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rs11655505 (c.-2265 C/T) Variant in *BRCA1* Promoter is not associated with Breast Cancer Risk in South India

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Authors' contributions

This work was carried out in collaboration between all authors. Author TNH did sample collection, laboratory work and statistical analysis. Author BLG designed the work and manuscript preparation. Author GS designed the work and critical review of the manuscript. Author RS completed laboratory work and manuscript preparation. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: Breast cancer is leading cause of cancer deaths in women, globally. Recently a study on a Chinese population suggested that there is a protective role of rs11655505 (c.-2265 C/T) in the *BRCA1* promoter whereas, the same in Caucasian population showed no effect on breast cancer prevalence. This study was undertaken to find out, if there is any association between rs11655505 (c.-2265 C/T) and breast cancer in an Indian population.
Methodology: Blood samples were collected from 352 female breast cancer patients and 380 healthy women of age range 18-68 years. DNA was extracted from blood through standard salting out procedure. All the DNA samples were genotyped by using TaqMan® SNP Genotyping Assays. Data obtained was analyzed statistically to find out the possible association between the variation and predisposition of breast cancer
Results: No association was found between rs11655505 and breast cancer occurrences in familial (p=0.61), non-familial (p=0.45) and premenopausal patients

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(p=0.52) groups. Menopausal group had a significant association (p=0.01) with studied SNP.
Conclusion: Present study failed to confirm an association between of rs11655505 and breast cancer. Larger studies are required to consolidate if there is any little association of rs11655505 with breast cancer risk.

Keywords: BRCA1; promoter; rs11655505; breast cancer.

1. INTRODUCTION

Breast cancer is the most common cancer and the main cause of cancer deaths for women, worldwide. Incidences count per annum is increasing [1]. For year, 2010 it was 1.5 million [2]. About half of the breast cancer incidences and 60% of concerned deaths are occurring in economically developing countries. In many African and Asian countries, like Uganda, South Korea, and India, incidence and mortality rates have been increasing [2,3].

In general, highest rate of incidences are in Western and Northern Europe, Australia/New Zealand, and North America. South America, the Caribbean, and Northern Africa are the region where rate of breast cancer incidence are at intermediate range whereas, in sub-Saharan Africa and Asia that is low. However, in many African and Asian countries, like Uganda, South Korea, and India, incidence and mortality rates have been increasing [2,3].

Age-adjusted breast cancer incidence rates in less developed countries are $1/5^{th}$ time lower than the rates in Western countries [4]. However, among all types of cancers diagnosed in Indian women, the cancer of the breast is the most common cancer in many regions and has overtaken cervix cancer [5]. Breast cancer shows geographical variation in its incidence, even within areas of ethnic homogeneity. In India, over past few years, witnesses increase in incidence of breast cancer in its different ethnic population. It is estimated that approximately 80,000 cases occur annually; the age adjusted incidence rates varying between 16 and 25/ 100,000 population. Breast cancer ranks first in incidence out of all cancers among females in Mumbai and Thiruvananthapuram and constitutes 26.2% and 25.4% of all cancers respectively. The peak incidence of breast cancer is in the age group of 45-49years in females [6].

BRCA1 gene is located at chromosome 17q12-21. It is an autosomal dominant gene, codes for a tumor suppressor protein “breast cancer type 1 susceptibility protein” (BRCA1), which plays a crucial role in breast cancer risk. Germ line mutations in *BRCA1* are associated with approximately 20% of familial breast cancers in Caucasian women [7]. There is an 81% risk of developing breast cancer to the women carrying loss of functional *BRCA1* mutations [8]. Interestingly, *BRCA1* mutations account for only 5-10% of all breast cancers [9]. In addition of *BRCA1* mutations, promoter hypermethylation was also found to be involved in to reduce BRCA1 expression in some breast cancer patients, including the sporadic breast cancer cases too [10,11,12].

Activity of a promoter can be affected by the single nucleotide polymorphisms (SNPs) in promoter sequence of a gene, as the change in nucleotide may affect the affinity between transcription factors involved in regulation of gene expression and promoter [13]. Study of Freedman et al. on *BRCA1* promoter included two promoter SNPs, c.-2613G>C (rs799907: G>C) and c.-2004G>A (rs799906: G>A) and deduced no substantial risk of sporadic breast cancer [14]. Another study of (c.-2265C/T) rs11655505 in the BRCA1 promoter of Australian,

German and Italian populations reports for no association between rs11655505 and breast cancer risk [15]. Interestingly, in a Chinese population (c.-2265C/T) rs11655505 variation has been found to be associated with increased promoter activity and has a protective effect on breast cancer risk [16].

So far no study has comprehensively investigated the *BRCA1* promoter SNPs and (c.-2265C/T) rs11655505 variation in particular for their functional roles and contribution to risk in developing breast cancer in any Indian population. Hence objective of present study is to examine in a South India population for the functional role and contribution of (c.-2265C/T) rs11655505 variation in developing breast cancer.

2. MATERIALS AND METHODS

2.1 Patients

Screening for *BRCA1* (c.-2265C/T) rs11655505 variation was performed in 352 unselected female breast cancer patients with histological verification and diagnosis at Indo-American Cancer Institute and Research Center, Hyderabad, India. Mean age of breast cancer diagnosis in patients were 44.86 ±10.53 years, ranging from 18 to 68 years. Total 139 (39.48%) patients had attained menopause while rest 213 (60.51%) were in pre-menopausal status. Breast cancer family history was reported from 73 (20.73%) patients while rest of the patients had no familial history of breast cancer. Only the patients had at least one additional first- or second-degree relative with breast cancer diagnosed before 50 years were regarded as familial breast cancer patients. For control, 380 healthy women with no history of breast cancer up to first- or second-degree relative also participated in this study voluntarily. The mean age of healthy women was 43.81±10.33 years. Blood samples from healthy women were used as control for the study. Informant consent was taken from patients and healthy volunteers.

2.2 Sample Collection

About 5 mL of venous blood was drawn from patients and healthy women. Samples were then transferred to sterile, lavender-top vacutainer containing anticoagulant. Samples were stored at 4ºC until processed for DNA extraction from them. Mostly DNA was extracted within 24h of collection of samples.

2.3 DNA Extraction

Blood samples were subjected to the genomic DNA extraction by using a standard salting out procedure [17]. The quantity of extracted DNA (ng/µL) was determined by UV absorbance using a NanoDrop 1000 (Thermo Fisher Scientific, MA, USA), and agarose gel electrophoresis was performed for the quality determination. Extracted DNA was stored at -80ºC until used for genotyping.

2.4 Mutation Analysis

For the single nucleotide polymorphism (SNP) analysis TaqMan® SNP Genotyping Assays were performed with a slight modification. TaqMan® SNP Genotyping Assays is a real-time PCR allelic discrimination technique. The assay accounts for non-labeled forward and reverse primers and two fluorescent TaqMan oligonucleotide probes. For the ease of allelic

discrimination, allele 1-specific probe labeled with VIC fluorophore, allele 2-specific probe labeled with FAM (6-carboxy- fluorescein fluorophore). Both the reporter dyes, VIC and FAM are covalently attached to the 5' terminal base of the two respective probes and the nonfluorescent quencher dye is attached near the 3' ends. The probes differentially bind to the amplicons generated during PCR and selectively report their respective alleles [18]. All PCRs were run in duplicate and contained 50 ng (10 µL) DNA, 9 µL TaqMan genotype PCR master mix, and 1 µL allelic discrimination mix. Real-time PCR was performed on an ABI 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Condition for the allelic discrimination PCRs were; 50ºC for 2 min, 95ºC for 10 min, and then 40 cycles of amplification (92ºC denaturation 15 s, 62ºC annealing, extension for 60 s). The annealing temperature was empirically determined (data not shown) to promote high binding specificity of the probes without the loss of assay sensitivity. For each cycle, the SDS software determined the ΔRn, which is the normalized (i.e., compared with a passive reference fluorophore) florescent signal from the VIC- or FAM-labeled probe. For our analysis, we used the ΔRn value after the final cycle because it proved more reliable than the cycle at which the threshold was crossed (CT value, data not shown).

2.5 Statistical Analysis

For each SNP, deviation of genotype frequencies in cases and controls were compared by χ^2 test for heterogeneity (two degrees of freedom) and test for trend (one degree of freedom), in order to evaluate the ethnicity-specific effect. Genotype specific risks were estimated as odds ratio (ORs) using unconditional logistic regression. If no statistically significant differences were found (data not shown) and so the results have been combined and the risk associated with each SNP was estimated by allelic, dominant and recessive OR and associated 95% confidence intervals (CI). ORs with 95% confidence intervals (CIs) were calculated to assess the strength of the association between polymorphism and breast cancer risk. We explored the association for co-dominant model, dominant model, recessive model and allele versus allele, respectively. All statistical tests were based on two-sided probabilities using IBM SPSS v.19. The priori *P* value for an association was considered to be $P \leq 0.05$.

3. RESULTS

In total 352 breast cancer patients and 380 healthy women samples were analyzed for (c.-2265C/T) rs11655505 variations. Summary of statistical analysis for *BRCA1* (c.-2265C/T) rs11655505 had been shown in Table 1 to 6. Overall, there was no evidence of association between (c.-2265C/T) rs11655505 SNP and the breast cancer risk in the studied population (p=0.35 and 0.34 for genotypes and alleles respectively). Similarly in the grouped analysis for premenopausal, familial and non-familial breast cancer patients *P*-values for genotype were 0.52, 0.61, and 0.45, respectively and that for the allele *P*-values were 0.24, 0.96 and 0.25. Odds ratio (OR) (CI=95%) for T allele as independent risk factor was 1.11 (0.89-1.37). In the same fashion, ORs (CI=95%) for T allele as independent risk factor among premenopausal, familial and non-familial breast cancer patients groups were 1.11 (0.84-1.47), 0.99 (0.68-1.45) and 1.14 (0.90-1.43). A significant association was found between (c.-2265C/T) rs11655505 variation and risk of breast cancer in menopausal group. *P*-value for genotype and allele were 0.01 and 0.001, respectively. OR (CI=95%) for CT and TT genotypes were 0.81 (0.49-1.32) and 1.38 (0.69-2.75) respectively and that of T allele as independent risk factor was 1.08 (0.78-1.50).

No evidence was found for the association between *BRCA1* (c.-2265C/T) rs11655505 SNP and breast cancer risk up to the genotypic and allelic analyses for total patients-control as well as different groups of patients, except for menopausal patients. Test of two models TT+CT vs. CC and CC+CT vs. TT were performed as described earlier [19]. Total patients-control analysis it was found that CC+CT vs. TT model was not significantly associated with breast cancer risk (*p*=0.15) and OR=1.33 (0.88-2.00). Rest other groups did not shown any association between breast cancer risk and either of the models, except menopausal patients where TT+CT vs. CC and CC+CT vs. TT were 1.08 (0.67-1.72); *p*=0.01 and 2.12 (1.08-4.21); *p*=0.004 (Table-6).

Table 1. Distribution of BRCA1 rs11655505 (c.2265C/T) genotypes and allelic frequencies of the studied population

Study group	BRCA1 rs11655505 (c.2265C/T) genotypes			Allelic frequency	
	CC	CT	TT	C	T
Patients *n* (%)	133 (37.78)	167 (47.44)	52 (14.77)	0.61	0.38
Control *n* (%)	140 (36.84)	169 (44.47)	71 (18.68)	0.59	0.4

χ^2 (2 d. f.) = 2.06, p=0.35 for genotypes; χ^2 (1 d. f.) = 0.9, p=0.34 for allelic frequency. Odds ratio (CI=95%) for CT genotype = 0.96 (0.69-1.34), TT genotype =1.30 (0.83-2.04) where OR of CC genotype considered as 1 (reference).Odds ratio (CI=95%) for T allele = 1.11 (0.89-1.37) as independent risk factor.

Table 2. Distribution of BRCA1 rs11655505 (c.2265C/T) genotypes and allelic frequencies of the premenopausal patients

Study Group	BRCA1 rs11655505 (c.2265C/T) genotypes			Allelic frequency	
	CC	CT	TT	C	T
Patients *n* (%)	80 (36.69)	104 (47.70)	34 (15.59)	0.6	0.39
Control *n* (%)	77 (36.66)	94 (44.76)	39 (18.57)	0.58	0.41

χ^2 (2 d. f.) = 1.7, p=0.55 for genotypes; χ^2 (1 d. f.) = 0.53, p=0.46 for allelic frequency. Odds ratio(CI=95%) for CT genotype = 0.96 (0.62-1.50), TT genotype =1.29 (0.71-2.32) where OR of CC genotype considered as 1 (reference). Odds ratio (CI=95%) for T allele = 1.11 (0.83-1.47) as independent risk factor.

Table 3. Distribution of BRCA1 rs11655505 (c.2265C/T) genotypes and allelic frequencies of the menopausal patients

Study Group	BRCA1 rs11655505 (c.2265C/T) genotypes			Allelic frequency	
	CC	CT	TT	C	T
Patients *n* (%)	49 (35.25)	72 (51.79)	18 (12.94)	0.61	0.38
Control *n* (%)	63 (37.05)	75 (44.11)	32 (18.82)	0.55	0.44

χ^2 (2 d. f.) = 9.35, P=0.01 for genotypes; χ 2 (1 d. f.) = 11.69, P=0.001 for allelic frequency. Odds ratio (CI=95%) for CT genotype = 0.81 (0.49-1.32), TT genotype =1.38 (0.69-2.75) where OR of CC genotype considered as 1 (reference). Odd ratio (CI=95%) for T allele = 1.08 (0.78-1.50) as independent risk factor.

Table 4. Distribution of BRCA1 rs11655505 (c.2265C/T) genotypes and allelic frequencies of the familial patients

Study Group	BRCA1 rs11655505 (c.2265C/T) genotypes			Allelic frequency	
	CC	CT	TT	C	T
Patients *n* (%)	23 (31.50)	40 (54.79)	10 (13.69)	0.58	0.41
Control *n* (%)	140 (36.84)	169 (44.47)	71 (18.68)	0.59	0.4

χ^2 (2 d. f.) = 0.25, p=0.61 for genotypes; χ^2 (1 d. f.) = 0.0, p=0.96 for allelic frequency. Odds ratio (CI=95%) for CT genotype = 0.69 (0.38-1.26), TT genotype =1.17 (0.50-2.79) where OR of CC genotype considered as 1 (reference). Odds ratio (CI=95%) for T allele = 0.99 (0.68-1.45) as independent risk factor.

Table 5. Distribution of BRCA1 rs11655505 (c.2265C/T) genotypes and allelic frequencies of the non-familial patients

Study Group	BRCA1 rs11655505 (c.2265C/T) genotypes			Allelic frequency	
	CC	CT	TT	C	T
Patients *n* (%)	110 (39.42)	127 (45.51)	42 (15.05)	0.62	0.37
Control *n* (%)	140 (36.84)	169 (44.47)	71 (18.68)	0.59	0.4

χ^2 (2 d. f.) = 1.56, p=0.45 for genotypes; χ^2 (1 d. f.) = 1.3, p=0.25 for allelic frequency. Odds ratio (CI=95%) for CT genotype = 1.05 (0.73-1.49), TT genotype =1.33 (0.82-2.15) where OR of CC genotype considered as 1 (reference). Odds ratio (CI=95%) for T allele = 1.14 (0.90-1.43) as independent risk factor.

Table 6. Odds ratio with CI 95%BRCA1 rs11655505 (c.2265C/T)

Groups	Genotype	OR(CI=95%)	*p*-value
All Patients	TT+CT vs. CC	0.96 (0.70-1.31)	0.79
	CC+CT vs. TT	1.33 (0.88-2.00)	0.15
Premenopausal Patients	TT+CT vs. CC	0.88 (0.60-1.31)	0.55
	CC+CT vs. TT	1.20 (0.72-1.99)	0.47
Menopausal Patients	TT+CT vs. CC	1.08 (0.67-1.72)	0.01
	CC+CT vs. TT	1.55 (1.83-2.91)	0.004
Familial Patients	TT+CT vs. CC	1.27 (0.72-2.25)	0.38
	CC+CT vs. TT	1.45 (0.68-3.17)	0.3
Non-familial Patients	TT+CT vs. CC	0.90 (0.64-1.25)	0.49
	CC+CT vs. TT	1.30 (0.84-2.01)	0.22

4. DISCUSSION

It is a well established fact that the *BRCA1* mutations are prevalent in breast cancers in high-incidence, low-incidence and racially diverse populations [20]. Racial differences in breast cancer incidence and mortality exist throughout different populations [21]. However, the information regarding the contribution of these *BRCA1* mutations in the incidence of and predisposition to breast cancer in ethnic South Indian population, particularly in AP is lacking. A Tamil family had been studied for a deletion of nucleotide “T” at np 1307 codon 396 which cause a frame shift mutation in *BRCA1* and a loss of BRCT domain of BRCA1 protein [21]. Another hospital based population study from Bangalore reports for three novel mutations in the coding region of *BRCA1* which leads to the frame shifts [22]. Saxena et al. [23] had identified two novel splice variants (331+1G>T; 4476+2T>C) in *BRCA1* gene in a North Indian population [23]. There are a few records for the studies about the *BRCA1*

variation in South India populations. Most of them are exploring the coding region of the gene. For study of *BRCA1* promoter region variations no record had been found.

A recent study of Chinese breast cancer patients advocated that the minor allele "T" of rs11655505 in the *BRCA1* promoter (c.2265C/T) enhances promoter activity. Thus *BRCA1* promoter variation rs11655505 (c.2265C/T) has a protective effect on breast cancer risk [16]. Another study of larger populations from Australia, Germany and Italy for rs11655505 (c.2265C/T) showed no significant association of concerned variation and the risk of cancer [15].

To our knowledge present study of rs11655505 (c.2265C/T) in breast cancer in South Indian population of AP is the first study on promoter region SNP of *BRCA1*. On statistical analyses OR (CI=95%) was found to be 0.96 (0.69-1.34) for CT genotype and 1.30 (0.83-2.04) TT genotype and no significant association was found between rs11655505 (c.2265C/T) and breast cancer risk. To elucidate the possible risk in premenopausal, menopausal, familial and non-familial cancers, data from the patients were categorized accordingly and further analyzed statistically. Again there were no associations between rs11655505 (c.2265C/T) and breast cancer risk in different groups. Only menopausal patients group showed a significant association with the rs11655505 (c.2265C/T) where there was a protective effect on breast cancer. As in the control group TT genotypes accounted for 22.3% and11.9% in menopausal patients. It was also deduced that neither CC+CT vs. TT nor TT+CT vs. CC models were associated to the breast cancer risk in premenopausal, familial and non-familial cancers patients. These findings are in agreement with that of Verderio and co-worker [15]. Interestingly, the variation rs11655505 (c.2265C/T) showed a protective effect on menopausal breast cancer, our findings are in partial agreement with Chan and coworker [16].

5. CONCLUSION

Since, present study explores only one ethnic population of India. Further this study was comprised of only 352 patients and 380 healthy women. Hence there is a need of larger studies to determine if there is a weak association between rs11655505 (c.2265C/T) and breast cancer risk in general as well as among the different patients group on the basis of family history and physiological conditions.

ETHICAL APPROVAL

The study was approved by the ethical committee of IACIRC, Hyderabad, India and the Review Board of Bharathiar University, Coimbatore, India.

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COMPETING INTERESTS

There was no competing interest among authors and with any funding agency.

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