

Novel Sequence Variants and a High Frequency of Recurrent Polymorphisms in *BRCA1* Gene in Breast Cancer Women of North Coastal Andhra Pradesh

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ABSTRACT Owing to its high incidence coupled with relatively good prognosis, breast cancer is the most prevalent cancer in the world today. Germ line mutations in the susceptibility gene *BRCA1* in hereditary breast/ovarian cancer, though low in prevalence, are highly penetrant and show geographical variations. Most cancer-associated *BRCA1* mutations identified to date result in the premature translational termination of the protein. However, the molecular and genetic effects of missense mutations remain largely unknown. There have been only a few reports from India on mutations in *BRCA1* and none from North Coastal Andhra Pradesh. We have analyzed 114 breast cancer patients with (N = 9) and without (N = 105) a family history of breast cancer, 22 at risk relatives from familial (n=11), sporadic (n=11) cases and 97 control subjects for mutations in exons 2 and 11 and their intron-exon boundaries of *BRCA1* gene by direct sequencing. Sequence alignment was carried by CLUSTAL W and PSI-BLAST. Out of eight sequence variants found, one novel deleterious frame-shift mutation (c.2717insA), one novel polymorphism (c.1400A>G), five previously reported common polymorphisms in exon 11 and one intronic (intron 1) variant (base1822C>T) were observed. All the identified polymorphisms in exon 11 fall in DNA binding domain of *BRCA1* protein.

INTRODUCTION

Breast Cancer is the most common malignancy in women worldwide, accounting for 23 percent of all cancer across genders (Parkin et al. 2005). Germline mutations within the breast and ovarian cancer susceptibility gene *BRCA1* predispose carriers to early-onset breast and breast-ovarian cancers (Nathanson et al. 2001). Tumor-associated mutations occur throughout the *BRCA1* coding sequence, but cluster to sequences encoding the N-terminal RING finger domain and the BRCT1 domains (Couch and Weber 1996; Shattuck et al. 1995; Shen and Vadgama 1999). An increased relative risk for the development of colon, cervix, uterus, pancreas and prostate cancer has been suggested in *BRCA1*-mutation carriers (Shih et al. 2000). Accumulating evidence points to a role for the *BRCA1* protein product in the regulation of multiple nuclear functions including transcription, recombination, DNA repair and checkpoint control (Chapman and Verma 1996; Monteiro 2000; Scully and Livingston 2000; Venkitaraman 2002).

To date, more than 1600 sequence variants of *BRCA1* have been described (Breast Cancer Information Core database 2006). The prevalence of *BRCA1* mutations is variable among different populations (Neuhausen 1999) and varies from 1.8 – 13 percent (Bonadona et al. 2005; Esther et al. 2007; Southey et al. 1999) in developed countries. The incidence of breast cancer is high among Indian females in metropolitan cities (ICMR Delhi 2001; ICMR Bangalore 2001, 2002, 2006; Jussawalla and Jain 1977) and the pathogenic mutations account for 16-25 percent at a median age of 35-40 years in familial breast cancer patients (Hedau et al. 2002; Kumar et al. 2002; Pestonjamas and Mittra 2000; Saxena et al. 2002, 2006; Rajkumar et al. 2003; Valarmathi et al. 2003, 2004).

Information on *BRCA1* mutations in north coastal Andhra Pradesh, India is still lacking. In this study, we carried a detailed analysis of exons 2 and 11 of *BRCA1* gene by direct sequencing, as this approach would cause the least bias in the type of mutation identified while theoretically being the most sensitive method.

MATERIALS AND METHODS

Patient/Family Series

Of 10,128 cancer patients who were registered in the three main cancer hospitals - Lion's Cancer Hospital (2001-2008), King George Ho-

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spital (2001-2004) and Mahatma Gandhi Cancer Hospital (2006-2008), Visakhapatnam during August 2001 to September 2008, 1920 (19%) were cytologically and histologically confirmed breast cancer patients. All Patients were from three districts of North Coastal Andhra Pradesh viz., Visakhapatnam, Vizianagaram and Srikakulam. About 80% of the patients had low socioeconomic background.

The sporadic breast cancer patients were categorized with respect to their family history of with/without any other cancers (18 with a history and 87 without any history of cancer) (Table 1). Age of the patients' ranged from 25 to 89 years with 54.8 percent being premenopausal.

Blood samples from multiple affected individuals and unaffected relatives were collected with informed consent. A total of 136 samples were analyzed which included 9 primary familial breast cancer patients, 105 sporadic breast cancer patients (index cases) and 22 at risk individuals (includes relatives of index cases with a family history of breast and/or other cancers) derived from first-/second-/ third degree relative(s).

Control Population

Ninety-seven age and sex matched healthy unrelated individuals with no history of cancers were selected from Visakhapatnam as the control group to estimate the frequency of the detected *BRCA1* variants.

Molecular Studies

Genomic DNA was extracted from peripheral blood mononuclear cells (Lahiri and Nurnberger 1991). Exons 2 and 11 of *BRCA1* and their intron/exon splice junctions were amplified by polymerase chain reaction (Denaturation 95°C; annealing 58°C; extension 72°C). Six sets of primers were designed using primer3 software (<http://www.genome.wi.mit.edu/cgi-bin/primer/primer3-www.cgi>) for exons 2 and 11 of human *BRCA1* gene (accession # U14680; version # U14680.1 GI: 555931). Primers were supplied by Sigma Genosys. The primer sequences were as follows.

Exon 2: F. 5'-AGCTAAGGCTACCAACACCTAC-3'
R. 5'-CAGAGTGGATGGAGAACAAGG-3'
Exon 11: F. 5'-GTGAAAGAGTTCCTCCAAATCAG-3'
R. 5'-TTGGGGTCTTCAGCATTATTAG-3'
F. 5'-AGACATGACAGCGATACTTTCC-3'
R. 5'-CATTTCCCATTTCTCTTCAGG-3'
F. 5'-AGAAAGGAGAGCTTAGCAGGAG-3'
R. 5'-ACGTCCTAGCTGTGTGAAGG-3'
F. 5'-GCAACGAACTGGACTCATTAC-3'
R. 5'-CCAAATAACAAGTGTGGAAGC-3'
F. 5'-AGAAAGGAGAGCTTAGCAGGAG-3'
R. 5'-ACGTCCTAGCTGTGTGAAGG-3'

BRCA1 Mutation Analysis

Direct Sequencing

Direct sequencing was carried out on amplified PCR products by forward and when necessary reverse sequencing by ABI 3730 automated

Table 1: Characteristics of breast cancer patients and at risk individuals

Characteristics	Familial breast cancer patients (N = 9)	Sporadic breast cancer patients (N=105)	At risk individuals (N = 22)
<i>Age (years) at Diagnosis (patients)/Sample Collection (at risk individuals)</i>			
Mean± SD	57.4±4.97	50.2±10.43	36.4±9.67
≤ 40	0	23	18
> 40	9	82	4
<i>Number of Family Members With Breast Cancer</i>			
N=0	0	105	0
N=1	5 (1 st ; 5)	0	13
N=2	3 (1 st ; 1, 1 st and 2 nd ; 1, 1 st and 3 rd ; 1)	0	2
N=3	1 (1 st and 3 rd ; 1)	0	7
<i>Number of Family Members with Other Cancers*</i>			
N=1	3	10	2
N=2	1	4	5
N=3	1	2	4
N=4	2	2	1

* ovarian, uterine, colon, kidney, brain, bone, lung, oral, ovarian, thyroid, uterine cervix cancers

1st, 2nd and 3rd degree relative affected with breast cancer.

Table 2: Mutation and polymorphisms in breast cancer patients

Category	No. of screen ed pa-tients	No. of rela-tives sc-reened	BRCA1 mutations				BRCA1 polymorphisms			
			Frequency		%		Frequency		%	
			PT	RT	PT	RT	PT	RT	PT	RT
Familial breast cancer cases	9	11	0	0	0	0	8	7	88.9	63.64
Sporadic cases with family history of other cancers	18	5	1	0	5.6	0	14	4	77.8	80.0
Sporadic cases without any family history of other cancers	87	6	0	0	0	0	58	3	66.7	50.0
Total summary	114	22	1		0.9	0.0	80	14	70.2	63.64

PT- patients; RT-relatives

sequencer (version 2.5 - SeqScape), to ascertain the position and type of sequence variation for 233 individuals including 105 sporadic, 9 familial, 22 at risk individuals and 97 control group.

Mutation Nomenclature

Approved nomenclature for the description of sequence variants was adopted. All nucleotide numbers refer to the wild type cDNA sequence of BRCA1 (accession # U14680; version # U14680.1GI: 555931) as reported in Gen Bank. The approved nucleotide numbering system uses the A of the ATG translation initiation start site as nucleotide +1.

Multiple Sequence Alignment

The multiple sequence alignment (MSA) of orthologous BRCA1 gene from six species including Homo sapiens (Gene Bank accession number U14680), Pan troglodytes (AF207822), Mus musculus (U68174), Ratus norvegicus (AF036760), Gallus gallus (AF3552273), Canis familiaris (U50709), were obtained by using program ClustalW (Thompson et al. 1994). According to PSI-BLAST (Altschul et al. 1997),

these five sequences are the only sequences in the nonredundant protein sequence data based at National Center for Biotechnology Information (NCBI) that have 90% sequence identity to the human BRCA1 DNA-binding domain (residues 452-1092).

RESULTS AND DISCUSSION

This is the first report on BRCA1 mutations in breast cancer patients from north coastal Andhra Pradesh, India. The number of breast cancer cases screened and the mutation status in each category are shown in Table 2. We have identified eight sequence variants of which two are novel variants: one deleterious frame-shift mutation at c. 2717-2718insA and one polymorphism at c.1400 A>G in exon 11 (Fig. 1). Five previously reported polymorphisms (c.2082C>T), (c.2311T>C) (c.2612C>T), (c.3113A>G), (c.3548A>G) in exons 11, and one known intronic variant at base 1822C>T in intron1 of BRCA1 were found (Table 3). These sequence variations were identified in eight out of 9 (89%) familial and 72 out of 105 (68.6%) sporadic breast cancer patients analyzed in this study.

Table 3: BRCA1 gene mutations/polymorphisms in North Coastal Andhra Pradesh

Exon/ Intron	NT*	Basechange	Codon	Amino acid change	Designation	Variation	BIC entry
11	c.2717	+A	906	fs 914stop	-	Frame shift	Novel
	c.1400	A>G	467	Lys - Arg.	K467R	polymorphism	Novel
	c.2082	C>T	694	Ser - Ser	Silent	polymorphism	Reported
	c.2311	T>C	771	Leu - Leu	Silent	Polymorphism	Reported
	c.2612	C<T	871	Pro - Leu	P871L	polymorphism	Reported
	c.3113	A>G	1038	Glu - Gly	E1038G	polymorphism	Reported
Int.1	c.3548	A>G	1183	Lys - Arg	K1183R	polymorphism	Reported
	1822	C>T	-	-	-	polymorphism	Reported

*approved nucleotide number

Electropherogram of *BRCA1* showing c. 2717insA codon in 906:fs914 stop/polimorphism c. 1400A>G

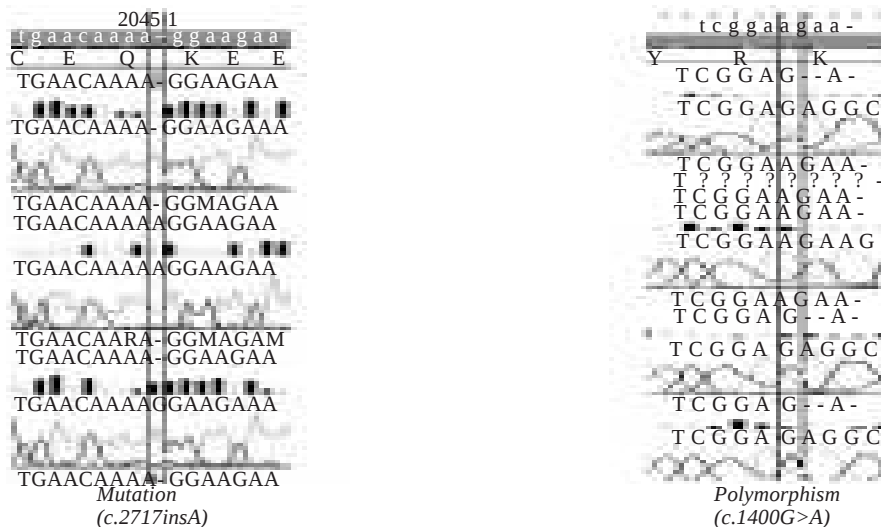


Fig. 1. Electropherogram of *BRCA1* gene showing frameshift mutation.

Frameshift Mutation

The frameshift mutation at c.2717insA (codon 906:fs914stop) occurs due to an insertion (A) in the normal sequence AA⁺G GAA GAA AAT CAA GGA AAG AAT GAG at codon 906 in exon 11 of *BRCA1*. This mutation changes the reading frame of the mRNA and causes a premature termination codon at position 914. This mutation was detected in one of the sporadic patients of late-onset breast cancer (aged 64) with only one daughter (aged 40) affected with uterine cancer. The c.2717-2718insA frameshift mutation occurs at a frequency of 0.9% and was observed only in a sporadic breast cancer patient but not in familial cases, at risk individuals or in control chromosomes studied. This mutation has not been reported in Breast cancer Information Core database (BIC) or Human Gene Mutation Database (HGMD).

Neither mutations nor polymorphisms were observed in exon 2 in the present study. The founder Ashkenazi Jewish mutation, 185delAG, in exon 2 was reported in four Indian studies (Kumar et al. 2002 in South India and Valarmathi et al. 2002; Hedau et al. 2004; Saxena et al. 2006 in North India) and missense mutations at 145 G>A, 186G>A in exon 2 were observed by Valarmathi et al. (2004) in North India. Lack of mutations in exon 2 may be because of small sample size or due to ethnic diversity.

Polymorphisms

Common polymorphisms in *BRCA1* gene appear to be highly prevalent in north coastal Andhra Pradesh breast cancer patients. In this study, the polymorphism at c.1400 A>G (p. K467R-28%) in exon 11 (Fig. 1) has not been previously reported. The six known nucleotide substitutions which include two synonymous (c.2082C>T: p. S694S, 24.3%), (c.2311T>C: p.L771L, 17.6%), three non synonymous (c.2612C>T: p.P871L, 25%), (c.3113A>G: p.E1038G, 39%), (c.3548A>G: p.K1183R, 22%) amino acid changes of unknown functional importance in exon 11, and one nucleotide substitution (base 1822 C>T (20.8%); rs.37655640) in intron-1 of *BRCA1* were detected in breast cancer patients. The six amino acid changes in exon 11 have occurred in N-terminus DNA-binding domain (residues 452-1092), that bind to different proteins such as P53, RAD50, RAD51, c-Myc (Aihara et al. 1999; Narod and Foulkes 2004) and also function as nuclear localization signal domain.

The polymorphism at c.1400A>G leads to the substitution of Arginine to Lysine at codon 467 (K467R). Arginine is the evolutionarily conserved amino acid observed in all other species aligned excluding *Homo sapiens* (U14680) where it differs by having Lysine at c.1400 position (Fig. 2). The reverse change from Lysine to

query	R—KASLPNLSHVTENLIIGAFVTEPQIIQERPL—TNKLKRRRPTSGLHPEDFIKKA225
U68174	R—KGSRPHLNHVTE—IIGTFITEPQITQEPPF—TNKLKRRR—STSLQPEDFIKKA 514
AF036760	R—KGSRPHLNHVTE—IIGTFITEPQIIQEPPF—TNKLKRRR—STCLHPEDFIKKA515
U14680	K—KASLPNLSHVTENLIIGAFVTEPQIIQERPL—TNKLKRRRPTSGLHPEDFIKKA521
AF207822	R—KASLPNLSHVTENLIIGAFVTEPQIIQERPL—TNKLKRRRRATSGLHPEDFIKKA521
U50709	R—KASLPKVSHTTEVLTIGACAIEPQTMQTHPF—MNKAEHKRRRTSSLHPEDFIKKV518
AF355273	RGRKSNPSTILRDILPATKKEDAAAEEGCLNNSR—KDRLKRRKRSACILQPEDFIKKK534
AF416868	REKAMPNNITCVAEVHDSALETGKENMTLEYGTGMSHLSKRRKMVYSLNPENTSKKN492
:	*, . : ** *:** **

Fig. 2. Protein sequence alignment of BRCA1 DNA binding domain

CLUSTAL W (1.83) multiple sequence alignment [Mus musculus (U68174); Ratus norvegicus (AF036760); Homo sapiens (Gene Bank accession number U14680); Pan troglodytes (AF207822); Canis familiaris (U50709); Gallus gallus (AF3552273)].

the conserved amino acid (Arginine) was observed in breast cancer patients. This polymorphism was identified in 28 percent of the sporadic, 22 percent of familial cases, 36 percent of at risk individuals and 15.5 percent of control population. The frequency of this novel variant is 23.2 percent. The distribution of allele frequency of the polymorphisms was summarized in Table 4.

Polymorphism at c.3113A>G in exon 11 of *BRCA1* at codon 1038 (E1038G), was identified in 27.6 percent in sporadic cases without any other cancers in the family, 44.4 percent in sporadic cases with a family history of other cancers, 78 percent of familial breast cancer patients, 36.4 percent in at risk individuals and only 0.02 percent out of 97 in control group of this series. Interestingly only E1038G polymorphism was

observed to be transmitted to at least a few members of all the families in familial cases and to daughters in two sporadic cases. The frequency of E1038G was found to be increasing with an increasing association with cancer (Table 5) suggesting that it does not represent a common polymorphism. There is a significant discernible difference ($P = 0.0000$) in the allele frequencies of this polymorphism between familial breast cancer, sporadic breast cancer with and without a history of other cancer, at risk individuals and control population. When physiochemical properties of the amino acids concerned are taken into account, this alteration would be expected to be significant, since it results in the replacement of a large negatively charged amino acid (Glutamic acid) by smaller uncharged (Glycine) amino acid. The low frequency of this variant in

Table 4: Allele frequencies of BRCA1 mutation/polymorphisms in North Coastal Andhra Pradesh

<i>BRCA1 mutations</i>							
Exon / Intron	Designation approved [†]	Predicted effect	Age* Yrs.	Method of detection	No. detected		
					Cases n = 136	Controls n = 97	Pvalue
11	c.2717(+A)	Fs914stop	64	DS	1	0	0.3889
<i>BRCA1 polymorphisms</i>							
Exon / Intron	Designation approved	Predicted effect	Method of detection	Allele frequency [§] %			BIC entries
				Cases n = 136	Controls n = 97	pvalue	
11	c.1400A>G	Lys467– Arg	DS	14.0	7.7	0.02	No
11	c.2082C>T	p.Ser694Ser	DS	12.1	7.7	0.0943	Yes
11	c.2311T>C	p.Leu771Leu	DS	8.8	6.2	0.1944	Yes
11	c.2612C>T	p.Por871Leu	DS	12.5	7.7	0.0738	Yes
11	c.3113A>G	Glu 1038Gly	DS	17.0	1.0	0.0000	Yes
11	c.3548A>G	Lys1183Arg	DS	11.0	11.9	0.8938	Yes
Int.1	c.1822C>T	-	DS	20.8	8.2	0.0006	Yes

[†]The GenBank reference sequences: *BRCA1* ~ accession # U14680; version # U14680.1 GI: 555931; *Age at diagnosis of proband; DS: direct sequencing; [‡]The Breast Cancer Information Core Database (BIC); [§] Allele frequency was expressed as the prevalence among 466 chromosomes; **Bold face = Novel variants.**

Table 5: Distribution of non synonymous polymorphisms in exon-11 of *BRCA1* gene

Category	N	K467R%	P871L%	E1038G%	K1183R%
Sporadic cases without family history of cancers	87	25.3	17.2	27.6	26.4
Sporadic cases with a family history of other cancers	18	39.0	16.7	44.4	5.6
Familial	9	22.2	44.4	77.8	0.0
At risk individuals	22	15.5	22.7	36.4	9.1
Controls	97	15.5	6.2	2.0	23.7

the control group, lack of similarity between normal and the variant amino acid and location in putative functional domain may be indicative of a biologically deleterious effect.

The allele frequencies of some of the polymorphisms observed in this study were higher than that reported by north Indian studies (Saxena et al. 2002; Valarmathi et al. 2004). However, the frequencies reported in a study from south India (Nagaswamy et al. 2009), Malaysia (Balraj et al. 2002; Toh et al. 2008) and Sri Lanka (Wasanthi et al. 2008) were higher than that observed in the present study.

All the six polymorphisms were found to co-exist only in one at risk individual (daughter of a sporadic case) but not in cases or in controls. However, polymorphisms c.1400A>G and c.3113A>G co-existed in 9 sporadic breast cancer patients of which five have family history of other cancers, one familial and two at risk individuals with a family history of breast and uterine/ovarian cancers. Of 18, seventeen sporadic breast cancer patients with a family history of cancers (either/or uterine, ovarian, brain, head and neck, cervix, thyroid in first and second degree family members) had only c.1400A>G polymorphism. Twelve sporadic cases (with either/or bone, throat uterine cancers in five of the families), three familial cases (with uterine cancer in the family) had only c.3113A>G polymorphism. All these six polymorphisms were found to co-occur in other south Indian (Nagaswamy et al. 2009), Malaysian (Balraj et al. 2002; Lim and Halimah 2004; Tang et al. 1999; Toh et al. 2008; Wasanthi et al. 2008) studies as well as Caucasian populations (Durocher et al.1996).

CONCLUSION

Considering the Indian history and taking into account the multi ethnic, multi religion status and population structure of this country, the results of this initial study suggest that the mutational spectrum in exons 2 and 11 of *BRCA1* gene

in this population may differ from what has been observed in other Indian populations. Further studies on mutational analysis of the gene will help in identifying the relevant mutation/polymorphisms and the multiple structure-based alignments of the native structure of these polymorphisms may provide the knowledge of the biological effects of the protein corresponding to polymorphisms to help predict the aetiology of breast cancer in different geographical regions. Accompanied by proper counselling, patients and pre-symptomatic mutation carriers would be able to make better decisions about medical and surgical preventive options.

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REFERENCES

- Aihara H, Ito Y, Kurumizaka H, Yokoyama S, Shibata T 1999. The N-terminal domain of the human Rad51 protein binds DNA: Structure and a DNA binding surface as revealed by NMR. *J Mol Biol*, 290: 495-504.
- Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z et al.1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res*, 25: 3389-402.
- Balraj P, Khoo ASB, Volpi L, Tan JAMA, Nair S et al. 2002. Mutation analysis of the *BRCA1* gene in Malaysian breast cancer patients, Kuala Lumpur Malaysia. *Singapore Med J*, 43(4): 194-197.
- Bonadona V, Sinilnikova OM, Chopin S, Antoniou AC, Mignotte H et al. 2005. Contribution of *BRCA1* and *BRCA2* germ-line mutations to the incidence of breast cancer in young women. Results from a prospective population-based study in France. *Genes, Chromosomes Cancer*, 43: 404-413.
- Breast Cancer Information Core (BIC) 2006. From <http://research.nhgri.nih.gov/bic/ (Retrieved on, September 19, 2007.)
- Chapman MS, Verma IM 1996. Transcriptional activation by *BRCA1*. *Nature*, 382: 678-679.

- Couch FJ, Weber BL 1996. Mutations and polymorphisms in the familial early-onset breast cancer (BRCA1) gene. *Hum Mutat*, 8: 8-18.
- Durocher F, Shattuck-Eidens D, McClure M, Labrie F, Skolnick MH et al. 1996. Comparison of BRCA1 polymorphisms, rare sequence variants and/or missense mutations in unaffected and breast/ovarian cancer populations. *Hum Mol Genet*, 5: 835-842.
- Esther M John, Alexander Miron, Gail Gong, Amanda I Phipps, Anna Felberg et al. 2007. Prevalence of pathogenic BRCA1 mutation carriers in 5 US racial/ethnic groups. *JAMA*, 298(24): 2869-2876.
- Hedau S, Jain N, Husain SA, Mandal AK, Roy G et al. 2002. Novel germline BRCA1 mutation analysis in Indian breast/ovarian families. *Cancer Biol Ther*, 1: 18-21.
- Hedau S, Jain N, Husain SA, Mandal AK, Ray G, Shahid M, Kant R, Gupta V, Shukla NK, Deo SS, Das BC 2004. Novel germline mutations in breast cancer susceptibility genes BRCA1, BRCA2 and p53 gene in breast cancer patients from India. PMID: 15564800 [PubMed - indexed for MEDLINE]
- ICMR. National Cancer Registry Programme 2001. *Consolidated Report of the Population Based Cancer Registries 1990-1996*. New Delhi: ICMR.
- ICMR. National Cancer Registry Programme 2001, 2002, 2006. *Annual Report*. Bangalore: ICMR.
- Jussawalla DJ, Jain DK 1977. Breast cancer and religion in greater Bombay women: An epidemiological study of 2130 women over a 9-year period. *Br J Cancer*, 36: 634-8.
- Kumar BV, Lakhotia S, Ankathil R, Madhavan J, Jayaprakash PG et al. 2002. Germline BRCA1 mutation analysis in Indian breast/ovarian cancer families. *Cancer Biol Ther*, 1: 18-21.
- Lahiri DK, Nurnberger JI, Jr. 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res*, 19(19): 5444.
- Lim GCC, Halimah Y (Eds.) 2004. *Cancer Incidence in Malaysia (2003)*. Second Report of the National Cancer Registry. Kuala Lumpur, Malaysia
- Monteiro AN 2000. BRCA1: Exploring the links to transcription. *Trends Biochem Sci*, 25(10): 469-474.
- Nagaswamy Soumitra, Balaiah Meenakumari, Tithi Parija, Veluswami Sridevi, Karunakaran N Nancy et al. 2009. Molecular genetics analysis of hereditary breast and ovarian cancer patients in India. *Hered Cancer Clin Pract*, 7(1): 13.
- Narod SA, Foulkes WD 2004. BRCA1 and BRCA2: 1994 and beyond. *Nat Rev Cancer*, 4: 665-676.
- Nathanson KL, Wooster R, Weber BL, Nathanson KN 2001. *Nat Med*, 7: 552-556.
- Neuhausen SL 1999. Ethnic differences in cancer risk resulting from genetic variation. *Cancer*, 86: 2575-2582.
- Parkin DM, Bray F, Ferlay J, Pisani P 2005. Global cancer statistics CA. *Cancer J Clin*, 55: 74-108.
- Pestonjamasp PH, Mittra I 2000. Analysis of BRCA1 involvement in breast cancer in Indian women. *J Biosci*, 25(1): 19-23.
- Rajkumar T, Soumitra N, Nancy NK, Swaminathan R, Sridevi V et al. 2003. BRCA1, BRCA2 and CHEK2 (1100 del C) germline mutations in hereditary breast and ovarian cancer families in South India. *Asian Pac J Cancer Prev*, 4(3): 203-208.
- Saxena S, Szabo CI, Chopin S, Barjhoux L, Sinilnikova O et al. 2002. BRCA1 and BRCA2 in Indian breast cancer patients. *Hum Mutat*, 20(6): 473-474.
- Saxena S, Anurupa Chakraborty, Mishi Kaushal, Sanjeev Kotwal, Dinesh Bhatnager et al. 2006. Contribution of germline BRCA1 and BRCA2 sequence alterations to breast cancer in Northern India. *BMC Medical Genetics*, 7: 75.
- Shattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, Couch F et al. 1995. A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene: Implications for presymptomatic testing and screening. *JAMA*, 273: 535-541.
- Shen D, Vadgama JV 1999. BRCA1 and BRCA2 gene mutation analysis. *Oncol Res*, 11: 63-69.
- Shih HA., Katherine L Nathanson, Sheila Seal, Nadine Collins, Michael R. Stratton et al. 2000. BRCA1 and BRCA2 mutations in breast cancer families with multiple primary cancers. *Clin Cancer Res*, 6: 4259-4264.
- Scully R, Livingston DM 2000. In search of the tumour-suppressor functions of BRCA1 and BRCA2. *Nature*, 408(6811): 429-432.
- Southey M, Tesoriero AA, Andersen CR, Jennings KM, Brown SM et al. 1999. BRCA1 mutations and other sequence variants in a population-based sample of Australian women with breast cancer. *Br J Cancer*, 79: 34-39.
- Tang NL, Pang CP, Yeo W, Choy KW, Lam PK et al. 1999. Prevalence of mutations in the BRCA1 gene among Chinese patients with breast cancer. *J Natl Cancer Inst*, 91: 882-5.
- Thompson JD, Higgins DG, Gibson TJ 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, 22: 4673-80.
- Toh GT, Kang P, Lee SSW, Lee DS-C, Lee SY, et al. 2008. BRCA1 and BRCA2 germline mutations in Malaysian women with early-onset breast cancer without a family history. *PLoS ONE*, 3(4): e2024.
- Venkitaraman Ashok R 2002. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell*, 108(2): 171-182.
- Valarmathi MT, AA, Deo SS, Shukla NK, Das SN 2003. BRCA1 germline mutations in Indian familial breast cancer. *Hum Mutat*, 21(1): 98-99.
- Valarmathi MT, Sawhney M, Deo SS, Shukla NK, Das SN. 2004. Novel germline mutations in the BRCA1 and BRCA2 genes in Indian breast and breast-ovarian cancer families: *Hum Mutat*, 23(2): 205.
- Wasanthi De Silva, Eric H Karunanayake, Kamani H Tennekoon, Marie Allen, Indrani Amarasinghe et al. 2008. Novel sequence variants and a high frequency of recurrent polymorphisms in BRCA1 gene in Sri Lankan breast cancer patients and at risk individuals. *BMC Cancer*, 8: 214.