

RESEARCH COMMUNICATION

BRCA1, BRCA2 and CHEK2 (1100 del C) Germline Mutations in Hereditary Breast and Ovarian Cancer Families in South India

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Abstract

Cancer of the breast is the second most common cancer seen among Indian women. This study describes the use of DHPLC for mutation analysis for *BRCA1*, *BRCA2* and *CHEK2* (1100delC) in 22 patients with a family history of breast and/or ovarian cancer and early onset breast cancer (<35 years of age). Three of the 22 patients were found to have a non-sense mutation or a deletion, resulting in a premature stop codon, potentially leading to a truncated protein. Two of these were in *BRCA1* (one was a novel 5 base deletion) and one in the *BRCA2* gene. No patient was found in our series to have the *CHEK2* (1100delC) mutation. DNA from a healthy blood donor and all but one of the 22 patients, demonstrated polymorphisms in *BRCA1* and/or *BRCA2* genes. This is the first study from South India, on *BRCA1*, *BRCA2* & *CHEK2* (1100 del C) mutations in patients with a family history of breast and/or ovarian cancer and early onset breast/ovarian cancer, using the sensitive DHPLC approach.

Key Words: *BRCA1* - *BRCA2* - *CHEK2* (del1100) - hereditary breast &/or ovarian cancer - DHPLC - India

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Introduction

Cancer of the breast is the second most common cancer seen in South Indian women with crude incidence rate (CIR) of 20/100,000 in the Madras Metropolitan Tumour Registry (MMTR). There has been a gradual increase in the CIR for breast cancer over the past several years. As per the MMTR, between the period of 1984-1988 and 1994-1998, there has been a 33% rise in the crude incidence rate (CIR) for breast cancer (Shanta et al. 2001; Gajalakshmi et al., 2001).

Hereditary breast cancers account for only 5-10% of breast cancers. The *BRCA1* and the *BRCA2* have been associated with hereditary breast and ovarian cancers. The *CHEK2* gene is considered to be a low risk breast cancer susceptibility gene, with the characteristic 1100delC being associated with altered function for this gene involved in the DNA repair pathway (The *CHEK2* breast cancer consortium 2002). With the availability of technologies for identification of the mutations in these genes, it is now possible to offer predictive testing to unaffected women in families that are known to carry a deleterious mutation, with

an intent to introduce cancer prevention and/or early detection strategies. In addition, it is also becoming clear that there may be implications in the management of the patients with these deleterious mutations (Pierce 2002). In view of the entire breast tissue being at risk, breast conservation approaches are associated with second primary tumours in the ipsilateral and contralateral breast (Haffty et al., 2002).

There has been a paucity of information on the status of these genes in Indian patients. In a recently published study (Saxena et al., 2002) done in collaboration with IARC, Lyon, 20 North Indian breast cancer patients with a family history of breast and/or ovarian cancer and early onset breast cancer (<35 years of age) were analyzed for *BRCA1* and *BRCA2* gene mutations. Two of the 20 patients were found to have splice junction mutation in *BRCA1*. Polymorphisms in *BRCA2* were also detected in two cases. In the only study from South Indian patients, 3/15 cases were found to harbor disease causing mutations in *BRCA1* (Kumar et al., 2002). Our study presents the clinico-pathologic and mutation data for *BRCA1*, *BRCA2* and *CHEK2* 1100delC on 22 patients

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with family history of Hereditary breast and ovarian cancer (HBOC)/Hereditary breast cancer (HBC) or early onset breast cancer (<35 years of age).

Materials and Methods

Patients

This study was approved by the Institutional Ethics committee. The 22 patients selected for the genetic testing were referred to Hereditary Cancer Clinic, functioning under the Hereditary Cancer Detection and Prevention Programme at the Cancer Institute (WIA), Chennai, India and were found to fall within the criteria used for considering them for genetic analysis. The criteria used was as follows: Early onset of breast cancer (at or less than 35 years of age); Two cases of breast cancer diagnosed under the age of 50 years; Three or more cases of breast cancer diagnosed at any age; Presence of breast and ovarian cancer in the family or in the same individual; Male breast cancer with a relative (of either sex) with breast cancer.

Thirteen patients were from HBC families, 6 from HBOC families and there were 3 cases of early onset breast cancer (≤ 35 years of age). Detailed questionnaire regarding the family history, personal history and dietary habits were collected from the patients. Blood sample and where feasible, tumor tissue sample were collected from the patients after obtaining an informed consent. One age matched healthy donor without a family history of cancer, provided blood sample for use as normal control.

DNA extraction and PCR conditions

Genomic DNA from the lymphocytes was isolated using QIAamp DNA Blood Mini Kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions. The entire coding sequence and the splice junctions of the *BRCA1* and *BRCA2* genes were PCR amplified using 35 and 48 primer pairs respectively, and using the PCR conditions as described (Wagner *et al.*, 1999). PCR was performed in a 30 μ l reaction containing 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5-4.5mM MgCl₂, 100 μ M dNTP's each, 0.8 μ M of each primer and 1U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). The annealing temperature was changed for some exons in the *BRCA1* (exons 2 and 11K at 54°C; exons 14, 20, 23 at 59°C) and *BRCA2* (exon 2 at 54°C and exon 11P at 68-61/61°C with 3.5mM MgCl₂) genes. Positive controls for some of the exons were included in the PCR and the subsequent DHPLC run (kind gift of Dr. T.Wagner). Negative control (no DNA) for the PCR was included in all runs.

The functional copy of the exon 10 of *CHEK2* gene was PCR amplified using primers and conditions published elsewhere (The *CHEK2* breast cancer consortium 2002).

Denaturing High Performance Liquid Chromatography (DHPLC)

DHPLC was carried out in the automated DHPLC instrument (Varian Analytical Instruments, USA). The PCR product was denatured at 95 °C for 3 minutes followed by

gradual re-annealing from 95 to 65 °C over a period of 30 minutes before subjecting to DHPLC analysis. All the samples were run at the non-denaturing temperature of 50°C. The temperature required for heteroduplex analysis of *BRCA1* and *BRCA2* genes was obtained from Wagner *et al.*, 1999. The PCR product was eluted using 3% lower acetonitrile gradient at a flow rate of 0.45 mL/min. pUC18 DNA HaeIII digest (Sigma) was run at the start and after every 200 runs to validate the system's performance. The doublet peaks, (257 and 267 bp peaks, and the 434 and 458 bp peaks) were used to assess the resolution of the system. In addition, positive control samples with known mutations were run to check the performance of the system.

Direct DNA sequencing

Fragments showing hetero-duplex in DHPLC were directly sequenced with DNA sequencing kit v3.0 (Applied Biosystems, Foster City, CA) according to manufacturer's instructions, using the corresponding forward and reverse primers in individual reactions. The samples were analyzed with ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Exon 10 of the *CHEK2* gene was directly sequenced after PCR, as described above. All the samples that were found to have a mutation were rechecked with fresh DNA prepared from an aliquot of frozen lymphocytes from the same patient.

Results

The *BRCA1* and *BRCA2* mutation data and polymorphisms in the coding region is given in Table 1. Three of the 22 patients studied were found to carry a deleterious mutation - two in *BRCA1* and one in *BRCA2*. The pedigree chart, DHPLC graph and the sequencing data of the three patients are given in Figure 1. One of the *BRCA1* mutation was a novel 5 base deletion (Ex12 1386 delCTCTC Stop 1389), resulting in premature stop codon (GenBank Accession Number - AY144588). This was seen in the daughter and her mother, both of whom had breast cancer (Figure 1 A). The other deleterious mutations seen in *BRCA2* (Ex110 1235delCTTAA stop 1237) (Figure 1 B) and *BRCA1* (Ex13 CGA→TGA Arginine1443 Stop) (Figure 1C) have been described already (BIC database).

The 1100delC mutation in the *CHEK2* gene was not detected in any of our patients or in the lone control. Except one patient, all the others showed polymorphisms in *BRCA1* and/or *BRCA2*, either in the coding region (Table 1) or in the introns (data not shown). The patient, who did not show an abnormality in either *BRCA1* or *BRCA2*, was from a different country and ethnically different. The one control sample (a healthy blood donor with no family history of cancer) showed polymorphisms in the *BRCA2* gene.

One variant of unknown significance was also detected in a patient. The Methionine 1652 to Isoleucine has been described by different authors as polymorphism or as a variant of unknown significance (Blesa *et al.*, 2002, Schoumacher *et al.*, 2000). The amino- acid is in the BRCT

Table 1. BRCA1 & BRCA2 Status in Patients Studied (Only Mutations & Polymorphisms in the Coding Region Shown)

No	Site	Age at Dx	Family members affected (age in yrs)	BRCA1	BRCA2
1	BC (R)	28	M-BC (44); MGM-BC (50), MA-BC	Ex12 1386 delCTCTC Stop 1389 11S GAA→GGA E1038G 11N CCG→CTG P871L	10BCAT→AAT H372N
2	BC (L)	41	M-BC (35y); MGM-Uts; MGF-Throat.	11S GAA→GGA E1038G Stop1237	Ex11O 1235 delCTTAA
3	BC (R)	40	3S-BC (40,41,35) PA-BC (64)	Ex13 CGA→TGA Arginine1443 Stop 11S GAA→GGA E1038G	NAD
4	BC (Bil)	30	S-BC (44); PA- BC	NAD	10B CAT→AAT H372N
5	BC (L)	39	S-BC (36)	NAD	NAD
6	BC (L)	46	S-BC (51); D-BC (27)	NAD	10B CAT→AAT H372N
7	BC (Bil)	35	S-BC (38); PA- BC, 2PCS-BC	11S GAA→GGA E1038G	NAD
8	BC (R)	40	S-BC (36)	11S GAA→GGA E1038G 11N CCG→CTG P871L	11B GAT→TAT D1420Y
9	BC (R)	51	M-OC (65); S-BC (43)	11S GAA→GGA E1038G	10B CAT→AAT H372N
10	BC (R)	40	M-BC; S-BC (40)	11S GAA→GGA E1038G 16 AGT→GGT S1613G 16 ATG→ATA M1652I	10B CAT→AAT H372N
11	BC (R)	48	D-OC (22)	NAD	10B CAT→AAT H372N
12	BC (R)	67	PCS-BC; Niece- OC Niece- BC (54);	11S GAA→GGA E1038G 11N CCG→CTG P871L	NAD
13	BC (R)	37	S-BC (33)	11N CCG→CTG P871L	10B CAT→AAT H372N
14	BC (R)	50	M-BC (50); S-BC (50) MA-BC (30)	11N CCG→CTG P871L	10B CAT→AAT H372N
15	BC (R)	50	S-BC (42), MGM-Uts	NAD	11I AAC→GAC N991D
16	BC (R)	23	PGM- BC	NAD	NAD
17	BC (L)	33	-	11S GAA→GGA E1038G 11N CCG→CTG P871L	10B CAT→AAT H372N
18	BC (R)	26	-	NAD	10B CAT→AAT H372N
19	BC (Bil)	23	-	NAD	11I AAC→GAC N991D
20	OC	46	S-BOC; 2MC-BC	11S GAA→GGA E1038G 11N CCG→CTG P871L	NAD
21	OC	22	M-BC (46)	11S GAA→GGA E1038G	10B CAT→AAT H372N
22	OC	50	S-BC (27); N-BC (30)	11S GAA→GGA E1038G	10B CAT→AAT H372N 26 CTT→CGT L3180R
Control			-	NAD	10B CAT→AAT H372N

Abbreviations used in the Table: BC= Breast Cancer; OC= ovarian Cancer; Uts= Uterine cancer; (L)= Left; (R)= Right; (Bil)= Bilateral; M= Mother; S= Sister; B= Brother; D= Daughter; MGM= Maternal Grandmother; MA= Maternal aunt; PA= Paternal aunt; PCS= Paternal Cousin sister; PGM= Paternal Grand Mother.

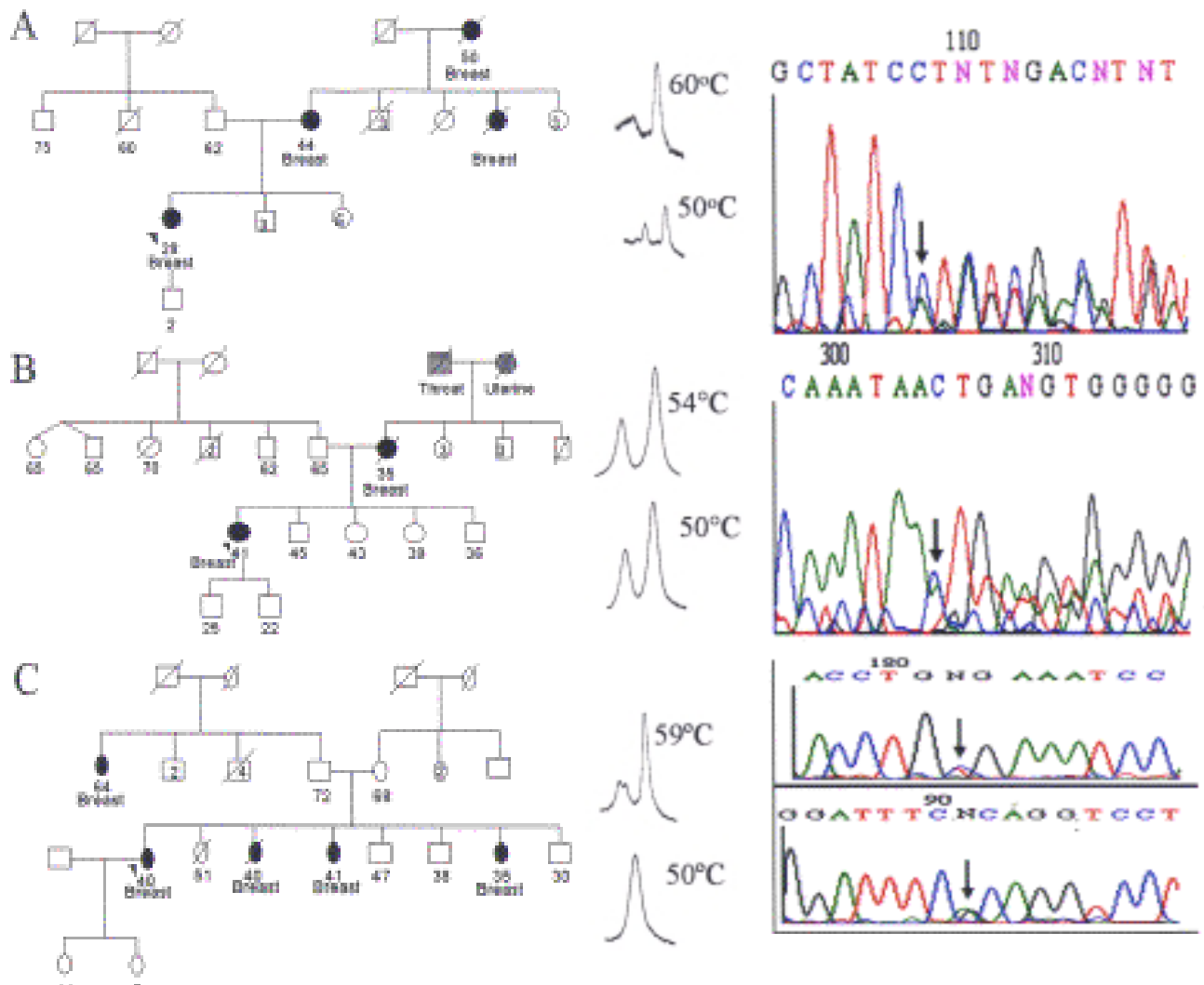


Figure 1. Pedigree, DHPLC trace and Sequencing data in the patients with deleterious mutation.
1A: Pedigree chart, DHPLC trace and DNA Sequencing data from the patient with the 5 base pair deletion in Exon 12 of BRCA1;
1B: Pedigree chart, DHPLC trace and DNA Sequencing data from the patient with the 5 base pair deletion in Exon 110 of BRCA2;
1C: Pedigree chart, DHPLC trace and DNA Sequencing data from the patient with the point mutation CGAÆTGA in Exon 13 of BRCA1.

domain of the *BRCA1* gene. On comparison of the human sequence of the BRCT region with chimpanzee, dogs and mice, the Methionine residue was found to be conserved.

The control patient was found to have polymorphism in the *BRCA2* gene.

Among the 3 breast cancer patients with deleterious mutations, one of the patient declined further investigation and treatment; the remaining two patients with the deleterious mutations were Estrogen Receptor (ER) and HER2 (c-erbB2) negative. In four breast cancer patients, in whom no deleterious mutation was detected, tumour diagnosis was based on hematoxylin and eosin stained slides, brought after having had the primary surgery done elsewhere, and hence ER, HER2 and p53 analysis could

not be done. In the remaining 12 patients with breast cancer, in whom molecular markers were done, positivity for ER was 42% (5/12), HER2 in 17% (2/12) and p53 in 25% (3/12). There was no association of the molecular markers with any of the polymorphisms detected.

Discussion

This study describes the mutation profile in *BRCA1*, *BRCA2* and *CHEK2* (1100delC) in 22 women either with a family history of HBOC/HBC or early onset breast cancer, detected using DHPLC. Except one (from a neighboring country), all the other women were of South Indian origin. This is the first study to describe the mutation profile in the

above three genes, in this population.

Deleterious mutations in *BRCA1* and *BRCA2* were seen in 13.6% (3/22) of patients studied. This compares with other studies which have shown a rate of around 16% in women with breast cancer at any age and with a strong family history of breast cancer (Wagner *et al.*, 1998, Couch *et al.*, 1997). In fact, the study by Couch *et al.* (1997) had found only 7% of women from families with a history of breast cancer but not ovarian cancer as having mutations in the *BRCA1* gene. Their study did not find an association between the presence of bilateral breast cancer or the number of breast cancers in a family and *BRCA1* mutation. In our limited series, 2 of the 3 patients with deleterious mutations were from families with 4 or more breast cancers (Figure 1 A and C) and only one was from a family with 2 breast cancers (Figure 1B). None of the three patients with bilateral breast cancer (2 with a family history of breast cancer and 1 with an early onset breast cancer) were found to carry deleterious mutations in *BRCA1* or *BRCA2*.

In our series, none of the 3 early onset breast cancer (≤ 35 years of age) patients with no family history were found to carry deleterious mutations. Peto *et al.* (1999) have also reported a lower rate of mutation in early onset breast cancer (< 36 years of age) in *BRCA1* (5.9%) and *BRCA2* (2.4%). There were 6 patients with HBOC family history in our series and none were found to have any deleterious mutation in *BRCA1* and *BRCA2*.

This is the first study from India to report the use of DHPLC in the mutation analysis. The use of DHPLC in the mutation analysis has been found to be a reliable approach with sensitivity approaching 99% or more and a specificity of 98% or more (Gross *et al.* 1999, Choy *et al.*, 1999, Speigelman *et al.*, 2000). Among the limitations of this method, is its inability to detect gross gene deletions. For reliable detection of all mutations, analysis will have to be performed at multiple temperatures or use GC-clamped primers (Marsh *et al.*, 2001). In our study, two to three different temperatures were used for analysis of the different exons of *BRCA1* and *BRCA2*.

None of the samples studied were found to be positive for the *CHEK2* 1100delC, including those that were negative for *BRCA1* and *BRCA2* deleterious mutations. The *CHEK2*-Breast cancer consortium (2002) had estimated the above variant to confer approximately two fold increase in risk of breast cancer in women and a ten fold increase of risk in men. In our series there were no male breast cancers. We did not look for the other known *CHEK2* mutations, the 1422delT (which results in a truncated protein) (Bell *et al.*, 1999), isoleucine to threonine in the fork head homology-associated-domain (Bell *et al.*, 1999) and Arg145Trp (which could lead to accelerated degradation) (Lee *et al.*, 2001) all of which were seen in Li-Fraumeni syndrome (LFS) and LFS variants. The 1100delC has also been reported in LFS and LFS variants (Bell *et al.*, 1999).

Eight different polymorphisms were seen in the coding region of *BRCA1* and *BRCA2*. One of them was the M1652I in the BRCT domain, which has been described as a

polymorphism in the BIC database. Comparison of the human sequence with that of the chimpanzee, canine and murine sequences, show that this Methionine is conserved. This could suggest that the variant may better be classified as a variant of unknown significance.

Breast cancer patients carrying mutations in *BRCA1* are more likely to have a higher grade of tumour, more lymphocytic infiltration, more likely to be estrogen receptor negative (Verhoog *et al.*, 1998, Hedenfalk *et al.*, 2001). The two tumours from patients who were found to have deleterious mutation in the *BRCA1* gene were high grade and ER & HER2/c-erbB2 negative. One of them had histopathological features of Medullary carcinoma.

This study describes the *BRCA1*, *BRCA2* and *CHEK2* 1100delC mutation data in South Indian women, using the highly sensitive DHPLC technique. One of the mutation has been found to be a novel 5 base deletion, resulting in a truncated BRCA1 protein. A larger study will be needed to identify any founder mutations in this population.

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