# Independent origin of 185delAG *BRCA1* mutation in an Indian family

K. KADALMANI[1], S. DEEPA[3], S. BAGAVATHI[1], SHARMILA ANISHETTY[2], K. THANGARAJ[3], AND P. GAJALAKSHMI[1]

*[1]Lifesciences Division, AU-KBC Research Centre, Anna University, Chennai, e-mail: gaja@au-kbc.org, India 600 044, [2]Centre for Biotechnology, Anna University, Chennai, India 600 032, [3]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad, India 500 007.*

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*BRCA1* and *BRCA2* are the two major breast cancer susceptibility genes. We tested members of a family with multiple cases of breast cancer, for mutations in the *BRCA1* gene. Analysis of the PCR amplicons of all the exons (22) of the *BRCA1* gene using conformation sensitive gel electrophoresis (CSGE) revealed a heteroduplex band pattern in exon 2 of the proband (III-3) in this family. The amplicon was further sequenced to assess the nature of the mutation, which revealed a deletion of AG nucleotides at the 185$^{th}$ position (185delAG). The two base pair deletion introduces a stop codon at the 39$^{th}$ amino acid residue. A similar analysis was carried out on other extended family members to evaluate their allelic status. We detected the same deletion in 7 of the 19 members tested. Two of them are males. Haplotype analysis suggested an independent origin for this mutation. Our study highlights the importance of testing hereditary cases of breast/ovarian cancer for *BRCA1* mutations in extended families in order to identify high-risk individuals at a pre-clinical stage and provide genetic counseling.

*Key words: BRCA1- 185delAG-haplotype-Indian-origin-hereditary*

Breast cancer is the most common malignancy and is the second leading cause of cancer death among women [1]. The age-adjusted annual incidence rate (AAR) has increased from 17.9 to 24.9 per 100,000 women from 1965 to 1985 among Indian women [2]. The incidence of the disease varies among different ethnic groups [3]. In the South Indian city of Chennai, breast cancer is the second most common type of malignancy - the first being cervical cancer [4].

The first susceptibility gene for breast cancer was mapped to the long arm of chromosome 17 (17q12-21) using linkage analysis [5]. The identified gene namely, BRCA1 spans 117 kb of genomic DNA, which codes for a 5.7 kb transcript composed of 24 exons. Twenty-two of these exons translate into a 220 kd protein comprised of 1,863 amino acids [6]. Family studies suggest that women carrying mutations in the BRCA1 gene have a potential risk of developing breast/ovarian cancer. This risk varies between 26% and 80% and depends upon modifying factors such as genetic, reproductive, or lifestyle characteristics [7, 8, 9, 10]. Till now, about 730 unique variants have been reported in the Breast Cancer Information Core (BIC) database (http://www.nhgri.nih.gov/ Intramural_research/ Lab_transfer/Bic/). However, as many of these variants are polymorphic, they do not exhibit a phenotypic effect. Among Ashkenazi Jews, two predominant mutations in BRCA1 (185delAG and 5382insC) account for the majority of germ line mutations in high-risk breast and/or ovarian cancer families [11]. However, there have been very limited studies on the prevalence of BRCA1 mutations in Indian breast cancer patients [12, 13]. Here, we report a BRCA1 185delAG mutation with a unique haplotype in a South Indian family.

## Subjects And Methods

The described study is part of an ongoing research project aimed at screening for BRCA1 mutations in familial breast cancer patients in Tamilnadu, India. Clinical information about the proband and her affected sister was obtained from clinical records with due consent. The study was approved by the institutional biosafety and ethical committee (IBEC-8) and informed written consent was obtained from all individuals who participated in the study. The proband (III-3) had unilateral breast cancer (left side; clinical details not available), diagnosed at the age of 53 (Figure 1). She had recurrence (5x5 cm) in the same breast at the age of 55. Axillary node dissection showed an infiltrating ductal carcinoma with one

Correspondence to

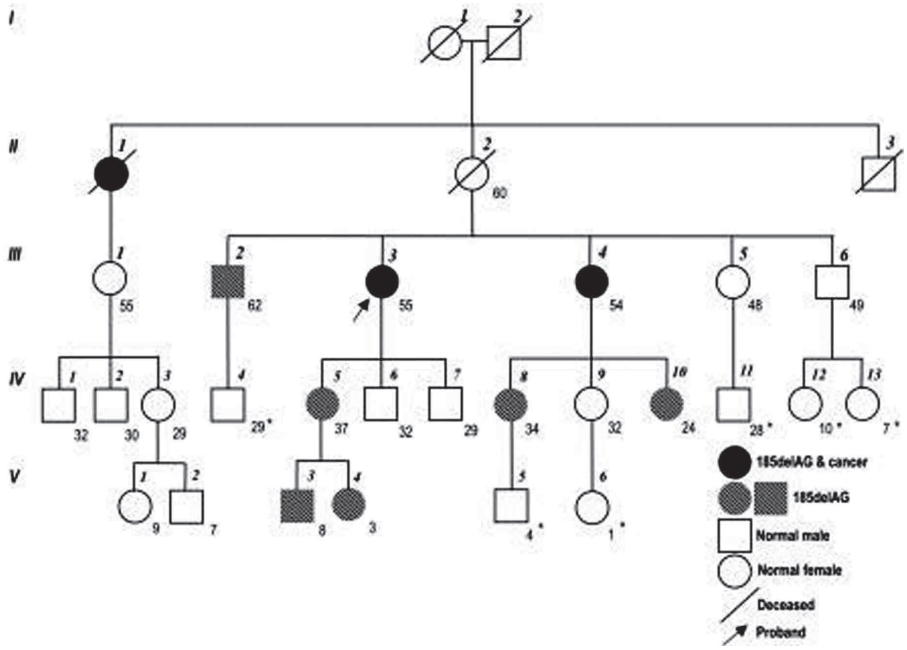


**Figure 1. Pedigree of a family with 185delAG mutation in the *BRCA1* gene. The proband (III-3) was diagnosed with breast cancer at the age of 53 years. Individual III-4 developed breast cancer at the age of 47 years. Numbers immediately above the symbols are individual identification numbers. Numbers below symbols indicate the current age for the individuals in the pedigree. The symbol * indicates the individuals who did not participate in the study.**

out of two regional lymph nodes affected, with a diagnosis of grade IIIA metastatic carcinoma. The proband underwent revision mastectomy and axiliary lymph node dissection and was treated with a CMF drug regimen followed by twenty sittings of radiotherapy. At present, the individual is on a regular follow up and is free from disease. The sister (individual III-4) of the proband was also diagnosed with breast cancer at the age of 47 after an initial symptom of swelling in the right breast for three months. The lump was located in the upper outer quadrant and measured 3x4 cm. The clinical diagnosis was $T_2N_0M_0$ Stage IIA with all four lymph nodes negative and no metastasis. Histopathological examination revealed an infiltrating ductal carcinoma. After complete presurgical investigations, the individual underwent modified radical mastectomy followed by chemotherapy and radiotherapy for twenty sittings. This individual is also now on a regular follow up and is free from disease. There is also a history of breast cancer in the proband's maternal aunt; however, there is no history of any type of cancer in the proband's paternal side. The elder sister (III-3) attained menopause at the age of 53 when she was diagnosed with disease and the younger sister was diagnosed with cancer in the premenopausal stage. The members of this family generally reside in and around Vellore district, North Tamil Nadu, South India. They belong to the Hindu Senguntha Mudaliar caste and speak the Tamil language.

*Screening of BRCA1 mutation by PCR-CSGE*

DNA was isolated from blood samples of the proband and her family members using the QIAamp DNA minikit (Qiagen, USA). All the coding exons of *BRCA1* were amplified and the amplicons were subjected to CSGE technique to detect mutations. CSGE is a simple and effective technique for detecting mutations in larger-size fragments of up to 450bp without loss of sensitivity. This method basically identifies heteroduplex bands, which arise due to annealing of complementary strands, one each from mutant and wild-type alleles. Primer sequences, PCR conditions and the heteroduplex detection protocols were performed according to Kumar *et al* [13].

*Sequencing of PCR amplicons showing heteroduplex band*

PCR amplicons, which showed a heteroduplex band in PCR-CSGE, were sequenced using the Big Dye terminator cycle sequencing kit and an ABI 3730 automated DNA analyzer (Applied Biosystems Inc., Foster City, CA, USA) following the previously described procedure [14].

*Cloning of BRCA1 exon 2*

Since the sequence electropherogram of the PCR amplicons with heteroduplex band showed overlapping peaks after 200 bp, we cloned the PCR amplicons to separate normal and mutant alleles. PCR amplicons of exon 2 of proband III-3 were ligated in pMOS*Blue* vector (Amersham Biosciences, Buckinghamshire, UK) according to the manufacturer's instructions. The recombinant plasmids were transformed into DH5a cells and transformed cells were plated on IPTG–X-gal and Ampicillin-treated agar plates for blue-white and antibiotic selections, respectively.

*Plasmid DNA preparation and sequencing*

Twenty isolated white colonies were inoculated in LB broth medium along with one blue colony as a control. Plasmid DNA was isolated using the protocol described elsewhere [15]. DNA isolated from all twenty colonies were subjected to DNA sequencing. About 150ng of DNA from each clone was sequenced (both forward and reverse strands) using the Big Dye terminator ready reaction kit (Perkin Elmer, Foster City, USA), U19 and M13 primers, with an ABI 3730 automated DNA analyzer.

*Genotyping and haplotyping*

Haplotype analysis among the family members was performed using five STR markers, of which three are intragenic to the *BRCA1* gene (D17S855, D17S1322, D17S1323) and

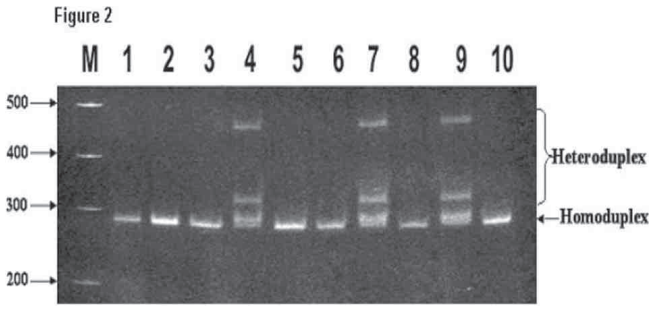


**Figure 2. An ethidium bromide – stained CSGE gel of exon 2 (258 bp) in individuals of generation IV. Lane M is a 100bp DNA ladder while lane 10 is a control sample from an unaffected, unrelated person. Lanes 1-9 represents the carriers of 185delAG mutation (lanes 4, 7, and 9) and noncarriers of the mutation (lanes 1, 2, 3, 5, 6 and 8) of generation IV.**

two are additional extragenic, flanking markers (D17S1321, D17S1327). Primer sequences and amplification conditions for all five STR markers were obtained from the Genome database (http://www.gdb.org). The forward primers of each pair of primers were labeled with FAM for the analysis of the amplicons. Two microlitres of each PCR product were mixed with 0.2 µl of the LIZ 500 internal size standard (Applied Biosystems Inc., Foster City, CA, USA), and 7.8 µl of 50% Hi-Di formamide. The GeneScan raw data were analyzed using the GeneMapper software (version 3.1) to obtain the allele repeat in base pairs. Alleles obtained from the samples were used to construct the haplotype.

## Results

The amplicon of exon 2 of *BRCA1* of the proband III-3 showed heteroduplex bands on the CSGE gel (Figure 2). Direct sequencing of the PCR amplicons showed an overlapping of the electrophoretogram from the middle of the sequence, suggesting a heterozygous allele (ie one allele having either deletion or duplication; data not shown). However, sequencing of the cloned amplicons showed an AG deletion at the 185$^{th}$ nucleotide position in one of the chromosomes (Figure 3). The normal *BRCA1* sequence has two adjacent AG nucleotides (TTA GAG) in codons 22-23. Deletion of one of the AG's introduces a frameshift mutation, which leads to a premature termination codon at position 39, in turn resulting in the formation of a truncated protein.

For this mutation we screened all members of the family (Figure 1). Nineteen out of twenty five individuals spread over three generations participated in the study. Our analysis revealed the presence of a heterozygous 185delAG mutation in six unaffected individuals of varying age and an affected sister of the proband (Figures 1 and 2). We then tested available family members for the haplotype associated with this particular 185delAG-mutation. STR analysis using three intragenic and two flanking markers revealed a unique haplotype (Figure 4). Individuals who did not carry the 185delAG did not have the disease-associated haplotype. The disease-associated haplotype observed in the present study was different (see Table 1) from the haplotype observed in Ashkenazi mutation carriers and non-Ashkenazi mutation carriers and provided evidence that the observed 185delAG mutation is an independent mutational event.

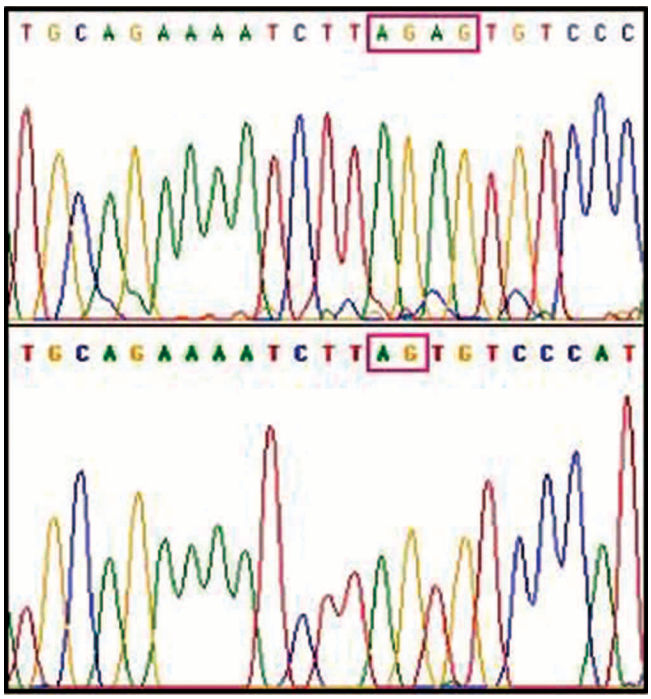


**Figure 3. Sequence electropherogram of cloned exon 2 of the *BRCA1* gene. The upper panel shows the presence of 2 copies of AG (boxed) and is from a healthy normal individual, while the lower panel that shows deletion of one copy of AG (boxed) is from the proband.**

## Discussion

In this study, we demonstrate for the first time the occurrence and segregation of 185delAG *BRCA1* mutation in

**Table 1: Haplotype analysis of the 185delAG mutation carriers in Indian, Ashkenazi Jewish and non-Ashkenazi Jewish chromosomes**

| Markers | Alleles linked to 185delAG | | |
|---|---|---|---|
| | Present study | Ashkenazi mutation carriers* | Non – Ashkenazi mutation carriers* |
| D17S1321 | 14 | 14 | 14 |
| D17S855 | 7 | 7 | 4 |
| D17S1322 | 8 | 3 | 5 |
| D17S1323 | 8 | 3 | 7 |
| D17S1327 | 6 | 6 | 12 |

* data from Neuhausen ***et al***., 1996.

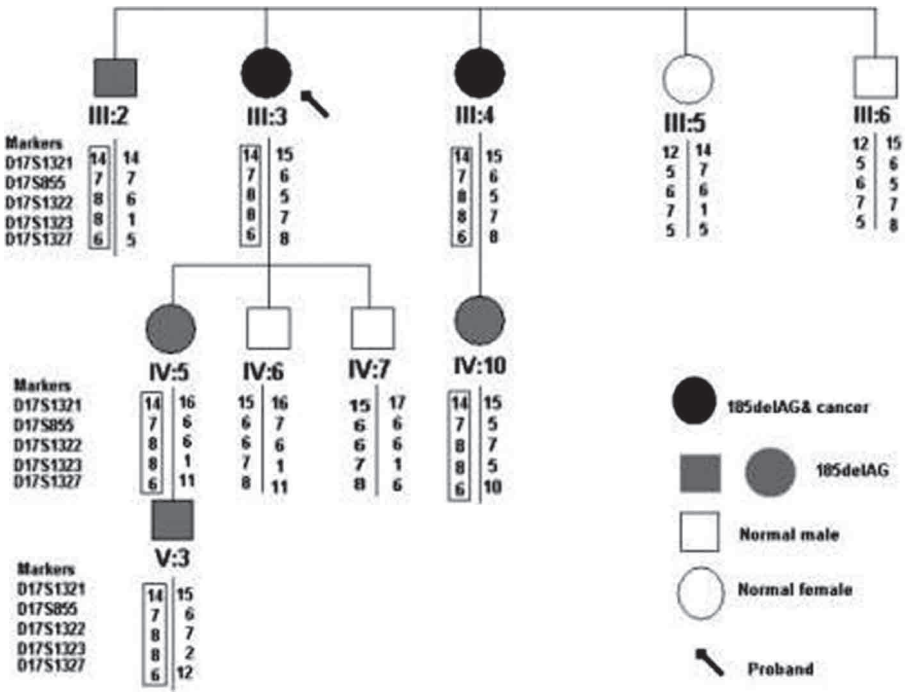


**Figure 4. Haplotype analysis among the selected members of the family (see figure 1). The STR markers that were used (indicated on the left) and the alleles observed are given below the symbols for the individuals. The haplotype linked to the mutation is shown in the box.**

a multiple generation Indian family with breast cancer (Fig. 1). Ours is the first study to demonstrate 185delAG in a familial case belonging to Tamil Nadu, South India. Kumar *et al* [13] found 3 different mutations (a missense mutation in exon 7, a base pair deletion in exon 11 and 2 base pair deletion in exon 2) in *BRCA1* gene of the 14 families studied. This study also detected a 185delAG mutation in one of the families from the state of Kerala in South India. Subsequent studies on Indian patients also revealed the presence of the 185delAG mutation in patients belonging to Goa and Kerala state [12, 16]. However, these groups did not carry out an extended family analysis or a haplotype analysis to trace the origin of the 185delAG mutation.

The 185delAG mutation is one of the common, ancient mutations; it is located at the 5' end of the gene and is predicted to cause truncation at the beginning of the zinc-binding region of the RING of the putative polypeptide [17]. Ruffner *et al* [18] have proposed that mutations within the *BRCA1* RING domain predispose to cancer by inactivating *BRCA1* ubiquitin protein ligase activity. There is no report on the specific mechanism of 185delAG mutation, although it may be noted that it occurs in an area of multiple adenosines, which is prone to DNA slippage.

Analysis of the extended family revealed the presence of 185delAG *BRCA1* heterozygous mutation in seven more individuals as shown in Figure 1, of which five (III-4, IV-5, 8, 10 and V-4) were females, including an affected sister and two (III-2 and V-3) were males. However, none of them have developed breast cancer so far except the proband's (III-3) immediate sister (III-4). Individual II-1 (deceased) was reported to have had breast cancer. However, the only daughter (III-1) of this affected woman (II-1), aged 55, did not inherit the mutation and is normal. Individual II-2, who died at the age of 60 years, seems to be an apparent carrier of the 185delAG mutation. Although her disease status could not be evaluated, we presume that breast cancer may be the primary cause for her death. The penetrance of 185delAG *BRCA1* mutation in the pedigree shown in Figure 1 is varied. The manifestation of tumors varies among siblings (III-3, 4) who have the same 185delAG *BRCA1* mutation. However, risk-modifying factors can explain inter-individual variability in cancer risk [19]. The average age of onset in this family is 50 years although it is estimated to be 44.7 years in carriers of 185delAG who belong to the Ashkenazi Jewish population [11]. Incomplete development of breast tissue in males, may partially explain the non-expression of the 185delAG mutation (for example, individual III-2) of *BRCA1*.

The lifetime risk of developing breast cancer and ovarian cancer in *BRCA1* carriers are 60% to 85% and 20% to 50%, respectively, in contrast to the 2% to 3% risk in the general population [20]. Therefore, the individuals IV-5, IV-8, IV-10 and V-4, who possess the 185delAG *BRCA1* mutation, have a higher probability of getting breast cancer during their lifetime.

The mutation 185delAG has been coined as the "Ashkenazi mutation" because it was predominantly detected among Ashkenazi Jews. An association of 185delAG in the *BRCA1* gene with breast cancer has also been found in Australian Jews [21] and Moroccan Jews [22]. However, it has also been reported in people of non-Jewish origins [23, 24]. Haplotype analysis has shown that there is likely a single origin for this mutation since all tested Ashkenazi mutation carriers displayed the same allelic pattern [25]. A similar study on several populations suggests that most of the populations have the Ashkenazi founding mutations, as they have similar haplotype. Ah Mew *et al* [26] reported the Ashkenazi origin of 185delAG in non-Jewish Chilean family. Similar observation was also made in Spanish families [27, 28].

Our haplotype analysis (Fig 4 and Table 1) indicates that the 185delAG mutation in the family considered in the present

study may have an independent origin. The haplotypes constructed from our samples is based on the STR markers flanking 185delAG, and does not match the haplotype in Ashkenazi Jewish or any of the non-Ashkenazi population (see Table 1). This suggests strongly that the deletion event is unique. Bar-Sade *et al*. [29] have reported that the Indian samples analysed by them have a non-Ashkenazi haplotype; however, their ethnic background is not known. This is relevant as India has a high ethnic diversity, consisting of about 5,000 anthropologically well-defined populations [30]. Interestingly, the Indian family in the present study belongs to one of the ancient linguistic (Dravidian) families. Based on the markers for the Y chromosome and mitochondrial DNA (mtDNA), it has been estimated that the coalescence time of Dravidians is >40,000 years [31]. Therefore, the deletion observed in this study may have an old and independent origin. However, comparison of a haplotype between the present family and other Indian families with 185delAG *BRCA1* mutation may give us more insights about the origin of this mutation.

Women from families with an identified *BRCA1* mutation usually benefit from being found not to carry the alteration (Example: III-1, 5; IV- 9 in this family), but women with a positive test (Example: III-3, 4; IV-5, 8, 10; V-3, 4 in this family) face different psychological and even social problems. Extensive education and genetic counseling are needed for women who undergo such testing in view of the fact that the benefits of presymptomatic testing and identification of carriers to determine susceptibility to common cancers, such as those of the breast, are potentially substantial. In the long run, identification of *BRCA1* mutations and other cancer-susceptibility genes should permit the development of new and more effective therapies, so that physicians can not only predict future risks, but also reduce those risks reliably and safely before disease occurs.

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