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

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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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**

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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-penetrance 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,

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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	GAGCCACAGATAATACAAGAGCGT C	GCAGATTCTTTTTCGAGTGATTCTATTGGG	60
c.3333_3333del A	TTGAATGCTATGCTTAGATTAGGGG	GACGCTTTTGCTAAAAACAGCAG	60
c.5164_5165delAG	CAG CTA GCG GGA AAA AAG TTA	TTC GGA GAG ATG ATT TTT GTC	55
c.182_182delT	TGTCTGTCACTGGTTAAAACTAAG	TAGTTTGTAGTTCTCCCCAGTC	60
c.981_982delAT	AACACC ACT GAG AAG CGT GCAG	CTC ACA CAG GGG ATC AGC ATT C	60
c.3109C>T	TTT GGA GGT AGC TTC AGA AC	TTCTGCAATATGTAGCTTGG	50
c.2806_2809delAAAC	ATGGAAAAGAATCAAGATGTAT	CTT AAT GTT ATG TTC AGA GAG	55
c.5718_5719delCT	CCA TTA AAT TGT CCA TAT CTA	TCA AAT TCC TCT AAC ACT CC	55
c.5959C>T	CCA TTA AAT TGT CCA TAT CTA	TCA AAT TCC TCT AAC ACT CC	55
c.9400_9400delG	CTA TTT TGA TTT GCT TTT ATT ATT	GCT ATT TCC TTG ATA CTG GAC	55
c.1299_1300insC	GGT TCT GAT GAC TCA CAT GAT GGG	TCT GTG GCT CAG TAA CAA ATG CTC	60
c.469_473delAAGTC	TGT GTTGGCATT TTA AAC ATC A	CAG GGC AAA GGT ATA ACG CT	55
c.1934_1934delC	AGG CTG AGG AGG AAG TCT TCT ACC	CAG CTC TGG GAA AGT ATC GCT G	60
c.304_304delA	TGTCTGTCACTGGTTAAAACTAAG	TAGTTTGTAGTTCTCCCCAGTC	60
c.3214_3214delC	TCAATG TCA CCT GAA AGA GAA ATGG	CAG GAT GCT TAC AAT TAC TTC CAG G	60
c.5510G>A	ATG AAT TGACACTAA TCTCTG C	GTA GCC AGG ACA GTA GAA GGA	55
c.7480C>T	ATT TCA ATT TTA TTT TTG CT	ATG AAA TAA AAT TAC ACT CTG TC	50
c.3559G>T	AAGTGCCTGAAAACCAGATG	CAACAAAAGTGCCAGTAGTCA	58
c.8955_8956insA	ATC ACT TCT TCC ATT GCA TC	CCG TGG CTG GTA AAT CTG	55
c.1961_1961delA	AGG CTG AGG AGG AAG TCT TCT ACC	CAG CTC TGG GAA AGT ATC GCT G	60
c.3352C>T	TTG AAT GCT ATG CTT AGA TTA GGG G	GAC GCT TTT GCT AAA AAC AGC AG	60
c.8827C>T	TTT GTT GTA TTT GTC CTG TTT A	ATT TTG TTA GTA AGG TCA TTT TT	50
c.464_468delGAGAT	TGT GTT GGC ATT TTA AAC ATC A	CAG GGC AAA GGT ATA ACG CT	55
c.8517C>A	TGA ATG TTA TAT ATG TGA CTT TT	CTT GTT GCT ATT CTT TGT CTA	52
c.5574_5577delAATT	CCA TTA AAT TGT CCA TAT CTA	TCA AAT TCC TCT AAC ACT CC	55
c.376C>T	CACAACAAAGAGCATACATAGG	AGAAGAAGAAGAAAACAAATGG	55
c.3163_3166delAATC	TTT GGA GGT AGC TTC AGA AC	TTC TGC AAT ATG TAG CTT GG	50
c.5353C>T	TCC CAT TGA GAG GTC TTG CT	GAG AAG ACT TCT GAG GCT AC	55
c.5900_5901insG	CCA TTA AAT TGT CCA TAT CTA	TCA AAT TCC TCT AAC ACT CC	55
c.3472G>T	TTG AAT GCT ATG CTT AGA TTA GGG G	GAC GCT TTT GCT AAA AAC AGC AG	60
c.1012A>T	CAA CAT AAC AGA TGG GCT GGA AG	ACG TCC AAT ACA TCA GCT ACT TTG G	60
c.8576_8576delA	TGA ATG TTA TAT ATG TGA CTT TT	CTT GTT GCT ATT CTT TGT CTA	52
c.4222C>T	AAT GGA AAG CTT CTC AAA GTA	ATG TTG GAG CTA GGT CCT TAC	55
c.1439_1440insA	GAA AAC CTA TCG GAA GAA GGC AAG	TCA TCA CTT GAC CAT TCT GCT CC	60
c.283_286delCTTG	CTT ATT TTA GTG TCC TTA AAA GG	TTT CAT GGA CAG CAC TTG AGT G	55
c.5521_5521delA	CAG AGC AAG ACC CTG TCT C	ACT GTG CTA CTC AAG CAC CA	57
c.9317G>A	CTA TTT TGA TTT GCT TTT ATT ATT	GCT ATT TCC TTG ATA CTG GAC	55
c.8951C>G	TTT GTT GTA TTT GTC CTG TTT A	ATT TTG TTA GTA AGG TCA TTT TT	50
c.1301_1304delAAAG	AAC AGT TGT AGA TAC CTC TGA A	GAC TTT TTG ATA CCC TGA AAT G	55
c.6952C>T	TTT ATG CTG ATT TCT GTT GTA T	ATA AAA CGG GAA GTG TTA ACT	50
c.5718_5721delCTCT	CCA TTA AAT TGT CCA TAT CTA	TCA AAT TCC TCT AAC ACT CC	55
c.7562_7563delTC	ATT TCA ATT TTA TTT TTG CT	ATG AAA TAA AAT TAC ACT CTG TC	50

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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. Grantham reflects greater evolutionary distance with a higher score, which is considered to be more deleterious.

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

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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	3	13 (14.44%)	*P*=0.001
No	461	3	20	23 (4.99%)	
Bilateral					
Yes	8	-	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.Leu502Ala fs	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.Ser645fs	N	FH+TNBC
NF48	c.3214_3214delC	52	11	FS	p.Leu1072fs	Y	
SD33	c.5510G>A	46	24	NS	p.Trp1837Ter	Y	TNBC
GZ54	c.1961_1961delA	61	11	FS	p.Lys654fs	Y	
GZ63	c.3352C>T	49	11	NS	p.Gln1118Ter	N	TNBC
SD136	c.376C>T	32	7	NS	p.Gln126Ter	N	FH+E+TNBC
SD257	c.5353C>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.Lys338Ter	N	
ZJ116	c.4222C>T	–	13	NS	p.Gln1408Ter	Y	
ZJ123	c.1439_1440insA	–	11	FS	p.Asn480fs	Y	
ZJ1760	c.283_286delCTTG	–	6	FS	p.Leu95fs	N	
ZJ10040	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.

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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	c.3109C>T	49	11	NS	p.Gln1037Ter	Y	
MBC27		42					FH
CQ66		43					FH
NF23		42					
NF54		44					
NF98		55					
LHY027	c.2806_2809delAAAC	43	11	FS	p.Lys936_Gln937?fs	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_9400delG	66	25	FS	p.Gly3134fs	N	TNBC
CQ145		32					
CQ69	c.469_473delAAGTC	46	5	FS	p.Val159fs	N	
CQ116	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_8956insA	32	23	FS	p.Ile2986fs	N	E
GZ174	c.8827C>T	35	22	NS	p.Gln2943Ter	N	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_3166delAATC	36	11	FS	p.Gly2281fs	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	
ZJ1212	c.8951C>G	–	22	NS	p.Ser2984Ter	Y	
ZJ1776	c.1301_1304delAAAG	–	10	FS	p.Lys437fs	Y	
ZJ4611	c.6952C>T	–	13	NS	p.Arg2318Ter	Y	
ZJ7730	c.5718_5721delCTCT	–	11	FS	p.Ser1907fs	Y	
ZJ10024	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.

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Table 5. Spectrum of uncertain significant variants.

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

* PD – probably damaging; # D – deleterious.

cancer. The prevalence of the high-risk group was significantly higher than that of the low-risk group (p=0.001). Moreover, *BRCA1/2* mutations were determined to be significantly associated with early-onset breast cancer (p=0.01), a family history (p=0.014), and TNBC (p=0.001).

BRCA1 deleterious mutations

Seventeen deleterious *BRCA1* mutations were detected in our cohort, including 10 frameshift mutations (Table 3). Six novel pathogenic mutations (c.1299_1300insC, c.1934_1934delC, 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 *BRCA1* gene. Eleven mutations were located in exon 11, and the majority (58.82%) of the samples with mutations were from TNBC patients.

BRCA2 deleterious mutations

Twenty-five deleterious *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 (c.182_182delT, c.9400_9400delG, c.304_304delA, c.3559G>T, c.8955_8956insA, c.8827C>T, c.464_468delGAGAT, c.8517C>A, c.5574_5577delAATT, c.3163_3166delAATC, c.5900_5901insG, c.8576_ 8576delA, and c.7562_7563delTC) were identified, accounting for 52% (13/25) of all mutations in the *BRCA2* gene. Two recurrent mutations (c.3109C>T and c.9400_9400delG) were detected in this cohort. c.3109C>T was found in 6 unrelated patients, whereas c.9400_9400delG was found in 2 unrelated cases.

Variants of uncertain significance in *BRCA1/2* genes

According to the sequencing data from 18 000 normal Chinese females, 20 *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 *BRCA* mutations using Sanger sequencing

Excluding the insufficient samples, 40 deleterious *BRCA* mutations were identified by Sanger sequencing. All were true-positive mutations. Sanger sequencing chromatograms of 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

BRCA1 and *BRCA2* play important roles in the development of breast cancer. The prevalence of *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 *BRCA*-associated breast cancer in China remain

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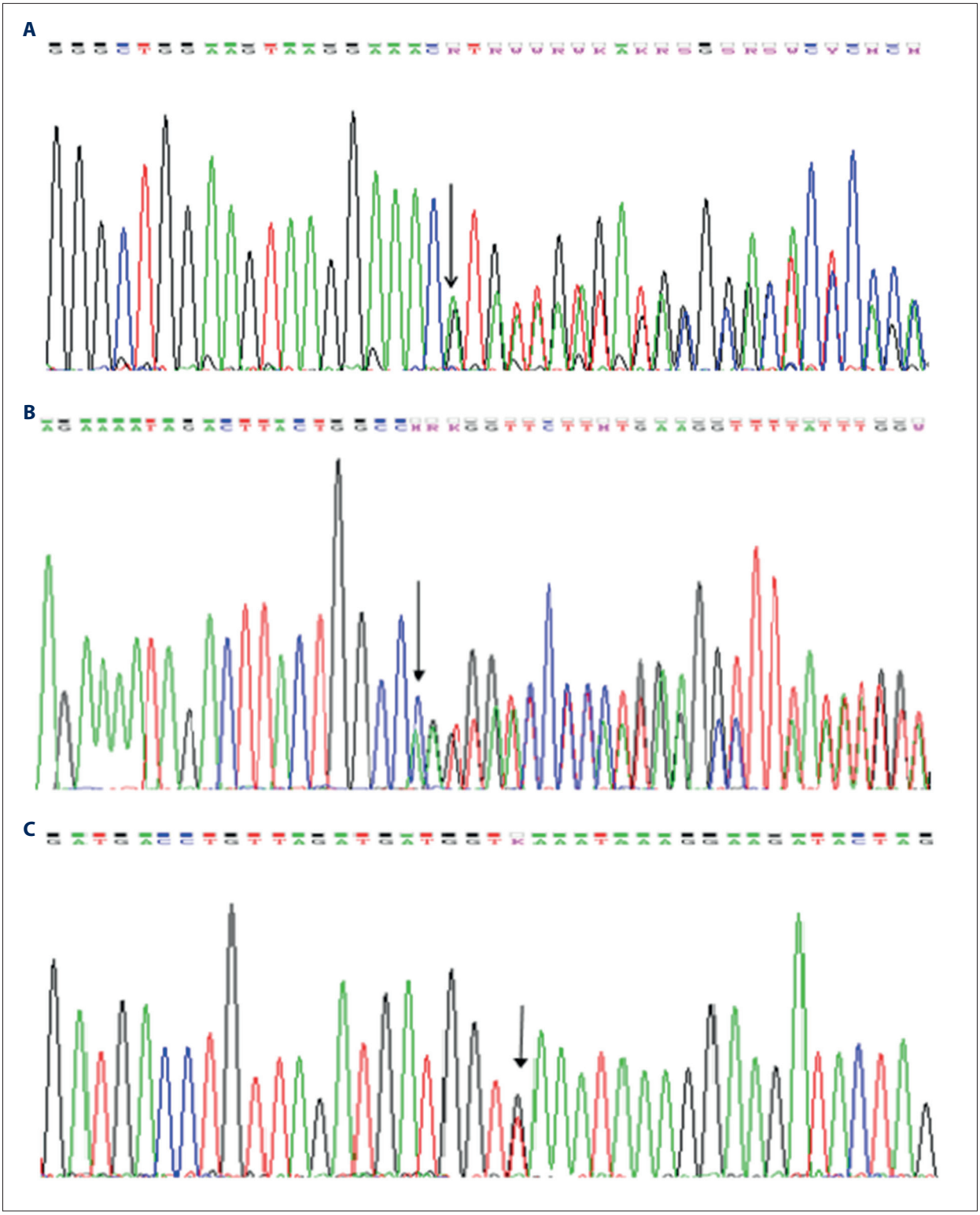

Figure 1. Sanger sequencing chromatograms. (**A–C**) Show *BRCA1*, c.981_982delAT; BRCA1, c.1299_1300insC and BRCA1, c.3472G>T, respectively. The variant position are indicated by arrows.

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limited. With complex climate and geographical environment, it is necessary to study *BRCA1/2* mutations in Chinese populations comprehensively. The aim of this study was to use an NGS-based panel to detect *BRCA1* and *BRCA2* mutations to assess the characteristics of *BRCA1/2* mutations in Chinese populations. This study is the first to perform *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 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 *BRCA*-associated breast cancer. Data analysis of 595 breast cancer patients revealed that the prevalence of *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 *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 *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 *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 *BRCA1* c.981_982delAT and *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 *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 *BRCA1* mutation is more common in eastern Chinese populations [14], we found that the prevalences of *BRCA1* and *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 *BRCA* mutation remains controversial. It has been reported that 17% of bilateral breast cancer patients harbor *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 *BRCA* mutations [18], as the proportion of a *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 (14.44%). Interestingly, 76.92% (10/13) of mutations detected in the TNBC cases were in the *BRCA1* gene, indicating that *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 *BRCA1* mutations, while the remainder of cases harbored *BRCA2* mutations. According to the BIC database, the pathogenic *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.376C>T, c.3472G>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 *BRCA1* mutations. Moreover, the pathogenic *BRCA2* mutations c.2806_2809del AAAC, 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, c.5959C>T, c.8951C>G, c.1301_1304del AAAG, and c.5718_5721delCTCT in a Chinese population. The detection of c.2806_2809del AAAC, 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_468delGAGAT, c.8517C>A, c.5574_5577delAATT, c.3163_3166delAATC, c.5900_5901insG, c.8576_ 8576delA, and c.7562_7563delTC) have never been reported in the BIC database. Notably, c.469_473delAAGTC was reported in a study of *BRCA1/2* mutations in Zhejiang, China [33]. Therefore, these 14 *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

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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.

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.

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