

## RESEARCH ARTICLE

# BRCA1 Gene Exon 11 Mutations in Uighur and Han Women with Early-onset Sporadic Breast Cancer in the Northwest Region of China

Yu-Wen Cao<sup>1,2&</sup>, Xin-Ge Fu<sup>3&</sup>, Guo-Xing Wan<sup>2</sup>, Shi-Ying Yu<sup>1</sup>, Xiao-Bin Cui<sup>2</sup>, Li Li<sup>2</sup>, Jin-Fang Jiang<sup>2</sup>, Yu-Qin Zheng<sup>2</sup>, Wen-Jie Zhang<sup>2</sup>, Feng Li<sup>2\*</sup>

### Abstract

The prevalence of BRCA1 gene mutations in breast cancer differs between diverse ethnic groups. Relatively little information is known about patterns of BRCA1 mutations in early-onset breast cancer in women of Uighur or Han descent, the major ethnic populations of the Xinjiang region in China. The aim of this study was to identify BRCA1 mutations in Uighur and Han patients with early-onset (age <35 years), and sporadic breast cancer for genetic predisposition to breast cancer. For detection of BRCA1 mutations, we used a polymerase chain reaction single-stranded conformation polymorphism approach, followed by direct DNA sequencing in 22 Uighur and 13 Han women with early-onset sporadic breast cancer, and 32 women with benign breast diseases. The prevalence of BRCA1 mutations in this population was 22.9% (8/35) among early-onset sporadic breast cancer cases. Of these, 31.8% (7/22) of Uighur patients and 7.69% (1/13) of Han patients were found to have BRCA1 mutations. In 7 Uighur patients with BRCA1 mutations, there were 11 unique sequence alterations in the BRCA1 gene, including 4 clearly disease-associated mutations on exon 11 and 3 variants of uncertain clinical significance on exon 11, meanwhile 4 neutral variants on intron 20 or 2. None of the 11 BRCA1 mutations identified have been previously reported in the Breast Cancer Information Core database. These findings reflect the prevalence of BRCA1 mutations in Uighur women with early-onset and sporadic breast cancer, which will allow for provision of appropriate genetic counseling and treatment for Uighur patients in the Xinjiang region.

**Keywords:** Uighurs - ethnicity - BRCA1 mutation - early onset - sporadic breast cancer - Xinjiang, China

*Asian Pac J Cancer Prev*, 15 (11), 4513-4518

### Introduction

Breast cancer was documented as the most frequently diagnosed cancer in women by the National Central Cancer Registry of China for the year 2009, with an age-standardized rate of incidence of 23.16 per 100,000 based on the Chinese standard population, a rate lower than in North America and Europe (Ferlay et al., 2004; Parkin et al., 2004; Chen et al., 2013). Approximately 2% of breast cancer patients are diagnosed with early-onset (<35 years old) disease, a subtype believed to possess a more aggressive biological behavior and to be associated with a more unfavorable prognosis than the disease in older patients (Colleonim et al., 2002). However, there is considerable geographical variation in incidence rates of early-onset breast cancer. In the United States, the proportion of early-onset (<40 years old) breast cancer was reported as approximately 6% among all breast cancers

(Buchanan et al., 2013).

The Xinjiang region in China is a multi-ethnic region consisting of approximately 45.7% Uighurs and 39.8% Han Chinese, as well as other minority indigenous groups. Currently, there is little information about the precise incidence rates of breast cancer in the Uighur and Han nationalities, particularly with regard to early-onset breast cancer. As reported in 2010, approximately 10.99% and 15.22% of breast cancer cases were diagnosed as early-onset cases in Han and Uighur women, respectively (Li et al., 2012). Some studies on Uighur and Han women in the Xinjiang region have also shown striking differences in breast cancer patterns. Additionally, the peak age of onset for breast cancer was 30-44 in Uighur women versus 45-59 in Han women in 2012. Of note, some of these studies demonstrated that the observed increase in breast cancer incidence in the Uighur population was partially attributed to a specific genetic background (Cheng et al., 2010).

<sup>1</sup>Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, <sup>2</sup>Department of Pathology and Key Laboratory of Xinjiang Endemic and Ethnic Diseases (Ministry of Education), Shihezi University School of Medicine, Shihezi, Xinjiang, <sup>3</sup>Department of Pathology, The First Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China <sup>&</sup>Equal contributors \*For correspondence: [fengli7855@126.com](mailto:fengli7855@126.com)

Taken together, in the Xinjiang region, breast cancer in Uighur patients is characterized by both an apparent lower peak age of onset and a proportionately higher incidence of early-onset breast cancer. The typical Xinjiang multi-ethnic population provides an excellent opportunity to understand genetic predispositions to breast cancer among the different ethnicities.

It is widely accepted that BRCA1 mutations are responsible for a significant proportion of hereditary breast and ovarian cancer cases, particularly those of early-onset. The lifetime risk of breast cancer for Caucasian BRCA1 mutation carriers is 60–85%, while the lifetime risk of ovarian cancer in the same population is 15–40% (Couch, 2004). To date, studies of BRCA1 mutation spectrum in Chinese populations are limited and most of these studies were performed in single institutions or a small number of medical centers. The mutation rates of BRCA1 in familial breast cancer ranged from 3.9% to 6.9% (Cao et al., 2013). This is consistent with rates in other Asian ethnic groups (Hall et al., 2009). Another study, by Suter et al. (2004) found that the rates of BRCA1 mutations in sporadic breast cancers were 0.7% (4/590) in Shanghai of China. Moreover, in early-onset breast cancer, mutation rates of these genes were 5.1% in east regions of China (Li et al., 2008). Recently reported (Ou et al., 2013), in Northwest China, among 79 patients with hereditary predisposition to breast cancer, 3 (3.8%) had a BRCA1 mutation. The results are consistent with the previous reports (Cao et al., 2013).

Diagnostically, BRCA1 mutations have facilitated the identification of women at risk for cancer in many developed countries. Although predictive genetic testing is becoming a part of clinical practice in some high-resource regions of China, the lack of information concerning the prevalence of BRCA1 mutations in different ethnic groups has limited the availability of such testing BRCA1. Xinjiang in China is a low-resource and multi-ethnic region where Uighur and Han nationalities comprise the majority of the population. Relatively little is known about the presence of BRCA1 mutations in Uighur and Han women with early-onset breast cancer without a family history. Point mutations are the most common type of mutation and have been frequently reported in the Chinese population. Mutations within exon 11 of BRCA1 is the most common and accounted for 56.3% (58/103) of all mutations (Cao et al., 2013). We therefore set out to determine the contribution of mainly exon 11 mutations in the BRCA1 gene to the susceptibility to early-onset and sporadic breast cancer in these populations. The aim of the present study was to identify BRCA1 exon 11 mutations that could be used in diagnosis to provide appropriate genetic counseling and treatment for patients in the Xinjiang region.

## Materials and Methods

### *Tissue specimens*

A total of 35 tissue specimens from patients with operable breast cancer and no evidence of prior chemotherapy were randomly collected for this study. The pathologically confirmed breast cancer patients with early-

onset and sporadic disease were screened in the Xinjiang region. Early-onset cases were identified as patients who had been diagnosed with breast cancer before the age of 35. Early-onset disease was defined to include patients without any first- and second-degree relatives affected with breast and/or ovarian cancers. The 35 early-onset and sporadic cases were divided into 2 groups: Uighur and Han. Twenty-two individuals were Uighur women with age range of 16 to 33 years (the mean age of 27.3 years) recruited from 1 major hospital in the Xinjiang region, The People's Hospital of Xinjiang Uygur Autonomous Region, between January 2003 and December 2006. Due to its demographics, a larger proportion of patients treated at this hospital were ethnically Uighur. Thirteen cases of breast cancer in Han women with age range of 18 to 34 years (the mean age of 33.6 years) were identified at the First Affiliated Hospital of Shihezi University School of Medicine, between January 2003 and December 2006. The comparisons of the onset ages showed higher proportion of younger patients in Uighur than that in Han. Thirty-two benign breast lesion (fibroadenoma and adenosis) control tissue samples were acquired during mastectomy. All patients provided written informed consent.

### *Mutation detection*

DNA extraction from formalin-fixed paraffin-embedded breast cancer tissue was performed as follows. Tissue specimens were fixed in 10% (v/v) neutral buffered formalin for up to 24 h, dehydrated in 70% ethanol, and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin. The histopathological features were reviewed by an experienced breast pathologist. Ten slices of 5- $\mu$ m thickness were cut and placed in 1.5-ml centrifuge tubes. The paraffin was removed by placing the specimen in xylene for 30 min, followed by dehydration with 100% ethanol and drying in a Speed-Vac concentrator (Savant Instruments, Farmingdale, NY). The preparations were then incubated with 240  $\mu$ l of Tris-disodium ethylenediaminetetraacetate (Tris-EDTA, pH 9.0), 10% sodium dodecyl sulfate, and 20 g/L of proteinase K at 55°C with shaking for 1 day. DNA was extracted using the phenol/chloroform method. We identified sequence variants within 3 exons of BRCA1 using polymerase chain reaction single-stranded conformation polymorphism (PCR-SSCP) followed by direct DNA sequencing.

Oligonucleotide primers mainly for 11 of the BRCA1 gene were constructed for use in PCR amplification (Table 1). The mutations of exon 11 of BRCA1 is the most common in Chinese patients with breast cancer (Cao et al., 2013), and the mutation of exon 2 and 20 of BRCA1 had also been reported in population of China (Huang et al., 2008; Meng et al., 2009). Moreover, the mutations of the five regions within exon 11, 2 and 20 (three regions within exon 11, one region within exon 2 and one region within exon 20) of BRCA1 had been detected in American women with breast and ovarian cancer (Friedman et al., 1994; Miki et al., 1994). Accordingly, the five regions of BRCA1 were selected and sequenced for the purpose of this study. The primers were synthesized by Shanghai Sangon Biotech Company and purified on an oligonucleotide purification column. PCR was carried

out with 35 cycles, each consisting of amplification for 3 min at 94°C, 45 s at 94°C, and 30 s at 57°C, followed by 1 min of extension at 72°C. Separate experiments were performed for analyzing mutations in the respective target exons. To confirm the presence of the expected PCR products, 5 µl of the products were identified by 1.5% gel electrophoresis. Eight microliter of the PCR products was diluted 2-fold with a solution consisting of 95% formamide, 0.5 mol/L EDTA, and 0.25% bromophenol blue. One microliter of the diluted sample was denatured at 100°C for 10 min and electrophoresed on an 8% neutral polyacrylamide gel, with or without 1–5% glycerol at 100 V for 5–7 h. The gel was dried on filter paper and subjected to autoradiography. A band showing a difference in conformation of the PCR products in the polyacrylamide gel was considered to indicate BRCA1 mutation.

PCR products were analyzed for sequence variants by PCR and direct nucleotide sequencing (GeneCore BioTechnologies Co., Ltd, Shanghai).

To confirm the mutations, sequences were compared to the BRCA1 cDNA reference sequences (GenBank accession number U14680) using the BLAST tool. All mutations were compared to sequences in the Breast Cancer Information Core (BIC) database for determining polymorphisms, novel mutations, or disease-associated mutations.

#### *In silico prediction*

Potential clinical effect of variants of uncertain significance (VUS) was evaluated by analyses of the severity of the amino acid changes and their conservation

across species. Relevant analyses were performed with prediction analysis web tools Alignment-Grantham variation Grantham deviation (International Agency for Research on Cancer, World Health Organization, <http://agvgd.iarc.fr/alignments.php>), Polymorphism Phenotyping (PolyPhen) and Sorting Intolerant from Tolerant (SIFT) score.

#### *Statistical analyses*

Statistical analysis used the Fisher exact test, with  $p < 0.05$  as the threshold for detecting statistical significance.

## Results

BRCA1 mutation detection led to the discovery of 8 cases of mutations in 35 breast cancer patients with early-onset disease (8/35, 22.86%). Of these, 7 cases of BRCA1 mutations were observed in Uighur women (n=22, 31.82%), and only 1 case of mutation was found in the Han women (n=13, 7.69%). The BRCA1 mutation rate in early-onset breast cancer seen in the Uighur women (31.82%) was higher than that seen in the Han women (7.69%). There were 7 single-nucleotide polymorphisms among the 35 cases of early-onset breast cancer (Table 2).

Nineteen sequence alterations of the BRCA1 gene were detected in 11 early-onset breast cancer patients (Table 2). Four of these sequence alterations were considered clinically significant or clear disease-associated mutations. All patients were classified as having a deleterious mutation if the BRCA1 protein terminated

**Table 1. PCR Reaction Condition of BRCA1 Gene**

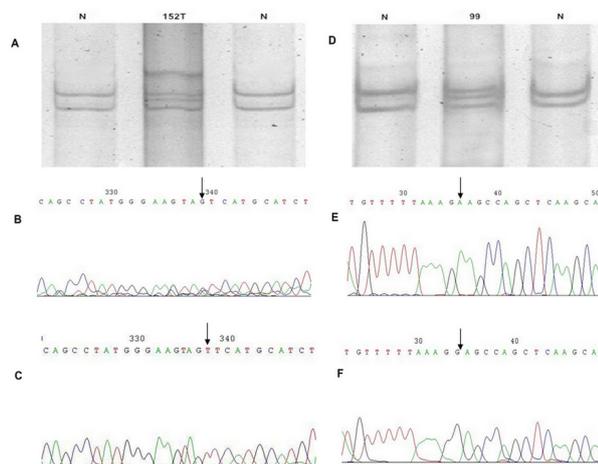
Primer pair	size (bp)	DNA	U14680 (bp)	Forward primer	Reverse primer	Tm (°C)
Exon11.1	244	cDNA	1731-1975	CAAACGGAGCAGAATGGTCA	GCCTGGTAGAAGACTTCCCTCCTC	58.9
Exon11.2	378	cDNA	3171-3549	AACATTTCCAAGTACAGTCAGCAC	AGATGCATGACTACTTCCCATAGG	61.1
Exon11.3	274	cDNA	3686-3960	TCCTAGCCCTTTCACCCATACA	AGATGCCTTTGCCAATATACCTG	58.5
Exon2	250	genomic	101-199	GAAGTTGTGCTTTTATAAACCTTT	TGTCCTTTCCCTAGTATGT	57
Exon20	220	genomic	5311-5396	ATATGACGTGTCTGCTCCAC	AATGAAGCGGCCCATCTC	59.9

**Table 2. Sequence Variants Identified in the BRCA1 Gene in Early-onset and Sporadic Breast Cancer**

Case	Clinical Relevance	Type	Exon/Intron	Nucleotide Change	AA Change	Novel/Reported (BIC)	Ethnicity
152T	Deleterious	FS	11 Exon	3180insA	stop1026	Novel	Uighur
	Deleterious	FS	11 Exon	3538insT	stop1147	Novel	Uighur
99	Deleterious	FS	11 Exon	3694insAA	stop1209	Novel	Uighur
221	Deleterious	FS	11 Exon	1963insT	stop623	Novel	Uighur
99	VUS	MS	11 Exon	3948G>C	Ala1277Pro	Novel	Uighur
146T	VUS	MS	11 Exon	3182A>C	Ser1021Arg	Novel	Uighur
	VUS	MS	11 Exon	3538G>T	Ser1140Ile	Novel	Uighur
221	Neutral	SP	20 intron	IVS20-68insA		Novel	Uighur
12	Neutral	SP	20 intron	IVS20+78G>T		Novel	Han
192	Neutral	SP	20 intron	IVS20-68insA		Novel	Uighur
17	Neutral	SP	2 intron	IVS2-55insG		Novel	Uighur
85	Neutral	SP	2 intron	IVS2-55insTG		Novel	Uighur
11	Polymorphism	MS	11 Exon	3232A>G	Glu1038Gly	Reported	Han
18	Polymorphism	MS	11 Exon	3232A>G	Glu1038Gly	Reported	Han
99	Polymorphism	MS	11 Exon	3232A>G	Glu1038Gly	Reported	Uighur
146T	Polymorphism	MS	11 Exon	3232A>G	Glu1038Gly	Reported	Uighur
152T	Polymorphism	MS	11 Exon	3232A>G	Glu1038Gly	Reported	Uighur
208	Polymorphism	MS	11 Exon	3232A>G	Glu1038Gly	Reported	Uighur
221	Polymorphism	MS	11 Exon	3232A>G	Glu1038Gly	Reported	Uighur

\*VUS, variant of uncertain significance; FS, frame-shift; MS, missense; SP, splice site variants; BIC, Breast Information Core

prematurely at least 10 amino acids from the C terminus (Thirthagiri et al., 2008). These deleterious mutations were all frame-shift variants in exon 11, including 3180insA, 3538insT (Figure 1A-C), 3694insAA, and 1963insT, which cause translation of the BRCA1 protein to terminate prematurely at 1026, 1147, 1209, and 623 amino acids, respectively. Of note, all the three cases with deleterious mutations were diagnosed with poorly differentiated cancer at high pathological stage. Additionally, all the 4 deleterious mutations were not described in the BIC database. Eight sequence variations, which were not considered clinically important, were also observed (Table 2). Of these, 5 BRCA1 splice site variants were identified in intron 20 or 2, which were neutral and novel variants. Three missense variants of uncertain significance were found in exon 11 and novel. The most frequent mutation in the BRCA1 gene was IVS20-68insA on intron 20, which were found in 2 cases. There were 7 other single-nucleotide polymorphisms (Table 2), which have been reported previously in BIC and are classified as neutral



**Figure 1. DNA sequencing for BRCA1.** A) Single-strand conformation polymorphism (SSCP) of BRCA1 at Exon 11. N shows the normal electrophoresis strip in a benign breast lesion control, and an abnormal electrophoresis strip was observed in breast cancer sample 152T. B) DNA sequencing electropherogram of BRCA1 for a benign breast lesion control. The base 3538 detected was G at Exon 11 (marked with arrowhead). C) DNA sequencing electropherogram of BRCA1 for breast cancer sample 152T. An insertion was noted in base 3538 (marked with arrowhead) at Exon 11 (3538insT). D) SSCP of BRCA1 at Exon 11. N shows the normal electrophoresis strip in a benign breast lesion control, and an abnormal electrophoresis strip was observed in another benign breast lesion control 99. E) DNA sequencing electropherogram of BRCA1 at Exon 11 for a benign breast lesion sample. F) DNA sequencing electropherogram of BRCA1 for control 99. A substitution of A>G was noted in base 3232 at Exon 11 (marked with arrowhead)

polymorphisms. The 7 single-nucleotide polymorphisms 3232A>G were missense alterations in exon 11 (Figure 1D-F), which lead to a Glu for Gly substitution at codon 1038 (Glu1038Gly).

We next analyzed the incidence of cancer identified as carrying BRCA1 mutations in the 2 ethnic groups, patients of Uighur and Han descent (Table 2). With regard to BRCA1 sequence variants, 4 deleterious variants were found in 16 sequence alterations of the BRCA1 gene in patients of Uighur ethnicity (4/16, 25%), and none deleterious variants were detected in 3 sequence alterations in patients of Han descent (0/3, 0%). Obviously, the prevalence of deleterious mutation in Uighur patients is significantly higher than that in Han ( $P=0.036$ ). Moreover, three BRCA1 variants of uncertain significance were identified in Uighur breast cancer cases. Four of 5 neutral variants were detected in Uighur women, and the neutral variants, IVS20-68insA, was detected in 2 Uighur cases. Concerning the single-nucleotide polymorphisms of BRCA1, the missense variant 3232A>G was found in 5 Uighur cases and 2 Han cases.

In consideration of the existence of VUS, we conducted *in silico* prediction for the potential clinical effect using Align-GVGD, which was also predicted using the bioinformatics tools PolyPhen and SIFT, the results were shown in Table 3.

## Discussion

Hereditary breast carcinomas are predominantly a consequence of loss-of-function germline mutations in 1 of 2 major breast cancer susceptibility genes, BRCA1 and BRCA2 (Tohgt et al., 2008). Deleterious mutations in either of these genes were suggested to confer a significant increase in the lifetime risk of developing breast cancer (Ford et al., 1994; Wooster et al., 2003). BRCA1 mutations were reported to be associated with 12% of breast cancer cases diagnosed at or before the age of 35 in Caucasian women (Fitzgerald et al., 1996). However, our current understanding of BRCA1 mutations and their contribution to sporadic early-onset breast cancer (<35 years old) has arisen primarily from studies conducted in Caucasian women. To date, the little knowledge we have of BRCA1 germline mutations in Chinese women with early-onset breast cancer is mainly derived from Shanghai and Hong Kong (Tang et al., 1999; Suter et al., 2004). However, information on BRCA1 mutations in Uighur women with sporadic early-onset breast cancer is largely lacking.

In the present study, a total of 35 proband patients diagnosed with early-onset breast cancer (<35 years) and no family history of the disease were selected from the Xinjiang region. Within this cohort, BRCA1 sequence

**Table 3. Predicted Effect of Unclassified Missense Variants of BRCA1**

genetic variant	consequence	GV	GD	Align-GVGD class	Polyphen	SIFT
c.3948G>C	p.Ala1277Pro	0	26.87	C25	0.13 (benign)	0.03 (not tolerated)
c.3182T>G	p.Ser1021Arg	0	109.21	C65	0.948 (probably damaging)	0.00 (not tolerated)
c.3538G>T	p.Ser1140Ile	0	141.8	C65	0.819 (probably damaging)	0.01 (not tolerated)

\*Align-GVGD was used to further assess the functional effect of missense VUS, with alignment to 13 BRCA1 and 12 BRCA2 ortholog sequences down to sea urchin (<http://agvgd.iarc.fr/alignments.php>). Align-GVGD, Align Grantham Variation Grantham Deviation; GV, Grantham Variation score, GD, Grantham Deviation score, PolyPhen, Polymorphism Phenotyping; SIFT, Sorting Intolerant from Tolerant. c.3182T>G, namely, c.3182A>C displayed in Table 2

variants were examined in 22 Uighurs and 13 Han Chinese. BRCA1 mutations were identified in 8 cases of the total 35 patients analyzed (22.86%). Of these, the BRCA1 mutation rate in Uighur women was 31.82% (7/22), and the mutation rate in Han women was 7.69% (1/13). Evidently, the incidence of BRCA1 mutation in Uighur women is much higher than that in Han women. Based on vast published data, there are considerable ethnic variations of BRCA1 in early-onset and sporadic breast cancer. In developing countries, such as Algeria, 9.8% (5/51) of sporadic and early-onset cases were found to be associated with BRCA1 mutations (Uhrhammer et al., 2008). However, in Morocco, only 1% (1/102) of early-onset sporadic breast cancer (<45 years) was found to be associated with BRCA1 mutations (Laraqui et al., 2013). In sporadic breast cancer patients ( $\leq 40$  years) from northeast of India, the presence of BRCA1 mutations was 9.37% (3/32) (Jagadish et al., 2012). A 1.5% (1/69) mutation detection rate in BRCA1 was seen in Iranian women with early-onset breast cancer without a family history (Yassae et al., 2002), and the carriers of BRCA1 founder mutations have a high lifetime risk of breast cancer in Iran (Mohammad et al., 2013). Although the BRCA1 mutation rate of Han Chinese in the Xinjiang region falls within the range reported in these developing countries, the BRCA1 mutation rate of Uighur women is much higher and has similar distributions to developed countries. In Italy, BRCA1 mutations were detected in 15 (22.7%) out of 66 tested women diagnosed with breast cancer before the age of 40 who were unselected for family history (Musoplino et al., 2007). A previous study analyzing the prevalence of BRCA1 and BRCA2 mutations in German women with early-onset breast cancer (aged  $\leq 35$ ) estimated a mutation frequency of 8% for BRCA1 (Meindl., 2002). Consequently, a given explanation for the variation in the BRCA1 mutation rate is the varying contribution of BRCA1 mutations to sporadic early-onset breast cancer in populations that vary from one another in their racial and ethnic backgrounds. Alternative possibilities are the differences in the selection criteria, the sensitivity of the mutation detection, geographical variation, sample size, or all of these in combination. Nevertheless, these findings are more likely to reflect the genetic heterogeneity of the Uighur population and are relevant to providing appropriate genetic counseling and clinical management of early-onset breast cancer in the Uighur population.

BRCA1 mutation detection led to the discovery of 12 unique mutations in 8 cases of the total 35 patients analyzed. According to the BIC database and data in the literature, all the 12 BRCA1 mutations were novel and had not been previously reported. The clinical relevance of BRCA1 sequence variants were interpreted as previously described (Cui et al., 2000). Of the 12 novel BRCA1 mutations, 4 variants were clearly disease-associated mutations found in Uighur women, and no variants were found in Han women. Eight mutations were identified as variants of uncertain significance or neutral variants in this cohort, and 7 of 8 mutations were detected in the Uighur population, with only 1 detected in the Han population. In 8 BRCA1 variants of uncertain significance or neutral variants, the most frequent mutation was IVS20-68insA,

which was found in 2 Uighur cases and was highly prevalent among the Uighur population. Taken together, 11 of the 12 novel BRCA1 mutations were detected in the Uighur population, and all 4 of the deleterious mutations were found in the Uighur population, which suggests that the mutation pattern of BRCA1 is more characteristic of Uighur women. Therefore, presence of BRCA1 mutations put Uighur women at a relatively increased risk for sporadic early-onset breast cancer.

We identified 3 different BRCA1 VUS in this study. All VUS were unclassified missense variants. It is possible that these sequence variants have no clinical relevance, and the few that may be deleterious are unlikely to change the basic conclusions of this study. For instance, although the c.3182T>G and c.3538G>T that both occurred at highly conserved (GV=0) and suggested by Align-GVGD as candidate risk variant were defined as interfering with function (A-GVGD class C65), no clinical interest was observed. The patient with the two variants was diagnosed with moderately differentiated invasive ductal carcinoma at II stage with metastasis-free node, which implies that interpretation of VUS still remains problematic. Prediction of the effect of VUS in functional analysis studies and by prediction software may generate results as the expectation.

We also observed 1 common polymorphism in 7 cases; the polymorphism had been previously reported in BIC and was classified as a neutral polymorphism. The polymorphism was a missense substitution in exon 11 leading to a Glu for Gly substitution at codon 1038 and was identified in 5 Uighur patients and 2 Han patients.

In summary, we analyzed BRCA1 DNA from a total of 35 women with sporadic early-onset breast cancer from the Xinjiang region in China, performing a screen for mutations in the BRCA1 gene. BRCA1 mutations were identified in 8 of the 35 patients analyzed (22.86%). In Uighur women, the BRCA1 mutation rate was 31.82% (7/22), and 12 mutations in 7 cases were novel. Therefore, we report on the high prevalence of BRCA1 mutations in the analyzed cases of early-onset breast cancer, particularly in patients of Uighur descent. The Xinjiang of China, the low-resource region, has limited health and medical care resources and infrastructures to meet the needs of these patients. Thus, it is important to obtain a better understanding of the causes of breast cancer among Uighur and Han populations so as to improve prevention and cancer risk assessment efficiently. These findings highlight the prevalence of BRCA1 mutations in Uighur women with early-onset and sporadic breast cancer, which will allow for provision of appropriate genetic counseling and treatment for Uighur patients in the Xinjiang region.

## Acknowledgements

This work was supported by the Natural Science Foundation of China (Grant No. 81160322)

## References

Buchanan N, Roland KB, Rodriguez JL, et al (2013). Opportunities for public health communication, intervention,

- and future research on breast cancer in younger women. *J Womens Health*, **22**, 293-8.
- Cao WM, Wang XJ, Li JC (2013). Hereditary breast cancer in the han Chinese population. *J Epidemiol*, **23**, 75-84.
- Chen W, Zheng R, Zhang S, et al (2013). The incidences and mortalities of major cancers in China, 2009. *Chin J Cancer*, **32**, 106-12.
- Cheng F, Zhang Q, Liu WH, et al (2010). Clinicopathologic analysis of 2019 breast cancer patients with Han and Uygur nationalities in Xinjiang areas. *Cancer Res Prev Treat*, **7**, 1312-4.
- Colleoni M, Rotmensz N, Robertson C, et al (2002). Very young women (<35 years) with operable breast cancer: features of disease at presentation. *Ann Oncol*, **13**, 273-9.
- Couch FJ (2004). Genetic epidemiology of BRCA1. *Cancer Biol Ther*, **3**, 509-14.
- Cui J, Hopper JL (2000). Why are the majority of hereditary cases of early-onset breast cancer sporadic? A simulation study. *Cancer Epidemiol Biomarkers Prev*, **9**, 805-12.
- Ferlay J, Bray F, Pisani P, et al (2004). GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC Cancer Base No. 5 version 2.0, IARC Press, Lyon.
- Fitzgerald MG, Macdonald DJ, Krainer M, et al (1996). Germline BRCA1 mutations in Jewish and non-Jewish women with early-onset breast cancer. *N Engl J Med*, **334**, 143-9.
- Ford DI, Easton DF, Bishop DT, Narod SA, Goldgar DE (1994). Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. *Lancet*, **343**, 692-5.
- Friedman LS, Ostermeyer EA, Szabo CI, et al (1994). Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nature Genetics*, **8**, 399-404.
- Hall MJ, Reid JE, Burbidge LA, et al (2009). BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast ovarian cancer. *Cancer*, **115**, 2222-33.
- Hansa J, Kannan R, Ghosh SK (2012). Screening of 185DelAG, 1014DelGT and 3889DelAG BRCA1 mutations in breast cancer patients from North-East India. *Asian Pac J Cancer Prev*, **13**, 5871-4.
- Huang J, Tang LL, Hu Z, et al (2008). BRCA1 and BRCA2 gene mutations of familial breast cancer and early-onset breast cancer from Hunan province in China. *Zhongguo Ai Zheng Za Zhi*, **18**, 566-72.
- Laraqui A, Uhrhammer N, Lahlou-amine I, et al (2013). Mutation screening of the BRCA1 gene in early onset and familial breast/ovarian cancer in Moroccan population. *Int J Med Sci*, **10**, 60-7.
- Li SW, Lu Y, Yang XY, et al (2012). Analysis of clinical characteristics and related factors of breast cancer patients with different nationalities in Xinjiang areas. *J Xinjiang Med University*, **35**, 895-8.
- Li WF, Hu Z, Rao NY, et al (2008). 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*, **110**, 99-109.
- Meindl A (2002). Comprehensive analysis of 989 patients with breast or ovarian cancer provides BRCA1 and BRCA2 mutation profiles and frequencies for the German population. *Int J Cancer*, **97**, 472-80.
- Meng J, Shi YR, Niu RF, et al (2009). Relationship between mutation of BRCA1 and susceptibility to early onset of breast cancer. *Zhonghua Yi Xue Za Zhi*, **89**, 79-82.
- Miki Y, Swensen J, Shattuck-Eidens D, et al (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science*, **266**, 66-71.
- Mohammad Mahdi Kooshyar, Mohammadreza Nassiri, Morteza Mahdavi, et al (2013). Identification of germline BRCA1 mutations among breast cancer families in northeastern Iran. *Asian Pac J Cancer Prev*, **14**, 4339-45.
- Musopolino A, Bella MA, Bortesi B, et al (2007). BRCA mutations, molecular markers, and clinical variables in early-onset breast cancer: a population-based study. *Breast*, **16**, 280-92.
- Ou JH, Wu T, Sijmons R, et al (2013). Prevalence of BRCA1 and BRCA2 germline mutations in breast cancer women of multiple ethnic region in Northwest China. *J Breast Cancer*, **16**, 50-4.
- Parkin DM (2004). International variation. *Oncogene*, **23**, 6329-40.
- Suter NM, Ray RM, Hu YW, et al (2004). BRCA1 and BRCA2 mutations in women from Shanghai China. *Cancer Epidemiol Biomarkers Prev*, **13**, 181-9.
- Tang NL, Pang CP, Yeo W, et al (1999). Prevalence of mutations in the BRCA1 gene among Chinese patients with breast cancer. *J Natl Cancer Inst*, **91**, 882-5.
- Thirthagiri E, Lee SY, Kang P, et al (2008). Evaluation of BRCA1 and BRCA2 mutations and risk-prediction models in a typical Asian country (Malaysia) with a relatively low incidence of breast cancer. *Breast Cancer Res*, **10**, 59.
- Tohg T, Kang P, Lee SS, et al (2008). BRCA1 and BRCA2 germline mutations in Malaysian women with early-onset breast cancer without a family history. *PLoS One*, **3**, 2024.
- Uhrhammer N, Abdelouahab A, Laffarge I, et al (2008). BRCA1 mutations in Algerian breast cancer patients: high frequency in young, sporadic cases. *Int J Med Sci*, **5**, 197-202.
- Wooster R, Weber BL (2003). Genomic medicine: breast and ovarian cancer. *N Engl J Med*, **348**, 2339-47.
- Yassaee VR, Zeinali S, Harirchi I, et al (2002). Novel mutations in the BRCA1 and BRCA2 genes in Iranian women with early-onset breast cancer. *Breast Cancer Res*, **4**, 6.