RESEARCH ARTICLE

p.R72P, PIN3 Ins16bp Polymorphisms of TP53 and *CCR5*Δ32 in North Indian Breast Cancer Patients

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Abstract

Background: The present study aimed to find the prognostic implications of two polymorphisms in TP53 (p.R72P, PIN3 Ins16bp) and one in *CCR5* (*CCR5*Δ32) in sporadic breast cancer patients. Methods: DNA samples of 80 breast cancer patients and 80 age and gender matched unrelated healthy control individuals from Punjab, North West India were analyzed. Results: For p.R72P, the genotype frequency was 13.8% (RR), 58.8% (RP), 27.5% (PP) in patients and 33.9% (RR), 40.0% (RP), 26.5% (PP) in controls. For PIN3 Ins16bp, the genotype frequencies were 53.75% (A1A1), 37.5% (A1A2), 8.75% (A2A2) in patients and 66.3% (A1A1), 31.3% (A1A2), 2.5% (A2A2) in controls. Only 4 (5%) breast cancer patients were heterozygous for *CCR5*Δ32 deletion. Common RR-A1A1-WT/WT genotype was lower while RP-A1A2-WT/WT genotype was higher in patients as compared to controls. RP-A1A1-WT/WT genotype was significantly higher in patients as compared to control individuals (p = 0.008). Conclusion: Though a clear association of any particular genotype with sporadic breast cancer or stage was not apparent, the results of present study were suggestive that sporadic breast cancer patients with RR-A1A1-WT/WT genotype might have a better response to chemotherapy, thus improving their chances of survival.

Keywords: Breast cancer - polymorphism - TP53 - *CCR5*Δ32

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Introduction

The development of many diseases including cancer, often involves the interaction of relatively common polymorphisms combined with specific environmental insults. TP53 (OMIM 191170) a classic tumor suppressor gene localized on 17p13.1, has 11 exons and codes for 53 kDa nuclear phosphoprotein that plays a critical role in the complex signal transduction network, regulating the cell cycle arrest, apoptosis, senescence and DNA repair in response to cellular stress of different etiology (Vousden and Lane, 2007). TP53 is mutated in the majority of human cancers (Vogelstein et al., 2000; Vousden and Lu, 2002). A nonsynonymous single nucleotide polymorphism (SNP) p.R72P (rs1042522) located in exon 4 results either in arginine (R) or proline (P) at amino acid position 72 of TP53. Thus, p53 protein exists in two polymorphic forms (p53-Pro or p53-Arg) in the general population (Matlashewski et al., 1987; Beckman et al., 1994) with different structural and functional properties (Thomas et al., 1999). The Arg variant suppresses effectively cellular transformation and is more efficient than the Pro variant in inducing apoptosis (Dumont et al., 2003). The allelic distribution of p.R72P varies in different ethnic groups and geographic locations; P-encoding allele is more prevalent in African populations whereas the R-encoding allele is more common in Caucasians. The frequency of p.R72P polymorphism in the population varies from the equator to higher latitudes, suggesting a selection pressure upon these two forms of p53 protein (Beckman et al., 1994). p.R72P has been associated with risk for developing various cancers but different genotypes have been associated with predispositions to different cancers including lung, breast, colon, and prostate cancers with conflicting results (Whibley et al., 2009).

PIN3 Ins16bp polymorphism (rs17878362) is 16 base pair duplication in intron 3 of the TP53 which has been reported to affect mRNA splicing, altering the coding regions. It is therefore implicated in regulation of gene expression and DNA protein interactions, resulting in a defective protein (Mattick, 1994, 2004). The intron 3 duplication have been correlated with an increased risk of various cancers, including the ovary (Runnebaum et al., 1995), lung (Wu et al., 2002), colon (Gemignani et al., 2004) and breast (Costa et al., 2008). Association of PIN3 Ins16bp polymorphism with higher incidence of lymph node metastases has also been reported (Costa et al., 2008; Hrstka et al., 2009). However, in breast cancer other groups have failed to confirm these results (Khaliq et al., 2000; Osorio et al., 2008).

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Human and murines epithelial cancers express a complex network of cytokines and chemokines (Wilson and Balkwill, 2002). A negative correlation has been reported between *CCR5* expression and the growth of human breast tumors expressing wild type TP53 (Manes et al., 2003). CCR5 is a chemokine receptor localized on chromosome 3, comprising three exons; encodes protein *CCR5* which is a member of ß-chemokine receptors family of integral membrane proteins. A 32 base pair deletion in *CCR5* leads to the formation of non functional receptor that causes significant defects in the chemotaxis mediated by these ligands and has been implicated in a variety of immune-mediated diseases (Yang et al 2004; Kaimen-Maciel et al., 2007). In cervical cancer, individuals with Δ32/Δ32 genotype have been reported to have 4.58% increased risk for HPV (Human Papillomavirus) infection as compared to *CCR5*/*CCR5* genotype (Zheng et al., 2006). *CCR5* may have an indirect effect on cancer progression by controlling the antitumor immune response. *CCR5*Δ32 has been studied in various cancers including skin cancer and bladder cancer (Zafiropoulos et al., 2004), cervical cancer (Zheng et al., 2006), osteosarcoma (Luettichau et al., 2008), gall bladder cancer (Srivastava et al., 2008), breast cancer (Manes et al., 2003; Aoki et al., 2009) and oral cancer (Weng et al., 2010) with contradictory results. It has been reported that some polymorphisms can influence the treatment outcome as well as survival of cancer patients (Tommiska et al., 2005; Toyama et al., 2007; Vannini et al., 2008; Xu et al., 2005, 2008). A few studies have investigated the influence of p.R72P and PIN3 Ins16bp polymorphism of TP53 and *CCR5*Δ32 polymorphism on drug sensitivity. It has been reported that breast cancer patients with the Pro/Pro genotype of TP53 have poor survival than with Pro/Arg and Arg/Arg genotypes (Tommiska et al., 2005). Patients with Pro/Pro genotype were also less sensitive to anthracycline based neoadjuvant chemotherapy than with Pro/Arg and Arg/Arg genotypes (Xu et al., 2005, 2008). The disease free survival was found to be shorter in the *CCR5*Δ32 individuals than in *CCR5* wild type patients with wild type TP53 (Manes et al., 2003). It has been documented that head and neck cancer (HNC) cells expressing the wild-type arginine (72R) were more sensitive to a variety of anti-cancer drugs as compared to proline (72P) and had a longer survival (Bergamaschi et al., 2003; Sullivan et al., 2004). For PIN3 the patients with A2A2 genotype were reported to have better survival when treated with anthracycline containing chemotherapy (Bisof et al., 2012).

The estimated number of Breast cancer cases in India for the years 2010, 2015 and 2020 are 90,659, 106,124 and 123,634 respectively (Takiar et al., 2010). In Amritsar, the third largest city of Punjab state in North West part of India, an increasing number of sporadic breast cancer patients have been observed (personal communication, SGRD Rotary Cancer Hospital, Vallah, Sri Amritsar). In view of the role that TP53 and *CCR5* play in carcinogenesis and response to therapy, the present study aimed to find the possible prognostic implications of TP53 p.R72P, PIN3 Ins16bp and *CCR5*Δ32 polymorphisms in sporadic breast cancer patients of Amritsar. It might serve as a useful platform against which clinical data could be systematically compared, hence used for genotype-specific treatment of breast cancer. To the best of our knowledge it is the first report in Breast cancer on TP53 p.R72P, PIN3 Ins16bp and *CCR5*Δ32 polymorphisms in this population.

Materials and Methods

Clinical evaluation and collection of genetic material

The study protocols adhered to the tenets of the Declaration of Helsinki and were approved by the institutional ethical committee of Guru Nanak Dev University, Amritsar, Punjab, India. Clinically confirmed Breast cancer patients were selected from Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Sri Amritsar, Punjab after informed consent. For each subject, a detailed history relating to demographic particulars, family history of breast cancer or any other disease and clinical details was collected in a pre-tested structured questionnaire. Patients who had received chemotherapy, radiotherapy or blood transfusion before surgery or had prior history of any cancer were excluded from the study. After informed consent, 5 ml peripheral venous blood sample from 80 breast cancer patients and 80 age and gender matched unrelated healthy control individuals from same geographical region was collected. Individual who had family history of any type of cancer or any other chronic disease and on regular medications were not included in the control group. Genomic DNA was extracted from peripheral blood leucocytes using standard phenol chloroform method. To ensure quality control, genotyping was performed without knowledge of case/control status.

Analysis of TP53 Codon 72 Arg/Pro Polymorphism

An allele specific PCR assay was used to detect either the arginine (Arg) or the proline (Pro) allele using published primer sequences (Kazemi et al., 2009). A negative control without DNA template was included in each reaction. The PCR conditions were initial denaturation at 95 °C for 5 min followed by 35 cycles with denaturation at 95 °C for 45 sec, annealing at 59 °C for 30 sec and extension at 72 °C for 45 sec, and final extension at 72 ˚C for 10 min in a Mastercycler gradient (Eppendorf, Germany). The PCR product of the Arg allele was 136bp, while the product of Pro allele was 178bp (Figure 1). The allele specific PCR results were revalidated in 10% of randomly selected DNA samples using Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and the results were 100% concordant. PCR products of 279bp were analyzed on 2% ethidium bromide stained agarose gel. Amplified products were digested with BstUI restriction enzyme following the manufacturer instructions (New England Biolabs, Beverly, MA). The restriction digestion reaction products were analyzed on 2.3 % ethidium bromide stained agarose gel. The presence of the Arg allele was indicated by bands of 160 and 119 base pairs, whereas undigested product of 279bp indicated the Pro allele. Heterozygous Arg/Pro variant displayed three bands of 279, 160 and 119 base

pairs (Figure 2).

Analysis of TP53 PIN3 Ins16bp Polymorphism

TP53 PIN3 Ins16bp polymorphism was detected by amplifying genomic DNA using published primer sequences (Costa et al., 2008). The PCR conditions were denaturation at 95 ˚C for 5 min, 35 cycles of 45 sec at 95 ˚C, 30 sec at 55 ˚C and 45 sec at 72 ˚C, and 10 min extension at 72 ˚C in a Mastercycler gradient (Eppendorf, Germany). A negative control without template DNA was included in each reaction. PCR products were analyzed on 2.4% ethidium bromide stained agarose gel. Wild type allele, designated A1 allele (no duplication) resulted in 119bp fragment and the variant allele, designated A2 allele (with 16bp duplication) resulted in 135bp fragment (Figure 3).

Analysis of CCR5Δ32

DNA samples were amplified using previously published primers (Apostolakis et al., 2005). PCR conditions were denaturation at 95 ˚C for 5 min, 35 cycles of 45 sec at 95 ˚C, 30 sec at 59 ˚C and 45 sec at 72 ˚C, and 10 min extension at 72 ˚C in a Mastercycler gradient (Eppendorf, Germany). A negative control without template DNA was included in each reaction. PCR products of 320bp and 288bp were analyzed on 2.3% ethidium bromide stained agarose gel (Figure 4).

Statistical analysis

The statistical analysis was done to evaluate association of screened polymorphisms with breast cancer risk. Hardy Weinberg equilibrium (HWE) was tested by comparing the observed to expected genotype frequencies in controls using a χ^2 test. Genotype frequencies were calculated for the cases and controls to determine their association with breast cancer. The odds ratio (OR) and its 95% confidence interval (CI) were calculated as a

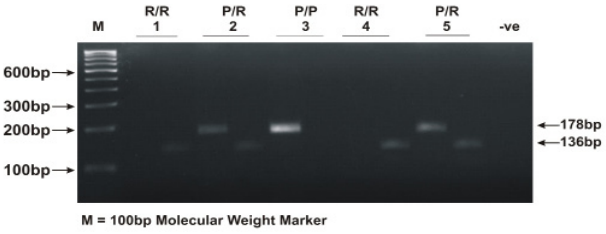

Figure 1. Photograph of 2.3% Ethidium Bromide Stained Agarose Gel Showing Allele Specific PCR Amplified Products. Lanes 1 and 4 show homozygous arginine, lanes 2 and 5 show heterozygous and lanes 3 shows homozygous proline

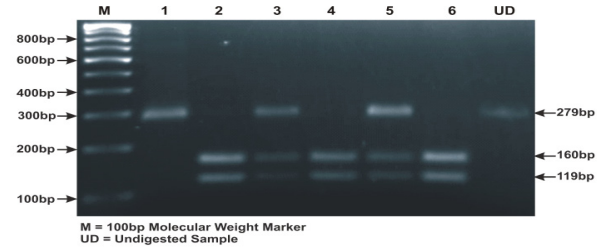

Figure 2. Photograph of 2.3% Ethidium Bromide Stained Agarose Gel Showing BstUI Digested Products of the p.R72P Polymorphism of TP53. Lane 1 shows homozygous proline, lanes 2, 4 and 6 show homozygous arginine and lanes 3 and 5 show heterozygous form

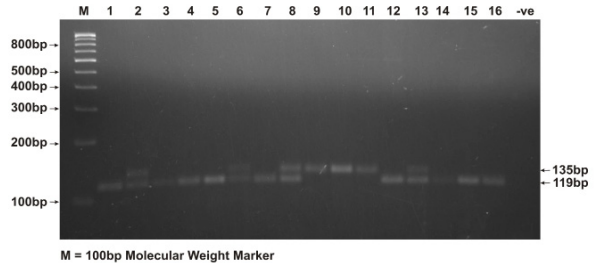

Figure 3. Photograph of 2.4% Ethidium Bromide Stained Agarose Gel Showing Amplified PCR Products. Lanes 1, 3, 4, 5, 7, 12, 14, 15 and 16 show A1A1 genotype, lanes 2, 6, 8 and 13 show A1A2 and lane 9-11 show A2A2 genotype of PIN3 Ins16bp polymorphism

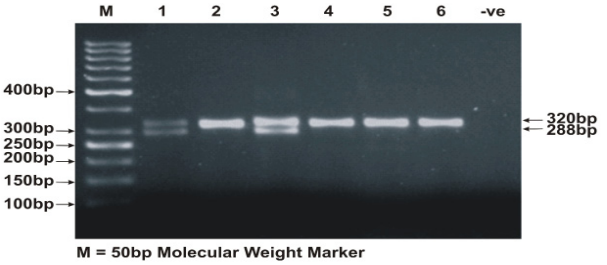

Figure 4. Photograph of 2.3% Ethidium Bromide Stained Agarose Gel Showing CCR5 Genotypes. Lane M: 50bp molecular weight marker, Lane 1 and 3: heterozygous, Lane 2, 4, 5 and 6: wild type homozygous

Table 1. Genotype Distributions of p.R72P, PIN3 Ins16bp Polymorphisms of TP53 and CCR5Δ32 in Breast Cancer Patients and Controls

Polymorphism	p.R72P			PIN3 Ins16bp			CCR5Δ32		
Patients	RR n (%)	RP n (%)	PP n (%)	A1A1 n (%)	A1A2 n (%)	A2A2 n (%)	WT/WT n (%)	WT/Δ32 n (%)	Δ32/Δ32 n (%)
Stage I (n = 7)	1(1.3)	6(7.5)	-	3(3.8)	3(3.8)	1(1.3)	7(8.8)	-	0(0)
Stage II (n = 42)	7(8.8)	25(31.3)	10(12.5)	24(30.0)	15(18.8)	3(3.8)	39(48.8)	3(3.8)	0(0)
Stage III (n= 25)	3(3.8)	12(15.0)	10(12.5)	14(17.5)	8(10.0)	3(3.8)	24(30.0)	1(1.3)	0(0)
Stage IV (n = 6)	-	4(5.0)	2(2.5)	2(2.5)	4(5.0)	0(0)	6(7.5)	0(0)	0(0)
Total n (%)	11(13.8)	47(58.8)	22(27.5)	43(53.8)	30(37.5)	7(8.8)	76(95.0)	4(5.0)	0(0)
Controls n (%)	27(33.8)	32(40.0)	21(26.3)	53(66.3)	25(31.3)	2(2.5)	80(100)	0(0)	0(0)
OR		3.61	2.57		1.48	4.31	-	-	-
(95% Cl)	Reference	(1.57-8.29)	(1.02-6.46)	Reference	(0.76-2.88)	(0.85-21.85)			
p value		0.008*			0.109			-	

n- Number of subjects, Figures in parentheses represents frequency of each genotype; WT, Wild type; OR, Odds Ratio; CI, Confidence interval; *p < 0.05 was considered significant

Table 2. Genotype Distributions and Genetic Models for p.R72P, PIN3 Ins16bp Polymorphisms of *TP53* and *CCR5*Δ32 in Breast Cancer Patients and Controls

TP53 p.R72P polymorphism													
Study group	Genotypes n(%)			Allele n(%)		p value		Dominant model RP/PP vs RR		Co-dominant model PP vs. RP = RP vs. RR		Recessive model PP vs. RR/RP	
	RR	RP	PP	R	P	Genotype	Allele	OR(95% Cl)	p value	OR(95% Cl)	p value	OR(95% Cl)	p value
Patients	11(13.8)	47(58.8)	22(27.5)	69(43.1)	91(56.9)	0.008*	0.05*	3.2(1.45-7.02)	0.003*	1.53(0.98-2.39)	0.06	1.07(0.53-2.14)	0.86
Controls	27(33.8)	32(40.0)	21(26.3)	86(53.8)	74(46.3)								
TP53 PIN3 Ins16bp polymorphism													
Study group	Genotypes n(%)			Allele n(%)		p value		Dominant model A1A2/A2A2 vs. A1A1		Co-dominant model A2A2 vs. A1A2 = A1A2 vs. A1A1		Recessive model A2A2 vs. A1A1/ A1A2	
	A1A1	A1A2	A2A2	A1	A2	Genotype	Allele	OR(95% Cl)	p value	OR(95% Cl)	p value	OR(95% Cl)	p value
Patients	43(53.8)	30(37.5)	7(8.8)	116(72.5)	44(27.5)	0.109	0.045*	1.69(0.89-3.20)	0.106	1.70(1.00-2.91)	0.047*	3.74(0.75-18.59	0.078
Controls	53(66.3)	25(31.3)	2(2.5)	131(81.9)	29(18.1)								
CCR5Δ32 polymorphism													
Study group	Genotypes n(%)			Allele n(%)		p value		Dominant model WT/Δ32/Δ32/Δ32 vs. WT/WT		Co-dominant model Δ32/Δ32 vs. WT/Δ32 = WT/Δ32 vs. WT/WT		Recessive model Δ32/Δ32 vs. WT/WT/ WT/Δ32	
	WT/WT	WT/Δ32	Δ32/Δ32	WT	Δ32	Genotype	Allele	OR(95% Cl)	p value	OR(95% Cl)	p value	OR(95% Cl)	p value
Patients	76(95.0)	4 (5.0)	0(0)	156(97.5)	4(2.5)	-	-	-	-	-	-	-	-
Controls	80(100)	0(0)	0(0)	160(100)	0(0)								

n- Number of subjects, Figures in parentheses represents frequency of each genotype and allele; WT, Wild type; OR, Odds Ratio; CI, Confidence interval; *p < 0.05 was considered significant

Table 3. Genotype Combinations of p.R72P, PIN3 Ins16bp Polymorphisms of TP53 and CCR5Δ32 in Breast Cancer Patients and Controls

R72P-PIN3-CCR5	No. of patients n(%)	No. of controls n(%)	OR (95% Cl)	p value
RR-A1A1-WT/WT	11(13.8)	26(32.5)	Reference	Reference
RR-A1A2-WT/WT	0(0)	1(1.3)	-	-
RP-A1A2-WT/WT	18(22.5)	15(18.8)	2.84(1.06-7.58)	0.035*
RP-A2A2-WT/WT	1(1.3)	0(0)	-	-
PP-A1A1-WT/WT	6(7.5)	10(12.5)	1.42(0.413-4.87)	0.578
PP-A1A2-WT/WT	9(11.3)	9(11.3)	2.36(0.74-7.6)	0.143
PP-A2A2-WT/WT	6(7.5)	2(2.5)	7.09(1.23-4.75)	0.039*
RP-A1A1-WT/WT	25(31.3)	17(21.3)	3.47(1.36-8.86)	0.008*
RP-A1A2-WT/Δ32	2(2.5)	0(0)	-	-
RP-A1A1-WT/Δ32	1(1.3)	0(0)	-	-
PP-A1A2-WT/Δ32	1(1.3)	0(0)	-	-

n- Number of subjects, Figures in parentheses represents frequency; WT, Wild type; OR, Odds Ratio; CI, Confidence interval; *p < 0.05 was considered significant

measure of the association between the different genotypes and Breast cancer risk. Analyses were also performed assuming dominant, co-dominant and recessive genetic models. The odds ratios (ORs), their 95% CI ranges and corresponding P-values were calculated using the Web-Assotest program (http://www.ekstroem.com). A cut off p value of 0.05 was adopted for all the statistical analyses.

Results

A total of 80 sporadic breast cancer patients (2 males and 78 females) and 80 age and gender matched unrelated healthy individuals (2 males and 78 females) were analyzed in this study. The age of the patients ranged from 30-75 years. Seven patients had stage 1, 42 had stage II, 25 had stage III and 6 had stage IV tumor (Table 1). The proportion of RR, PP and RP genotypes in breast cancer patients was found to be 13.75%, 58.75% and 27.5% respectively, as compared to 33.75%, 40.0% and 26.5% in the control individuals. The genotype and allele distribution for p.R72P polymorphism were different significantly between patients and controls (p = 0.008 and 0.05 respectively). The heterozygous RP genotype was more common in patients than in controls (58.75 vs 40.0%) and there was suggestive evidence of an association in a dominant model (RP/PP vs RR; OR 3.2, 95% CI 1.45-7.02, p=0.003, (Table 2). The frequencies of minor allele (P allele) in patients and controls were 0.568 and 0.462 respectively.

The frequencies of TP53 PIN3 Ins16bp polymorphism genotypes A1A1, A1A2 and A2A2 in breast cancer patients was found to be 53.75%, 37.5% and 8.75% respectively, as compared to 66.25%, 31.25% and 2.5% in the unrelated healthy control individuals (Table1). No significant difference was observed in the genotype frequency between patients and controls (p = 0.109). The heterozygous A1A2 and homozygous A2A2 genotypes were more common in patients than in controls (37.5%, 8.75% vs 31.25%, 2.5%) and there was suggestive evidence of an association in a Co-dominant model (A2A2 vs. A1A2 = A1A2 vs. A1A1; OR 1.70, 95% CI 1.00-2.91, p=0.047 (Table 2).

In the present study, only four of the breast cancer patients (5.0%) were found to be heterozygous for *CCR5*Δ32 deletion (Table 1). Out of 4 heterozygous *CCR5*Δ32 patients, 2 patients had RP genotype for p.R72P polymorphism and A1A2 genotype for PIN3 Ins16bp polymorphism (Table 3).

Comparison of the genotypes of p.R72P, PIN3 Ins16bp and *CCR5*Δ32 polymorphism showed that 13.75% patients and 32.5% of controls had common RR-A1A1-WT/WT genotype whereas 22.5% of patients and 18.75% controls had RP-A1A2-WT/WT genotype. RP-A1A1-WT/WT genotype was significantly higher (p = 0.008) in breast cancer patients as compared to control individuals (Table 3).

Discussion

The state of Punjab in plains of North West India is inhabited by a mixed population of Caucasian and Indoscythian racial stock (Bhasin et al., 1992). Asians

have been reported to express the Pro allele, whereas Caucasians preferentially express the Arg allele; however, ~75% of heterozygote Chinese breast cancer patients expressed the Arg allele (Siddique et al., 2005). In the present case control study, frequency of Pro allele of p.R72P polymorphism was 56.88% in patients and 46.25% in control individuals. From North India, association of Pro/Pro genotype with the increased risk of breast cancer (Sayeed et al., 2010), colorectal cancer (Sameer et al., 2010) and urinary bladder cancer (Pandith et al., 2010) has been previously reported in Kashmiri population inhabiting a mountainous region. Association of Pro allele with increased risk of colorectal cancer has been reported in Japanese population (Hamajima et al., 2002), Korean population (Cao et al., 2009), in Chinese (Zhu et al., 2007) and in Malaysian population (Aizat et al., 2011). But for breast cancer risk no association of p.R72P polymorphism have been reported in Tunisian (Mabrouk et al., 2003) and Russian subjects (Suspitsin et al., 2003).

In present study, Arg/Arg genotype was also significantly lower in patients (13.75%) than control individuals (33.75%). Arg allele has been reported as a risk factor for developing breast cancer in Greece (Papadakis et al., 2000) and Turkish population (Buyru et al., 2003). A previous study which analyzed the correlation between the p.R72P polymorphism and p53 mutation in breast cancer patients, has reported that p53 mutation was more prevalent in the Arg/Arg genotype than those of the Pro/Pro genotype (Langerod et al., 2002). The Arg allele at codon 72 of the TP53 has been suggested to affect the risk of UV-induced basal cell carcinoma (Pezeshki et al., 2006) as the frequency of the Arg allele was significantly higher in sun-exposed patients compared to controls.

Polymorphisms in the non-coding region of TP53 could also play an important role in the regulation of gene expression. Several studies have correlated the intron 3 duplication with an increased risk of various cancers, including the ovary (Runnebaum et al., 1995), lung (Wu et al., 2002), colon (Gemignani et al., 2004), breast (Costa et al., 2008), esophageal cancer and gastric cancer (Malik et al., 2011). In the present study, higher frequency of PIN3 Ins16bp A2A2 genotypes has been observed in breast cancer patients (8.75%) as compared to control individuals (2.5%). While A2A2 genotype of PIN3 polymorphism has been associated with increased risk for breast cancer (Weston et al., 1997; Wang-Gohrke et al., 2002; Costa et al., 2008), on the other hand, six fold higher risk for breast cancer has been reported in Slovak population who had wild-type intron 3 (A1A1) genotype as compared to A2A2 genotype (Franekova et al., 2007). Association of PIN3 Ins16bp polymorphism with higher incidence of lymph node metastases has also been reported (Costa et al., 2008; Hrstka et al., 2009).

In the present study, 37.5% breast cancer patients and 31.25% of controls had A1A2 genotype. Co-dominant model (A2A2 vs. A1A2 = A1A2 vs. A1A1) analysis revealed a significant difference between patients and controls ($p = 0.047$). In Iranian patients association of A1A2 genotype with high risk of breast cancer has been documented (Faghani et al., 2011). Though PIN3 Ins16bp was not associated with increased breast cancer risk (Fiszer-Maliszewska et al., 2004; De Vecchi et al., 2008), PIN3 Ins16bp A2A2 genotype was found to confer significant high risk for both esophageal cancer and gastric cancer in north Indian patients (Malik et al., 2011). The authors also suggested that PIN3 A2A2 genotype could be a useful genetic marker in predicting high-risk individuals for the development of esophageal cancer and gastric cancer and an early diagnosis. No correlation has been reported between PIN3 genotypes and TP53 mRNA expression levels in primary blood lymphocytes of prostate cancer patients (Woelfelschneider et al., 2008).

For *CCR5*, the prevalence of Δ32 allele in Europe was approximately 10% and it was low or almost absent in most of Asian and African populations (Samson et al., 1996). In India *CCR5*Δ32 allele was absent in most of the populations of India, except some populations of Northern/western India where it could have been introduced by Caucasian gene flow (Majumder and Dey, 2001). *CCR5*Δ32 deletion might alter the expression or function of the protein (Sidoti et al., 2005). In the present study, only four (5.0%) of the breast cancer patients were reported to be heterozygous for *CCR5*Δ32 mutation similar to 3.47% *CCR5*Δ32 heterozygous breast cancer patients reported in Brazilian population (Aoki et al., 2009). Out of 4 heterozygous *CCR5*Δ32 patients, 2 patients had RP genotype for p.R72P polymorphism and A1A2 genotype for PIN3 Ins16bp polymorphism. *CCR5* activity probably influences progression of human breast cancer in p53-dependent manner as disease free survival was shorter in the *CCR5*Δ32 individuals than in *CCR5* wild type patients with wild type TP53 (Manes et al., 2003). It has been suggested that Δ32 mutation may confer significant risk for gall bladder cancer in north Indian patients with early onset of disease (Srivastava et al., 2008). No association of Δ32 deletion with breast cancer (Aoki et al., 2009), bladder cancer and non melanoma skin cancer has also been reported (Zafiropoulos et al., 2004). But mice expressing *CCR5* showed enhanced local tumor growth and an impaired response to vaccine therapy as compared to knockout mice (van Deventer et al., 2005).

In the present study, 13.75% patients and 32.5% of controls had common RR-A1A1-WT/WT genotype of p.R72P, PIN3 Ins16bp and *CCR5*Δ32 polymorphism whereas 22.5% of patients and 18.75% controls had RP-A1A2-WT/WT genotype. RP-A1A1-WT/WT genotype was observed in 31.25% of breast cancer patients and 21.25% of control individuals. It has been suggested that worse survival in patients with PP genotype was largely due to resistance to adjuvant chemotherapy in Japanese breast cancer patients as PP genotype was associated with poorer disease-free survival (DFS) in patients who received adjuvant chemotherapy. In contrast, there was no association between the PP genotype and survival in patients who received tamoxifen treatment or who did not receive adjuvant therapy (Toyama et al., 2007). The Proline allele was also shown to be associated with increased apoptotic capacity whereas the arginine allele enhanced cell survival (Vannini et al., 2008). The TP53 Pro allele was also associated with a poorer prognosis in ovarian cancer patients who received adjuvant cisplatin and paclitaxel chemotherapy (Santos et al., 2006). In

gastric cancer patients, it has been reported that Arg/Pro and Pro/Pro genotypes of TP53 codon 72 showed lower response to paclitaxel and cisplatin chemotherapy as compared to the Arg/Arg genotype (Kim et al., 2009). Patients with PIN3 A2A2 genotype were reported to have better survival when treated with anthracycline containing chemotherapy (Bisof et al., 2012).

In the present study, an association of Pro allele of TP53 with breast cancer was observed but a clear association of a particular genotype with Breast cancer or its stage was not apparent probably due to small sample size. The chemotherapy regimen of these patients consists of cyclophosphamide, 5-fluorouracil, and Adriamycin. Thus, on basis of previous reported studies on drug response (Bergamaschi et al., 2003; Sullivan et al., 2004; Tommiska et al., 2005; Xu et al., 2005; Santos et al., 2006; Toyama et al., 2007; Vannini et al., 2008; Xu et al., 2008; Kim et al., 2009; Bisof et al., 2012), the sporadic breast cancer patients in present study with RR-A1A1-WT/WT genotype might have a better response to chemotherapy, thus improving their chances of survival. A follow up of the patients has been initiated to assess their response to chemotherapy.

Future studies are needed to investigate the potential function of these polymorphisms in response to different types of drug regimen being used in Breast cancer apart from their role in tumor behavior. Such studies would serve as a useful platform against which clinical data can be systematically compared, hence used for genotype-specific treatment of breast cancer.

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