BRCA1 and *BRCA2* germline mutation analysis among Indian women from south India: identification of four novel mutations and high-frequency occurrence of 185delAG mutation

KANNAN VAIDYANATHAN[1,#], SMITA LAKHOTIA[1,#], H M RAVISHANKAR[1,#], UMAIRA TABASSUM[2], GEETASHREE MUKHERJEE[2,*] and KUMARAVEL SOMASUNDARAM[1,*]

[1]*Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India*
[2]*Department of Pathology, Kidwai Memorial Institute of Oncology, Bangalore 560 068, India*
[#]*Contributed equally*

**Corresponding author (Fax, 91-80-23602697; Email, skumar@mcbl.iisc.ernet.in)*

Mutations in the *BRCA1* and *BRCA2* genes profoundly increase the risk of developing breast and/or ovarian cancer among women. To explore the contribution of *BRCA1* and *BRCA2* mutations in the development of hereditary breast cancer among Indian women, we carried out mutation analysis of the *BRCA1* and *BRCA2* genes in 61 breast or ovarian cancer patients from south India with a positive family history of breast and/or ovarian cancer. Mutation analysis was carried out using conformation-sensitive gel electrophoresis (CSGE) followed by sequencing. Mutations were identified in 17 patients (28.0%); 15 (24.6%) had *BRCA1* mutations and two (3.28%) had *BRCA2* mutations. While no specific association between *BRCA1* or *BRCA2* mutations with cancer type was seen, mutations were more often seen in families with ovarian cancer. While 40% (4/10) and 30.8% (4/12) of families with ovarian or breast and ovarian cancer had mutations, only 23.1% (9/39) of families with breast cancer carried mutations in the *BRCA1* and *BRCA2* genes. In addition, while *BRCA1* mutations were found in all age groups, *BRCA2* mutations were found only in the age group of ≤40 years. Of the *BRCA1* mutations, there were three novel mutations (295delCA; 4213T→A; 5267T→G) and three mutations that have been reported earlier. Interestingly, 185delAG, a *BRCA1* mutation which occurs at a very high frequency in Ashkenazi Jews, was found at a frequency of 16.4% (10/61). There was one novel mutation (4866insT) and one reported mutation in *BRCA2*. Thus, our study emphasizes the importance of mutation screening in familial breast and/or ovarian cancers, and the potential implications of these findings in genetic counselling and preventive therapy.

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1. Introduction

Genetic predisposition for familial early-onset breast cancer accounts for approximately 5–10% of all breast cancers and 7–10% of all ovarian cancers (Emery *et al.* 2001). Mutations in two autosomal dominant genes, *BRCA1* and *BRCA2*, have been linked to familial breast or breast and ovarian cancer (Hall *et al.* 1990; Miki *et al.* 1994; Wooster *et al.* 1995). Women who carry *BRCA1* or *BRCA2* mutations have an estimated lifetime risk of between 60% and 85% for developing breast cancer, and a lifetime risk of between 26% and 54% for developing ovarian cancer for *BRCA1*, and between 10% and 23% for *BRCA2* (Easton *et al.* 1993; Brose *et al.* 2002; Antoniou *et al.* 2003; King *et al.* 2003).

Although their mechanism of action is not yet fully elucidated, it is assumed that these genes play a key role in important cellular pathways including response to DNA damage, transcription, and interaction with other proteins involved in DNA repair and apoptosis (Somasundaram 2003; Narod and Foulkes 2004). Genetic testing helps in identifying high-risk individuals in families with

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inherited breast and/or ovarian cancer, and there are various management options available for mutation carriers.

Breast cancer is the most commonly occurring cancer among Indian women with a relative proportion ranging from 19.3% to 27.5% (ICMR 2006). Significant variations in the relative contribution of mutations in *BRCA1* and *BRCA2* to the development of inherited familial breast and/or ovarian cancer have been reported (Liebens *et al.* 2007). However, the contribution of mutations in these two genes to breast cancer patients from Indian familial breast or ovarian cancer families remains relatively unexplored apart from a few small studies (Kumar *et al.* 2002; Saxena *et al.* 2002; Rajkumar *et al.* 2003; Valarmathi *et al.* 2003, 2004; Hedau *et al.* 2004; Saxena *et al.* 2006). Hence, there is a need to screen a large number of samples to investigate the role of *BRCA1/BRCA2* gene mutations in the high-risk group of familial breast or ovarian cancer families. In this study, we report the screening for BRCA1 and *BRCA2* mutations in 61 breast and/or ovarian cancer patients with a positive family history of breast and/or ovarian cancer.

2. Materials and methods

2.1 *Patient selection*

All the patients included in this study were treated at the Kidwai Memorial Institute of Oncology, Bangalore, India. Sixty-one patients with breast or ovarian or breast/ovarian cancer, each from a different family, were recruited for the study. These families were found to be not related to each other as per the records. The families were derived from different parts of states located in south India including Kerala, Karnataka, Andra Pradesh and Tamil Nadu. Selected patients had at least one first-degree relative affected with breast, ovarian or breast/ovarian cancer. There were 39 families with breast cancer (median age of patients 42 years), 10 families with ovarian cancer (median age of patients 33 years) and 12 families with breast and ovarian cancer (median age of patients 43.5 years). The age distribution of all patients ranged from 16 to 68 years with a mean of 42.5 years. Out of the 61 patients studied, there were 4 patients less than 25 years of age (6.55%), 26 patients in the age group of 25–40 years (42.6%) and 31 patients above 40 years (50.8%). Blood samples were collected from the patients and stored in acid–citrate–dextrose solution at –70°C. Blood samples collected from 100 age-matched, unrelated normal individuals without a family history of breast or ovarian cancer were used as controls. This study was approved by the Institutional Review Board of Kidwai Memorial Institute of Oncology (IRB No: PER/CAB-I/D-1-13/01).

2.2 *Isolation of DNA from blood samples*

Genomic DNA was isolated from 200 μl of each blood sample using a commercial DNA isolation kit (Qiagen, USA). For polymerase chain reaction (PCR), the DNA was diluted to 25 ng/μl and 4 μl was used in a 25 μl PCR reaction.

2.3 *Preparation of PCR products*

The sequence of primers for genomic DNA amplification of *BRCA1* and *BRCA2* has already been described (Ganguly *et al.* 1997). A total of 33 and 52 PCR reactions were carried out to screen for *BRCA1* and *BRCA2*, respectively. The primers for each exon were located at least 50 bp away from exon–intron boundaries. Non-coding exons were excluded from the study. The composition of the 10X buffer used except for exon 12 of *BRCA1* was 100 mM Tris-HCl pH 9.0, 15 mM $MgCl_2$, 500 mM KCl, 0.1% gelatin; for exon 12, the 10X buffer contained 100 mM Tris-HCl pH 8.8, 15 mM $MgCl_2$, 750 mM KCl. PCR was performed in 25 μl solutions containing 100 ng of genomic DNA, 1X PCR buffer, 100 μM dNTPs (Gibco BRL), 10 pmol of each primer (Sigma) and 0.5 U of *Taq* DNA polymerase (Bangalore Genei). Reactions were carried out in a thermal cycler (MyCycler, Biorad) as follows: 94°C for 5 min, 35 cycles (94°C for 1 min, 55°C for 1 min, and 72°C for 30 s) and 72°C for 10 min. For exon 13 and 11J of *BRCA2*, the annealing temperature was 56°C and 58°C, respectively.

2.4 *Conformation-sensitive gel electrophoresis*

For mutation analysis, we adopted conformation-sensitive gel electrophoresis (CSGE) as our preliminary screening method as described below. The CSGE method involves heteroduplex analysis of PCR products in a novel, mildly denaturing polyacrylamide gel matrix using a different cross-linker, bis-acrolyl piperazine, instead of the conventional bis-acrylamide (Ganguly *et al.* 1993; Williams *et al.* 1995; Lakhotia and Somasundaram 2003). PCR products were denatured by heating at 98°C for 5 min and reannealed at 68°C for 1 h to generate heteroduplexes. The samples were loaded onto a polyacrylamide gel. The 1 mm thick 10% polyacrylamide gel contained acrylamide (Sigma) and 1,4-bis acryloyl piperazine (Fluka) cross-linker in the ratio of 99:1, 10% ethylene glycol (Sigma) and 15% formamide (Sigma) in 0.5X Tris taurine–EDTA buffer. 1X Tris taurine–EDTA buffer contains 89 mM Tris, 28.5 mM taurine and 0.2 mM EDTA pH 9.0. The samples were subjected to electrophoresis at 400 v for 16 h. The gels were stained with ethidium bromide (1 μg/ml) for 10 min and destained for 10 min in double distilled water. PCR products were visualized by ultraviolet light and photographed. Samples displaying

abnormal CSGE profiles compared with that of the controls were identified.

2.5 *Direct sequencing of PCR products*

Samples that showed an aberrant heteroduplex pattern were reamplified from genomic DNA, the amplicons were purified using QIAquick PCR purification kit (Qiagen) and subjected to automated DNA sequencing (ABI 377; Applied Biosystems) using the manufacturer's suggested protocols. Sense and antisense strand sequencing were done to confirm all mutations.

3. Results

We screened the genomic DNA derived from 61 independent familial cases of both breast and/or ovarian cancer by a combination of heteroduplex analysis using CSGE and subsequent DNA sequencing. We found mutations in 17/61 patients, thus bringing the total contribution of *BRAC1* and *BRCA2* to 28.0%. The total DNA from one hundred age-matched, unrelated normal controls was tested by CSGE to confirm the absence of the identified mutations in the normal population.

In the *BRCA1* gene, 15 mutations were identified; thus, the mutation frequency of *BRCA1* was 24.6% (15/61) (table 1). Of the *BRCA1* mutations identified, there were three novel mutations which have not been reported earlier. The first novel mutation 295delCA has a deletion of two base pairs resulting in a frame shift with the generation of a translation stop at downstream codon 64 (figure 1 panels A, B and C). The second novel mutation 4213delT (L1365X) has a single base deletion, which results in the conversion of the leucine codon (TTA) at position 1365 to a stop codon TAG (figure 1 panels D, E and F). The third novel mutation

Table 1. *BRCA1* and *BRCA2* mutations in Indian families with familial breast and/or ovarian cancer

Patient ID	Nucleotide change	Exon	Codon affected	Termination codon	Mutation type	Breast/ovarian cancer	BIC entry
BRCA1 mutations							
KP-2	185delAG	2	23	39	FS	Breast/ovarian cancer	Yes
KP-14	185delAG	2	23	39	FS	Breast cancer	Yes
KP-17	185delAG	2	23	39	FS	Ovarian cancer	Yes
KP-21	185delAG	2	23	39	FS	Breast/ovarian cancer	Yes
KP-29	185delAG	2	23	39	FS	Breast cancer	Yes
KP-36	185delAG	2	23	39	FS	Breast cancer	Yes
KP-37	185delAG	2	23	39	FS	Breast cancer	Yes
KP-39	185delAG	2	23	39	FS	Breast cancer	Yes
KP-58	185delAG	2	23	39	FS	Breast cancer	Yes
KP-60	185delAG	2	23	39	FS	Ovarian cancer	Yes
KP-13	295delCA	5	60	64	FS	Breast/ovarian cancer	No
KP-19	2983C→A	11	S955X	955	NS	Breast cancer	Yes
KP-30	4213delT	11	L1365X	1365	NS	Breast cancer	No
KP-1	5267T→G	18	Y1716X	1716	NS	Breast/ovarian cancer	No
KP-9	3450delCAAG	11	1111	1115	FS	Ovarian cancer	Yes
BRCA2 mutations							
KP-7	4866InsT	11	D1547X	1547	FS	Ovarian cancer	No
KP-8	6079delAGTT	11	1951	1961	FS	Breast cancer	Yes

FS, frame shift mutation; NS, nonsense mutation; BIC, breast cancer information core.

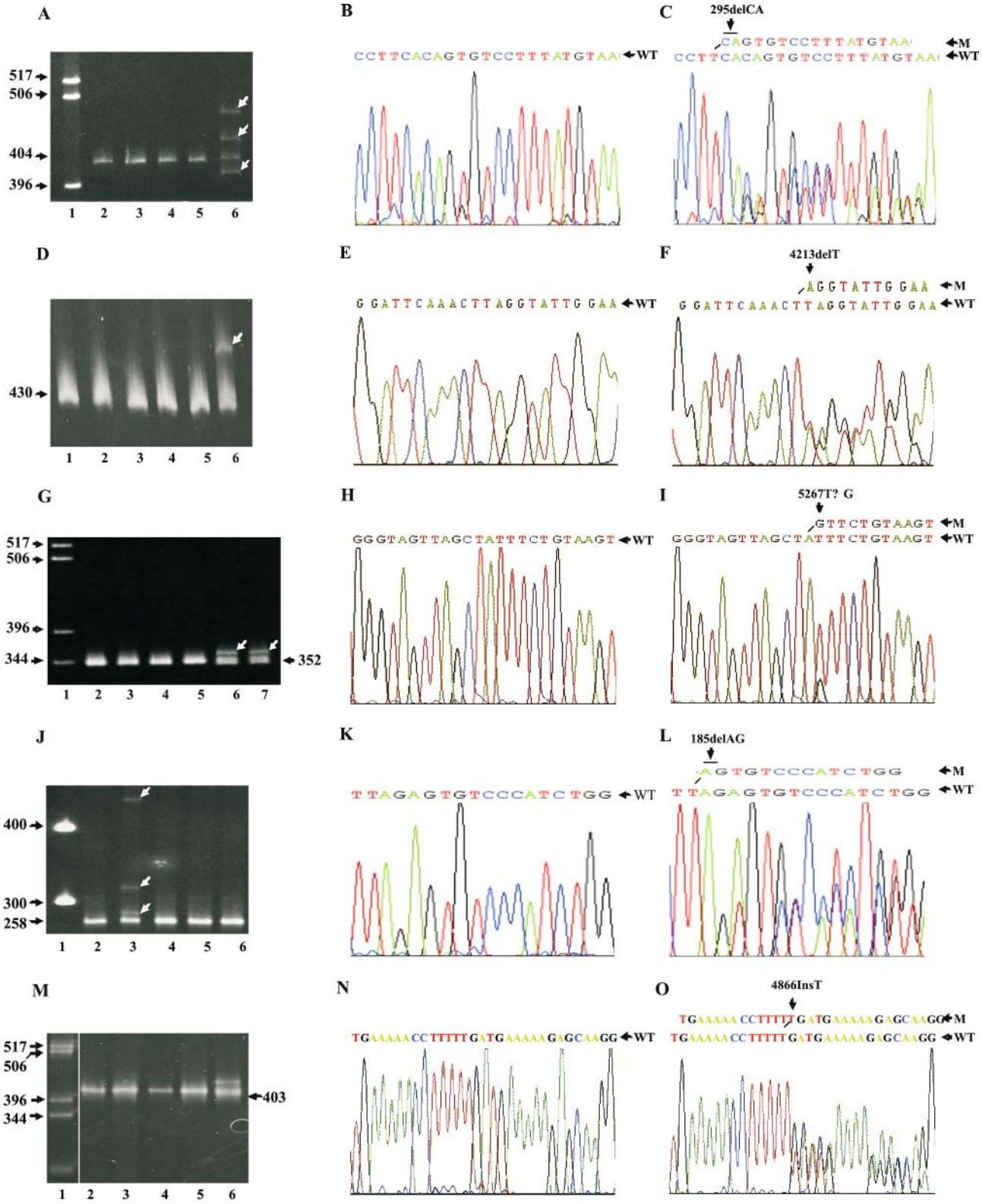

Figure 1. For caption, *see* page No. 419.

5267T→G (Y1716X) is a transversion mutation resulting in the conversion of the tyrosine codon (TAT) at position 1716 to a stop codon TAG (figure 1 panels G, H and I). Three more mutations were identified in *BRCA1*, which have previously been reported. These are 185delAG, 2983C→A and 3450delCAAG. Of these, 185delAG was found in 10 patients, thus occurring at a very high frequency of 16.4% (10/61) (figure 1 panels J, K and L). It is interesting to note that this mutation occurs at a very high frequency of 18.0% among breast/ovarian cancer families of Ashkenazi Jews and among 1% of Ashkenazi Jews in the general population (Struewing *et al.* 1995; Phelan *et al.* 2002).

In the *BRCA2* gene, two mutations were identified, thus the mutation frequency was 3.28% (2/61) (table 1). Of these two *BRCA2* mutations, one is novel while the other is a reported mutation. The novel mutation is an insertion mutation (4866insT) which results in the conversion of an aspartic acid codon (GAT) to a stop codon TGA at position 1547 (D1547X) (figure 1 M, N, and O). The other previously reported mutation found in *BRCA2* is a four-base pair deletion 6079delAGTT.

We further analysed the frequency of *BRCA1* and *BRCA2* mutations among different types of cancer as well as age groups. Of the families with ovarian cancer, 40.0% had mutations, while in families with breast cancer, mutations were present in 23.7% of the families. In families with breast and ovarian cancer, mutations were present in 30.8%, suggesting that *BRCA1* and *BRCA2* mutations are more often associated with ovarian cancer families. Upon analysis of different age groups, we found that 23.33% of mutations occurred in those ≤40 years and 32.25% in those >40 years. While *BRCA1* mutations were found in both the age groups, *BRCA2* mutations were found only in those ≤40 years.

4. Discussion

In this study, 61 breast/ovarian cancer patients with a family history of breast/ovarian cancer were studied for *BRCA1* and *BRCA2* mutations by CSGE. Screening for mutations carried out by several groups worldwide suggests a significant variation of the relative contribution of *BRCA1* and *BRCA2* genes to hereditary cancer between populations (Levy-Lahad and Friedman 2007). The contribution of *BRCA1* and *BRCA2* mutations pertaining to familial breast and/or ovarian cancer in Indian women remains largely unexplored. The few studies that have been reported, including one from our laboratory, are small studies, and suggest a need to screen for mutations in a large number of families with familial breast and ovarian cancer (Kumar *et al.* 2002; Saxena *et al.* 2002; Rajkumar *et al.* 2003; Valarmathi *et al.* 2003, 2004; Hedau *et al.* 2004; Saxena *et al.* 2006). Even though Saxena *et al.* (2006) screened 204 patients, this study included only 34 families with a positive family history. This set also included 105 families with early-onset breast cancer. Since early onset is a feature of inherited breast cancer involving *BRCA1* and *BRCA2* mutations, families with an early onset of breast cancer are generally considered for *BRCA1* and *BRCA2* mutation screening programmes. However, it is not ideal to include such families in *BRCA1* and *BRCA2* mutation screening in India because of the following reason. The average age of breast cancer patients in various population-based registries in India has been reported to be 50–53 years compared with 61 years among American women (ICMR 2001; Parkin *et al.* 2002). This is a very important point, considering the fact that approximately 90% of breast cancer is of the sporadic type. Therefore, including patients with early-onset breast cancer in mutation screening studies may undermine estimations of the frequency of *BRCA1* and *BRCA2* mutations. Thus, our report becomes the largest Indian study with 61 patients from families with a positive history of breast and/or ovarian cancer.

Based on this study, the frequency of *BRCA1* and *BRCA2* mutations among Indian women with familial breast and ovarian cancers is found to be 24.60% (15/61) and 3.28% (2/61), respectively. Reports worldwide suggest that mutations in *BRCA1* and *BRCA2* are responsible for 20% of familial cases of breast and/or ovarian cancer (Wooster and Weber 2003). While the contribution of *BRCA1* mutations to familial breast cancer is in the same range as those of other reports published worldwide, the contribution of *BRCA2* mutations seems rather low among Indian women. However, the lower contribution of *BRCA2* mutation to familial breast cancer development is supported by earlier studies from India (Saxena *et al.* 2002; Rajkumar *et al.* 2003; Valarmathi *et al.* 2004; Saxena *et al.* 2006).

All *BRCA1* mutations identified were truncating mutations. While three of them were deletion mutations leading to the generation of a stop codon further downstream,

Figure 1. Panels **A, D, G, J** and **M**. Ethidium bromide-stained gel for conformation-sensitive gel electrophoresis of PCR products. Panel **A**. *BRCA1* exon 5 of normal (lane 2), KP2 (lane 3), KP3 (lane 4), KP4 (lane 5) and KP13 (lane 6). Panel **D**. *BRCA1* exon 11 of KP25 (lane 1), KP26 (lane 2), KP27 (Lane 3), KP28 (lane 4), KP29 (lane 5) and KP30 (lane 6). Panel **G**. *BRCA1* exon18 of normal (lane 2 and 3), KP3 (lane 3), KP10 (Lane 4) and KP1 (lane 6 and 7). Panel **J**. *BRCA1* exon 2 of normal (lane 2), KP2 (lane 3), KP3 (lane 4), KP4 (lane 5) and KP5 (lane 6). Panel **M**. *BRCA2* exon 11 of normal (lane 2), KP4 (lane 3), KP5 (Lane 4), KP6 (lane 5) and KP7 (lane 6). White arrows indicate the heteroduplex bands. Panels **B, E, H, K** and **N**. Electropherogram of DNA sequencing of *BRCA1* exons 5, 11, 18, 2 and *BRCA2* exon 11, respectively, from normal individuals. Panels **C,F, I, L** and **O**. Electropherogram of DNA sequencing of *BRCA1* exon 5 from KP13 (panel **C**), *BRCA1* exon 11 from KP30 (panel **F**), *BRCA1* exon 18 from KP1 (panel **I**), *BRCA1* exon 2 from KP2 (panel **L**) and *BRCA2* exon 11 from KP7 (panel **O**). The mutation type and location are shown.

the other three were nonsense mutations resulting in the generation of a stop codon at the mutation site. Interestingly, three of the *BRCA1* mutations found in this study were novel mutations. In the case of *BRCA2* as well, the two mutations identified were of the truncating type. While one of them is a four base-pair deletion resulting in the generation of stop codon downstream, the other is a one base-pair insertion leading to the formation of a stop codon at the mutation site itself. One of the *BRCA2* mutations was found to be a novel type not reported earlier.

We also found that families with ovarian cancer had mutations in the *BRCA1* and *BRCA2* genes more often than families with breast cancer. *BRCA1* and *BRCA2* germline mutations were found in 43.0% of families with ovarian cancer, with *BRCA1* mutations being four-fold more common than *BRCA2* mutations (Gayther *et al.* 1999). While *BRCA1* mutations were found in all age groups, *BRCA2* mutations were found only in the age group of ≤40 years, suggesting perhaps that *BRCA2* defects more often lead to early-onset breast and ovarian cancer. In contrast to this observation, *BRCA2* mutations were found to contribute to fewer cases of breast cancer among young women than mutations in *BRCA1* (Krainer *et al.* 1997).

Interestingly, we found the *BRCA1* mutation 185delAG among 10/61 patients; the frequency of this mutation was thus 16.4%. Different specific mutations in *BRCA1* and *BRCA2* occurring at a high frequency in various ethnic groups have been reported. In Israel, three specific mutations: 185delAG, 5382insC in *BRCA1*, and 6174delT in *BRCA2* were reported to occur in 36% of breast/ovarian cancer families (Levy-Lahad *et al.* 1997). Of these, 185delAG was found to occur at a very high frequency of 18.0% in families of Ashkenazi Jews with breast/ovarian cancer (Phelan *et al.* 2002). This mutation also occurs at a frequency of 1% among the Ashkenazi general population, thus making it one of the founder mutations responsible for its increased association with inherited breast/ovarian cancer (Struewing *et al.* 1995).

However, 185delAG mutation has been reported to occur at varying frequencies among families with breast/ovarian cancer in different populations. A very high frequency of 31.6% has been reported among non-Jewish Americans of Spanish ancestry from the San Luis Valley, Colorado (Mullineaux *et al.* 2003). However, this mutation has been found to occur at a varying low frequency (1.13–5.9%) among white Americans, the Spanish from Spain, Polish, Iranian, Pakistani and Turkish women (Grzybowska *et al.* 2002; Shih *et al.* 2002; Guran *et al.* 2005; Weitzel *et al.* 2005; Mehdipour *et al.* 2006; Rashid *et al.* 2006). Interestingly, the 185delAG mutation was not found among Chinese and Japanese families with breast cancer (Ikeda *et al.* 2001; Zhi *et al.* 2002). Although previous studies, including one from our laboratory, reported the occurrence of 185delAG mutation among Indian women, the high frequency of occurrence of 185delAG mutation reported in this study, similar to that of Ashkenazi Jews, is notable. Haplotype analysis is required to identify the origin of this mutation among Indian women. Preliminary haplotype analysis revealed that 185delAG mutation among Indian women may have an independent origin as their haplotype was different from that of Ashkenazi Jews (data not shown). However, these data remain to be confirmed by studying a larger number of individuals. In addition, it would be interesting to test the carrier frequency of 185delAG among different religious/ethnic/geographical groups of the general Indian population to further understand its prevalence and origin.

Thus, this study suggests that the mutation spectrum and prevalence of the *BRCA1* and *BRCA2* genes in the south Indian population have some similarities and differences between what is observed in other populations. This study also emphasizes the importance of a positive family history as the basis for *BRCA1* and *BRCA2* mutation screening, particularly among patients with early-onset disease. With appropriate genetic counselling, patients and pre-symptomatic mutation carriers would be able to make better medical and surgical decisions.

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