Deleterious effect of BRCA1 mutations in familial breast cancer: A SSCP analysis

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ABSTRACT

The frameshift mutation 185delAG, in the BRCA1 gene, which results in the generation of a stop codon at position 39 in exon 2 was detected in 6 patients, 5 of the patients were with stage III, with age group of 62, 44, 29, 41 and 56 years, and one patient with stage IV, with the age of 36 years. SSCP is most often used to analyze the polymorphisms at single loci.

INTRODUCTION

BRCA1, a tumor suppressor gene, as it inhibits breast and ovarian cancer cell proliferation[1] is mapped to chromosome 17q21[2]. It is a large gene and spans over 100Kb. It has 24 exons of which only 22 are coding. Exon 11 of BRCA1 is the largest exon, encoding over 60% of the protein, and contains two nuclear localization signals[3].

The gene encodes a protein of 1863 amino acids (220kDa). It contains a RING finger domain near the N-terminus and an acidic carboxy terminal domain characteristic of many transcription factors. RING finger domain is critical for the function of BRCA1. It serves as an interface for recognition of DNA or protein-protein interaction. Several reports have demonstrated that BRCA1 physically associates with the p53 tumor suppressor protein and functions as a transcriptional coactivator for p53[4].

Mutations in two autosomal dominant genes, BRCA1 and BRCA2 have been linked to familial breast cancer[5].

Both prospective and retrospective genetic epidemiological studies have demonstrated that women who carry mutations in either BRCA1 or BRCA2 genes are at very high risk of developing both breast and ovarian cancers[6]. Although a woman in the general population aged 70 years has a 3% risk of breast cancer in the next 10 years; a woman with a BRCA1 mutation has as much as a 15% risk for the next 10 years when aged only 30 years[7].

BRCA1 and BRCA2 are the main susceptibility genes for hereditary breast cancer[8]. Among women with breast cancer, mutations in BRCA1 gene are more common than in BRCA2, suggesting that BRCA1 mutations may favor early-onset breast cancer[9].

Germline mutations of BRCA1 in families are esti-
mated to increase the risk of developing breast cancer for the first time for BRCA1 carries at 73% by age of 50 and 87% by 70 years. Young women with germ line mutations in BRCA1, BRCA2, p53 (Li Fraumeni syndrome), Muir syndrome or PTEN (Cowden’s syndrome) are at increased risk for breast cancer. More than 600 different mutations have been described in BRCA1 gene. They are scattered throughout the coding region of the gene and more than 50% of them have been described only once. 95% of mutations result in the truncation of protein. 70% of the mutations are frame shift mutations, 20% are nonsense. Splice site and missense mutations constitute about 5% each. Life time risk for mutation carriers of BRCA genes are 60-85% for breast cancer. About 10% of breast cancer cases are related to inherited mutations BRCA1 and BRCA2.

Germline mutations on chromosome 17q, located on the breast cancer gene-1 (BRCA1), have been reported to be responsible for many inherited breast and ovarian cancers. In the United States, it is estimated that as many as 1 in 200 women carry BRCA1 genetic mutations, making it one of the most common gene markers for which genetic testing could be feasible. The present study is hence done for the genotypic analysis of breast cancer BRCA1 gene (Exon 2 and Exon 20) for Single strand conformation polymorphism or single strand chain polymorphism (SSCP).

**EXPERIMENTAL**

**Study population:** The study subjects were selected from female patients attending the Oncology Departments of Tertiary Hospitals in and around Coimbatore City. 64 female subjects who displayed chromosomal aberrations were selected for SSCP analysis out of 97 subjects screened.

**Blood sample collection:** 5.0 ml of blood samples were collected in EDTA coated collection tubes by venepuncture from subjects and controls aseptically by using heparinised polypropylene tubes. The tubes were immediately placed vertically in a sterile ice packed plastic container.

**Whole blood DNA extraction:** 300μl of blood was centrifuged at 5000rpm for 5 minutes at room tempera-

SSCP analysis: SSCP was performed following the protocol of Orita et al., 1989. A 6% acrylamide glycerol SSCP gel was used to analyze PCR amplified products. The gel consisted of 6% polyacrylamide gel. After degassing the acrylamide solution, 150μl of 10% ammonium persulphate and 30μl of N,N,N',N'-tetramethylethlenediamine (TEMED) were added and
mixed to induce gel polymerization prior to the gels being poured. The solidified gels were set into the buffer chamber containing 1X TBE buffer (89mM tris-boric acid and 2mM EDTA). 5μl of PCR products (exon 2 and 20) diluted 1/20 in 95% formamide (containing 20mMEDTA, 0.05% bromophenol blue and 0.05% xylene cyanol) were heated to 90°C for 3 minutes, chilled on ice and immediately loaded on to a 6% acrylamide gel containing 5% (vol/vol) glycerol. Gels were run at 60mA for 4-6 hrs on a minislab gel (90x80x1mm) at 4°C until the bands migrated to the middle of the gel.

**Silver staining:** The gel was first fixed in washing solution (10% glacial acetic acid) for 5 minutes with gentle shaking followed by 3 minutes in 1% nitric acid. The washing solution was decanted and the gel was rinsed with distilled water for 3 minutes in 1% nitric acid. The gel was rinsed with double distilled water for about 10 seconds. Then the bands were developed with 0.25molL⁻¹ Na₂CO₃ in dark and final fixing with 10% acetic acid followed by washing with double distilled water. When the bands appeared, photos were taken and the gel was dried wrapped with a membrane and air dried.

### RESULTS

According to the stages of breast cancer, 97 study subjects were grouped into 4 based on their stages of breast cancer. Among them 1 subject was found with breast cancer stage I. 12 breast cancer subjects with stage II, 78 and 6 subjects with breast cancer stages III and IV respectively (TABLE 1).

**TABLE 1 : Characteristics of patients studied in breast cancer with different stages**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Breast cancer stages</th>
<th>Number of patients</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Stage I</td>
<td>1</td>
<td>0.97%</td>
</tr>
<tr>
<td>02.</td>
<td>Stage II</td>
<td>12</td>
<td>11.64%</td>
</tr>
<tr>
<td>03.</td>
<td>Stage III</td>
<td>78</td>
<td>75.66%</td>
</tr>
<tr>
<td>04.</td>
<td>Stage IV</td>
<td>6</td>
<td>5.82%</td>
</tr>
</tbody>
</table>

**TABLE 2 : BRCA1 (185delAG) mutation in familial breast cancer patients**

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Stage</th>
<th>Age</th>
<th>Exon</th>
<th>Nucleotide</th>
<th>Type of mutation</th>
<th>Mutation Family history</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stage III</td>
<td>62</td>
<td>2</td>
<td>185</td>
<td>FS</td>
<td>delAG-ter39</td>
</tr>
<tr>
<td>2</td>
<td>Stage III</td>
<td>44</td>
<td>2</td>
<td>185</td>
<td>FS</td>
<td>delAG-ter39</td>
</tr>
<tr>
<td>3</td>
<td>Stage III</td>
<td>29</td>
<td>2</td>
<td>185</td>
<td>FS</td>
<td>delAG-ter39</td>
</tr>
<tr>
<td>4</td>
<td>Stage III</td>
<td>41</td>
<td>2</td>
<td>185</td>
<td>FS</td>
<td>delAG-ter39</td>
</tr>
<tr>
<td>5</td>
<td>Stage III</td>
<td>56</td>
<td>2</td>
<td>185</td>
<td>FS</td>
<td>delAG-ter39</td>
</tr>
<tr>
<td>6</td>
<td>Stage IV</td>
<td>36</td>
<td>2</td>
<td>185</td>
<td>FS</td>
<td>delAG-ter39</td>
</tr>
</tbody>
</table>

Br – Breast cancer  
Ov – Ovarian cancer  
Lc – Lung cancer  
Pc – Prostrate cancer  
FS – Frame shift mutation

Figure 1 : PCR amplification with 185delAG(259bp) primers(Lane 1); 5382 inc(401bp) primers(Lane 2); 100bp DNA ladder (Lane 3)
Deleterious effect of BRCA1 mutations in familial breast cancer

**DISCUSSION**

BRCA1 associated mammary gland tumors in humans and murine cells deficient in BRCA1 exhibit genomic imbalances and chromosomal aberrations that are hallmarks of genetic instability. Dissection of the role of BRCA1 in maintaining genome integrity has been complicated by studies that link BRCA1 to multiple DNA repair pathways, such as homologous recombination repair, non-homologous end joining and nucleotide excision repair.

The N-terminus of BRCA1 interacts with BARD1, BAP-1, E2F-1. Cellular proteins that interact with the exon 11 of BRCA1 either directly or indirectly are RAD51, RAD50, p53, RB, c-Myc. The C terminus of BRCA1, which contains transcription activation domain and two BRCT domains, interacts with RNA polymerase II, p300/CBP, BRCA2, RNA helicase and CtIP.

BRCA1 binds to DNA without any specificity. BRCA1 over-expression also resulted in the transcriptional activation of Gadd 45. BRCA1 is likely to participate as a sensor or transducer rather than directly as a repair factor (effector). BRCA1 has been shown to associate with many cell cycle proteins during late G1 and S phases, BRCA1 protein increases and becomes phosphorylated. Upon exiting from M phase BRCA1 is dephosphorylated and its expression decreases. This expression pattern prompted intensive studies of BRCA1 in cell cycle checkpoints, leading to the discoveries of its important roles in centrosome duplication as well as G2/M and S checkpoints.

Ample experimental evidences indicate that BRCA1 plays an essential role in maintaining genome integrity in vivo. The study of BRCA1 function in vivo is complicated by its essential role in cellular viability, in that disruption of BRCA1 in the absence of additional compensatory mutations results in lethality.

BRCA1 regulates the activity of the RAD50-MRE11-NBS1 complex. BRCA1 has been reported to associate in vivo to the RNA polymerase II holoenzyme complex. In addition to a possible function of BRCA1 proteins, BARD1 and BAP1 that interact with Zinc-RING domain of BRCA1 protein. These findings suggest that BRCA1 is a tumor suppressor gene responsible for both normal development and carcinogenesis of the breast.

The number of mutations of BRCA1 and BRCA2 were estimated to be 718 and 458 respectively. A large scale mutation analysis of these two genes indicated that in many populations only 30-60% of familial breast cancers are attributable to BRCA1 and BRCA2 gene mutations. Mutation carriers also have a statistically increased risk for several other cancers; i.e. pancreatic cancer and carcinoma of the uterine body and cervix for BRCA1.

About 1200 mutations and polymorphisms are known in the BRCA1 gene. The ancient mutation 185delAG has been estimated to have approximately 46 generations for 1000-1500 years. It is also guessed that mutations 5382insC were originated in the Baltic.
38 generations ago[25]. Two mutations in BRCA 1, 185delAG and 5382insC, are common in Ashkenazi Jews with a combined frequency of above 1.0 %[28]. In contrast, up to 41% of Ashkenazi Jewish breast cancer patients with a family history of breast and/or ovarian cancer have either 185delAG or 5382insC BRCA1 founder mutations[29].

In India, 185delAG has been reported in all populations studied[30]. This deleterious frame shift mutation was first reported in a family residing in a part of Trivandrum not far from the small towns with the settlement of Jewish people[31]. It was later reported in two South Indian families in Kerala province[30]. BRCA1 mutations are most common in Russia accounts for 79% of hereditary cancer of breast while the prevalence is 47% in Israel and 27% in Italy[25]. Various Indian studies have reported BRCA mutations in 9-25% of familial breast cancer patients[30]. Breast tumors of BRCA1 mutation carriers generally occur at an earlier age and have distinct pathological features when compared with non-familial breast cancers[32]. Tumors are generally higher grade, highly proliferative and aneuploid and are predominantly ER2- and PR-negative compared with non-familial cases[33].

In the present study the clinicopathological characteristics of familial breast cancer patients with BRCA1 mutations, 5 of the patients were of tumor grade 3 and one patient having the tumor grade of 4 and four of the patients were having the tumor size of above 5 cms and two of them with tumor size of 2-5 cms. Our studies show clinical significance in certain families if the risks are associated with specific mutations. The main conclusions in our study, are that BRCA1 associated breast cancer has a serious prognosis, and this is especially so for early stages.

REFERENCES

Full Paper