RESEARCH ARTICLE

Screening of 185DelAG, 1014DelGT and 3889DelAG BRCA1 Mutations in Breast Cancer Patients from North-East India

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Abstract

Around 1.35 million people of worldwide suffer from breast cancer each year, whereas in India, 1 in every 17 women develops the disease. Mutations of the Breast Cancer 1 (BRCA1) gene account for the majority of breast/ovarian cancer families. The purpose of study was to provide a prevalence of BRCA1 germline mutations in the North-East Indian population. In relation to the personal and family history with the breast cancer, we found mutations in 6.25% and 12.5% respectively. Three mutations, 185DelAG, 1014DelGT and 3889DelAG, were observed in our North-East Indian patients in exons 2 and 11, resulting in truncation of the BRCA1 protein by forming stop codons individually at amino acid positions 39, 303 and 1265. Our results point to a necessity for an extensive mutation screening study of high risk breast cancer cases in our North-East Indian population, which will provide better decisive medical and surgical preventive options.

Keywords: Breast cancer cases - BRCA1 - mutations - North-East India

Introduction

Globally, breast cancer is the most common cause of cancer-related death in women, with around 327,000 deaths each year. Around 1·35 million cases of breast cancer have been found each year and 4·4 million women are believed to be live with breast cancer worldwide. It has been speculated that in 2020, around 1·7 million women will be diagnosed with breast cancer, which is an increased of about 26% in the developing world from current levels (Wong et al., 2009). In India, almost 100,000 women are diagnosed every year with breast cancer, and a rise to 131 000 cases is predicted by 2020 (Agarwal et al., 2008; Mangtani et al., 2010). And in North-East India breast cancer has always been a hotspot in comparison to rest part of the India because of genotoxic stress from tobacco exposure (Sunita et al., 2010).

Several environmental risk factors that may contribute to or hasten the development of breast cancer have been identified, including mainly lifestyle and reproductive factors. The factor with the strongest breast cancer risk association is a family history of breast and/or ovarian cancer, the associated risk being even higher for family history of early-onset disease (≤ age 40) (Datta et al., 2009). Genetic susceptibility to breast cancer is triggered in several ways; the best understood causal mechanism being due to germline mutations in tumor suppressor genes. Together, mutations in BRCA1 and BRCA2 genes account for the great majority of families with hereditary susceptibility to breast and ovarian cancer (Ford et al., 1998).

Among breast cancer patients, up to 5%~10% are considered directly relating to the inheritance of mutation in BRCA1 (MIM 113705, Genbank accession no. U14680) and BRCA2 (MIM 600185, Gene bank accession no. U43746), which accounts for most of the hereditary breast cancers (Claus et al., 1994). Moreover, women carrying these mutations have 60%~80% prone to breast cancer and ovarian cancer (Wooster et al., 2003). The BRCA1 gene is located on long arms of chromosomes 17 and it encodes a protein of 1863 amino acids (Hall et al., 1990). The protein physically associates with p53 and involved in homologous recombination (HR) and double-strand break repair in response to DNA damage (Greenberg 2008; Zhang et al., 2010). Miki et al. describes that BRCA1 is a strong candidate for the breast and ovary cancer (Miki et al., 1994). The spectrum of BRCA1 mutations has been characterized in different populations worldwide, with significant variation of the relative contribution of these genes to hereditary cancer between populations (Brozek et al., 2011). However, the contribution of mutations in these two genes to breast cancer patients in the Indian population remains relatively unexplored apart from a few small studies (Saxena et al., 2006). Thus, the screening of prevalence of mutations in BRCA1 gene will serve as a molecular predictor for women with breast cancer along with ovarian cancer in North-East Indian population.

Accumulation of various environment and genetic factors during lifespan of an individual can combine to form pathogenesis in breast cancer. Direct analysis of the tumor genome can reveal the genomic events accumulated during tumor progression. Hence, we investigated genomic alterations in thirty two breast cancer participants of North-East India with consideration to the exon 2 and

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exon 11 of BRCA1 gene. The main focus behind the study is to estimates genetic influence of BRCA1 in North-eastern population, which would be able to make better decision about medical and surgical preventive options.

Materials and Methods

Case selection

Thirty-two breast cancer tissues of female patients' (aged 29-73 years) were randomly chosen and used in the study from the CCHRC in 2009-2011 along with their consent letter. To examine the population frequency of any sequence variants identified in the patients, a series of age matched control samples were also collected from women. The genetic background of the population studied was unknown prior to study. Patients' selection was mainly based on following observation: 1) Younger women effected from breast cancer (≤40). 2) Previous personal history of a patient related to breast cancer. 3) Family history of the breast cancer patients.

Methods

Fresh cancerous and matched normal (adjacent non-neoplastic) tissue's specimen as well as blood samples were collected into EDTA vials from thirty two randomly selected patients from Cachar Cancer Hospital and Research Centre (CCHRC). The study was carried out after taking written informed consent from the participant. The extraction of DNA from tissue and blood were done by phenol/chloroform method (Ghosh et al., 2011). The supernatant containing total genomic DNA was aliquot and stored at -20°C. The isolated DNA was checked by the spectrophotometer and gel electrophoresis; by the help of its purity and quantity, the DNA was processes for the PCR amplification. The quantity of DNA used for PCR reaction was about 50-100 ng. The coding regions of BRCA1 gene were screened for DNA sequence variants by PCR techniques using exon specific primers (Table 1). It was performed and monitored in Veriti PCR machine (Applied Biosystems Inc., CA, USA) for 30 cycles (Table 2). The reaction's mixture contained total volume of 30 μl. Then PCR products were eluted from the agarose gel by the help of QIA kit® (Cat. No. 28704, USA) and processed for bidirectional Sanger sequencing at Bose Institute, India

Bio-informatics Tools

Raw nucleotide sequences from the sequencing results were processed through Sequence Scanner v1.0 (Applied Biosystem). The exported sequences were analyzed by using BLASTN software at NCBI site. According to the highest similarities, we depict the peak through the Chromas 2 software, and show the variations among sequences in figure panel.

Results

This is the first report in BRCA1 mutations from breast cancer patients of North-East India. We have designed our experiment to perceive the spectrum of mutation in breast cancer patients as well as the age matched controls. In the present investigation, we had selected three patterns of observation, age, personal history and family history of the patient.

In consideration to the studied data, the most common age group consisting of women 46-57 years around 90.62%, with the mean age of onset for 32 women diagnosis with breast cancer surveyed during 2009-2011 was 48 years (Table 2). Approximately 9.37% of cases were diagnosed under the age of 40 years. In relation to the age and breast cancer, the percentage of breast cancer in the younger women is quite high in this region. 6.25% patients have the personal history of breast cancer, and they found out with the breast cancer disease (Table 2). Reminiscent of this, the personal history of a patient with breast cancer in one breast or related cancer in other parts of the body has a 3 to 4 fold increased risk of developing a new cancer in other breast or in another part of the same breast. From the present exploration of mutational research study, we have found that 12.5 \% of the patients, whose have breast cancer in first-degree relative (Table 2).

By the help of three sets of primer, we amplified the particular regions of the BRCA1 gene and screened for alterations of the particular position of 185DelAG, 1014DelGT and 3889DelAG of exon 2, 11a and 11d respectively (Figure 1). To facilitate the objective of this study, we used the PCR technique and DNA sequencing technology for screening of all patients for the particular region of mutation with this population. The mutation rate is high, more than 40% were found to be positive with

Table 1. Primer pairs for the amplification of desire nucleotide bases

Primers Name	Primers Sequence (5'-3')	Tm (^{0}C)	Ta (^{0}C)	Amplicon size (bp)
BRCA1-185DelAG-F	ATT GGA ACA GAA AGA AAT GG	51.2	50	180
BRCA1-185DelAG-R	CCT AGT ATG TAA GGT CAA TTC T	54.7		
BRCA1-1014DelGT-F	ACA GCA TGA GAA CAG CAG	53.7	54	195
BRCA1-1014DelGT-R	CAC AGG GGA TCA GCA TTC AGA	59.8		
BRCA1-3889DelAG-F	TCT ACT AGG CAT AGC ACC GTT	57.9	53	192
BRCA1-3889DelAG-R	CTT CCA ATT CAC TGC ACT GTG	57.9		

*Tm - Melting Temperature, Ta - Annealing Temperature

Table 2. Characteristics of Breast Carcinoma Patients

Age	Total No. of patients	Mutation Positive cases	Family History and Personal History of patients			
			F.H	No F.H	P.H	No P.H
≤40	3 (9.37%)	1(33.33%)	1 (3.12%)	2 (6.25%)	0	3 (9.37%)
≥40	29(90.62%)	12(37.5%)	3 (9.37%)	26 (81.25%)	2(6.25%)	27(84.37%)
Total	32	13(40.62%)	4(12.5%)	28 (87.5%)	2(6.25%)	30(93.75%)

*F.H: family history, No F.H: without reported family history, P.H: Personal history, No P.H: without reported personal history

Table 3. *BRCA1* Deleterious Mutation in North-Eastern Breast Cancer Patients, India

Gene	Exon	NT	Base change	Codon	AA change	BIC Designation	Variation Type	Reported	Cases found	Mutation Rate
BRCA1	2	185	DelAG	23	Stop 39	185DelAG	Truncated protein	Ashkenazi, Jews, others	1	3.12%
BRCA1	11a	1014	DelGT	299	Stop 303	1014DelGT	Truncated protein	Various, Pakistani	3	9.37%
BRCA1	11d	3889	DelAG	1257	Stop 1265	3889DelAG	Truncated protein	Various, Chinese	9	28.12%

*NT: Nucleotide Position, AA Change: Amino Acid Change, BIC Designation: Breast Cancer information core Designation
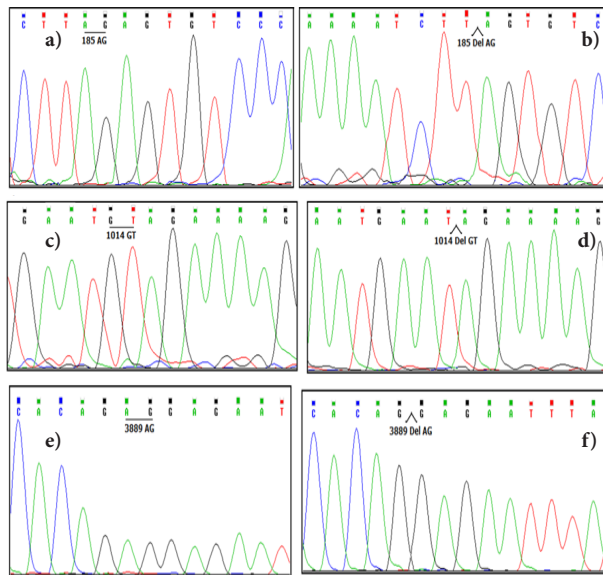
Figure 1. Variation in the Sequence Chromatograms of BRCA1 Gene Amplified and Screened for Alterations of the Specific Position at 185 Del AG, 1014 Del GT and 3889 Del AG of Exon 2, 11a and 11d Respectively from Breast Cancer Patients. a) Normal sequence, no deletion at 185 AG position from exon 2. b) Mutated sequences, deletion at 185 AG position, from exon 2. c) Normal sequence, no deletion at 1014 GT position from exon 11a. d) Mutated sequence, deletion at 1014 GT position from exon 11a. e) Normal sequence, no deletion at 3889 AG position from exon 11d. f) Mutated sequence, deletion at 3889 AG position from exon 11d.

these three variations of the BRCA1 after screening. And from that, most of the mutation occurrences were from the exon 11, out of the 32 patients 12 have the mutation which was around 37.5% and in exon 2, the incidences of mutations were quite low (around 3.12%) to the other part of world ethnicity. In commencing exon 11, we have screened two parts of the partial sequence; those were exon 11a and 11d of 192 and 194 bp product of amplicon respectively (Table 1). The mutation rate of 1014DelAG and 3889DelAG was 09.37% and 28.12% correspondingly (Table 3).

In summary, we found two mutations of two base pair deletion in exon 11 among 12 patients (1014 Del GT {3 Patients} and 3889DelAG {9 patients}) which results in protein truncation of BRCA1 protein by forming stop codons at 303 and 1265 position of amino acid respectively and one mutation of two base pair deletion at exon 2 (185DelAG) results in a stop codon at 39 position of amino acid in BRCA1 protein. Cachar Cancer Hospital and Research Centre support the observations, evaluations and findings of the research study undertaken among breast cancer patients from the North-East India.

Discussion

In the last few years many studies have focused in screening of mutations in breast/ovarian cancer. In this study majority of patients were at the fourth stage and large percentage of the patients who come to the CCHRC seeking care owing to their nominal incomes. Generally, breast cancer can occur at any age but younger women are less susceptible to ward's breast cancer (Mathew et al., 2004). Our study comprises a lower mean value of age, which is revealed that the disease occurs a decade earlier, as compared to western countries (Sandhu et al., 2010). The probable reason for the early onset of this dreaded disease in the younger women may be due to personal history with a breast cancer/ovary cancer (Liang et al., 2011), family history of breast cancer, particularly in a mother, sister and daughter (Metcalfe et al., 2010), history of radiation therapy to the chest before age 40. (Narod, 2011)

The aim of the mutational research analysis was to study the incidences and distribution of mutations in North-Eastern region of India concerning the other factors relating for breast cancer. For the diagnosis of breast carcinoma, FNAC technique was used as it is a useful diagnostic tool because of its cost efficient and rapidness (Sandhu et al., 2010). Three deleterious nonsense mutations resulting in a premature termination codon were identified in BRCA1: 185DelAG in exon 2; 1014DelGT and 3889DelAG in exon 11, rather absent in the observed control group. Nonsense mutations of these three specific mutations are very detrimental to the protein; it can render the resulting protein non-functional due to formation of stop codon at the early stage. Unexpectedly, we have gathered an 185DelAG in North-East Indian Hindu patient residing in Cachar district who claimed to have family history but not to Jewish ancestry (Figure 1b). In India, 185DelAG has been reported in all populations studied (Kumar et al., 2002; Hedau et al., 2004; Valarmathi et al., 2004; Saxena et al., 2005). Similarly, Lakhotia et al. found the same mutation by the help of conformation sensitive gel electrophoresis in four Indian breast cancer families (Lakhotia et al., 2010). Worldwide population studies have revealed that the 185DelAG mutation predates the severance of Sephardi and Ashkenazi Jewish populations and is probably 2000 years old (Bar-Sade et al., 1998). BRCA1 1014DelGT was detected in two Muslim index cases of without any family history from Karimganj District, but both have the personal history of ovary cancer (Figure 1d). Interestingly, the same mutation was reported in a heterogeneous Pakistani population of Muslim religious (BIC-NHGRI). The specific position of 1014DelGT comes under the part of a DNA binding region where other tumor suppressor proteins could not bind and unable to form complex protein for the downstream act of protein. These observations suggest that 1014DelGT might be a common mutation in the Muslim community and might have migrated to the Indian population through a pool of Muslim immigrants (Liede et al., 2009). In North-Eastern region, the mutation of 3889DelAG is higher than the rest of the mutation found (Figure 1f), out of nine, three have the family history and found scattered in studied population; it also found in various populations of the world (Thirthagiri et al., 2008; Farooq et al., 2011). The location of 3889AG is towards the C terminus of BRCA1, within the transcriptional activation domain, a region as well reported to interact with the BRCA2 protein, which plays an important role in double stranded break (DSB) repair (Roy et al., 2012). Nevertheless, the number of mutations identified in the studied North-East Indian population is higher; it may be due to the selected

candidate who comes under the three patterns of the study. This significant proportion of mutation from BRCA1 suggests one of the several possibilities for genetic predisposition in the North-East Indian population.

The results of this primary study put forward that the mutational spectrum in exons 2 and exon 11 of BRCA1 gene in this population may be at variance from what has been observed in other Indian populations. Further studies or mutational screening of the whole BRCA1 gene from different geographical regions of India will help in identifying the mutations may provide the knowledge of biological properties of the protein corresponding to polymorphism. Through this aspect of proper counselling, patients and pre-symptomatic mutations carriers' studies would be able to make better decision about medical and surgical preventive options.

Acknowledgements

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