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Original Article

BRCA1/2 gene mutations in Uyghur and Han women with sporadic breast cancer in the xinjiang region of China

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Abstract: Objective: This study investigated the prevalence of *BRCA1/2* gene mutations and their relationship with clinical pathological parameters in Uyghur and Han sporadic breast cancer patients in the Xinjiang Uyghur Autonomous Region. Methods: Polymerase chain reaction (PCR) and DNA sequencing were used to detect mutations of the *BRCA1* (exons 2, 11 (11A and 11B) and 20) and *BRCA2* (exon 11) genes in paraffin-embedded tissues obtained from 170 sporadic breast cancer patients (85 Uyghur and 85 Han) in the Xinjiang Uyghur Autonomous Region. Results: The prevalence of *BRCA* mutations in sporadic breast cancer cases was 9.41% (16/170). Of these, 8.24% (14/170) harbored *BRCA1* mutations and 1.18% (2/170) *BRCA2* mutations. Among the 14 patients with *BRCA1* mutations, the detection rates of mutations in exons 2, 11 and 20 were 21.43% (3/14), 64.29% (9/14) and 14.29% (2/14), respectively. One of the sixteen mutations was at the -5382 locus in *BRCA1* (a hot-spot mutation site in Ashkenazi Jews); seven cases exhibited novel mutations. There were two germline mutations in exon 11 of the *BRCA2* gene. The rates of *BRCA* gene mutations in Uyghur and Han patients were 10.59% (9/85) and 8.24% (7/85), respectively; this difference was not significant ($P > 0.05$). Patients with *BRCA* gene mutations were on average 41.31±6.34 years old, and those without mutations were 49.67±12.63 years old. Thus, the mutation group was younger than the no-mutation group, and the age of onset of breast cancer in the total population was ≤ 50 years. Patients with amenorrhea comprised 18.75% (3/16) of the mutation group, and 81.25% (13/16) of patients were premenopausal; this difference was significant ($P < 0.05$). Analysis of the relationship between *BRCA* gene mutation status and clinical pathological features showed no significant difference ($P > 0.05$) in clinical staging, lymph node metastasis, tumor diameter, fertility status and age at menarche. *BRCA* mutations showed no significant differences among the patients of different molecular classifications ($P > 0.05$). Conclusion: The prevalence of *BRCA1* mutations was significantly higher than that of *BRCA2* mutations in sporadic breast cancer patients in Xinjiang. This study detected one mutation of the *BRCA1* gene at the -5382 locus (a hot spot in Jewish individuals) and seven novel mutations, perhaps is the founder mutation of the local area in patients with breast cancer mutations. The *BRCA* mutations detected did not differ markedly between Uyghur and Han patients. Pre-menopausal patients with breast cancer < 50 years old in Xinjiang may be at high risk of *BRCA1/2* mutations, and screening of the *BRCA* gene should be performed in such individuals.

Keywords: Sporadic breast cancer, *BRCA1/2* gene, mutation, DNA sequencing

Introduction

Breast cancer is one of the most common malignant tumors and a serious threat to the health of women [1]. At present, the mechanism of the occurrence and development of breast cancer are unknown, but the genetic background of individuals exposed to identical environmental conditions is thought to determine their susceptibility to breast cancer [2]. The *BRCA1/2* genes have been demonstrated to be associated with breast cancer, particularly hereditary breast cancer [3]. Understanding the mutation status of the *BRCA* gene can help predict the prognosis of patients and to change the development of BRCA-associated cancer. Furthermore, the prevalence of *BRCA1* mutations in breast cancer differs among ethnicities [4].

According to the latest international consensus in 2013, breast cancer can be classified into

Table 1. PCR conditions for amplification of BRCA1 and BRCA2 and the expected products

Primer name	Primer sequence (5'→3')	Tm (°C)	Size (bp)
BRCA1	F: GAAGTTGTCATTTTATAAACCTTT	58.0	258
exon2*	R: TGTCTTTTCTTCCCTAGTATGT		
BRCA1	F: AACATTCCAAGTACAGTGAGCACA	58.0	378
exon11 (A)	R: AGATGCATGACTACTTCCCATAGG		
BRCA1	F: TCCTAGCCCTTTCACCCATACA	58.0	274
exon11 (B)	R: AGATGCCTTTGCCAATATTACCTG		
BRCA1	F: ATATGACGTGTCTGCTCCAC	55.0	249
exon20**	R: AATGAAGCGGCCCATCTC		
BRCA2	F: CTTGTGGGATTTTTAGCACAGC	57.0	159
exon11***	R: GTGAGCTGGTTGACATGTTCG		

*, *BRCA1* -185delAG; **, *BRCA1* -5382insC; ***, *BRCA2* -6174delT.

the following four molecular subtypes [5]: (1) luminal A subtype, ER + and/or PR+, HER2-, Ki67 low expression (< 14%); (2) luminal B subtype, ER + and/or PR+, HER2-, Ki67 high expression (≥ 14%) and ER + and/or PR+, HER2-enriched, Ki67 any level; (3) HER2-enriched subtype, ER and PR deletion, HER2-enriched; and (4) basal-like subtype, ER and PR deletion, HER2-. The fourth breast cancer subtype has 80% similarity to triple-negative breast cancer (TNBC). We report here the relationships between *BRCA1* and *BRCA2* mutations and the clinical and pathological parameters of Xinjiang Uyghur and Han patients with sporadic breast cancer.

Materials and methods

Tissue specimens

A total of 170 tissue specimens from patients with invasive breast cancer (85 Han and Uyghur women each) who had not undergone chemotherapy were collected for this research. Patients were recruited from The First Affiliated Hospital of Xinjiang Medical University between January 2003 and December 2012. Inclusion criteria were primary unilateral breast cancer without a family history of breast or ovarian cancer. The histopathological features were reviewed by two experienced breast pathologists who had access to the patients' complete clinical and pathological data, including general information, pathological diagnosis, tumor staging and molecular typing. All patients provided written informed consent.

Mutation detection

PCR was performed on paraffin-embedded sections from 170 sporadic breast cancer patients. Total DNA was extracted from tissues using the QIAamp DNA FFPE Tissue Kit (50) (Bio-Rad, USA No. 56404), according to the manufacturer's instructions. DNA was diluted serially to obtain standard solutions (20 ng/μl). The primers were synthesized by Shenzhen City Kai Bio Technology Co., Ltd. Primer sequences and annealing temperatures are shown in **Table 1**. PCR was performed over 30 amplification cycles, consisting of 5 min at 94°C, 45 s at 94°C, the appropriate primer annealing temperature for 45 s, and 1 min at 72°C, followed by 7 min of extension at 72°C. To confirm the presence of the expected PCR products, 5 μl of the products were resolved by 1.5% gel electrophoresis. PCR products were stored at -20°C until required.

PCR products were analyzed for sequence mutations by PCR and direct nucleotide sequencing (Shenzhen City Kai Bio Technology Co., Ltd). To confirm the mutations, the sequences were compared with the non-mutated sequences using the Chromas Pro 1.33 software.

Data analysis

To confirm the mutations, sequences were compared with the cDNA reference sequences of *BRCA1* (GenBank accession number U146-80.1) and *BRCA2* (GenBank accession number U43746.1) from the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/). The Breast Cancer Information core (BIC) database was used to identify novel mutations.

Statistical analyses

This experimental data are quantitative data and the Fisher's exact or x^2 test was used to analyse the different clinicopathological parameters between patients with and without mutation respectively. All statistical tests were performed by the bilateral probability test using SPSS 17.0 software. And the difference was statistically significant when P value was < 0.05.

Table 2. Sequence variants of the *BRCA1/2* genes identified in sporadic breast cancer patients

Base change	Exon	Mutation type	Amino acid change	Ethnicity	Age of onset	Tumor grade	BIC
BRCA1							
50A > G	exon11	MS	Ala 17 Thr	Uyghur	47	II	Novel
63A > G	exon11	MS	Glu 21 Gly	Uyghur	50	I	Novel
79C > T	exon11	SY	Asn 25 Asn	Han	36	II	Novel
130C > T	exon11	SY	Gly 43 Gly	Uyghur	47	II	Novel
87A > G	exon11	MS	Glu 1038 Gly	Han	36	II	Reported
1616C > T	exon11	MS	Thr 539 Met	Han	42	III	Reported
1100delAT	exon11	SP	Stop 328	Uyghur	50	I	Reported
230A > G	exon11	MS	Lys 1183 Arg	Uyghur	45	II	Reported
4193insA	exon11	FS	Stop 1254	Uyghur	47	II	Reported
109C > T	exon20	NS	Stop 28	Han	38	III	Reported
5358C > T	exon20	NS	Stop 1747	Uyghur	40	II	Reported
60C > T	exon2	MS	Gln 12 Stop	Han	36	II	Novel
176G > C	exon2	MS	Arg 20 Thr	Han	38	III	Novel
162A > T	exon2	MS	Ile 15 Phe	Uyghur	44	III	Novel
BRCA2							
2971A > G	exon11	MS	Asn 991 Asp	Han	33	II	Reported
3036delA	exon11	FS	Stop 959	Uyghur	43	I	Reported

*BIC: Breast Cancer Information Core. MS: missense mutation. SY: synonymous mutation. NS: nonsense mutation. FS: frame shift mutation. SP: splice-site variant.

Results

BRCA gene mutation

BRCA1 exon 11 mutations are the most common in Chinese patients with breast cancer [6]; mutations in exons 2 and 20 have also been reported [7, 8]. Moreover, mutations in five regions within exons 11, 2 and 20 (three regions within exon 11, one region within exon 2 and one region within exon 20) of *BRCA1* have been detected in American women with breast and ovarian cancer [9, 10]. Accordingly, these five regions of *BRCA* were selected and sequenced in this study (**Table 1**).

BRCA1/2 mutation detection led to the discovery of 16 mutations in 170 Uyghur and Han sporadic breast cancer patients (16/170, 9.41%; **Table 2**). These comprised 14 cases of *BRCA1* mutations and 2 of *BRCA2* mutations. In the 14 patients with *BRCA1* mutations, the rates of exon 2, 11 and 20 mutations were 21.43% (3/14), 64.29% (9/14) and 14.29% (2/14), respectively. There were nine missense mutations (**Figures 1**, **2**), two synonymous mutations (**Figures 3**, **4**), two nonsense mutations, two frameshift mutations, and one splice-site variant. Seven cases were not present in the BIC database. In this study, we found a mutation at the -5382 locus of *BRCA1* in a Han female, aged 38 years; this mutation was not found in Uyghur patients. This mutation site is a hot spot in Ashkenazi Jews. Two other common mutations in Ashkenazi Jews, *BRCA1* -185delAG and *BRCA2* -6174delT, were not found in this study.

Relationships between BRCA gene mutations and clinical pathological parameters

The average age of the *BRCA* mutation-associated breast cancer patients was 42.8±8.8 years, which was significantly younger than those without *BRCA* mutations (mean age, 48.2±11.0 years). Nine *BRCA* mutations were seen in Uyghur women (n = 85, 10.59%): eight *BRCA1* and one *BRCA2* mutation. Seven mutations were found in Han women (n = 85, 8.24%): six *BRCA1* and one *BRCA2* mutation. The mutation rate in the Uyghur women was higher than that in the Han women, albeit not significantly so ($x^2 = 0.276$, $P = 0.599$).

The comparison of clinical and pathological parameters between the *BRCA* gene mutation and no-mutation groups showed that the muta-

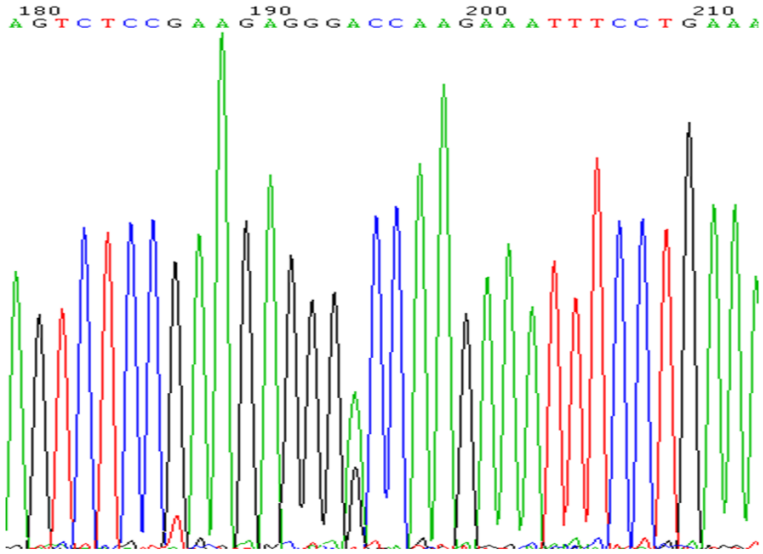

Figure 1. *BRCA1* 11E 50A > G missense mutations.

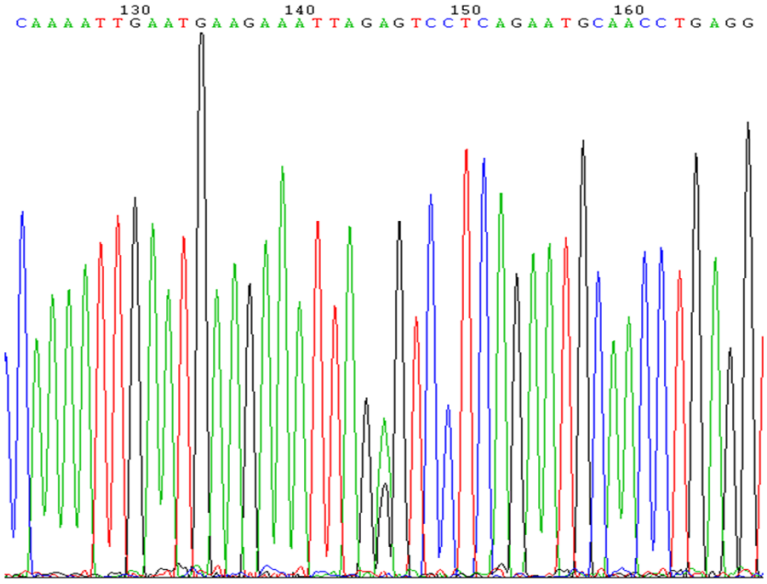

Figure 2. *BRCA1* 11E 63A > G missense mutations.

tion group (n = 16) comprised three amenorrheic patients (18.75%) and 13 premenopausal patients (81.25%). The mutation rate of premenopausal patients was significantly higher than that of amenorrheic patients. The ratio of amenorrheic and premenopausal patients in the no-mutation group was the same and the difference was statistically significant (P = 0.017). Comparison of other clinical and pathological parameters between the two groups showed that the mutation group exhibited earlier clinical stages, smaller tumor diameters, multiple births and late menarche. However, the differences were not statistically significant (P > 0.05) (**Table 3**).

Relationship between BRCA gene mutations and molecular subtype

The 170 patients were classified into breast cancer molecular subtypes according to the latest consensus breast cancer typing method, released in 2013. There were 63 luminal A subtype cases of which five had *BRCA* mutations (7.94%); 48 luminal B subtype cases, of which five harbored *BRCA* mutations (10.42%); 26 Erb-B2 overexpression cases, of which one exhibited a *BRCA* mutation (3.85%); and 33 basal-like subtype cases, of which five harbored *BRCA* mutations (15.15%). The mutation rate of the basal-like subtype was higher than that of the other subtypes, albeit not significantly (x^2 = 2.437, P = 0.487).

Discussion

The *BRCA1* gene, which is closely related to breast cancer development, was first reported by Hall *et al*. in 1990 [11] and first successfully cloned and separated using positional cloning technology by Miki and coworkers in 1994 [10]. The *BRCA2* gene was subsequently identified by analysis and location cloning of successive generations of families with breast cancer. *BRCA1/2* genes have been confirmed to be associated with susceptibility to breast cancer, particularly hereditary breast cancer [3]. The mutation rate of the *BRCA* gene is very low in healthy individuals, but the lifetime risk of developing a first primary breast cancer among *BRCA/BRCA2* mutation carriers ranges from 26 to 84% [12-14]. *BRCA*

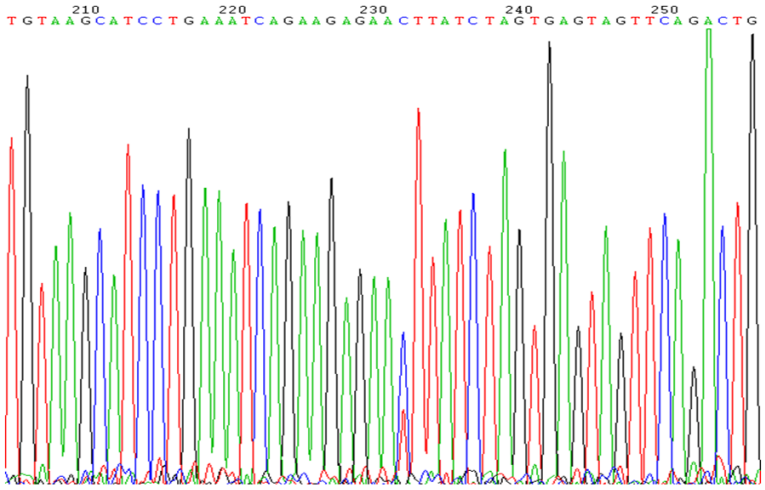

Figure 3. *BRCA1* 11E 79C > T synonymous mutations.

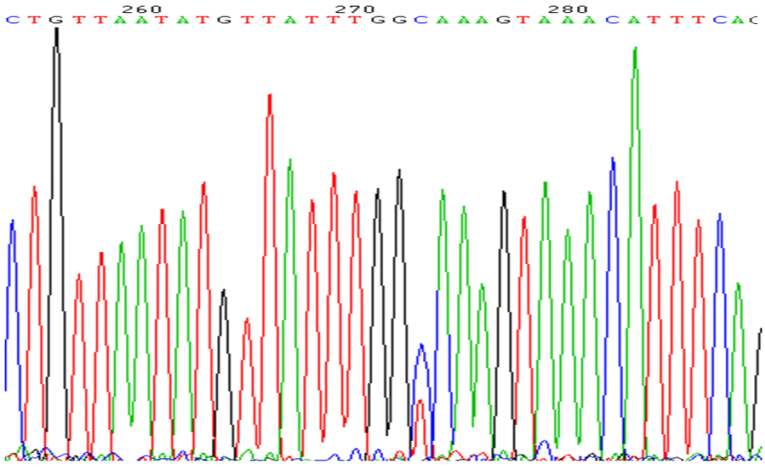

Figure 4. *BRCA1* 11E 130C > T synonymous mutations.

genes can promote the development of breast cancer upon mutation, promoter methylation and loss of gene heterozygosity [15].

BRCA gene mutation frequency and type exhibit marked geographical and ethnic differences. Kwong *et al*. [16] reported that the incidence of *BRCA1/2* mutation was 15.3% among 651 patients with familial breast or ovarian cancer in Hong Kong. Moreover, the mutations were enriched in exons 8, 11 and 22 of *BRCA1* and in exons 11 and 23 of *BRCA2*. Our data indicated that 9.41% (16/170) of 170 female breast cancer cases in the Xinjiang Region harbored *BRCA1/2* mutations. The differences between the two studies may be due to the different sample sizes. The mutation detection rates were 21.43% (3/14), 64.29% (9/14) and 14.29% (2/14) in exons 2, 11 and 20, respectively, in the 14 patients with *BRCA1* mutations. This suggests that *BRCA1* mutation is associated with sporadic breast cancer in Xinjiang. However, because of the difference of the research objects and methods, the study results of the *BRCA* gene mutation are not all the same. Studies performed in other countries have reported marked ethnic differences in *BRCA1/2* mutations. Ayraktar *et al*. [4] evaluated the *BRCA* genes in 445 patients of various nationalities; the pathogenic mutation rate in exon 2 of *BRCA1* in Ashkenazi Jews was significantly higher than that in European-Americans.

The Xinjiang Uyghur Autonomous Region is populated predominantly by the Uyghur nationality, which has a different genetic background from that of the Han population. Consequently, a likely explanation for the difference in *BRCA* mutation rate and subtype distribution is the different ethnic backgrounds between the Uyghur and Han populations. The mutation rate in Uyghur women was reported to be higher than that in Han women, albeit not significantly ($P > 0.05$). Of note, this study detected one *BRCA1* gene mutation at the -5382 locus in a 38-year-old Han woman; this mutation was not found in Uyghur patients. Because this site is a mutation "hot spot" in Ashkenazi Jews, further research is needed to determine whether it is also a mutation hot spot in Han women with sporadic breast cancer in the Uyghur Autonomous Region of Xinjiang. Two other common mutations in Ashkenazi Jews, *BRCA1*-185delAG and *BRCA2*-6174delT, were not found in this study and therefore are likely not associated with sporadic breast cancer in females in the Xinjiang Uyghur Autonomous Region. In the

Table 3. Relationships between BRCA gene mutations and clinical pathological parameters

Clinical pathological parameter	Mutation (n = 16) n, %	No mutation (n = 154) n, %	x^2	P
Nationality				
Uyghur	9 (56.25)	76 (49.35)	0.276	0.599
Han	7 (43.75)	78 (50.65)		
Clinical stage				
I-II	12 (75.00)	94 (61.04)	1.203	0.273
III	4 (25.00)	60 (38.96)		
Lymph nodes				
Positive	10 (62.50)	95 (61.69)	0.004	0.949
Negative	6 (37.50)	59 (38.31)		
Tumor diameter (cm)				
< 5	15 (93.75)	123 (79.87)	1.827	0.176
≥ 5	1 (6.25)	31 (20.13)		
Birth status				
≤ 2	9 (56.25)	99 (64.29)	0.404	0.525
> 2	7 (43.75)	55 (35.71)		
Menstrual condition				
Amenorrhea	3 (18.75)	77 (50.00)	5.681	0.017
Premenopausal	13 (81.25)	77 (50.00)		
Menarche (years)				
≤ 12	3 (18.75)	45 (29.22)	0.784	0.376
> 12	13 (81.25)	109 (70.78)		
Molecular typing				
Luminal A	5 (31.25)	58 (37.66)	2.437	0.487
Luminal B	5 (31.25)	43 (27.92)		
Non luminal	1 (6.25)	25 (16.23)		
Basal-like	5 (31.25)	28 (18.18)		

present study, mutations in *BRCA1* accounted for 14 of the 16 *BRCA* mutations (87.5%), while mutations in *BRCA2* comprised 2 of the 16 (12.5%). Therefore, the incidence of *BRCA1* mutation is considerably higher than that of *BRCA2*. The mutation detection rates in exons 2, 11 and 20 were 21.43% (3/14), 64.29% (9/14) and 14.29% (2/14), respectively, in the 14 patients with *BRCA1* mutations. It is possible that *BRCA1* mutation is closely related to the occurrence of sporadic breast cancer in the Xinjiang Region, and that exon 11 of *BRCA1* plays an important role. According to the BIC database and data in the literature, 7 of the 16 *BRCA* mutations were novel and had not been reported previously, suggesting they are "hot spot" mutations in sporadic breast cancer patients in the Xinjiang Uyghur Autonomous Region. This matter warrants further research. In our study, the *BRCA* mutation rate in Uyghur women was 10.59% (9/85), compared with 8.24% (7/85) in Han women; the difference was not significant (P > 0.05). Scant information regarding *BRCA* germline mutations in Uyghur women with sporadic breast cancer is available; indeed, most research has been performed in Han women in mainland China. Therefore, our results should be verified by further, larger-scale research.

The average age of the 16 *BRCA* mutation-associated breast cancer patients was 42.8±8.8 years, which was significantly lower than that of patients without *BRCA* mutations (48.2±11.0 years). Therefore, screening for *BRCA* mutations at a younger age and intervention could reduce the incidence of breast cancer. The National Comprehensive Cancer Network (NCCN) suggested that *BRCA* gene mutation should be screened for in TNBC patients < 50 years old, which would enable prediction of the risk of developing breast and ovarian cancer. At the same time, prophylactic resection can reduce the risk of contralateral breast cancer in 23% of patients with breast cancer and a decrease of 41% patients with ovarian cancer risk [17].

Regarding clinical and pathological parameters, there were 3 cases (18.75%) of amenorrhea among the 16 patients with *BRCA* mutations; the other 13 patients were premenopausal (81.25%). The mutation rate of premenopausal patients was significantly higher than that of amenorrheic patients. The ratios of amenorrheic to premenopausal patients in the no-mutation group was the same and the difference was statistically significant (P = 0.017). Comparison of other clinical and pathological parameters between the two groups showed an earlier clinical stage, smaller tumor diameter, multiple births and late menarche in the mutation group; however, the differences were not statistically significant (P > 0.05). To summarize, patients who are < 50 years old and premenopausal could be a high-risk group for

sporadic breast cancer in the Xinjiang Region. Therefore, *BRCA* mutation screening should be performed in the future as part of counseling.

In our study, the *BRCA* mutation rates in the luminal A, luminal B, Erb-B2 overexpression and basal-like subtypes of breast cancer were 7.94% (5/63), 10.42% (5/48), 3.58% (*1/26*) and 15.15% (5/33), respectively. There was no significant difference in the *BRCA* composition rates among the breast cancer subtypes ($P > 0.05$). However, the mutation rate of the basal-like subtype was higher than that of the other subtypes. The basal-like subtype (ER, PR deletion and HER2 negative) had 80% similarity to TNBC; the latter also comprises various histological types [18]. Karama *et al*. [19] reported a *BRCA1/2* mutation rate of 18% among 122 cases of TNBC. Our findings showed that the mutation rate of the basal-like subtype was 15.15%; this is consistent with the above-mentioned reports.

In summary, we screened for mutations in the *BRCA1/2* gene in 170 women with sporadic breast cancer from the Xinjiang Region of China. Mutations in *BRCA1* were identified in 14 of the 170 patients analyzed (8.24%) and mutations in *BRCA2* in 1.18% (2/170). In Uyghur women, the *BRCA* mutation rate was 10.59% (9/85), and four mutations were novel. Moreover, we detected one mutation at the -5382 locus of the *BRCA1* gene (a hot spot in Ashkenazi Jews) and seven novel mutations, perhaps is the founder mutation of the local area in patients with breast cancer mutations. Therefore, we report herein a high prevalence of *BRCA1* mutations among sporadic breast cancer cases, particularly in patients of Uyghur descent. Thus, female patients in the Xinjiang Region < 50 years old and premenopausal could be at high risk of sporadic breast cancer, suggesting the need for *BRCA* mutation screening in the future as part of counseling. Xinjiang is located in the northwest of China and has a unique geographical environment, ethnic composition and living habits of its residents. At present, few studies have been performed on *BRCA* gene mutations in sporadic breast cancer women in the Xinjiang Region. Thus, it is important to understand the causes of, and risk factors for, breast cancer in women of different nationalities. Our aim in future work is to reduce the mortality rate of breast cancer patients and provide individualized treatment.

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Disclosure of conflict of interest

None.

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