BRCA1 Gene Mutations in Chinese Families with Breast Cancer

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OBJECTIVE To investigate the frequency of BRCA1 gene mutations in breast cancer families in China.

METHODS Genomic DNA was obtained by conventional techniques from the peripheral blood mononuclear cells collected from 94 persons derived from 45 breast cancer families. All participants gave written informed consent. The mutations in the BRCA1 gene were detected by the polymerase chain reaction and single stranded conformation polymorphism (PCR-SSCP). Then, the samples of interest were sent for direct DNA sequencing.

RESULTS No mutation sites were found in exon 2 or 20 by DNA sequencing. Eight sites were found in exon 11 such as 2201C>T (Ser694Ser), 3232A>G (Glu1038Gly), 2201C>A/G (Ser694Arg), 2731C>T (Pro871Leu), 2086A>T (Asn591Asp) and three sites of 1584G>T (Glu424Stop). Three mutation sites were found in exon 16 which included 5106A>G (Met1663Val), 5208delT (Stop1639) and 4956A>G (Ser1613Gly).

CONCLUSION These mutation sites may be related to breast cancer, but more investigation is needed to determine whether the mutation sites are hot spots of mutations in Chinese familial breast cancer patients.

KEYWORDS: breast cancer, BRCA1 gene, gene mutation, single stranded conformation polymorphism.

The breast cancer susceptibility gene BRCA1 which is associated with hereditary susceptibility to breast and ovarian cancer is one of the first tumor-suppressor genes to be identified. Mutations in the BRCA1 gene are highly penetrant and confer a 85% risk of hereditary breast cancer. There are some mutation sites that have been applied to screen the members of breast cancer families in Western countries in order to predict the risk of breast cancer. In Asia, the BRCA1 mutation rates in breast cancer families have been published only from Japan, Singapore and Taiwan and have been reported to be lower than those of Western countries. Some reports from China are either from sporadic breast cancer patients or from limited breast cancer families, but there have been only a few reports concerning large scale BRCA1 mutation analysis of breast cancer families in China. In this study, we collected data from 45 breast cancer families selected from patients who visited or revisited the Tianjin Medical University Cancer Hospital during 1997–2003. The BRCA1 gene mutation analyses...
were performed by the polymerase chain reaction and single conformational polymorphism (PCR-SSCP) and DNA sequencing in order to reveal the proportion of families who inherit the BRCA1 mutations, and the spectrum of the BRCA1 mutations in Chinese breast cancer families.

MATERIALS AND METHODS

Subjects
Families were identified who were willing to participate in a genetic screening study of BRCA1 and who fulfilled any one of the following minimum criteria: there were more than 2 first degree relatives or second degree relatives with breast cancer in one family; there was one family member with ovarian cancer in addition to breast cancer; there was one male breast cancer patient in the family. A total of 45 breast cancer families were involved in our study, consisting of 94 participants, among whom 56 were breast cancer patients, and 38 were either first or second-degree relatives. Included were 2 male breast cancer patients. Control samples from a series of 39 normal individuals enrolled in one breast exam study were analyzed to determine population frequencies of observed variants.

DNA extraction
Genomic DNA was extracted from peripheral blood lymphocytes using a E.Z.N.A. Blood DNA kit.

Mutation analysis
Exon 2, 11, 16 and 20 of the BRCA1 gene were amplified by using 19 pairs of primers described in Table 1. PCR was carried out in a total volume of 50 μl containing 10× buffer 5 μl, MgCl2 (25 mM)4 μl, dNTP (10 mM) 1 μl, Taq DNA polymerase 1U (Takara), each primer (50 μM) 1 μl and genomic DNA 1-1.5 μg. The mixture was cycled at 94℃ for 30s, 50℃ for 50s, 72℃ for a min, 35 times. Each 10 μl of the PCR products was diluted 1:1 in denaturing loading buffer (95% formamide, 0.05% bromophenol blue, 10 mM EDTA ), heated in boiling water for 10 min and cooled on ice for 10 min. Then a 20 μl sample was loaded on the gel (8%) for polyacrylamide gel electrophoresis (PAGE). The gel was run in 1× TBE (Tris-Borate EDTA) under 100-Volts for 4-6 h at 4℃. After electrophoresis, the gels were silver-strained and the shift bands were examined.

DNA sequencing
The shift bands were selected and the counterpart remaining PCR products were sent to the Takara Co. for completing bidirectional DNA sequencing.

Sequence analysis
DNA Star Mag-Align biology software was applied to compare our DNA sequences with the original DNA sequences of BRCA1 in order to find the mutation sites. Usage of BLAST 2 on the web of NCBI can reveal the alterations in proteins from the results of the DNA sequencing in order to ascertain whether the mutation sites can lead to amino acid changes (missense). Finally, the pathogenic mutations can be checked in the Breast Cancer Information Core database (Breast Cancer Information Core, http://www.ncbi.nlm.nih.gov/Intramural_research/Lab_transfer/Bic) so as to verify whether the mutations have been previously reported.

RESULTS

PCR-SSCP results
PCR products of all samples displayed single bands in PAGE compared to control samples, which indicated that the sequence of PCR products might have mutations (Fig.1). In this study, 24 single shifted bands in the PAGE gel were found and were therefore sent for DNA sequencing(Table 2).

Fig.1. The shifted bands in PAGE.