Original Article

BRCA1 germline mutations dominate familial breast cancer patients in Henan China

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Background: Gene mutations of *BRCA1* gene play a role in breast cancer. These mutations exist racial differences and can be inherited. The aim of the research is to study the relativity of *BRCA1* gene germline mutations with familial breast cancer patients in Henan, China.

Methods: Comprehensive *BRCA1* germline mutation analyses were performed through denaturing high-performance liquid chromatography (DHPLC) in a cohort of 59 breast cancer patients and 122 healthy donors with family history of breast cancer. The mutations detected by DHPLC were further validated by Sanger sequencing.

Results: About 52.54% (31/59) of familiar breast cancer patients showed *BRCA1* germline mutations, which is higher than other previous reports with Chinese patients. However, the mutation rate was only 5.74% (7/122) in healthy donors with family history of breast cancer, and also all these mutations were in *BRCA1* of all these mutations detected in both patients and healthy donors, mutation A3780G in *BRCA1* gene was reported for the first time. The mutation hotspots were A3113G and A3780G in *BRCA1* at least in this cohort of patients in Henan, China.

Conclusions: *BRCA1* germline mutations are related most closely to familial breast cancer patients in Henan China.

Keywords: Breast cancer; *BRCA*; germline mutation; denaturing high-performance liquid chromatography (DHPLC)

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Introduction

At present, the incidence of breast cancer in the world is obviously increasing, which seriously affects women's life and health. According to epidemiologic data, breast cancer exist familial clustering individual and race differences (1). These suggest that genetic factors play an important role in the initiation, promotion, and progression of mammary carcinogenesis. As rapid advances have been made in molecular biology and bioinformatic analyses, a lot of mutations were detected in *BRCA1* genes. One study has shown that gene mutations of *BRCA* could be inherited as an autosomal dominant (2). More than 80% of hereditary

Table 1 The family history of breast cancer patients in our study

Number of breast cancer patients	Quantity of breast cancer patients in the relatives	Relationship to patients
8	1	Aunt
1	1	Grandma
10	1	Sister
23	1	Mother
5	2	Mother, sister
5	2	Aunt, sister
4	2	Mother, grandmother
3	1	Grandmother

breast cancer cases carried gene mutations of *BRCA* by age 80, they had an early age at onset (3). Role of genetic testing for *BRCA* genes is to find hotspot pathogenesis and screen for high-risk groups. It is a manifestation of assessing the risk of familial breast cancer and its early diagnosis. Although a few studies have been carried out for investigating *BRCA* mutations in familial breast cancer in Chinese women (4,5), the spectrums of *BRCA* gene mutation are different due to other environmental and geographical factors (6,7). In this cohort study the aim was to analyze *BRCA1* gene mutations of familial breast cancer patients with Han nationality in Henan, China.

Methods

Study participants

Our study was approved by the Ethics Committee of the Institute for Henan Population and Family Planning (No. 2014YC005). Written informed consent was obtained for all patients.

This study enrolled 59 breast cancer patients and 122 healthy donors whom were unrelated to each other. All breast cancer samples were diagnosed according to histopathologic examination and came from the Center of Human Genetic Resources, Institute for Henan Population and Family Planning.

Blood sampling, DNA preparation

Venous blood was collected into two 4.5-mL sodium citrate anticoagulant tubes and stored at +4 ℃ until processing. Within 4 h after blood collecting, genomic DNA was extracted using QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) and stored at –80 ℃.

Gene mutations testing using denaturing high-performance liquid chromatography (DHPLC) assay

This assay included: all PCR primers were designed using the software PRIMER3-OUTPUT according to *BRCA1* and *BRCA2* gene exon sequences reported in Gene Bank. There were 24 primers on *BRCA1* gene. Multiplex PCR was performed with a thermal cycler and PCR products were purified. Purified PCR were denaturalized at 94 ℃ for 30 seconds and cooled down to room temperature within 30–40 minutes. This could contribute to forming hetero-duplex between normal *BRCA* gene and mutated gene. Denaturation temperature of target DNA fragments was predicted by WAVE Maker/Investigator software. Equal sample loading was 5 μL. According to changes of peak at 254 nm ultraviolet wavelengths, *BRCA* gene mutations were detected. Samples with *BRCA* gene mutations were PCR amplified again and confirmed by sequencing at Sangon Biotechnology Limited Company.

Statistical analysis

The difference in the rate of *BRCA* gene mutation between patient and healthy donor was analyzed χ^2 test. P values <0.05 was considered as statistically significant.

Results

The breast cancer patients were a mean age of 42.5± 7.9 years. They have at least one or more first-degree relatives affected with breast cancer (*Table 1*). Among these patients the percent that both mother and daughter had breast cancer was 39% and the percent that three generations all had breast cancer was 6.8%. These patients *BRCA1* gene mutations were detected in 31 of 59 breast cancer patients. These mutations are mostly located in exon 11 of *BRCA1* gene and the mutation rate is 52.54% (31/59). Mutation types are same sense mutations and missense mutation (*Table 2*). One novel missense mutation (3780 A>G) was found in this study.

BRCA1 mutations were detected in 7 of 122 healthy donors, and the mutation rate is 5.74%. Comparing to two rates of *BRCA* gene mutations between patients and

Table 2 *BRCA1* mutations in breast cancer patients

Gene	Base change	Effect on protein	Type of mutation
BRCA1	114 G>A	Lys38Lys	Same sense
	2082 C>T	Ser694Ser	Same sense
	2310 T>C	Leu771Leu	Same sense
	3113 A>G	Glu1038 Gly	Missense
	3780 A>G	Lys1183 Ary	Missense
	4308 T>C	Ser1436 Ser	Same sense

Table 3 Frequency of the variants detected in the *BRCA1* gene in breast cancer and control groups

Groups	Frequency in breast cancer groups	Frequency in healthy donors	HR (95% CI)	P value
Positive	31	7	18.19 (7.26–45.56)	0.0001
Negative	28	115	–	–
Total	59	122	–	–

Table 4 Pathological type of breast cancer which exist missense mutation in *BRCA* gene

Pathological type	Heterozygotes (n)	Homozygotes (n)	Percentage (%)
Invasive ductal carcinoma	10	13	74.19
Carcinoma *in situ*	1	1	6.45
Carcinoma *in situ*	2	0	6.45
Micro invasive carcinoma	0	1	3.23
Invasive lobular carcinoma	1	0	3.23
Mucinous carcinoma	0	1	3.23
Mucinous carcinoma	0	1	3.23

healthy donors there were statistically significant difference (P=0.0001) (*Table 3*).

The *BRCA1* mutation rates are varied regarding the subtypes of breast cancer. The mutation rate with *in situ* and tubule ductal carcinoma is 6.45% (2/31), respectively.

Table 5 Characteristics regarding the tumor type in the patients

Characteristics	Number of breast cancer patients (n)	Percentage (%)
Age		
≤35	8	13.5
36–45	27	45.8
≥45	24	40.7
Clinic stage		
I	7	11.9
II	20	33.9
IIb	15	25.4
IIIa	9	15.2
IIIb	2	3.4
IIIc	4	6.8
IV	2	3.4

One mutated case was found in micro invasive carcinoma, invasive lobular carcinoma, medullary carcinoma and mucinous carcinoma, and the mutation rate is 3.22% (1/31). Most significantly, 23 patients with invasive ductal carcinoma were shown *BRCA1* mutations and the mutation rate was 74.19% (23/31) (*Table 4*). According to AJCC Cancer Staging Manual (the sixth edition) clinic stage was shown in the *Table 5*.

Discussion

BRCA are tumor suppressors and play roles in DNA impair and posttranscriptional control. *BRCA* gene is closely related to the occurrence and development of breast cancer (8). They can inhibit cell proliferation and induce apoptosis (9,10). Loss of function of *BRCA* by mutations might lead to abnormal cell cycle and cell growth, and then cancers. Our result in this study shown the rate of *BRCA1* gene mutation is 52.54% among familiar breast cancer patients, which is higher than published result (11). A possible reason is that invasive ductal carcinoma is the main histopathologic types of breast cancer in this cohort of patients, in which the mutation rates in *BRCA1* even as high as 74.19%. One research have shown that *BRCA1* gene mutation invasive ductal carcinoma exist high correlations (4). So *BRCA1* gene mutation is more likely to harbor invasive ductal carcinoma. On the other hand, there was a high incidence of breast

cancer in the family the percent of *BRCA1* mutation was more than 80% (12). In our study the rate that two generations both had breast cancer were 39% and the rate that three generations all had breast cancer was 6.8%. So it had shown that family history was correlation to *BRCA1* gene mutation.

Our result had shown the rate of *BRCA* gene mutation among healthy donors was 5.74%, which was consistent with published result (4). There was statistical significance between breast cancer patients and healthy donors. So the result in this study suggested that women with familial breast cancer are good candidates for *BRCA* testing and women who carry a *BRCA* mutation should gain necessary inspections in order to find breast cancer.

Rebbeck *et al.*'s research (13) have shown that carcinogenesis of *BRCA* is associated with specific location and type of gene mutations. The localization of *BRCA* gene mutation in breast cancer cluster region (BCCR) will increase risk of breast cancer, and female carrier whose *BRCA1* gene mutation is located in EXON 11 will suffer from breast cancer in early youth. Our study had shown that *BRCA* 1 gene mutation was missense mutation at EXON 11 and located in BCCR. A mean age of patients group was 42.5±7.9 years. The hotspots of *BRCA1* gene mutation in familial breast cancer with Han nationality in Henan, China might locate in 3113 A>G and 3780 A>G. There was no *BRCA2* mutation in this study. So *BRCA1* mutation is more frequent than *BRCA2* mutation in Henan, China. Furthermore, we found one novel mutation 3780 A>G in *BRCA1* in this study.

In conclusion, in a cohort of 59 familiar breast cancer patients and 122 healthy donors in Henan China, we found that the mutation rate of *BRCA1* was as high as 52.54% in patients, and do not identify any mutation in *BRCA2*. Mutation rates were also varied in different subtypes of breast cancer. The subtype with the highest mutation rate was invasive ductal carcinoma, which was 74.19%. Our report here indicates that *BRCA1* is the major mutated gene in this cohort of patients. Therefore, the preventing and diagnosis of breast cancer with inherited patients should be mainly focus on detecting *BRCA1* germline mutations at least in Henan area of China.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: Our study was approved by the Ethics Committee of the Institute for Henan Population and Family Planning (No. 2014YC005). Written informed consent was obtained for all patients.

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