The prevalence of BRCA1/2 mutations of triple-negative breast cancer patients in Xinjiang multiple ethnic region of China

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Abstract

Background: The screening of BRCA1 and BRCA2 mutations is now an established component of risk evaluation and management of familial breast cancer, early-onset breast cancer and bilateral breast cancer patients. There is still some controversy about whether this screening should be done in triple-negative breast cancers. Therefore, we evaluated the BRCA mutation prevalence in patients with triple-negative breast cancer in a multi-ethnic region of China.

Methods: A total 96 women who were diagnosed with triple-negative breast cancer in the Xinjiang region of China were enrolled in this study. BRCA1 and BRCA2 screening was performed by polymerase chain reaction-denaturing high-performance liquid chromatography (PCR-DHPLC) sequencing analysis. All mutations were confirmed with direct sequencing.

Results: The prevalence of a BRCA1/2 germline mutation was about 25% (24/96) in the Xinjiang region of China. Among 35 selected cases with a family history and/or bilateral breast cancers, the BRCA1/2 mutation prevalence was 25.7% (9/35). Of the remaining 61 patients with unselected triple-negative breast cancer, the BRCA1/2 mutation prevalence was 24.6% (15/61), and all 15 individuals with these mutations were premenopausal patients.

Conclusions: These results suggest that premenopausal women with triple-negative breast cancer may be candidates for genetic testing for BRCA1/2 in the Xinjiang region of China, even in the absence of a family history or bilateral breast cancer.

Keywords: BRCA1/2, breast Cancer, triple-negative, genetic testing

Background

The two major contributors to hereditary breast cancer are the breast cancer susceptibility gene 1 (BRCA1) and BRCA2 [1]. Genetic testing for BRCA1 and BRCA2 mutations has been established throughout North America and most of Europe. Not all women are candidates for testing; in general, testing is only necessary for women who have a familial breast cancer, early-onset breast cancer or bilateral breast cancer.

China is a multi-ethnic country, but the majority of the population belongs to the Han ethnic group. Chinese breast cancer patients on average were younger than patients in America and Europe by about 10 years old, and premenopausal patients accounted for the majority [2]. The prevalence of BRCA1 and BRCA2 mutations among the Chinese Han population was less than 20%, mutation of BRCA2 gene was higher than that of BRCA1 [3,4], and this is significantly different than for America and Europe populations [5].

To date, only a few studies have been reported on BRCA-associated breast cancer in China; all the studies involved mainly ethnic Han Chinese. Our previous study showed that the prevalence of the BRCA1/2 germline mutation was about 7.6% (6/79) in the Xinjiang multi-ethnic region of China. Among six of these BRCA-related tumors, three BRCA1- and one BRCA2-associated tumors were in estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) triple-negative status [6].
Triple-negative breast cancer (TNBC) describes a subgroup of tumors that lack expression of ER, PR and HER-2. Due to the lack of effective targets for endocrinal and molecular treatment, it is considered a type of high risk breast cancer, and the prognosis is poor. Overall, TNBC accounts for about 15% [7,8] of all breast cancers, but occurs more frequently in younger women and is the predominant subtype in individuals with a germline BRCA1 mutation [9]. Therefore, we have evaluated the BRCA1 mutation frequency and the implications for the clinical practice of undertaking genetic testing in women with TNBC.

Methods
Cases
All patient cases were recruited from Xinjiang, China. They had a definite pathological diagnosis of breast cancer and received the standard treatment in our hospital during the period 2005 to 2013. At the beginning of this period, women diagnosed with breast cancer who had family history (FH), early onset, or bilateral breast cancer (BI-BC) were chosen for study. As the research developed, we found that the majority of patients with the BRCA1/2 germline mutation were in triple-negative status. Therefore, TNBC patients were included in our study. To date, a total of 214 patients were submitted for evaluation, of which 96 were TNBC.

The study was approved by the Ethics Committee (IRB approval number: XJYD1320) of the Cancer Hospital of Xinjiang Medical University, and informed consent was obtained from each participant.

Triple-negative breast cancer confirmed
ER, PR and HER-2 status were confirmed in a histopathology report of the tumor samples. HER-2 was regarded as negative when scored as 0 or 1 by immunohistochemistry (IHC) and/or when there was non-amplification of HER-2 mRNA by fluorescent in situ hybridization (FISH).

BRCA1/2 mutation analysis
Genomic DNA was isolated from 5 mL of 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). Denaturing high-performance liquid chromatography (DHPLC) analysis was used to screen BRCA1 and BRCA2 mutations; all variants identified were confirmed by direct sequencing.

Statistical analysis
To compare the BRCA1/2 mutation in unselected and selected TNBC, chi-square analysis was performed. A P value of <0.05 was considered statistically significant.

The SPSS statistical software system version 16.0 (SPSS Inc., Chicago, USA) was used.

Results
Patients' characteristics
A total of 96 women with TNBC were eligible for testing. Among them, 35 women were selected for study because they had FH and/or BI-BC (25 women had a FH of breast or ovarian cancer, 13 women were BI-BC, both a FH and BI-BC were identified in 3 women). The remaining 61 patients had unselected TNBC. The average age of the patients was 39.7 years old (range 24 to 64 years old).

BRCA1/2 mutations
The full results of all BRCA1/2 deleterious mutations are given in Additional file 1: Table S1, all mutations were protein-truncating. Both the BRCA1 and BRCA2 mutation was identified in one patient with a family history, and she was 47 years old. BRCA1/2 mutations were present in 24 out of 96 individuals with TNBC. The prevalence of the BRCA1/2 germline mutation was about 25% (24/96), 18 in BRCA1 and 7 in BRCA2 (Table 1). The prevalence of BRCA1 germline mutation was about 18.6% (18/96), and the prevalence of BRCA2 germline mutation was about 7.3% (7/96).

Eighty-one of the 96 women were premenopausal patients, The BRCA1/2 mutation prevalence was about 27.2% (22/81); among them, BRCA1 mutation prevalence was about 19.8% (16/81), and BRCA2 mutation prevalence was about 8.6% (7/81). The remaining 15 cases were postmenopausal patients, the BRCA1/2 mutation prevalence was about 13.3% (2/15), the BRCA1 mutation prevalence was about 13.3% (2/15), and BRCA2 had no mutation. If we remove the 35 cases with family history and/or bilateral breast cancers from the analysis, a total of 15 (24.6%) BRCA1/2 mutations were identified in the remaining 61 patients of unselected triple-negative breast cancer. All mutations in 15 individuals were premenopausal patients. Among the 15 patients, 11 (18%, 11/61) had BRCA1 mutations, and 4 (6.6%, 4/61) had BRCA2 mutations. Therefore, the prevalence of BRCA1 mutations is greater than the prevalence of BRCA2 in TNBC patients.

A total of 18 BRCA1 mutations were identified; 13 of these mutations (72.2%) were in exons 10 and 11. Eight BRCA2 mutations were identified; six mutations among them (75%) were in exons 10 and 11.

Comparison of BRCA1/2 mutation between unselected and selected triple-negative breast cancer
A total of 96 women with TNBC were analyzed: 35 cases were selected TNBC (25 women had FH of breast or ovarian cancer, 13 women had BI-BC, and 3 women had a FH and had BI-BC) and 61 cases were unselected TN cancers. A total of 7 (28%) mutations were identified in
25 patients who had a family history, 2 (15.4%) mutations were identified in 13 patients who had bilateral breast cancer, and 15 (24.6) mutations were identified in 61 patients who had unselected TNBC (Table 2). A comparison of the three kinds of patients by chi-square test showed no significant difference (\( P = 0.687 \)).

A total of 9 (25.7%) BRCA1 and BRCA2 mutations were identified in 35 selected patients, and 15 (24.65) BRCA1 and BRCA2 mutations were identified in the 61 unselected patients (Table 3). It makes no difference in the prevalence of TNBC whether the BRCA1/2 mutation is selected or not. When all 96 TNBC were broken down into different age groups, BRCA1 and BRCA2 mutations were 25% (5/20) in unselected women with TNBC ≤ 35 years old and 33.3% (2/6) in women with a FH and/or BI-BC, TNBC and ≤ 35 years old.

**Discussion**

These data support the position that premenopausal TNBC is an indicator that can be used to help to identify candidates for BRCA1/2 mutation testing. BRCA1/2 germline mutations are responsible for genetic predisposition and may increase the risk for breast and ovarian cancer [1]. The prevalence of BRCA mutations varies among different populations due to founder mutation effects and environmental factors [10,11]. Genetic cancer risk assessment guideline and genetic testing for breast cancer have become standard clinical management for selected patients in Western populations. But the situation in China is less managed. Only a few of studies reported BRCA mutations among breast cancer patients in China; the subjects were mainly familial breast cancer, early-onset breast cancer and bilateral breast cancer. Based on these studies, the BRCA mutation rate is generally less than 20% in China [3,4,12]. Our data show the prevalence of the BRCA1/2 mutation in 96 cases of TNBC was 25%, close to the previous studies.

TNBC is a subgroup of breast tumors with poor prognosis. TNBCs are the predominant cancer subtype with a germline BRCA mutation. The prevalence of the BRCA mutation was 10 to 30% [13-15]. A total of 15 (24.6%) mutations were identified in 61 patients who had unselected TNBC (Table 3). The frequency of unselected TNBC is similar to the frequency of BRCA1/2 mutations in individuals that were selected for inclusion because of a family history or bilateral breast cancer. Our study included samples of unselected TN cancers, which were primarily diagnosed in premenopause; only six cases were postmenopausal. All mutations were found in premenopausal patients. The prevalence of the BRCA1 germline mutation was about 18.6% (18/96), and the prevalence of the BRCA2 germline mutation was about 6.3% (6/96). Prevalence of BRCA1 mutations is more common than BRCA2 in TNBC patients, and BRCA1 mutation is mainly associated with TNBC. All the BRCA1 mutations are in TNBC cases with higher histological grades of invasive ductal carcinoma. These findings coincided with earlier findings for Asian populations, including Chinese [3,10-12], where BRCA1-mutated tumors conferred the following features: they were ER- or PR-negative and had a higher histological grade, but exhibited less medullary carcinoma compared to the Western population [16]. Whereas about 70% of the patients from this earlier study of TNBC in China were premenopausal [17], in our study, all BRCA mutations in TNBC were in premenopausal patients. TNBC with family history may influence the BRCA mutation; when the 96 TNBCs were broken down into different age groups, BRCA1 and BRCA2 mutations were 25% (5/20) in unselected women (≤ 35 years old) with TNBC and 33.3% (2/6) were in

| Cases classification       | BRCA1/2 Mutation | \( \chi^2 \) | \( p \) |
|----------------------------|------------------|--------------|--------|
| Family history             |                 | 0.752        | 0.687* |
| Bilateral breast cancer    |                 |              |        |
| Triple-negative cancer     |                 |              |        |

*There was no significant difference among the three kinds of patients (TNBC with family history, TN cancers with bilateral breast cancer and single TNBC) (\( P > 0.05 \)).

**Table 1 BRCA1 and BRCA2 mutation prevalence in premenopausal/postmenopausal triple-negative breast cancer (TNBC) patients**

| Number of cases | BRCA1 mutations (%) | BRCA2 mutations (%) | Total BRCA1/2 mutations (%) |
|-----------------|---------------------|---------------------|----------------------------|
| All             | 96                  | 18 (18.6)           | 7 (7.3)                    | 24 (25)*                   |
| Premenopausal   | 81                  | 16 (19.8)           | 7 (8.6)                    | 22 (27.2)*                 |
| Postmenopausal  | 15                  | 2 (13.3)            | 0                          | 2 (13.3)                   |
| All unselected TNBC | 61              | 11 (18)             | 4 (6.6)                    | 15 (24.6)                  |
| Premenopausal   | 55                  | 11 (20)             | 4 (7.3)                    | 15 (27.3)                  |
| Postmenopausal  | 6                   | 0                   | 0                          | 0                          |

*Both BRCA1 and BRCA2 mutations were identified in one patient with family history, and she was 47 years old.
women (≤35 years old) with FH and/or BI-BC TNBC, respectively. These results are consistent with those reported in [18]. Although this study is not a large-scale investigation (only 96 patients), our results suggest that diagnosis of premenopausal TNBC would be a suitable threshold for BRCA testing. It is also consistent with simulation data, suggesting that this testing threshold would be a cost-effective strategy [19].

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, and because BRCA mutations are also different, caution must be exercised when following the clinical guidelines of the high and familial risk assessment of breast cancer in the The National Comprehensive Cancer Network (NCCN) data. Most of those data come from Western populations, and in China, genetic testing should be modified to better assess the Chinese population. Our study only provides some preliminary information of BRCA mutation of TNBC in the multi-ethnic region of Xinjiang, China. Our dataset is relatively small, but we confirmed similar observations made by others. To date, no single study has been large or definitive in China for TNBC BRCA mutations, and therefore, it is important to consider the results of all studies in aggregate. More precise figures would be obtainable from larger, prospective studies. We also found a number of potential BRCA mutation hotspot regions in Xinjiang TNBC patients, such as the mutation mainly in exons 10 and 11, but our conclusions need further study and more data collection.

Conclusions
Premenopausal women with TNBC may be candidates for genetic testing for BRCA1/2 in the Xinjiang region of China, even in the absence of a family history or bilateral breast cancer.

Additional file

Additional file 1: Table S1. BRCA1 and BRCA2 mutation in triple-negative breast cancer patients.
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