Journal of Breast Cancer

J Breast Cancer 2013 March; 16(1): 50-54 http://dx.doi.org/10.4048/jbc.2013.16.1.50

ORIGINAL ARTICLE

Prevalence of *BRCA1* and *BRCA2* Germline Mutations in Breast Cancer Women of Multiple Ethnic Region in Northwest China

Jianghua Ou[1,2], Tao Wu[1], Rolf Sijmons[3], Duo Ni[1], Wenting Xu[1], Halmurat Upur[4]

[1]Department of Breast Surgery, Xinjiang Cancer Institute & Hospital, Xinjiang Medical University, Urumqi, [2]Postdoctoral Research Station of Clinical Medicine, Xinjiang Medical University, Urumqi, China; [3]Department of Genetics, Medical Center of Groningen University, Groningen, The Netherlands; [4]Xinjiang Medical University, Urumqi, China

Purpose: The aim of this study is to further understand the status of *BRCA1* and *BRCA2* mutation among Chinese high-risk breast cancer patients in multiple-ethnic regions of China. **Methods:** A total of 79 blood samples of high-risk breast cancer patients from Xinjiang Uyghur autonomous region were analyzed by PCR-DHPLC sequencing analysis. **Results:** Analysis with full length of the two genes identified a total of 6 deleterious mutations (2073delA, 2394C-T [Q759X] and IVS16+1G>A in *BRCA1*; 1627A-T [K467X], 6873delCTCC and 9481delA in *BRCA2*) in this cohort. The prevalence of *BRCA1/2* germline mutation was about 7.6% (6/79) in the Xinjiang multiple ethnic region of China. Among them, 3 novel deleterious mutations, 2073delA in *BRCA1* (Han ethnic Chinese) and *BRCA2* variants 6873delCTCC and 9481delA (both are Kazakh ethnic Chinese), were identified and they had never been reported in breast cancer information core (BIC) database before. 2394C-T (Q759X) and IVS16+1G>A, in *BRCA1* and *BRCA2* variants 1627A-T were previously reported in other populations but not Chinese. Among 6 of the *BRCA*-related tumors, three *BRCA1*- and one *BRCA2*-associated tumors were in triple negative (estrogen receptor, progesterone receptor, and HER2 negative expressed) status and exhibited a high tumor grade. So far none of these 6 deleterious mutations were reported in ethnic Han Chinese. **Conclusion:** *BRCA* germline mutation in Chinese multiple ethnicity region may exhibit different genotypes compared to ethnic Han Chinese in other regions. These differences may arise from interaction of genetic background and environmental factors.

Key Words: *BRCA1, BRCA2, Breast neoplasms, Chinese, Ethnic groups, Genetic variation*

INTRODUCTION

China is a multiple ethnic country comprising 56 ethnic groups. As the majority of the population belongs to the Han ethnic group, China's other 55 ethnic groups are customarily referred to as the national minorities. Xinjiang is the largest administrative region located in the northwest of China, spanning over 1.6 million km^2. It is home to a number of different ethnic groups including the Han, Uyghur, Kazakh, Hui, Kyrgyz, Mongol, etc. Three of the groups had a population of more than one million, including Han (8.75 million), Uyghur (8.8 million), and Kzakh (1.35 million) [1]. To date, only a few studies have been reported on *BRCA*-associated breast cancer in China; they included families with early-onset breast (under the age of 35 years) patients or with family history of breast/ovarian cancer or bilateral breast cancer [2-4]. These studies revealed some characteristics of *BRCA*-related cancer in Chinese: the prevalence in Chinese population was lower than those in Caucasian and other Asian patients. On the other hand, no characteristic mutation spots were reported in mainland China. All the above mentioned studies only involved ethnic Han Chinese. Up to now, few studies have investigated the prevalence of *BRCA1* and *BRCA2* germline mutation among patients in the multiple ethnic regions of China. We believe it is of great significance to have an overview of these two genes among the Chinese high-risk breast cancer patients in multiple ethnic regions of China, so as to develop genetic screening guidelines for the Chinese women.

Correspondence to: **Halmurat Upur**
Xinjiang Medical University, 393 Xinyi Road, Urumqi 830011, China
Tel: +86-22-2721-9052, Fax: +86-22-2721-9052
E-mail: neuroman@163.com

This research was supported by National Natural Science Foundation of China (Grant number: 30960435).

Received: October 16, 2012 **Accepted:** December 26, 2012

http://ejbc.kr | pISSN 1738-6756
eISSN 2092-9900

METHODS

Subjects

All patient cases were recruited from the Xinjiang Cancer Institute and Hospital of Xinjiang Medical University, Xinjiang, China. The patients were included if one of the following items was met: 1) at least one first or second degree relative with breast cancer and/or ovarian cancer, regardless of age; 2) at least one first-degree relative with malignant tumors other than breast cancer and ovarian cancer; 3) breast cancer onset age below 35 years; 4) bi-lateral breast cancer; or 5) triple negative breast cancer (estrogen receptor [ER], progesterone receptor [PR], and HER2/*neu* negative) with onset age below 45 years of age. Seventy-nine breast cancer patients, who received the standard treatment at the Xinjiang Cancer Institute and Hospital during 2005 to 2011, were finally included. Their family histories were retrieved from the medical records and ascertained by the families and/or patients in person with standard questionnaires procedure. All the cases were from different families. The study was approved by the Ethics Committee (IRB approval number: XJYD1320) of the Cancer Hospital of Xinjiang Medical University and informed consents were obtained from all participants.

Mutation analysis

Genomic DNA was isolated from 5 mL peripheral blood and stored in 10 mM Tris (pH 8) EDTA at 4°C. Specific *BRCA1* and *BRCA2* coding regions and intron-exon boundaries, ranging from 206 to 639 bp in length, were amplified by polymerase chain reaction (PCR) (primer sequences and conditions available upon request). PCR was carried out with a 25 μL system containing 100 ng genomic DNA, 1.5 mM $MgCl_2$, 50 mMKCl, 10 mM Tris-HCl (PH 8.3), 200 μM dNTPs, 0.5 μM of each primer, and 0.2 μL of Taq polyMerase (5 U/μL) (Clontech, Palo Alto, USA). The PCR conditions were: denaturing at 95°C for 5 minutes followed by 35 cycles of denaturing at 94°C for 30 seconds annealing at 55°C for 45 seconds and extension at 72°C for 1 minute; one final step at 72°C for 5 minutes.

Denaturing high-performance liquid chromatography

PCR-DHPLC analysis was used to screen mutations from all coding exons and adjacent intronic splice junction regions of the *BRCA1* and *BRCA2* genes as previously described [5]. The DHPLC gradients and temperatures were determined by WAVE maker software and were properly adjusted.

Assessment of clinicopathologic features

ER, PR, histological grade (Bloom-Richardson Grading System) and HER2 status were collected from the pathology reports of the tumor samples. A triple negative breast cancer is characterized by the lack of ER, PR, and HER2.

RESULTS

Patient's characteristics

A total of 79 patients were recruited in this study and were analyzed for both *BRCA1* and *BRCA2* mutation. In all the included patients, 32 early onset cases were diagnosed below 35 years of age, 18 had at least one affected relative, 16 had bilateral breast cancer, and 3 had both bilateral breast cancer and affected relatives. Twenty-four were triple negative breast cancer with onset age below 45 years. Eight patients reported family history of malignancies of other organs, including the stomach, pancreas, gallbladder, bladder and uterus, liver and bone.

Pathogenic mutations

Six deleterious mutations in *BRCA1/2* gene were found in the 79 patients (Table 1). Most of the *BRCA1/2* mutations were

Table 1. Disease associated *BRCA1* and *BRCA2*

Mutation	Exon	AA change	Age at diagnosis	Ethnic group	Family history	Inclusion reason	Previous report
BRCA1							
2073delA	Exon 11		36	Han	Mother (OC)	FH+TNBC	No
2394C-T	Exon 11	Q759X	44	Han		TNBC	Yes [11], see BIC database [20]
IVS16+1G>A	Exon 16, intron 16 junction	Splice site mutation	35	Uyghur		TNBC	Yes, see BIC database [20]
BRCA2							
1627A-T	Exon 10	K467X	42	Han		TNBC	Yes [12-14], see BIC database [20]
6873delCTCC	Exon 11	Frame shift	42	Kazakh		BI-BC+FH	No
9481delA	Exon 24	Frame shift	35	Kazakh		Early onset	No

A total of 6 disease-associated mutations were detected in this study. Three mutation in *BRCA1* and 3 in *BRCA2*. GeneBank reference sequence *BRCA1* version #U14680.1, *BRCA2* version #U43746.1.
OC = ovarian cancer; FH = family history; TNBC = triple negative breast cancer; BIC = breast cancer information core; BI-BC = bilateral breast cancer.

either frameshift or nonsense mutations, which resulted in premature truncation of the *BRCA1/2* protein (Table 1). Three novel deleterious mutations were identified: 2073delA in *BRCA1* (Han ethnic Chinese) and *BRCA2* variants 6873delCTCC and 9481delA (both are Kazakh ethnic Chinese). Among 6 of *BRCA*-related tumors, 3 *BRCA1*- and 1 *BRCA2*-associated tumors were all in triple negative status.

No *BRCA1/2* mutation was found in 18 patients with a family history of breast cancer. One pathogenic *BRCA* mutation (1/3, 33.3%) was detected in 3 breast cancer patients with family history of ovarian cancer. Two pathogenic *BRCA* mutations (2/8, 25%) were identified in 8 breast cancer patients with family history of other malignancies. In 32 breast cancer patients with onset under 35 years old, one pathogenic *BRCA* (1/32, 3.1%) mutation was detected. One pathogenic *BRCA* mutation (1/16, 6.25%) was detected in 16 patients with bilateral cancer. One *BRCA* pathogenic mutation (1/3, 33.3%) was detected in 3 cases with bilateral breast cancer and family history of breast cancer or other malignancies. Four pathogenic mutations (16.7%) (including 3 *BRCA1* mutation and 1 *BRCA2* mutation) were detected in 24 patients with triple negative breast cancer under 45 years old.

Variation of unknown significance

In our study, three unclassified variants were detected. These 3 novel variants were not previously reported in the breast cancer information core (BIC) database (Table 2).

DISCUSSION

According to segregation analysis studies, 5% to 10% breast and/or ovarian cancer cases arise from an autosomal dominant pattern of inheritance [6,7]. Germline mutations in *BRCA1* and *BRCA2* genes are responsible for the genetic predisposition and increased risk for breast and ovarian cancer [8,9]. The prevalence of *BRCA* mutations varies among different populations due to founder mutation effects and other environmental and geographical factors [10,11]. Genetic cancer risk assessment guideline and genetic testing for inherited breast cancer susceptibility have become standard clinical management for selected patients in Western populations. But the situation in China is less managed, with only a very limited number of studies focusing on *BRCA* mutations among hereditary predisposition to breast cancer patients in China.

A few studies have reported *BRCA1* and *BRCA2* in high-risk cohorts among Chinese in Malaysia, Singapore, and Mainland China [12-15] with the majority of published studies in the Caucasian population. Ethnic Han Chinese patients were enrolled in most of these studies, with no study focusing on multiple ethnic groups in China. Moreover, up to now there have been no studies on the prevalence of *BRCA* mutations in triple negative breast cancer in the Chinese population.

In this study based at our hospital, 79 patients with hereditary predisposition to breast cancer (including patients with early onset breast cancer, family history of breast, ovarian cancer or other malignant tumors, bilateral cancer, or triple negative breast cancer with onset age below 45 years old) were examined for both *BRCA1* and *BRCA2* mutation. Six (7.6%) of the patients were identified to harbor a deleterious *BRCA* mutation. Among the 79 patients, 3 (3.8%) had a *BRCA1* mutation and 3 (3.8%) had a *BRCA2* mutation. The results are consistent with the previous reports [3,15-17], showing *BRCA2* mutation may not be less prevalent at least in some Asian populations. In contrast, in most published data from the Caucasian population, *BRCA1* germline mutation is more prevalent than *BRCA2* [18]. One pathogenic *BRCA* mutation (1/16, 6.25%) was detected among 16 bilateral cancer cases. One *BRCA* pathogenic mutation (1/3, 33.3%) was detected in 3 bilateral breast cancer cases

Table 2. Variants of unknown significance

Patient initials	Mutation	Mutation position	AA change	Onset age	Ethnic group and FH	Previous report and comment
YXQ	-7 C-T *BRCA1*	5' UTR		23	Han, early onset BC, no FH	Not reported in BIC, this mutation is located in 5'UTR, CG-TG substitution, may influence CpG island function
MGL	2053C-A *BRCA1*	Exon 11	S645Y	47	Han, TNBC, no FH	Not reported in BIC
	6529del aatgta *BRCA2*	Exon 11	In frame deletion			Not reported in BIC, this mutation causes two amino acid in frame deletion
GZR	4864A-G *BRCA1*	Exon 16,	D1582G	44	Han, TNBC, no FH	Not reported in BIC, 3 missense mutation cluster in small region of exon 16 in *BRCA1*
	4912C-A *BRCA1*	Exon 16,	S1598Y			
	5010A-G *BRCA1*	Exon 16	S1631G			

GeneBank reference sequence *BRCA*1 version #U14680.1, *BRCA2* version #U43746.1.
FH = family history; BC = breast cancer; TNBC = triple negative breast cancer; BIC = breast cancer information core.

http://dx.doi.org/10.4048/jbc.2013.16.1.50

with a family history of breast cancer or other malignancies. One pathogenic *BRCA* mutation (1/4, 25%) was detected in 4 breast cancer patients with an ovarian cancer family history. Due to our limited sample size, more comprehensive data need to be collected especially for bilateral breast cancer with a family history and breast cancer with a family history of ovarian cancer in multiple ethnic regions in Xinjiang, China.

Four pathogenic mutations (16.7%) were detected in 24 patients with triple negative breast cancer under the age of 45. All the 3 *BRCA1* mutations are in triple negative breast cancer cases with higher histological grade of invasive ductal carcinoma (average score 7.7). It is consistent with the previous findings in the Asian populations, including Chinese [3,15-17] that *BRCA1* mutated tumor conferred such features: ER or PR negative, higher histological grade, but with less medullary carcinoma compared to the Western population [19]. Therefore it is suggested that triple negative breast cancer of onset age below 45 may be good indicator for genetic screening in Chinese population. It is also supported in the recent National Comprehensive Cancer Network (NCCN) guidelines published in March 2011: 'woman with breast cancer who was diagnosed up to age 45 years with or without triple negative breast cancer' (http://www.jnccn.org/content/8/5/562.full).

Three *BRCA1*- and three *BRCA2*-disease-associated mutations were found in our study. Among the 6 *BRCA* mutations, two *BRCA1* and one *BRCA2* sequence variants were found in BIC or published literature. *BRCA1* 2394 C-T (Q759X) was previously described in an Indian family and European population (BIC) [20], but not identified in the Chinese population before. In this study this mutation was found in triple negative breast cancer patients with onset age 44 in ethnic Han Chinese group without the family history. *BRCA1* IVS 16+1 G > A was previously reported in European and Latin American populations (BIC); in this study, it was found in an ethnic Uyghur triple negative breast cancer patient in China. *BRCA2* 1627A-T (K467X) was previously reported in a Korean population and identified as recurrent mutation in the Korean population [21-25]. In this study it was found in a triple negative breast cancer patient (onset age 42) of Han Chinese ethnicity; and it was also the first in China. Three novel deleterious mutations were firstly identified in this study, which had never been reported in BIC or published literatures. Three of the 6 deleterious mutations in this study were found in ethnic Han Chinese for the first time, 1 was from Uyghur Ethnic group (never before reported in the Uyghur population outside China) [20, 26-29], and 2 from Ethnic Kazak group (never before reported in the Kazak population outside China). It suggested that the *BRCA* mutation prevalence and spectrum in the Xinjiang multiple ethnic group region may arise from interaction of genetic background and environmental factors.

We detected three novel mutations of unknown significance (Table 2). All these mutations are from 3 independent Han Chinese families. One early onset breast cancer case (onset age: 23) harbors -7 C-T *BRCA1* mutation, which is located in 5'UTR with CG-TG substitution and may influence CpG island function. Patient MGL harbors one *BRCA1* missense mutation and in frame deletion in *BRCA2*; it is unknown whether these alterations influences the protein folding structure. Patient GZR harbors 3 missense mutation clustered in a small region of Exon 16 in *BRCA1*. Interestingly, D1582G changed aspartic acid (side-chain negative charge polarity) into Glycine (side chain neutral). Further functional assay should be done to classify the clinical significance of these variants. Some novel techniques could be considered for early breast cancer screening in China [30].

Our study only provides some preliminary information on the prevalence of *BRCA* in the Xinjiang multiple ethnic regions in China. Larger sample genetic testing should be done among the breast cancer patients with hereditary predisposition, especially bilateral breast cancer with a family history, breast cancer with a family history of ovarian cancer and triple negative breast cancer with onset age below 45 years. We did find some potentially 'hot' *BRCA* mutation regions or spots in the Xinjiang women, but a conclusion can not be made on larger sample study. No founder mutation has been found in Xinjiang and other parts of Mainland China as yet. More data should be collected on *BRCA* distribution in Chinese breast cancer patients, especially in multiple ethnic regions of China. China is a developing country with multiple ethnic groups. Due to variations in the breast cancer prevalence, molecular subtypes and onset age among the different races and ethnicities, caution must be exercised in the clinical guidelines of the genetic and high familial risk assessment of breast cancer in the NCCN data, mostly of the Western populations, in the Chinese genetic testing as modified for the Chinese population.

REFERENCES

1. The National Bureau of Statistics of China, Department of Population, Social, Science and Technology Statistics; The State Ethnic Affairs Commission of China, Department of Economic Development. Tabulation on Nationalities of 2000 Population Census of China. Beijing: Nationalities Publishing House; 2003.
2. Kwong A, Wong LP, Wong HN, Law FB, Ng EK, Tang YH, et al. Clinical and pathological characteristics of Chinese patients with BRCA related breast cancer. Hugo J 2009;3:63-76.
3. Zhang J, Pei R, Pang Z, Ouyang T, Li J, Wang T, et al. Prevalence and characterization of BRCA1 and BRCA2 germline mutations in Chinese wom-

en with familial breast cancer. Breast Cancer Res Treat 2012;132:421-8.
4. Cao AY, Hu Z, Shao ZM. Mutation screening of breast cancer susceptibility genes in Chinese high-risk families: the results will develop the genetic testing strategy in China. Breast Cancer Res Treat 2010;120:271-2.
5. Arnold N, Gross E, Schwarz-Boeger U, Pfisterer J, Jonat W, Kiechle M. A highly sensitive, fast, and economical technique for mutation analysis in hereditary breast and ovarian cancers. Hum Mutat 1999;14:333-9.
6. Breast Cancer Information Core. National Human Genome Research Institute. http://research.nhgri.nih.gov/bic/Member/index.shtml. Accessed February 26th, 2013.
7. Newman B, Austin MA, Lee M, King MC. Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. Proc Natl Acad Sci U S A 1988;85:3044-8.
8. Schildkraut JM, Risch N, Thompson WD. Evaluating genetic association among ovarian, breast, and endometrial cancer: evidence for a breast/ovarian cancer relationship. Am J Hum Genet 1989;45:521-9.
9. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990;250:1684-9.
10. Easton DF, Bishop DT, Ford D, Crockford GP. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1993;52:678-701.
11. Nurwidya F, Takahashi F, Takahashi K. Meeting report: current cancer perspectives from the 9th Annual Meeting of the Japanese Society of Medical Oncology. Thorac Cancer 2012;3:94-7.
12. Anglian Breast Cancer Study Group. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Br J Cancer 2000;83:1301-8.
13. Sng JH, Chang J, Feroze F, Rahman N, Tan W, Lim S, et al. The prevalence of BRCA1 mutations in Chinese patients with early onset breast cancer and affected relatives. Br J Cancer 2000;82:538-42.
14. Li SS, Tseng HM, Yang TP, Liu CH, Teng SJ, Huang HW, et al. Molecular characterization of germline mutations in the BRCA1 and BRCA2 genes from breast cancer families in Taiwan. Hum Genet 1999;104:201-4.
15. Song CG, Hu Z, Wu J, Luo JM, Shen ZZ, Huang W, et al. The prevalence of BRCA1 and BRCA2 mutations in eastern Chinese women with breast cancer. J Cancer Res Clin Oncol 2006;132:617-26.
16. Yip CH, Taib NA, Choo WY, Rampal S, Thong MK, Teo SH. Clinical and pathologic differences between BRCA1-, BRCA2-, and non-BRCA-associated breast cancers in a multiracial developing country. World J Surg 2009;33:2077-81.
17. Thirthagiri E, Lee SY, Kang P, Lee DS, Toh GT, Selamat S, et al. 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 2008;10:R59.
18. Ang P, Lim IH, Lee TC, Luo JT, Ong DC, Tan PH, et al. BRCA1 and BRCA2 mutations in an Asian clinic-based population detected using a comprehensive strategy. Cancer Epidemiol Biomarkers Prev 2007;16:2276-84.
19. Foulkes WD, Metcalfe K, Sun P, Hanna WM, Lynch HT, Ghadirian P, et al. Estrogen receptor status in BRCA1- and BRCA2-related breast cancer: the influence of age, grade, and histological type. Clin Cancer Res 2004;10:2029-34.
20. Armes JE, Egan AJ, Southey MC, Dite GS, McCredie MR, Giles GG, et al. The histologic phenotypes of breast carcinoma occurring before age 40 years in women with and without BRCA1 or BRCA2 germline mutations: a population-based study. Cancer 1998;83:2335-45.
21. Choi DH, Lee MH, Bale AE, Carter D, Haffty BG. Incidence of BRCA1 and BRCA2 mutations in young Korean breast cancer patients. J Clin Oncol 2004;22:1638-45.
22. Juwle A, Saranath D. BRCA1/BRCA2 gene mutations/SNPs and BRCA1 haplotypes in early-onset breast cancer patients of Indian ethnicity. Med Oncol 2012;29:3272-81.
23. Han SH, Lee KR, Lee DG, Kim BY, Lee KE, Chung WS. Mutation analysis of BRCA1 and BRCA2 from 793 Korean patients with sporadic breast cancer. Clin Genet 2006;70:496-501.
24. Seo JH, Cho DY, Ahn SH, Yoon KS, Kang CS, Cho HM, et al. BRCA1 and BRCA2 germline mutations in Korean patients with sporadic breast cancer. Hum Mutat 2004;24:350.
25. Ahn SH, Hwang UK, Kwak BS, Yoon HS, Ku BK, Kang HJ, et al. Prevalence of BRCA1 and BRCA2 mutations in Korean breast cancer patients. J Korean Med Sci 2004;19:269-74.
26. Yazici H, Bitisik O, Akisik E, Cabioglu N, Saip P, Muslumanoglu M, et al. BRCA1 and BRCA2 mutations in Turkish breast/ovarian families and young breast cancer patients. Br J Cancer 2000;83:737-42.
27. Manguoğlu E, Güran S, Yamaç D, Colak T, Simşek M, Baykara M, et al. Germline mutations of BRCA1 and BRCA2 genes in Turkish breast, ovarian, and prostate cancer patients. Cancer Genet Cytogenet 2010;203:230-7.
28. Liang Y, Fu D, Hu G. Metadherin: an emerging key regulator of the malignant progression of multiple cancers. Thorac Cancer 2011;2:143-8.
29. Chen WQ, Zheng RS, Zeng HM, Zhang SW, Li GL, Wu LY, et al. Incidence and mortality of breast cancer in China, 2008. Thorac Cancer. Epub 2012 Jul 11. DOI: http://dx.doi.org/10.1111/j.1759-7714.2012.00165.x.
30. Iglesias-Garcia J, Lindkvist B, Lariño-Noia J, Domínguez-Muñoz JE. Endoscopic ultrasound elastography. Endosc Ultrasound 2012;1:8-16.

 http://dx.doi.org/10.4048/jbc.2013.16.1.50