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Screening of *BRCA1* variants c.190T>C, 1307delT, g.5331G>A and c.2612C>T in breast cancer patients from North India

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Abstract

The polymorphic variants of *BRCA1*, which lead to amino acid substitutions, have a known pathogenic role in breast cancer. The present study investigated in North Indian breast cancer patients the association of risk with four reported pathogenic variants of *BRCA1*: c.190T>C (p.Cys64Arg), 1307delT, g.5331G>A (p.G1738R) and c.2612C>T (p.Pro871Leu). Genotyping was done by PCR-RFLP method in 255 clinically confirmed breast cancer patients and 255 age and gender matched healthy individuals. For c.190T>C, 1307delT and g.5331G>A, all the patients and controls had the wild-type genotype indicating no association with breast cancer risk. For c.2612C>T polymorphism, the frequency of the CC, CT, and TT genotypes was 14.5 vs 15.7%, 59.6 vs 53.7% and 25.9 vs 30.6% in breast cancer patients and controls respectively. The frequency of heterozygotes (CT genotype) was higher in cases than controls but the difference was not statistically significant. Genetic model analysis showed no association of the four analyzed *BRCA1* variants with breast cancer risk with any model. The studied variants were not associated with the risk of breast cancer in Punjab, North west India, suggesting a need for further screening of other *BRCA1* variants. It is the first reported study on these 4 variants from India.

Keywords: *BRCA1*, variants, breast cancer, North India.

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Introduction

Breast cancer is the most common cancer in the world (Ghoncheh *et al.*, 2016). In India, according to the National Cancer Registry Programme (NCRP 2012-2014), incidence of breast cancer ranks amongst the top in the following individual registries across India: Delhi (41.0%), Chennai (37.9%), Bangalore (34.4%) and Thiruvananthapuram district (33.7%). In the state of Punjab, the highest incidence rate of breast cancer has been reported in districts of Bathinda (37.3%), followed by Mohali (34.3%), Patiala (32.5%) and Faridkot (31.5%) (NCRP 2012-2013), with increasing frequency of sporadic breast cancer being reported from Amritsar (Batra *et al.*, 2010).

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One of the well-studied genes that has been associated with breast cancer, *BRCA1* (MIM 113705), is located at the chromosomal position 17q21 and contains 24 exons (Miki *et al.*, 1994). It encodes a multi-domain protein BRCA1, which has four major domains: a RING domain, the BRCA1 serine domain and two BRCA1 C terminus (BRCT) domains (Clark *et al.*, 2012). BRCA1 interacts with a variety of other proteins to carry out multiple functions at the cellular level, like controlling the cell cycle, DNA damage repair, regulation of transcription, replication, recombination and chromatin hierarchical control (Parvin, 2004).

Mutations in *BRCA1* and *BRCA2* genes are the most susceptible causes amongst the genetic risk factors that play a crucial role in familial breast cancer (Naga, 2011). A frequency of 2.9-24% of *BRCA1/2* mutations has been reported in familial breast cancer patients of South India (Vaidyanathan *et al.*, 2009). However, the reported fre-

quency of mutations in *BRCA2* is remarkably lower than in *BRCA1* in India (Kim and Choi, 2013).

Populations of Caucasian and Indoscythian mixed racial ancestry inhabit the state of Punjab in North India (Bhasin *et al.*, 1992). Therefore, the *BRCA1* variants were chosen for the study considering their prevalence in similar Caucasian and South Asian populations in other parts of the world. The four variants of *BRCA1* [c.190T>C (p.Cys64Arg), 1307delT, g.5331G>A (p.G1738R) and c.2612C>T (p.Pro871Leu)] were investigated in the current study. The variant c.2612C>T (p.Pro871Leu) is a nonsynonymous polymorphism of *BRCA1* that leads to the substitution of proline by leucine at position 871, a region where recombinase RAD51 interacts with BRCA1 to aid homologous recombination (Miao *et al.*, 2017). The T allele has been reported to show an association with breast cancer risk in Chinese populations (Xu *et al.*, 2018) though some studies have reported no significant association between the p.Pro871Leu variant of *BRCA1* and breast cancer risk. The 1307delT variant of *BRCA1*, present in codon 396 of exon 11, causes a frameshift mutation that introduces a premature stop codon at amino acid residue 409 and leads to protein truncation. It was reported in the South Indian population (Gajalakshmi *et al.*, 2007). The c.1907T>C variant of *BRCA1* is a missense mutation present in exon 5 (Cys64Arg). Molecular modelling indicated that the substitution of cysteine with an arginine probably disturbs the structure of the BRCA1 RING finger domain, which is responsible for the interaction with BARD1 and essential for the tumor-suppressor activity of the BRCA1-BARD1 complex (Karami and Mehdipour, 2013). g.5331G>A (p.G1738R) is a missense variant of *BRCA1* that affects the binding affinity of the protein by destabilizing it (Williams *et al.*, 2003; Glover, 2006). It has been reported as a pathogenic mutation (Chenevix-Trench *et al.*, 2006; Karchin *et al.*, 2007).

Although a few common variants of *BRCA1* have been repeatedly reported from South India, the dissimilarity between the populations of North India: Caucasians, Scythians and Indo-Europeans (Bhasin *et al.*, 1992; Reich *et al.*, 2009) and South India: Dravidian (Reich *et al.*, 2009), led us to consider studying the variants in North India. Thus, the current study aimed to screen the Punjabi population from Amritsar and adjoining regions of Punjab, North India, for the four variants of *BRCA1* [c.190T>C (p.Cys64Arg), 1307delT, g.5331G>A (p.G1738R) and c.2612C>T (p.Pro871Leu)] to investigate their association with breast cancer risk. Identifying *BRCA1* polymorphisms that are associated with breast cancer risk can also be further explored for involvement in the therapy response of patients. *BRCA1* c.190T>C (p.Cys64Arg) and g.5331G>A (p.G1738R) have never been reported from India in any of the studies so far. Also, these four variants together have not been investigated in Indian patients in any previous study.

Subjects and Methods

Study subjects

In this case-control study, patients were selected from a tertiary care hospital, Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar, Punjab. The majority of the patients (81%) belonged to the Majha region of Punjab, i.e., to the Amritsar district (34%) and its adjoining districts of Gurdaspur (32%) and Tarn Taran (15%). The rest of the patients belonged to the districts of Jalandhar (5%), Kapurthala (5%), Hoshiarpur (4%), Pathankot (3%), Ferozpur (2%) and Mansa (1%). Three patients, each belonging to the states of Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh, were also included. A total of 255 (5 males and 250 females) clinically confirmed breast cancer patients and 255 age and gender matched normal healthy individuals were recruited in the present study between January 2013 and March 2017. Patients who had received chemotherapy, radiotherapy or blood transfusion before surger, or had previous history of any malignancy were excluded from the study. Controls were biologically unrelated to cancer patients and were from the same geographical region as the patients. Individuals in the control group had no family history of any cancer or any other chronic disease. This study was undertaken after approval by the institutional ethics committee of Guru Nanak Dev University, Amritsar, Punjab, India. After informed consent, as per tenets of the Declaration of Helsinki, a 5 mL peripheral venous blood sample was collected in an EDTA vial from each subject.

DNA isolation and screening of *BRCA1* variants

Genomic DNA was extracted from peripheral blood lymphocytes by a standard phenol-chloroform method (Adeli and Ogbonna, 1990). Four variants of *BRCA1*, c.190T>C (p.Cys64Arg), 1307delT, g.5331G>A (p.G1738R) and c.2612C>T (p.Pro871Leu) were screened by a PCR-RFLP method using published primer sequences (Gajalakshmi *et al.*, 2007; Karami and Mehdipour, 2013; Xu *et al.*, 2018).

The PCR assays were set up in a 15 μL reaction volume containing 50 ng of DNA, 1X *Taq* buffer with 1.5 mM $MgCl_2$, 0.3 μL dNTPs mixture (Bangalore GeNei), 6 pmol of each primer (Sigma, St. Louis, MO, USA), 1U *Taq* DNA Polymerase (Bangalore GeNei). The PCR conditions were: an initial denaturation at 95 °C for 5 min, followed by 35 cycles with denaturation at 95 °C for 45 s, annealing at 58 °C for 30 s and extension at 72 °C for 45 s, and a final extension step at 72 °C for 10 min, run in a Mastercycler gradient (Eppendorf, Germany) machine. A negative control without template DNA was run in each reaction to check for contamination. The PCR products were analysed on 2% ethidium bromide-stained agarose gels. Amplified products were digested with the respective restriction enzymes following the manufacturer's instructions (New England

Biolabs, Beverly, MA), details of which are shown in Table 1. The restriction digestion reaction products were also analysed on 2.5% ethidium bromide-stained agarose gels. The genotype was assigned to each sample according to the size of the fragments obtained after digestion (Table 1). Genotyping was performed without knowing the status of the subjects.

Statistical analysis

A chi-square test was used to test for Hardy-Weinberg Equilibrium (HWE) and difference of genotype and allele frequencies between the breast cancer patients and normal controls. For determining the association between *BRCA1* variants with breast cancer risk, the odds ratio (OR) with 95% confidence interval (CI) was calculated. A genetic model analysis was done to check for an association of the *BRCA1* variants with breast cancer risk. A value of $p \leq 0.05$ was considered statistically significant. Statistical analysis was performed by using SPSS (Version 20.0; SPSS, Inc).

Results

The present study recruited 255 clinically and histopathologically confirmed breast cancer patients and 255 healthy controls. Out of the 255 patients, 250 (98%) were females and 5 (2%) were males. The mean age of the patients was 50.7 ± 11.43 years (range 27-85 years) and that of controls was 48.56 ± 14.26 (range 17-90 years). The frequency of patients diagnosed with stage I, stage II, stage III and stage IV breast cancer were 14.9%, 46.8%, 25.5% and 12.8%, respectively (Table 2). Out of these, 40% of the patients were triple negative for the receptor status. The majority of the patients had sporadic breast cancer (78.8%), while 7.5% reported a history of breast cancer in their family (Table 3). Another 13.7% of the patients had a history of cancer of other sites. Hence, 21.2% of the patients had a family history of any type of cancer.

The genotype and allele frequencies of the four analysed *BRCA1* variants in cases and controls are shown in Table 4. For the 3 variants c.190T>C (p.Cys64Arg), 1307delT and c.5331G>A (p.G1738R) all the patients and controls had the wild-type genotype (Figures S1-S4). The observed genotype frequencies for the c.2612C>T poly-

Table 2 - Clinical profile of breast cancer patients.

Parameter	Total n (%)	Females n (%)	Males n (%)
Stage			
I	35(14.9)	35(15.2)	0(0)
II	110(46.8)	105(46.7)	5(100)
III	60(25.5	60(26.1)	0(0)
IV	30(12.8)	30(13)	0(0)
Breast side			
Left	116(47.2)	113(46.9)	3(60)
Right	121(49.2)	120(49.8)	1(20)
Bilateral	9(3.6)	8(3.3)	1(20)
Histological Type			
DCIS	6(2.7)	6(2.8)	0(0)
DCIS-Papilary	3(1.3)	3(1.4)	0(00
IDC	205(91.9)	200(91.7)	5(100)
IDC-Mucinous	5(2.2)	5(2.2)	0(0)
IDC-Medulllary	1(0.5)	1(0.5)	0(0)
IDC-Pagets	1(0.5)	1(0.5)	0(0)
Invasive Lobular Carcinoma	2(0.9)	2(0.9)	0(0)

n: number of subjects

Table 3 - Breast cancer patients with family history of cancer.

Category	Cases	% of Total Cases
Sporadic cases	201	78.8
Familial cases	54	21.2
History of breast cancer		
1st Relatives	11	4.3
2nd Relatives	5	2.0
3rd Relatives	3	1.2
History of other cancer		
1st Relatives	23	9.0
2nd Relatives	12	4.7
3rd Relatives	0	0

Table 1 - Details of analyzed *BRCA1* variants and restriction conditions used for genotyping.

S. No.	Variant	PCR product size (bp)	Restriction enzyme used	Digestion temperature	Fragment size (bp) after digestion	Alleles
1.	c.190T>C (p.Cys64Arg)	193 bp	*SnaB*I	37 °C	193	T
					124 and 69	C
2.	g. 5331G>A (p.G1738R)	233 bp	*BsaX*I	37 °C	112, 91 and 30	G
					233	A
3.	1307delT	151 bp	*Dde*I	37 °C	151	WT
					96 and 55	MUT
4.	c.2612C>T (p.Pro871Leu)	125 bp	*Hpa*II	37 °C	99 and 26	C
					125	T

WT - wild type; MUT - mutant.

Table 4 - Association analyses of *BRCA1* variants with breast cancer risk.

Variant	Genotype	Patients n (%)	Controls n (%)	OR (95%CI)	*p*-value
c.190T>C (p.Cys64Arg)	TT	255(100)	255(100)	-	NC
	TC	0	0		
	CC	0	0		
1307delT	WT/WT	255(100)	255(100)	-	NC
	WT/MUT	0	0		
	MUT/MUT	0	0		
c.5331G>A (p.G1738R)	GG	255(100)	255(100)	-	NC
	GA	0	0		
	AA	0	0		
c.2612 C>T (p.Pro871Leu)	CC	37(14.5)	40(15.7)	1 (ref)	
	CT	152(59.6)	137(53.7)	1.19(0.73-1.98)	0.48
	TT	66(25.9)	78(30.6)	0.91(0.53-1.59)	0.75
	Allele				
	C	226(44.3)	217(42.5)	1 (ref)	
	T	284(55.7)	293(57.5)	0.93(0.73-1.19)	0.57
Genetic Models					
Dominant Model CT/TT vs CC				1.10(0.67-1.78)	0.71
Co-dominant Model TT vs CT =CT vs CC				0.92(0.70-1.20)	0.54
Recessive Model TT vs CC/CT				0.79(0.54-1.17)	0.24

n: number of subjects; OR: odds ratio; CI: confidence intervals; NC: not calculated.

morphism were in HWE in the controls (p=0.11). The frequency of the CC, CT and TT genotypes of c.2612C>T polymorphism were 14.5% vs. 15.7%, 59.6% vs. 53.7% and 25.9% vs 30.6%, respectively, in breast cancer patients and control individuals. No association was observed between the breast cancer risk and the CT (OR = 1.19, 95% CI: 0.73-1.98, p=0.48) and TT (OR = 0.91, 95% CI: 0.53-1.59, p=0.75) genotypes. The c.2612 C>T polymorphism did not show an association with breast cancer in any of the genetic models (Table 4). The T allele frequency was higher than that of the C allele in both patients and controls, but the difference between patients and controls was not significant (p=0.57). The difference in the frequency distribution of the C and T alleles in breast cancer patients with involvement of left, right or bilateral sides was statistically non-significant (p=0.55). This difference was also not significant (p=0.60) amongst patients at different stages of breast cancer.

To evaluate the relation between the parameters like age at diagnosis, menopausal history, habitat, diet and BMI with the *BRCA1* p.Pro871Leu variant, the cases and controls were stratified according to the aforementioned parameters. We found no significant difference in the genotypic frequencies among patients and controls. However, there was a significant difference in C and T allele frequencies among cases and controls for parameters like age at diagnosis in the group 36-50 years (OR = 0.47, 95% CI = 0.32-0.70, p=0.00), frequency of premenopausal women (OR = 0.56, 95% CI = 0.36-0.87, p=0.01), frequency of post-menopausal women (OR = 0.65, 95% CI = 0.46-0.90, p=0.01), rural habitat (OR = 0.51, 95% CI = 0.37-0.87, p=0.000), vegetarian diet (OR = 0.61, 95% CI = 0.44-0.85, p=0.003) and obesity (BMI ≥ 23 kg/m^2) (OR = 0.62, 95% CI = 0.46-0.84, p=0.002) (Table 5).

Discussion

In the present case-control study, we assessed the relationship of c.190T>C (p.Cys64Arg), 1307delT, g.5331G>A (p.G1738R) and c.2612C>T variants of *BRCA1* with breast cancer risk. The variants p.Cys64Arg and p.G1738R have never been reported from any Indian study so far, and also c.2612C>T had not yet been reported from India at the time when the present study was undertaken. It has been only recently reported from a population of West India (Gujarat), where 32 polymorphisms and two novel mutations in *BRCA1* were found in 35 breast cancer patients by an NGS method (Shah *et al.*, 2018). Among these, c.2612C>T was found in six out of the 35 patients.

The c.190T>C variant has been reported as a pathological mutation in families with HBOC (history of breast and ovarian cancer) in Polish (Jakubowska *et al.*, 2001), Italian (Willems *et al.*, 2009; Caleca *et al.*, 2014), Brazilian (Fernandes *et al.*, 2016), French (Azzollini *et al.*, 2017) and Japanese (Arai *et al.*, 2018) populations. A few *in-vitro* studies have also revealed its deleterious effects (Cochran *et al.*, 2015; Anantha *et al.*, 2017). In the present study, only the wild type genotype (TT) of c. 190T>C was found in all the patients and controls from Amritsar.

The 1307delT variant was first time reported in a South Indian family with Dravidian ancestry having breast

Table 5 - Correlation of *BRCA1* p.Pro871Leu variant with demographic parameters in subjects.

Parameter	Genotypes				OR (95% CI)	*p*-value	Allele				OR (95% CI)	*p*-value
	Cases		Controls				Cases		Controls			
	CC N (%)	CT+TT N (%)	CC N (%)	CT+TT N (%)			C N (%)	T N (%)	C N (%)	T N (%)		
Age at diagnosis (years)												
≤ 35	5(13.9)	26(10.9)	3(11.1)	45(20)	0.35(0.08-1.57)	0.17	26(11.7)	36(12.7)	34(19.9)	62(18.6)	0.76(0.39-1.46)	0.41
36-50	15(41.7)	118(49.6)	8(29.6)	96(42.7)	0.66(0.27-1.61)	0.36	106(47.5)	118(41.7)	62(36.3)	146(43.9)	0.47(0.32-0.70)	**0.00**
51-65	13(36.1)	77(32.4)	6(22.2)	66(29.3)	0.54(0.19-1.5)	0.24	73(32.7)	107(37.8)	45(26.3)	99(29.7)	0.67(0.42-1.06)	0.08
≥ 66	3(8.3)	17(7.1)	10(37.1)	18(8.0)	3.15(0.74-13.45)	0.12	18(8.1)	22(7.8)	30(17.5)	26(7.8)	1.41(0.62-3.19)	0.41
Menstrual history												
premenopausal	12(35.3)	66(32.0)	6(26.1)	88(42.3)	0.38(0.13-1.05)	0.06	71(33.3)	85(31.8)	60(39.5)	128(41.3)	0.56(0.36-0.87)	**0.01**
postmenopausal	22(64.7)	140(68.0)	17(73.9)	120(57.7)	1.46(0.56-3.18)	0.76	142(66.7)	182(68.2)	92(60.5)	182(58.7)	0.65(0.46-0.90)	**0.01**
Habitat												
rural	27(77.1)	163(76.9)	16(61.5)	162(73)	0.60(0.31-1.15)	0.12	167(77.0)	213(76.9)	101(60.8)	255(77.3)	0.51(0.37-0.69)	**0.000**
urban	8(22.9)	49(23.1)	10(38.5)	60(27)	1.02(0.37-2.78)	0.97	50(23.0)	64(23.1)	65(39.2)	75(22.7)	0.68(0.67-1.82)	0.68
Diet												
vegetarian	24(68.6)	136(64.5)	16(61.5)	134(61.5)	0.68(0.34-1.33)	0.26	146(67.3)	174(63.3)	102(61.8)	198(61.3)	0.61(0.44-0.85)	**0.003**
non-vegetarian	11(31.4)	75(35.5)	10(38.5)	84(38.5)	0.81(0.33-2.02)	0.65	71(32.7)	101(36.7)	63(38.2)	125(38.7)	0.72(0.47-1.10)	0.13
BMI												
non-obese	7(20.6)	60(29.9)	5(19.2)	42(19.0)	1.02(0.30-3.43)	0.97	56(26.9)	78(29.8)	30(18.1)	64(19.5)	0.65(0.38-1.14)	0.13
obese	27(79.4)	141(70.1)	21(80.8)	179(81.0)	0.61(0.33-1.23)	0.12	152(73.1)	184(70.2)	136(81.9)	264(80.5)	0.62(0.46-0.84)	**0.002**

n: number of subjects; OR: odds ratio; CI: confidence intervals; NC: not calculated; $p<0.05$ (significance).

cancer (Gajalakshmi *et al.*, 2007). The South Indian population represents a racial compositon different from that of North India. In the present study all the patients and controls, which were from the Northwest part of India, with Caucasian and Indoscythian ancestry (Reich *et al.*, 2009), had the wild type genotype for this variant.

The g.5331G>A (p.G1738R) missense mutation has been repeatedly found in Greek families with breast cancer (Belogianni *et al.*, 2004; Armaou *et al.*, 2009; Koumpis *et al.*, 2011), ovarian cancer (Stavropoulou *et al.*, 2013) or both (Konstantopoulou *et al.*, 2008; Karami and Mehdipour, 2013). It is also reported to be a deleterious mutation in *in-vitro* assays (Carvalho *et al.*, 2007; Chenevix-Trench *et al.*, 2016). None of the patients or controls in the present study carried this mutation.

The c.2612C>T (p.Pro871Leu) non-synonymous SNP causes an amino acid change from proline to leucine at position 871 in the BRCA1 protein. The previous published studies give contradictory reports on the role of p.P871L and the risk for various types of cancers. The wild type genotype (CC) was associated with increased risk of gastric cancer (Wang *et al.*, 2015), esophageal squamous cell carcinoma (ESCC) (Zhang *et al.*, 2013) and non-Hodgkin Lymphoma (NHL) (Chen *et al.*, 2013), but not associated with thyroid carcinoma (Xu *et al.*, 2012) and ovarian cancer (Wenham *et al.*, 2003; Auranen *et al.*, 2005). In a recent meta-analysis (Xu *et al.*, 2018), the p.Pro871Leu polymorphism was linked to decreased risk of various cancers like ESCC, cervical cancer, gastric cancer and NHL among Asians. But some studies from Chinese populations have reported significant associations between the *BRCA1* p.Pro871Leu variant and breast cancer, where the CT genotype provides increased risk to breast cancer in these populations (Huo *et al.*, 2009; Wang *et al.*, 2009). The homozygous variant TT genotype has also been reported to be associated with reduction in glioblastoma risk in Caucasians (Chang *et al.*, 2008) and NHL risk in Korea (Kim *et al.*, 2014). The T allele of the p.Pro871Leu polymorphism has been shown to be linked with miR-628 dependent BRCA1 reduction, indicating that cells with the T allele would express higher BRCA1 as compared to those with the C allele (Nicoloso *et al.*, 2010). In the present study, none of the genotypes of this variant were found to be associated with breast cancer risk. Similar findings have been reported in a Czech population (Soucek *et al.*, 2007), where no association was reported. Meta-analyses carried out in 2014 (Qin *et al.*, 2014) and 2017 (Miao *et al.*, 2017) reported no association of the p.Pro871Leu polymorphism and breast cancer risk. Contradictory results have also been reported for the *BRCA1* 871L allele, as significantly associated with increased risk of breast cancer in a Polish population (Smolarz *et al.*, 2017). An association of the TT genotype with reduced breast cancer risk in Chinese has also been reported (Zhou *et al.*, 2009).

When assessed for response to therapy, p.Pro871Leu showed no association with either advanced phases of CML or treatment response (Gutierrez-Malacatt *et al.*, 2016). The CT/TT genotypes have been reported to be associated with ovarian cancer progression in non-African American patients, but not with progression-free survival (PFS) and overall survival (OS) in patients treated with cisplatin and paclitaxel (Tian *et al.*, 2013). The TT genotype has been reported to not only contribute to increased breast cancer risk associated with combined estrogen monotherapy in postmenopausal women in Germany (Abbas *et al.*, 2010), but also with recurrence of TNBC after radiotherapy in Han Chinese (Shi *et al.*, 2017).

Understanding of the biological mechanisms underlying breast cancer predisposition by genomic profiling can help to define molecular targets for chemoprevention and biomarkers of breast cancer risk. India is the second most populous country in the world, but not much information on *BRCA1* is available as of now. The 185delAG variant has been found to be the most common mutation in BRCA1 in India, as revealed by a few reported studies of limited sample sizes (range 19 - 204). It has been found in variable frequencies in the studied groups from North India: 7.14% (Kumar *et al.*, 2002), 0.49% (Saxena *et al.*, 2006) and South India: 7 out of 19 members in a single family study (Kadalmani *et al.*, 2007), 54.5% (Vinodkumar *et al.*, 2007) and 16.4% (Vaidyanathan *et al.*, 2009). The present study is the first report providing baseline data on four variants of *BRCA1* from the Amritsar and adjoining regions of Punjab, Northwest India. The three mutations [c.190T>C (p.Cys64Arg), 1307delT and g.5331G>A (p.G1738R)], which have been widely known as pathogenic in Caucasians, have no mutant genotype in the studied population. For the c.2612C>T polymorphism also, the T allele frequency was slightly higher than that of the C allele, and there was a preponderance of the CT genotype, but no association with the breast cancer risk. A marked variation in the distribution of p.Pro871Leu in the Northwest Indian population from the previously reported populations of South India has been observed. The present study indicates the need of screening Indian populations for other mutations/variants in *BRCA1* gene for identifying breast cancer risk and possible targets for therapy.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

VS and KG conceptualized and designed the experiment. AK performed the experiments. AK and VS analysed the results and prepared the manuscript. NRS, MSU, MM and MS did diagnosis, clinical and histopathological classification and helped in acquiring blood samples of breast cancer patients. All the authors approved the final draft of the manuscript.

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Supplementary material

The following online material is available for this article:

Figure S1 – Agarose gel picture for *BRCA1* c.190T>C (p.Cys64Arg).

Figure S2 – Agarose gel picture for *BRCA1* 1307delT.

Figure S3 – Agarose gel picture for *BRCA1* c.5331G>A (p.G1738R).

Figure S4 – Agarose gel picture for *BRCA1* c.2612 C>T (p.Pro871Leu).

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