

RESEARCH COMMUNICATION

A Novel Polymorphism in BRCA2 Exon 8 and Breast Cancer Risk in South India

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Abstract

In India the incidence of breast cancer is on the rise and it is rapidly becoming the number one cancer in females, pushing cervical cancer to the second spot. The contribution of *BRCA2* to the development of the sporadic forms of breast cancer remains undefined. To assess the role of SNPs in exon 8 of the *BRCA2* gene in breast cancer development in India, a population-based study was here carried out on 107 breast cancer patients and 96 controls by PCR-RFLP and DNA sequencing. T-C transitions at positions 29 bp and 44 bp in relation to the total sequence of exon 8 were identified. Characterization of BRCA genes is relevant in a prevention setting as well as for the clinical management of hereditary breast cancer patients. The presently identified novel mutation in exon8 of the *BRCA2* gene might have clinical significance.

Keywords: *BRCA2* gene - breast cancer - SNP

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Introduction

The incidence of breast cancer is rising in every country of the world especially in developing countries such as India. *BRCA2*, located on chromosome 13q, has been identified as a breast cancer susceptibility gene (Wooster et al., 1994). Consistent with the tumor suppressor status of the gene, tumors that develop in carriers of heterozygous *BRCA2* mutations are frequently associated with loss of heterozygosity at the *BRCA2* locus (Venkitaraman, 2002). Inherited mutations in the gene continue to be associated with the familial form of breast, ovarian, and other types of cancer (Couch et al., 2007), which represent only a small proportion of the total cases.

The role of *BRCA2* in the development of the sporadic form of breast cancer remains undefined. Although loss of heterozygosity of the *BRCA2* locus has been detected in more than 50% of sporadic breast tumors (Bieche et al., 1994), somatic mutations (Valarmathi et al., 2004; Saxena et al., 2006) or inactivation by methylation has been either absent or rare (Collins et al., 1997). The involvement of altered expression of *BRCA2* in the development of sporadic breast cancer is a possibility. *BRCA2* gene expression, owing to its functional relevance, is tightly regulated by several known and unknown factors, which in turn could be candidates responsible for the deregulated expression of *BRCA2*, thus leading to cancer.

Not surprisingly, few studies have reported mutation frequencies for all of the coding exons of *BRCA2* because of its large size. Thus far only infrequent alterations

in *BRCA2* have been reported in ESCC (Harada et al., 1999). With the exception of alterations in the extreme carboxy-terminal of *BRCA2* (3' of the polymorphic stop codon K3326X), all *BRCA2* truncation mutations are considered to be deleterious (Mazoyer et al., 1996). The aim of this study was to detect the polymorphisms in exon 8 associated with the *BRCA2* gene.

Materials and Methods

Patients

The study population consisted of 107 patients who had received a histopathologically confirmed diagnosis of breast cancer from tertiary hospitals of Coimbatore city, Tamilnadu, South India. Each new patient was screened with a brief eligibility questionnaire that assessed various demographic features including prior cancer therapy and willingness to participate in the study. 96 healthy persons without a history of cancer were recruited as controls. The potential control subjects were first surveyed by using a short questionnaire to elicit willingness to participate in the study and to provide preliminary demographic data for matching.

DNA isolation

After informed consent was obtained, venous blood (3ml) was collected from all subjects using heparinized syringes. The samples were transported on ice to the laboratory and were processed. Genomic DNA was isolated from the lymphocytes of the peripheral blood samples using standard blood DNA isolation kit (Qiagen).

The DNA concentration was standardized by using a spectrophotometer (Shimadzu). DNA samples were stored at -20°C, and aliquots for immediate analysis were stored at 4°C.

PCR-RFLP

Polymorphism of exon 8 of *BRCA2* gene was analyzed by a PCR-RFLP procedure with the following oligonucleotide primers: FP: 5'-ATGGTTGGGTATGGGTA-3' RP: 5'-TGAAGGCCTTTCACA-3' which gave a 141 bp product. The PCR conditions were standardized as initial denaturation at 94°C for 3 min, followed by 38 cycles of denaturation at 94°C for 30 sec, annealing at 58.5°C for 30 sec, extension at 72°C for 8 sec and a final extension of 72°C for 10 min. A negative control without template DNA was used in each run. The amplified products were then resolved in 5% agarose gel stained with ethidium bromide.

The products were then digested with restriction endonuclease, MseI (Fermentas), as recommended by the manufacturer. The fragments obtained were analyzed in a 5% agarose gel stained with ethidium bromide and photographed under UV light. The bands obtained were confirmed in triplicates along with undigested sample.

DNA sequencing

The sequence variations in the samples were analysed by sequencing the amplified product. MinElute 96 UF PCR Purification Kit (Qiagen) was used to remove contaminating salts and unincorporated dye terminators before sequencing using a capillary sequencer (3730xl DNA Analyser, Applied Biosystems).

Results

The main characteristics showing the profile of the users and control subjects who took part in the study are presented in Table 1. For the exon 8 polymorphism, the controls produced three DNA bands at 102bp, 24bp and 15 bp, while in 36.8% of breast cancer samples, variations in DNA band size was observed due to the loss of restriction sites as a result of base modifications. Out of these, 52.4% cases showed modifications in both the restriction sites that resulted in a single band of 141 bp when digested, while two bands of sizes 126, 15 base pairs and 102, 39 base pairs were observed in 33.3% and 14.3% patients, respectively (Figure 1).

DNA sequencing results confirmed the presence of SNPs in exon 8 of the *BRCA2* gene. A T-C transition was found in 29th and 44th nucleotides in relation to the total sequence of exon 8 which resulted in the change of aminoacids from leucine to serine (Figure 2).

Table 1. Characteristics of Breast Cancer Cases and Controls

Variable	Cases (n=107)	Controls (n=96)
Age (mean ± SD)	45.9 ± 4.91	40.1 ± 4.90
Family history No (%)	57.3	62.1
of cancer		
Breast cancer (%)	12	6.9
Other cancer (%)	30.7	31.0



Figure 1. Agarose Gel Showing the Restricted Fragments M - 10bp marker; C - Control sample; 1,2,3 - Breast cancer samples

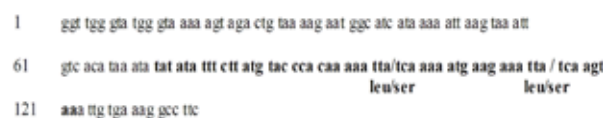


Figure 2. Flanked Exon 8 Sequence of BRCA2 Gene with SNPs and Aminoacid Substitutions

Discussion

Tumor suppressor genes play important roles in regulation of cell growth and differentiation. *BRCA2* mutations confer a greatly increased risk of breast cancer, some sequence variants in both genes might be candidates for moderate or low penetrance alleles. Most *BRCA* sequence variants are clearly deleterious and known to be unequivocally involved in the pathogenesis of breast cancer. A large number of genetic alterations are still classified 'variants of unknown significance' (Chenevix-Trench et al., 2006). All the germ line mutations in the *BRCA2* gene so far found in breast cancer families have been deletions and nonsense mutations (Thorlacius et al., 1996), leading to truncation and inactivation of the gene product.

According to the findings of Toyomasa et al. (Toyomasa et al., 1996), inactivation of *BRCA2* may play some role in development or progression of hepatocellular carcinoma and might predispose carriers of mutant alleles to liver malignancies. Researchers have identified more than 800 mutations in the *BRCA2* gene, many of which are associated with an increased risk of breast cancer. Most of these genetic changes disrupt protein production from one copy of the gene in each cell, resulting in an abnormally small, nonfunctional version of the *BRCA2* protein. As these defects accumulate, they can allow cells to grow and divide uncontrollably and form a tumor (<http://ghr.nlm.nih.gov/gene/BRCA2>).

Few data are available about the correlation between the site of the mutation and a specific cancer spectrum (Thompson et al., 1996). Risch et al., reported an increased risk of breast cancer associated with mutations downstream the *BRCA1* coding sequence and a peak in ovarian cancer risk associated with mutations in the middle of the coding sequence. Previous studies reported a higher risk of ovarian cancer for carriers of *BRCA2* mutations located in the ovarian cancer cluster region (OCCR) (Gayther et al., 1997; Mary-Claire et al., 2003).

In conclusion, the mutations in the exon 8 of *BRCA2* gene examined in the present study were rare among Indians, and were not associated with the risk of breast cancer in India. Although no associations were observed with the polymorphism, this study provided information useful to go on the next step of investigation, and this reporting has a meaning for the researchers to conduct the search for the same *BRCA2* exon specific polymorphisms. Our data showing *BRCA2* exon 8 polymorphism in breast cancer indicates that exon 8 is likely to play an important role in carcinogenesis. Insufficient data exist at this time to allow assessment of the clinical or functional implications of this sequence change. We are of the notion that sequence change of this type may interfere with gene function and could produce a risk in the development of cancer.

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