8956insA, c.8827C>T, c.464}_1\text{468delGAGAT, c.8517C>A, c.5574}_1\text{5577delAATT, c.3163}_1\text{3166delAATT, c.5900}_1\text{5901insG, c.8576}_1\text{8576delA, and c.7562}_1\text{7563delTC}) \) were identified, accounting for 52\% \( (13/25) \) of all mutations in the \( \text{BRCA2} \) gene. Two recurrent mutations \( (\text{c.3109C>T and c.9400}_1\text{9400delG}) \) were detected in this cohort. \( \text{c.3109C>T} \) was found in 6 unrelated patients, whereas \( \text{c.9400}_1\text{9400delG} \) was found in 2 unrelated cases.

### Table 5. Spectrum of uncertain significant variants.

| Mutations     | Gene  | Exon | AA change | Clinically Important (BIC) | Functional Prediction | Conservation prediction |
|---------------|-------|------|-----------|---------------------------|----------------------|------------------------|
| c.5504G>A     | \( \text{BRCA1} \) | 24   | p.Arg1835Gln | Unknown                   | PD(0.994) D(0.04)     | 2.77 43                |
| c.80G>A       | \( \text{BRCA1} \) | 2    | p.Cys27Tyr  | –                         | PD(0.972) D(0.00)     | 1.98 194               |
| c.733G>T      | \( \text{BRCA1} \) | 11   | p.Asp245Tyr | –                         | PD(0.933) D(0.00)     | 1.32 160               |
| c.3448C>T     | \( \text{BRCA1} \) | 11   | p.Pro1150Ser| Unknown                   | PD(0.968) D(0.01)     | 2.76 74                |
| c.8702G>A     | \( \text{BRCA2} \) | 21   | p.Gly2901Asp| Unknown                   | PD(0.999) D(0.00)     | 2.47 94                |
| c.8574A>T     | \( \text{BRCA2} \) | 20   | p.Gln2858His| –                         | PD(0.996) D(0.01)     | -0.44 24               |
| c.7522G>A     | \( \text{BRCA2} \) | 15   | p.Gly2508Ser| Unknown                   | PD(1.00) D(0.00)      | 2.73 56                |
| c.7857G>C     | \( \text{BRCA2} \) | 17   | p.Trp2619Cys| –                         | PD(1.00) D(0.00)      | 2.83 215               |
| c.9104A>G     | \( \text{BRCA2} \) | 23   | p.Tyr3035Cys| Unknown                   | PD(0.99) D(0.00)      | 1.05 194               |

*PD – probably damaging; D – deleterious.*

### Variants of uncertain significance in \( \text{BRCA1/2} \) genes

According to the sequencing data from 18 000 normal Chinese females, 20 \( \text{BRCA1/2} \) missense mutations with a frequency higher than 1\% were discarded. Four benign mutations were discarded according to the BIC database. Finally, 9 VUSs were predicted to be pathogenic by PolyPhen-2 and SIFT. All of them were evaluated for evolutionary conservation with PhyloP and Grantham (Table 5). Four novel mutations in 9 VUSs have not been reported in the BIC database.

### Identification of deleterious \( \text{BRCA} \) mutations using Sanger sequencing

Excluding the insufficient samples, 40 deleterious \( \text{BRCA} \) mutations were identified by Sanger sequencing. All were true-positive mutations. Sanger sequencing chromatograms of \( \text{BRCA1} \), c.981_982delAT, c.1299_1300insC, and c.3472G>T are shown in Figure 1. The rest of the sequencing chromatograms are presented in the Supplementary Material.

### Discussion

\( \text{BRCA1} \) and \( \text{BRCA2} \) play important roles in the development of breast cancer. The prevalence of \( \text{BRCA1/2} \) mutations varies among different populations due to founder mutation effects and other environmental and geographical factors \( [16,17] \). Although genetic risk assessment and genetic testing for breast cancer have become standard clinical management for high-risk families and patients in many Western countries \( [18] \), studies of \( \text{BRCA} \)-associated breast cancer in China remain...
Figure 1. Sanger sequencing chromatograms. (A–C) Show BRCA1, c.981_982delAT; BRCA1, c.1299_1300insC and BRCA1, c.3472G>T, respectively. The variant position are indicated by arrows.
limited. With complex climate and geographical environment, it is necessary to study \( \text{BRCA1/2} \) mutations in Chinese populations comprehensively. The aim of this study was to use an NGS-based panel to detect \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations to assess the characteristics of \( \text{BRCA1/2} \) mutations in Chinese populations. This study is the first to perform \( \text{BRCA1/2} \) mutation screening in breast cancer patients in different provinces including southern, eastern, and southwestern China using NGS. Patients from northern China were not enrolled in this study due to the long distance and other factors. The 40 deleterious mutations detected by this panel were confirmed by Sanger sequencing, exhibiting high accuracy and meeting the requirements for genetic diagnosis of \( \text{BRCA} \)-associated breast cancer. Data analysis of 595 breast cancer patients revealed that the prevalence of \( \text{BRCA1/2} \) germline mutations in breast cancer patients in China is 8.07%, which is within the worldwide average of 5–10% [5]. This is slightly lower than in a previous report on a larger Chinese cohort [19], but is higher than in Japanese cohort [20] using NGS. In our Chinese high-risk group, the proportion of \( \text{BRCA} \) mutations was 11.33%, which was 2.78-fold higher than that in low-risk patients. This coincides with previous reports for white cohorts, in which the prevalence of \( \text{BRCA} \) mutations was 5–13% [21,22]. Notably, a family history, early-onset breast cancer, and TNBC were important high-risk factors according to our analysis.

In Western populations with a family history of breast cancer, the prevalence of \( \text{BRCA1/2} \) mutations is 7.1–26.3% and 13.0%, respectively [23,24]; while in our group, the prevalence was 3.85% and 11.54%, respectively. This result may be due to ethnic differences in breast cancer genomics and variations in the selection criteria for family history. Previous studies have suggested that \( \text{BRCA1} \) c.981_982delAT and \( \text{BRCA2} \) c.3109C>T are founder mutations in Asian populations [13,25,26]. In this study, c.3109C>T was identified in 6 unrelated patients, while 1 patient with c.981_982delAT was identified. Furthermore, we found another recurrent \( \text{BRCA2} \) mutation, c.9400_9400delG, in 2 unrelated cases. This mutation has never been reported in the BIC database and may therefore be a specific recurrent mutation in the Chinese population.

Unlike reports in which the \( \text{BRCA1} \) mutation is more common in eastern Chinese populations [14], we found that the prevalences of \( \text{BRCA1} \) and \( \text{BRCA2} \) mutations for breast cancer patients diagnosed before the age of 35 were 2.63% and 9.21%, respectively. These values may be influenced by the detection method used. Whether there is a relationship between bilateral breast cancer and the \( \text{BRCA4} \) mutation remains controversial. It has been reported that 17% of bilateral breast cancer patients harbor \( \text{BRCA1/2} \) mutations [27]. In this study, only 1 mutation (12.5%) was identified in 8 bilateral cases. Although this prevalence is much higher than that in non-bilateral cases, it is difficult to infer any relationship from a limited number of patients.

TNBC is an important high-risk factor for \( \text{BRCA} \) mutations [18], as the proportion of a \( \text{BRCA} \) mutation is 10–30% [28–30]. This estimate is consistent with our findings, in which 13 deleterious mutations were identified from 90 TNBC cases (14.4%). Interestingly, 76.92% (10/13) of mutations detected in the TNBC cases were in the \( \text{BRCA1} \) gene, indicating that \( \text{BRCA1} \) mutations are associated with TNBC, similar to the case in Ashkenazi patients [31] and patients in the Xinjiang region of China [18].

In this study, we discovered 48 pathogenic mutation cases in 595 breast cancer patients; 35.42% (17/48) of cases harbored \( \text{BRCA1} \) mutations, while the remainder of cases harbored \( \text{BRCA2} \) mutations. According to the BIC database, the pathogenic \( \text{BRCA1} \) mutations c.1961_1961delA and c.4222C>T have been described only in Western populations. This is the first report in a Chinese cohort. The c.1504_1508delTTAAA, c.1961_1961delA and c.4222C>T mutations are so rare in Asia that those identified are probably due to migration. The frameshift mutation c.3214_3214delC has been found only in Asian cohorts and may be specific to Asian populations. Seven mutations (c.1299_1300insC, c.1934_1934delC, c.3352C>T, c.3765C>T, c.3742G>T, c.1012A>T, and c.283_286delCTTG) have never been reported in the BIC database. Interestingly, c.283_286delCTTG has been reported in a study of ovarian cancer in Chinese women [32]. These 7 mutations may represent Chinese-specific \( \text{BRCA1} \) mutations. Moreover, the pathogenic \( \text{BRCA2} \) mutations c.2806_2809delAAAC, c.5718_5719delCT and c.5959C>T have been described primarily in Western populations, while c.3109C>T has been described primarily in Asian populations. This is the first report of mutations c.2806_2809delAAAC, c.5718_5719delCT and c.5959C>T have been described in Chinese populations. The detection of c.2806_2809delAAAC, c.5718_5719delCT and c.5959C>T in Asian populations and that of c.3109C>T in Western populations are probably due to migration. The mutations c.5164_5165delAG and c.9317G>A have been found only in Asian cohorts and may therefore be specific to Asian populations. Thirteen mutations (c.182_182delT, c.9400_9400delG, c.304_304delA, c.3559G>T, c.8955_8956insA, c.8827C>T, c.464_468delAGAT, c.8517C>A, c.5574_5577delAAAT, c.3163_3166delATC, c.5900_5901insG, c.8576_8576delA, and c.7562_7563delCT) have never been reported in the BIC database. Notably, c.469_473delAAATC was reported in a study of \( \text{BRCA1/2} \) mutations in Zhejiang, China [33]. Therefore, these 14 \( \text{BRCA2} \) mutations may be specific to the Chinese population.

In the functional analysis of the 78 VUSs, 9 mutations were predicted to be deleterious by PolyPhen-2 and SIFT. All of them were evaluated by PhyloP and Grantham for evolutionary conservation. According to the BIC database, the only reports of mutations c.5504G>A and c.8702G>A were in 2 Malaysians and 3 Asians, respectively. This suggests that these 2 mutations
may be specific to Asian populations. Moreover, mutations c.80G>A, c.733G>T, c.8574A>T and c.7857G>C were novel, as they have never been reported before. Notably, the BRCA1 protein harbors an N-terminal RING-finger domain characteristic of ubiquitin E3 ligases, which is associated with tumor suppression [34–36]. The mutation c.80G>A (Cys27Tyr) is located in the RING-finger domain and removes the second cysteine of the putative C3HC4 zinc-binding motif, which would be expected to have a significant effect on the DNA binding properties of the BRCA1 protein.

Unlike Western populations in which BRCA1 mutations are reportedly more frequent [37], we found a predominance of BRCA2 mutations (64.58%), which is similar to most Asian studies [38,39]. This likely indicates that the epidemiology and biology of Chinese populations differ from those in the West. Additionally, we discovered BRCA1/2 mutation “hot” regions in exon 11, containing more than half of the mutations in our cohort. This finding is a good indication for a cost-effective screening strategy.

References:

1. Chen W, Zheng R, Baade PD et al: Cancer statistics in China 2015. Cancer J Clin, 2016; 66: 115–32
2. Porter P: “Westernizing” women’s risks? Breast cancer in lower-income countries. N Engl J Med, 2008; 358: 213–16
3. Miki Y, Swensen J, Shattuck-Eidens D et al: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science, 1994; 266: 66–71
4. Wooster R, Bignell G, Lancaster J et al: Identification of the breast cancer susceptibility gene BRCA2. Nature, 1995; 378: 785–92
5. Claus EB, Schildkraut JM, Thompson WD, Risch NJ: The genetic attributable risk of breast and ovarian cancer. Cancer, 1996; 77: 2318–24
6. Kainer M, Silva-Arrieta S, FitzGerald MG et al: Differential contributions of the BRCA1 protein. Cancer, 2005; 103: 110: 99–109
7. Tutt A, Robson M, Garber JE et al: Oral poly(ADP-ribose) polymerase inhibition for BRCA1 carrier breast cancer. N Engl J Med, 2010; 363: 1298–307
8. Rebbeck TR, Friebel T, Lynch HT et al: Bilateral prophylactic mastectomy re
9. Hu Z, Wu J, Liu CH et al: The analysis of BRCA1 mutations in a population-based series of breast cancer cases. J Med Res, 2014; 19: 35
10. Chen W, Pan K, Ouyang T et al: Bilateral prophylactic mastectomy re
11. Chen W, Zheng R, Baade PD et al: Cancer statistics in China 2015. Cancer J Clin, 2016; 66: 115–32
12. Porter P: “Westernizing” women’s risks? Breast cancer in lower-income countries. N Engl J Med, 2008; 358: 213–16
13. Miki Y, Swensen J, Shattuck-Eidens D et al: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science, 1994; 266: 66–71
14. Wooster R, Bignell G, Lancaster J et al: Identification of the breast cancer susceptibility gene BRCA2. Nature, 1995; 378: 785–92
15. Bu RS, Raj A, Obaisi KA et al: Identification of novel BRCA1 founder mutations in Middle Eastern breast cancer patients using capture and Sanger sequencing analysis. Int J Cancer, 2016; 139: 1091–97
16. Easton DF, Bishop DT, Ford D, Crockford GP: Genetic linkage analysis in familial breast and ovarian cancer: Results from 214 families. The Breast Cancer Linkage Consortium. Am J Hum Genet, 1993; 52: 678–701
17. Murwida F, Takahashi F, Takahashi K: Meeting report: Current cancer perspectives from the 9th Annual Meeting of the Japanese Society of Medical Oncology. Thorac Cancer, 2012; 3: 94–97
18. Li Y, Ni D, Yang L et al: The prevalence of BRCA1/2 mutations of triple-negative breast cancer patients in Xinjiang multiple ethnic region of China. Eur J Med Res, 2014; 19: 35
19. Lang CT, Shi JX, Hu X et al: The spectrum of BRCA1 mutations and characteristics of BRCA-associated breast cancers in China: Screening of 2,991 patients and 1,043 controls by next-generation sequencing. Int J Cancer, 2017; 141: 129–42
20. Hirotsu Y, Nakagomi H, Sakamoto I et al: Detection of BRCA1 and BRCA2 germline mutations in Japanese population using next-generation sequencing. Mol Genet Genomic Med, 2015; 3: 121–29
21. Anglian Breast Cancer Study Group: Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Br J Cancer, 2000; 83: 1301–8
22. Liade A, Narod SA: Hereditary breast and ovarian cancer in Asia: Genetic epidemiology of BRCA1 and BRCA2. Hum Mutat, 2002; 20: 413–24
23. Malone KE, Daling JR, Thompson JD et al: Identification of germline mutations and breast cancer in the general population: analyses in women before age 35 years and in women before age 45 years with first-degree family history. JAMA, 1998; 279: 922–29
24. Leknda N, Miyoshi Y, Yoneda K et al: Frequency of BRCA1 and BRCA2 germline mutations in Japanese breast cancer families. Int J Cancer, 2001; 91: 83–88
25. Lee AS, Ho GH, Oh PC et al: Founder mutation in the BRCA1 gene in Malay breast cancer patients from Singapore. Hum Mutat, 2003; 22: 178
26. Li WF, Hu Z, Rao NY et al: The prevalence of BRCA1 and BRCA2 germline mutations in high-risk breast cancer patients of Chinese Han nationality: Two recurrent mutations were identified. Breast Cancer Res Treat, 2008; 110: 99–109
27. Gryzbowksa E, Sieminska M, Zientek H et al: Germline mutations in the BRCA1 gene predisposing to breast and ovarian cancers in Upper Silesia population. Acta Biochim Pol, 2002; 49: 351–56

Conclusions

In conclusion, using an NGS panel, we identified 42 deleterious mutations in 48 of 595 breast cancer patients in China and 9 probable pathogenic missense mutations. The prevalence of BRCA1/2 germline mutations was 8.07% in this cohort. Compared with white populations, Chinese women exhibit unique characteristics. Specifically, BRCA2 mutations are more common than BRCA1 mutations. Additionally, the 19 novel mutations may be specific to Chinese women, and the recurrent mutations c.3109C>T and c.9400_9400delG may be founder mutations in this population. Our findings suggest that breast cancer patients with a family history, TNBC, or early-onset breast cancer are good candidates for BRCA1/2 testing.

Conflicts of interest

None.
28. Evans DG, Howell A, Ward D et al: Prevalence of BRCA1 and BRCA2 mutations in triple negative breast cancer. J Med Genet, 2011; 48: 520–22
29. Comen E, Davids M, Kirchhoff T et al: Relative contributions of BRCA1 and BRCA2 mutations to ‘triple-negative’ breast cancer in Ashkenazi Women. Breast Canc Res Treat, 2011; 129: 185–90
30. Gonzalez-Angulo AM, Timms KM, Liu S et al: Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. Clin Cancer Res, 2011; 17: 1082–89
31. Comen E, Davids M, Kirchhoff T et al: Prevalence of BRCA1 and BRCA2 mutations in Jewish women with triple negative breast cancer. J Clin Oncol, 2008; 26(Suppl.): 22002
32. Shi TY, Wang P, Xie CX et al: BRCA1 and BRCA2 mutations in ovarian cancer patients from China: Ethnic-related mutations in BRCA1 associated with an increased risk of ovarian cancer. Int J Cancer, 2017; 140: 2051–59
33. Cao WM, Gao Y, Yang HJ, Wang XJ: Novel germline mutations and unclassified variants of BRCA1 and BRCA2 genes in Chinese women with familial breast/ovarian cancer. BMC Cancer, 2016; 16: 64
34. Wu W, Koike A, Takeshita T, Ohta T: The ubiquitin E3 ligase activity of BRCA1 and its biological functions. Cell Div, 2008; 3: 1
35. Pujana MA, Han JD, Starita LM et al: Network modeling links breast cancer susceptibility and centrosome dysfunction. Nat Genet, 2007; 39: 1338–49
36. Boulton SJ: Cellular functions of the BRCA tumour-suppressor proteins. Biochem Soc Trans, 2006; 34: 633–45
37. Malone KE, Daling JR, Doody DR et al: Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. Cancer Res, 2006; 66: 8297–308
38. Fukutomi T, Ushijima T, Inoue R et al: BRCA1 and BRCA2 germline mutations in Japanese with hereditary breast cancer families. Breast Cancer, 1997; 4: 256–58
39. Choi DH, Lee MH, Bale AE et al: Incidence of BRCA1 and BRCA2 mutations in young Korean breast cancer patients. J Clin Oncol, 2004; 22: 1638–45