

Characterization of mutations in BRCA1/2 and the relationship with clinic-pathological features of breast cancer in a hereditarily high-risk sample of chinese population

MIN FANG^{1*}, LI ZHU^{2*}, HENGYU LI¹, XIZHOU LI¹, YANMEI WU¹,
KAINAN WU¹, JIAN LIN¹, YUAN SHENG¹ and YUE YU¹

¹Department of Breast and Thyroid Surgery, Changhai Hospital, The Second Military Medical University, Shanghai 200433;

²Department of General Surgery, Chinese People's Liberation Army General Hospital, Beijing 100853, P.R. China

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Abstract. The database of BRCA1/2 mutations in Chinese population remains incomplete at present. Therefore, the present study aimed to report specific harmful BRCA1/2 mutations in the Chinese population and discuss the clinic-pathological features in mutation carriers. BRCA1/2 germline mutation tests for 71 patients with breast cancer from a hereditarily high-risk Chinese population were performed using next-generation sequencing for identification of deleterious mutations. Furthermore, the clinicopathological features between BRCA1/2 mutation carriers and non-carriers were compared. A total of 13/71 (18.3%) patients carried a BRCA1 or BRCA2 mutation (7 BRCA1 and 6 BRCA2). The incidence of BRCA1/2 mutation in patients with bilateral breast cancer and patients with family history were 25, and 32.2%, respectively. Eleven pathogenic or likely pathogenic mutations were identified in 13 patients, among the mutation sites 7 were never reported before in Asian populations. The age at diagnosis of BRCA1/2 mutation carriers was older compared with non-mutation carriers (44.73 vs. 35.39 years; $P=0.001$) in this cohort. BRCA1/2 deleterious mutation carriers had a significantly lower chance of human epidermal growth factor receptor-2 (Her-2) positive status ($P=0.010$), higher tumor grade at diagnosis ($P=0.009$), higher probability to have a family history ($P=0.016$) and older age at diagnosis. Estrogen receptor (ER) and progesterone receptor

(PR) status were significantly different between BRCA1, and BRCA2 mutation carriers ($P=0.007$). The current interpretation of BRCA1/2 status can only explain a small part of hereditary high-risk breast cancer. However, BRCA1/2 gene testing should still be recommended for women with a family history of breast cancer, as well as patients with breast cancer with specific pathologic types, which may be useful to make appropriate clinical decisions for treatment and prevention.

Introduction

BRCA mutations occur frequently in breast cancer, which is a disease with considerable incidence in the Chinese population. Approximately 90% of hereditary breast cancers, which accounts for 5-10% of breast cancer, is related to BRCA1/2 mutations (1,2). BRCA1 and BRCA2 are located on 17q21 and 13q12, respectively, and have been widely accepted as the most important tumor suppressor genes associated with breast cancer (3-5).

Next-generation sequencing (NGS) technology offers a better choice over conventional BRCA1/2 mutation screening by providing additional information of non-coding regions and producing accurate variant results (6,7). The targeted sequencing strategy for both BRCA1 and BRCA2 frequently used currently are promising for characterizing BRCA1/2 mutation in a large population due to the decrease of costs in recent years.

Mutations of the BRCA genes and their associations with clinic-pathological features were reported in several studies (8-11). However, the status of BRCA1/2 mutation in Chinese population, including the incidence of gene mutation, founder mutation and clinic-pathological characteristics, still remains uncertain. In addition, breast cancer cluster regions (BCCRs) have recently been identified in both BRCA1 and BRCA2 based on large sample sets (12). BCCRs were considered to be associated with an increased likelihood of breast cancer compared to ovarian cancer (12). However, the correlation between the mutation locus of BRCA1/2 and tumor characteristics in the Chinese population remains to be obscure.

Correspondence to: Dr Yue Yu or Professor Yuan Sheng, Department of Breast and Thyroid Surgery, Changhai Hospital, The Second Military Medical University, 168 Changhai Road, Shanghai 200433, P.R. China
E-mail: dr_yuyue@163.com
E-mail: sshengyuan@163.com

*Contributed equally

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In this study, we performed gene testing in 71 hereditarily high-risk breast cancer patients, aiming at reporting the specific BRCA1/2 mutation patterns in Chinese population and discovering the clinic-pathological features of BRCA1/2-related breast cancer.

Materials and methods

Inclusion criteria. In this study, patients that were pathologically diagnosed with breast cancer and treated in Changhai Hospital Affiliated to Second Military Medical University between May 2015 and May 2016 were screened for BRCA1/2 germline mutation. The present study was approved by Shanghai Changhai Hospital Ethics Committee.

The inclusion criteria were: i) Patients diagnosed with breast cancer before the age of 50, with at least one first- or second-degree relatives diagnosed with breast cancer before the age of 50 or ovarian cancer at any age; or ii) patients diagnosed with bilateral breast cancers and the first diagnosis was made at age no more than 50; or iii) patients diagnosed with breast cancer at any age with at least two first- or second-degree relatives diagnosed with breast cancer or ovarian cancer at any age; or iv) patients diagnosed with breast cancer before age of 35; or v) patients with one or more first- or second-degree relatives diagnosed with male breast cancer; or vi) patients with both breast cancer and ovarian cancer.

Clinical and pathological data including age, sex, tumor type, lymph node status, immuno-histological characteristics and the family history of breast cancer were collected through medical records or telephone interviews.

Gene testing. For each patient, whole blood sample of 5 ml was extracted, and DNA was isolated from mononuclear blood cells using Qiagen DNeasy Blood & Tissue kit according to the manufacturer's instruction.

The DNA library was prepared according to the Illumina standard procedure: 1 μ g of genomic DNA was digested with a Biorupter contactless automatic ultrasonic disrupter (Diagenod) to 200-bp fragments, which were then amplified using KAPA HiFi DNA Polymerase after gene modification. The concentration of the sample was measured by Qubit 3.0 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and the size of the insert fragment was tested by Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA).

The exon library was prepared according to the Roche NimbleGen SeqCap EZ Choice standard procedure: Pooling hybridization of 12 to 16 DNA fragments was done, followed by target area capture, and the target fragment was enriched by LM-PCR using 1X KAPA HiFi Hot Start Ready Mix. The size of the insert fragment was detected by Agilent 2100 Bioanalyzer and the quantification of the library was tested by qPCR. Finally, PE100 sequencing was performed with the Illumina HiSeq 2500.

Mutation classification. Carriers with pathogenic/likely pathogenic BRCA1/2 mutations were analyzed in this study as deleterious mutations. Pathogenic mutations were defined as: i) Nonsense mutations generating a premature

termination codon; ii) large frame deletions; and iii) mutations in the transcription regulatory regions that are expected to influence the expression of mutant allele. In addition, mutations that were considered as pathogenic/likely pathogenic by the Breast Cancer Information Core Committee or that were classified as pathogenic/likely pathogenic by published evidence were also included. During analysis of clinic-pathological features among different BCCRs, people with nonsynonymous single nucleotide mutations were also enrolled.

Statistical analysis. All statistical analyses were performed with SPSS 22.0 software (IBM SPSS, Armonk, NY, USA). Differences between groups in categorical data were analyzed with Chi-square test with continuity correction or Fisher's exact test. Continuous variables were analyzed with t-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

In this study, BRCA1/2 germline mutations were tested in 71 hereditarily high-risk Chinese population with breast cancer, including 8 patients (11.3%) diagnosed with bilateral breast cancer. Deleterious BRCA1/2 mutation was found in 13 patients (18.3%), among which 7 had BRCA1 mutations and 6 had BRCA2 mutations. Furthermore, the incidence of BRCA1/2 mutation in patients with bilateral breast cancer and patients with family history were 25 and 32.2%, respectively. There were two duplicate mutations on BRCA1 and BRCA2, both from the same family. Frameshift mutation (8/13) was the predominant type, followed by splice site mutation (2/13), nonsense mutation (2/13) and nonsense mutation (1/13). Complementary DNA position and resulting amino acid change of each mutation can be found in Fig. 1. Clinical and pathological characteristics of BRCA1/2 mutation carries were listed in Table I.

In this study, we investigated the correlation of clinic-pathological features of breast cancer between BRCA1/2 mutation carriers and non-carriers (Table II) and between BRCA1 and BRCA2 mutations carriers as well (Table III). We also identified the difference in tumor features among people with mutations in different BCCRs (In BRCA1, BCCRs are located at c.179 to c.505, c.4328 to c.4945, c.5261 to c.5563, respectively; while in BRCA2, BCCRs are located at c.1 to c.596, c.772 to c.1806, c.7394 to c.8904, respectively) (12). We have also detected novel BCCRs that were not reported previously, which would influence the patient's age at diagnosis of breast cancer.

The clinical and demographic features of enrolled patients were listed in Table II. The average patient age at diagnosis was older in BRCA1/2 carriers than non-carriers (44.73 vs. 35.39 years, $P = 0.001$). No difference was found between two groups in terms of tumor size and lymph node status. However, the tumor grade at diagnosis of BRCA1/2 carriers was much higher than non-carriers ($P = 0.009$). BRCA1/2 carriers were more likely to have a family history than non-carriers (66.7% vs. 32.8%, $P = 0.016$). In addition, no correlation between mutation locus and clinic-pathological features nor novel BCCRs were found.

Table I. Clinical and pathological characteristics of BRCA1/2 mutation carriers.

Gene	Mutation	Protein expression	Clinical manifestations (age)	Family history
BRCA1	c.3442delG	E1148fs	BC, 52 years; OC, 42 years	Daughter, BC, 34 years
	c.3442delG	E1148fs	BC, 34 years	Mother, BC, 52 years; Mother, OC, 42 years
	c.485_486del ^a	V162fs	BC, 35 years	No family history
	c.212G>A	R71K	BC, 44 years	Mother, OC, 54 years; M aunt, OC, 50 years
	c.4676-1G>T ^a	E1559_Splice	BC, 34 years	Mother, BC, 50 years
	c.5278-1G>C ^a	I1760_Splice	BBC, 44 and 49 years	No family history
	c.3626T>G ^a	L1209X	BBC, 42 and 49 years	No family history
BRCA2	c.5753delA ^a	H1918fs	BC, 59 years	Sister, BC, 45 years; M cousin, BC, 37 years
	c.5753delA ^a	H1918fs	BC, 37 years	Mother, BC, 45 years; M aunt, BC, 59 years
	c.8400_8402delTT TinsAAAA	2800_2801del	BC, 34 years	No family history
	c.3883C>T ^a	Q1295X	BC, 53 years; OC, 58 years	No family history
	c.5495delC ^a	S1832fs	BC, 75 years	Sister, BC, 70 years
	c.2806_2809delAAAC	K936fs	BC, 30 years	No family history

BC, breast cancer; BBC, bilateral breast cancer; OC, ovarian cancer; M, maternal. ^aNot previously reported in Chinese population with BRCA-associated breast cancer.

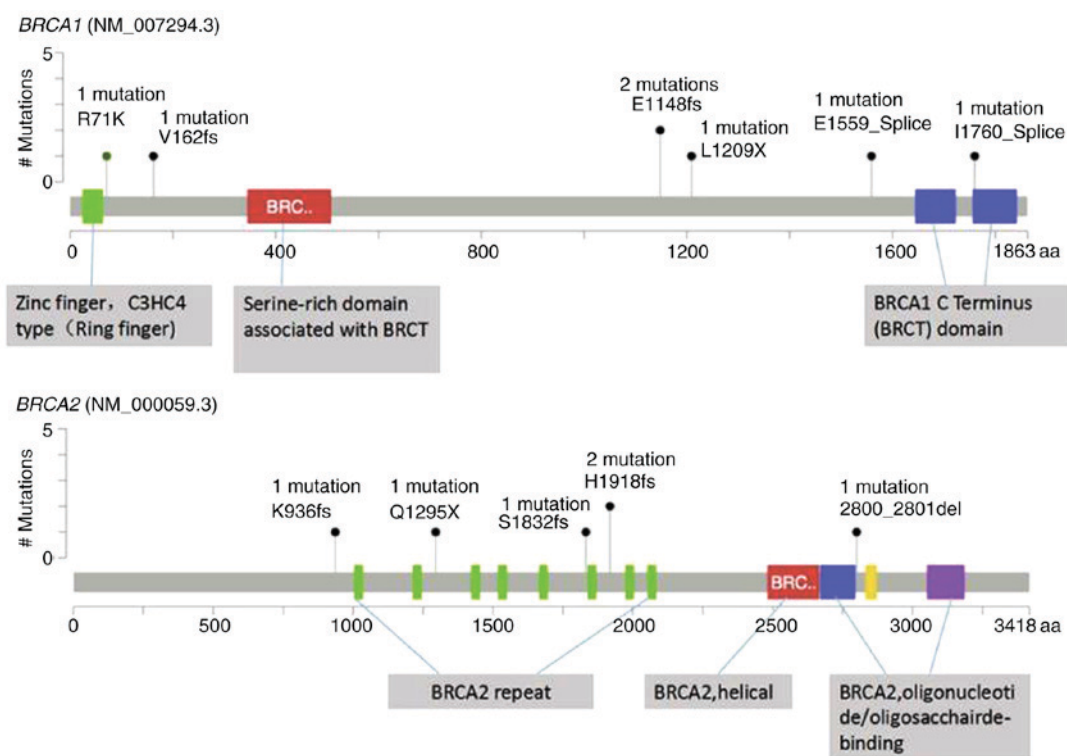


Figure 1. Germline pathogenic/likely pathogenic mutations in BRCA1/2. The total number of mutations identified and resulting acid change of each mutation is presented by a lollipop plot. BRCT, BRCA1 C-terminal domain.

Histologically, the most common type of breast cancer in both group was infiltrating ductal carcinoma (86.7 vs. 89.0%, $P=1.000$). However, there is no difference in histological type between two groups. Positive Her2 status was less frequently seen in BRCA1/2 carriers than non-carriers (6.7 vs. 42.2%, $P=0.010$). Similarly, no difference in ER and PR status was found between

groups. Triple negative (ER, PR and Her2 negative) ratio was mildly higher in BRCA1/2 carriers (46.7 vs. 20.3%, $P=0.075$).

The correlation between ER, PR and Her2 positivity and BRCA1/2 mutation status was analyzed in Table III with Fisher's exact test. ER and PR positivity was quite different between BRCA1 and BRCA2 mutation carriers (22.2

Table II. Correlation of clinicopathological features of breast cancer between BRCA1/2 mutation carriers and non-carriers.

Characteristic	BRCA1/2 mutation		P-value	OR (95% CI)
	Carriers (N=15)	Non-carriers (N=64)		
Age at diagnosis (years) (mean + SD)/(n, %)	44.7±12.0	35.4±8.6	0.023	0.138 (0.027-0.701)
≤50	11 (73.3)	61 (95.3)		
>50	4 (26.7)	3 (4.7)		
Age at menarche (years) (mean + SD)	14.9±2.2	14.6±1.6	0.542 ^b	
Age at first pregnancy (years) (mean + SD)	25.0±3.1	24.8±3.1	0.399 ^b	
BMI (kg/m ²) ^a (n, %)			0.912	1.382 (0.338-5.653)
≤25	10 (76.9)	41 (70.7)		
>25	3 (23.1)	17 (29.3)		
Family history (n, %)			0.016	4.095 (1.241-13.510)
Yes	10 (66.7)	21 (32.8)		
No	5 (33.3)	43 (67.2)		
Tumor localization ^a (n, %)			0.973	0.635 (0.113-3.571)
Unilateral	11 (84.6)	52 (89.7)		
Bilateral	2 (15.4)	6 (10.3)		
Histological type (n, %)				
Ductal	13 (86.7)	57 (89.0)	1.000	0.798 (0.148-4.296)
Lobular	1 (6.7)	1 (1.6)	0.826	4.500 (0.265-76.381)
Other	1 (6.7)	6 (9.4)	1.000	0.690 (0.077-6.207)
Grade at diagnosis (n, %)			0.009	0.182 (0.054-0.606)
I-II	7 (46.7)	53 (82.8)		
III	8 (53.3)	11 (17.2)		
Tumor size (n, %)				
T1	9 (60)	37 (57.8)	1.000	1.095 (0.384-3.443)
T2	6 (40)	20 (31.3)	0.731	1.467 (0.460-4.680)
T3	0 (0)	7 (10.9)	0.403	-
Lymph nodes status (n, %)			0.763	0.833 (0.254-2.731)
+	5 (33.3)	24 (37.5)		
-	10 (66.7)	40 (62.5)		
ER (n, %)			0.753	0.834 (0.270-2.580)
+	8 (53.3)	37 (57.8)		
-	7 (46.7)	27 (42.2)		
PR (n, %)			0.902	-
+	8 (53.3)	33 (51.6)		
-	7 (46.7)	31 (48.4)		
Her2 (n, %)			0.010	0.098 (0.012-0.790)
+	1 (6.7)	27 (42.2)		
-	14 (93.3)	37 (57.8)		
Triple negative (n, %)			0.075	3.433 (1.052-11.206)
Yes	7 (46.7)	13 (20.3)		
No	8 (53.3)	51 (79.7)		

^aThe statistics were cases of patients; ^bt-test; +, positive; -, negative. BRCA, breast cancer susceptibility gene; BMI, body mass index; ER, estrogen receptor; PR, progesterone receptor; OR, odds ratio; CI, confidence interval; SD, standard deviation.

vs. 100.0%, P=0.007). Furthermore, ER and PR statuses were identical in each patient between two groups. On the other

hand, Her2 positivity status was similar between two groups (11.1 vs. 0.0%, P=1.000).

Table III. Correlation between ER, PR and Her2 positivity and BRCA1/2 mutation status.

Characteristic	Mutation carriers		P-value
	BRCA1 n=9	BRCA2 n=6	
ER (n, %)			0.007
+	2 (22.2)	6 (100.0)	
-	7 (77.8)	0 (0)	
PR (n, %)			0.007
+	2 (22.2)	6 (100.0)	
-	7 (77.8)	0 (0)	
Her2 (n, %)			1.000
+	1 (11.1)	0 (0)	
-	8 (88.9)	6 (100.0)	

+, positive; -, negative; BRCA, breast cancer susceptibility gene; ER, estrogen receptor; PR, progesterone receptor; OR, odds ratio; CI, confidence interval.

Discussion

Mutations in several genes have been proved to be correlated with the pathogenesis of breast cancer, including p53, PTEN, CDH1, STK11, MLH1, MSH2, MSH6, PMS2, BRCA1 and BRCA2 (13). Many studies have reported the assessment and management of familial breast cancer risk based on family history or high-risk breast cancer susceptibility alleles (14). BRCA1 and BRCA2 are considered to be the two major tumor suppressor genes that are most closely related to familial breast cancer (15). In this study, pathogenic/likely pathogenic mutations of BRCA1/2 were identified and differences in clinic-pathological features of breast cancer between BRCA1/2 mutation carriers and non-carriers were analyzed in hereditarily high-risk Chinese patients. The association between BRCA1 and BRCA2 mutations was also studied.

In this study, a total of 11 deleterious BRCA1/2 mutations were found in 13 patients (there are two repeat mutation sites: BRCA1: c.3442delG and BRCA2: c.5753delA), among which 6 mutations were located on BRCA1 and 5 on BRCA2 loci. Four (BRCA1: c.3442delG and c.212G>A; BRCA2: c.8400_8402del3ins4 and c.2806_2809delAAAC) out of the 11 mutations have been reported in Asian populations (16-19). The other 7 (63.6%) mutations identified in this study, i.e., BRCA1: c.485_486del; c.4676-1G>T; c.5278-1G>T and c.3626T>G; BRCA2: c.5753delA; c.3883C>T and c.5495delC, have not been reported in any Asian population previously. Two recent studies on Chinese population with large sample numbers have reported the rates of novel BRCA1/2 mutations to be 41.4 and 40.0%, respectively (19,20). This indicates that the spectrum of BRCA1/2 mutation in the Chinese population is quite different from that in the Western population, although the database of BRCA1/2 mutation in Chinese population is not yet complete. Two of the eight newly identified mutations (BRCA1: c.3626T>G and BRCA2: c.3883C>T) were nonsense mutations, which result in a premature stop codon

and consequently a truncated, incomplete, and usually non-functional protein product.

Up until now, most data of BRCA1/2 mutations associated with high risk for hereditary breast cancer were derived from non-Asian cohorts (21). Some pilot studies also reported the unique pattern of BRCA1/2 mutations for hereditary breast cancer patients in China (18,22). Thus, it is of high necessity to profile large Chinese hereditary breast cancer cohorts so as to accurately describe the Chinese-specific variants. Our study demonstrated an efficient approach of characterizing mutations in hereditary breast cancer using NGS in a small number of Chinese patients.

BRCA1/2 mutation rate varies widely in different populations. As is reported in a study including 5,931 Chinese women with breast cancer, the mutation rate was 16.9% in familial breast cancers, 5.2% in early-onset breast cancers, and 2.0% in sporadic breast cancers, respectively (19). In another study based on NGS analyses, the mutation rate was 0.38% in healthy Chinese controls (20). In our study, the mutation rate was 18.3% in the hereditary high-risk patients, which was significantly higher than that in sporadic breast cancer group. It implicates that people with hereditary high risk are more likely to carry BRCA1/2 mutation. However, a large proportion of patients with familial breast cancer did not present specific harmful mutations in BRCA1/2. This may be attributed to various reasons: Firstly, the current NGS cannot fully identify the functions of variants of uncertain significance (VUS), single nucleotide polymorphism (SNP) and the interaction between multi-genes during the pathogenesis of hereditary breast cancer; Secondly, in addition to BRCA1/2, many other genes are associated with the onset of breast cancer. The BRCA1/2 mutation-based risk management depends a lot on the accurate interpretation of the specific mutation detected. In the clinical context, VUS in BRCA2 was more difficult to identify compared to that in BRCA1 (23,24). All of the 6 BRCA1 mutations detected in our study were identified as pathogenic while only 1 in 5 BRCA2 mutations was filtered as likely pathogenic according to ClinVar and BRCA mutation database. One feasible way to reduce the number of VUS would be integrating more sequencing data from different clinical centers into the public databases.

Previous studies have demonstrated that family history, age at diagnosis and race are predicting factors for the probability of an individual to carry a BRCA1/2 germline mutation. It is reported that there is a 45-80% lifetime breast cancer risk in BRCA1/2 mutation carriers (25). In fact, for women carrying BRCA mutations, the risk of developing breast and ovarian cancer increases by 10-15% annually after the age of 40 (26). The median ages at diagnosis of breast cancer in BRCA1 and BRCA2 mutation carriers are 39.9 and 42.8 years, respectively (12). In other words, BRCA1/2 gene mutations have a limited effect on the pathogenesis of early-onset breast cancer (diagnosed at and before the age of 35). In our study, we found that patient with a younger age at diagnosis was more likely to be a BRCA1/2 non-carrier rather than a carrier (44.73 vs. 35.39 years, P=0.001). This may be partly explained by the fact that patients selected in this study were in a hereditarily high-risk population with a relatively young age. Therefore, women with specific characteristics should be recommended for a genetic screening. BRCA1/2 mutation carriers identified

may thereby benefit from lifestyle modification, intensive screening, chemoprevention or risk-reducing surgery (27).

The association between BRCA and ER, PR and Her2 status remains complicated and controversial. Previous studies have shown that the BRCA1 gene can inhibit the transcriptional activity of ERs in human breast and prostate cancer cell lines (28). As a result, nearly 70% of BRCA1-associated breast cancer have negative ER expression whereas BRCA2-associated cancers are mainly ER positive (8,29). Sanford *et al* reported that the incidence of BRCA1/2 mutation is similar in patients with hormone receptor (HR)-positive breast cancer and Her2-negative breast cancer (30). BRCA1-related breast cancer is known to have different clinicopathological features from non-BRCA1-related cancer in several studies. Most BRCA1-related breast cancers have low expression of ER, PR and Her2, hence there is a much higher rate of Triple negative breast cancer (TNBC) (31,32). On the other hand, the immunophenotype of BRCA2-related breast cancer is often luminal with overexpression of ER and PR, which is similar to non-BRCA cancer or sporadic cancers (33). As is reported in Hispanic patients of Mexican origin, 72% of BRCA1/2 mutation carriers were diagnosed with breast cancer at ages <50 years, and 61% were TNBC with a significantly higher BMI (34). They also reported that the ER status was quite different between patients with BRCA1 and BRCA2 mutations, with ER positivity predominately seen in BRCA2 mutation carriers, which is consistent with our findings. In addition, BRCA1/2 mutation carriers are more likely to be Her2 negative ($P=0.010$), which was confirmed by two studies on the Chinese population (19,20). However, high incidence of BRCA1/2 mutation was not found in ER or PR negative patients in this study. Furthermore, we find that BRCA1/2 mutation carriers tend to have a higher tumor grade at diagnosis ($P=0.009$), which is in line with previous literature (8,35-37). Zhang *et al* reported a lymph node positivity rate of 34.1% in BRCA1/2 mutation carriers (19), which is similar to our result (33.3%). Furthermore, on-going clinical trials have shown that BRCA mutation-associated tumors tend to be sensitive to the targeted drugs, e.g., poly(ADP-ribose) polymerase (PARP) inhibitors (38). Moreover, BRCA1 mutation carriers may benefit from anthracycline-taxane-containing regimens (39). Therefore, it is essential for Her2-negative patients with higher tumor grade to undergo genetic testing, which may guide future clinical treatment and prognosis assessment.

No significant difference between BCCRs and clinicopathological features of the patients. A possible explanation would be that BCCRs may affect the risk of breast cancer but have a negligible effect on tumor characteristics.

Considering the retrospective single-center nature of the study, the bias may not be ignorable and further studies with larger sample size and other regional groups are warranted. Therefore, these results should be interpreted cautiously.

In conclusion, the current understanding of the BRCA1/2 mutation pattern can only explain a small part of patients with hereditarily high-risk breast cancer. BRCA1/2 gene mutations have a limited effect on the pathogenesis of early-onset breast cancer. However, BRCA1/2 testing should still be recommended for women with a family history of breast cancer, as well as breast cancer patients with specific pathologic types.

Such genetic testing may be useful to make appropriate clinical decisions for the treatment and prevention of breast cancer.

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