RESEARCH ARTICLE

TP53 Polymorphisms in Sporadic North Indian Breast Cancer Patients

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Abstract

Background: The purpose of this study was to evaluate the potential association of five (p.P47S, p.R72P, PIN3 Ins16bp, p.R213R and r.13494g>a) polymorphisms of TP53 with the risk of developing breast cancer in North Indian Punjabi population. Methods: We screened DNA samples of 200 sporadic breast cancer patients (197 females and 3 males) and 200 unrelated healthy, gender and age matched individuals for the polymorphisms. Results: For the p.P47S polymorphism, we observed the PP genotype in 99.5% of the patients and PS genotype in only 1 patient. All the controls had the wild type PP genotype. The frequency of RR, RP and PP genotype of p.R72P was 23.5% vs 33.5%, 51.5% vs 45.5% and 25% vs 21% in patients and controls respectively. Heterozygous (RP) genotype was increased in breast cancer patients as compared to controls (51.5 vs 45.5%) and showed 1.61 fold significantly increased risk for breast cancer (OR=1.61, 95% CI, 1.01-2.58, p=0.04). In breast cancer patients the frequencies of A1A1, A1A2 and A2A2 genotypes of PIN3 Ins16bp polymorphism were 67%, 26% and 7% respectively whereas in controls the genotype frequencies were 68.5%, 27.5% and 4% respectively, with no significant difference. For p.R213R (c.639A>G), all individuals had homozygous wild type genotype. The frequencies of GG, GA and AA genotypes of TP53 r.13494g>a polymorphism were 62 vs 67.5%, 33 vs 28% and 5 vs 4.5% in patients and controls respectively, again without significant difference. We observed that RP-A1A1 genotype combination of p.R72P and PIN3 Ins16bp and RP-GG combination of p.R72P and r.13494g>a polymorphism showed significant risk of breast cancer (OR=1.65, 95%CI: 0.98-2.78, p=0.05; OR=1.72, 95%CI: 1.01-2.92, p=0.04). Conclusion: The results of present study indicated that among the five TP53 polymorphisms investigated, the p.R72P polymorphism, and the RP-A1A1 and RP-GG genotype combination contribute to breast cancer susceptibility in North Indians.

Keywords: Breast cancer - TP53 polymorphisms - susceptibility - North Indians

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Introduction

Common genetic polymorphisms with possible effects on function and/or protein expression within genes involved in essential cellular pathways like carcinogen metabolism, DNA repair, cell cycle control and cell proliferation could predispose individuals to cancer (Medeiros et al., 2004; Pinto et al., 2004; Costa et al., 2005; Costa et al., 2007). Accumulation of various genetic alterations, including amplification of oncogenes and mutation or loss of tumor suppressor genes is reported to be among the causes of breast cancer (Wajapeye and Somasundaram, 2004). Breast cancer is one of the most common malignancies in women, causing over 4,00,000 deaths yearly worldwide (Walerych et al., 2012). Involvement of several breast cancer predisposition genes like TP53, FGFR, MYC, BRCA1 and BRCA2 in various cancers indicates that different cancer type may show common predisposition mechanisms which include commonly inherited SNPs.

Tumor suppressor gene TP53 (OMIM 191170) is a multifunctional tetrameric transcription factor involved in the control of cell cycle progression, DNA repair, apoptosis and senescence (Levine and Oren, 2009). Human p53 is a nuclear phosphoprotein of molecular weight 53 kDa, encoded by a 20kb gene located on 17p13.1 comprising 11 exons and 10 introns (Lamb and Crawford, 1986; Isobe et al., 1986). TP53 is often mutated in a variety of human cancers and is also highly polymorphic with more than 200 single nucleotide polymorphisms (SNPs) been identified in both coding and non-coding regions (http://www-p53.iarc.fr/). A large number of studies have documented the association of common TP53 polymorphisms with cancer risk (Whibley et al., 2009).

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The p.R72P (rs 1042522) the non-synonymous single nucleotide polymorphism is one of the most extensively studied polymorphisms of TP53 located in a proline-rich domain of the protein, which has been known to be important for the growth suppression and apoptotic functions (Soussi and Lozano, 2005). This polymorphism exists in two isoforms (Arg72 and Pro72) which differ in their biochemical and biological properties (Thomas et al., 1999). The TP53 Arg72 form induces apoptosis more efficiently than the Pro72 form (Dumont et al., 2003; Pim and Banks, 2004). However, the Pro72 variant is more efficient at inducing cell cycle arrest in the G1 phase, allowing better repair of damaged DNA (Oersted et al., 2007). Various meta-analyses studies reported the involvement of p.R72P in susceptibility to various cancer types including gastric (Zhou et al., 2007), lung (Dai et al., 2009) and breast (Zhang et al., 2010). However, studies on association of breast cancer risk with p.R72P polymorphism have not yielded consistent results.

Codon 47 (p.P47S (rs1800371)), is the second functionally significant polymorphism located in the N-terminal transactivation domain of p53 which leads to a non-synonymous amino acid substitution of proline with serine. It has been documented that Ser47 variant has upto 5-fold decreased ability to induce apoptosis compared with wild type Pro47 variant (Li et al., 2005). However, only small numbers of studies have been performed on p.P47S and its association with cancer due to the low frequency of this polymorphism (Murphy, 2006).

Another rare polymorphism pR213R (rs1800372) at codon 213 results from silent alteration of CGA to CGG within the coding region of the p53 gene. The rare polymorphic allele was observed in 3.2% of lung and breast cancer patients (Carbone et al., 1991). But in a study on healthy Turkish population and Turks with different types of tumours no association between this polymorphism and the development of tumours was observed (Iihan et al., 1995).

Intronic polymorphisms may influence coding-region sequence alterations that increase the likelihood of a deleterious phenotype (Malkinson and You, 1994). PIN3 Ins 16bp duplication (rs 17878362) in intron 3 and 13494 g to a transversion (rs 1625895) in intron 6 have been suggested to affect the function and expression of p53 (McDaniel et al., 1991; Chumakov and Jenkins, 1991; Lazar et al., 1993; Hillebrandt et al., 1997). PIN3 Ins16bp polymorphism has been associated with a reduced level of p53 mRNA and decreased apoptotic indices and DNA repair capacity in lymphoblastoid cell lines (Wu et al., 2002; Gemignani et al., 2004). Several case control studies have correlated the intron 3 duplication with an increased risk of various cancers, with the most consistent association reported for breast (Wang-Gohrke et al., 2002; Costa et al., 2008) and colorectal cancers (Gemignani et al., 2004; Perfumo et al., 2006). In a recent meta-analysis, association of A2A2 genotype of PIN3 Ins16bp polymorphism with increased cancer risk has been documented in Indian, Mediterranean and Northern European populations (Sagne et al., 2013).

Another intronic polymorphism r.13494g>a is localised in intron 6 of TP53, which is one of the hot spots region for TP53 muation. The r.13494g>a sequence variant was observed in six affected members of a Li-Fraumeni family, and in only one out of 184 healthy controls (Avigad et al., 1997). Significant association of this polymorphism with the risk of developing breast and colon cancers has been observed (Peller et al., 1995). These nucleotide changes may act via novel mechanisms of gene regulation that appears to be important for tumor formation (Surekha et al., 2011). On the contrary in another study, no difference in the distribution of r.13494g>a polymorphism was observed in the breast cancer patients and controls (Mavridou et al., 1998).

In Punjab an agragarian state in North India, increased cancer incidence possibly due to increased use of agricultural chemicals has been reported (The Tribune, 2013). According to a report from Department of Health and Family Welfare India, the cancer prevalence per million in the different regions of Punjab is: Malwa region 1089, Majha region 647 and Doaba region 881 (DHFW, 2013). Breast cancer is the most prevalent cancer among females in Punjab.

TP53 is a major tumor suppressor gene playing role in carcinogenesis and response to therapy. Among the TP53 polymorphisms there are more reported studies on the p.R72P, PIN3 Ins16bp in breast cancer but very few data is available for p.P47S, p.R213R and 13494g>a polymorphisms. So the present study was aimed to study the potential association of five (p.P47S, p.R72P, PIN3 Ins16bp, p.R213R and r.13494g>a) polymorphisms of TP53 with the risk of developing breast cancer in North Indian Punjabi population. The combined effects of these five polymorphisms on cancer risk could be more significant than the individual effect of any one of them alone.

Materials and Methods

Study subjects

This study was approved by the ethical committee constituted by Guru Nanak Dev University, Amritsar, Punjab, India. The patients were investigated at Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar, Punjab, India. Clinically confirmed breast cancer patients, who had not undergone blood transfusion and received any therapy were included in the study. Gender and age matched (±5 years) unrelated healthy individuals from the same geographical areas as that of patients, having no family history of cancer or other related comorbidities up to three generations were recruited as controls. All the subjects were from Majha region of Punjab. Relevant information including self reported personal history and disease history of each subject was recorded on a pre-tested structured questionnaire by interview or from medical records. After informed consent, 5 ml venous blood was collected from each subject in 0.5M EDTA.

DNA extraction and genotyping of TP53 polymorphisms

The genomic DNA was extracted from peripheral blood lymphocytes using standard phenol chloroform method (Adeli and Ogbonna, 1990). The quantity and

quality of DNA was checked by gel electrophoresis. Four polymorphisms (p.P47S, p.R72P, p.R213R and r.13494g>a) of TP53 were screened using PCR-RFLP method. PIN3 Ins16bp was analysed by direct PCR. The PCR reaction was carried out in a 15μl volume containing 1X PCR buffer, 0.4μl dNTPs mixture, 6 picomole primers (Sigma), 1 U Taq DNA Polymerase (Bangalore GeNei). DNA samples were amplified using specific primer sequences and conditions (Table 1). A negative control without DNA template was included in each reaction. The amplified PCR products were analysed on 2% ethidium bromide stained agarose gel. PCR products were digested with appropriate restriction enzymes (Table 1) following the manufacturer instructions (New England Biolabs, Beverly, MA). The restriction digestion products were analysed on 2.3% ethidium bromide stained agarose gel. The genotype of each sample was assigned on the basis of fragments obtained after digestion (Table 1).

The genotyping was performed without the knowledge of the clinical status of the subjects. Ten percent of DNA samples were reanalyzed and results of both sets of analyses were 100% concordant.

Table 1. Details of TP53 Polymorphisms and Screening Conditions

Variant (RefSNP)	Genotyping Method	Primers References	PCR product size (bp)	Annealing Temperature, $MgCl_2$	Restriction enzyme	Allele Size (bp)	Expected Fragment
p.P47S (rs1800371)	PCR-RFLP	Pinto et al., 2008	201/185*	59°C, 1.5 mM	MspI	S	201/185
						P	156/140 and 45
p.R72P (rs1800371)	PCR-RFLP	Kazemi et al., 2009	279	59°C, 1.5 mM	BstUI	P	279
						R	160 and 119
PIN3 Ins 16bp (rs17878362)	PCR	Costa et al., 2008	119 or 135	61°C, 1.5 mM	-	A1	119
						A2	135
p.R213R (rs1800372)	PCR-RFLP	Pilger et al., 2008	1621	59°C, 1.0 mM	TaqI	A	926, 383, 312
						G	926, 695
r.13494g>a (rs1625895)	PCR-RFLP	Pilger et al., 2008	1621	59°C, 1.0 mM	MspI	G	356, 299, 277, 277, 168, 124, 120
						A	633, 299, 277, 168, 124, 120

*Size divergence is due to 16bp ins/del polymorphism in intron 3

Table 2. Genotype and Allele Distribution of TP53 Polymorphisms in Breast Cancer Patients and Control Individuals

Polymorphism (RefSNP)	Genotype	Allele	Patients n (%)	Controls n (%)	OR (95% CI)	p-value
p.P47S	PP		199 (99.5)	200 (100.0)	-	-
(rs 1800371)	PS		1 (0.5)	0 (0.00)	-	-
	SS					
		P	399 (99.75)	400 (100.0)	-	-
		S	1 (0.25)	0 (0.00)	-	-
p.R72P	RR		47 (23.5)	67 (33.5)	Reference	
(rs1042522)	RP		103 (51.5)	91 (45.5)	1.61 (1.01-2.58)	**0.04**
	PP		50 (25.0)	42 (21.0)	1.70 (0.97-2.95)	0.84
	RP+PP		153 (76.5)	133 (66.5)	1.64 (1.06-2.54)	**0.02**
		R	197 (49.3)	225 (56.3)	Reference	
		P	203 (50.7)	175 (43.7)	1.32 (1.00-1.75)	0.04
PIN3 Ins16bp	A1A1		134 (67.0)	137 (68.5)	Reference	
(rs17878362)	A1A2		52 (26.0)	55 (27.5)	0.97 (0.62-1.51)	0.88
	A2A2		14 (07.0)	8 (04.0)	1.79 (0.73-4.40)	0.19
	A1A2+A2A2		66 (33.0)	63 (31.5)	1.07 (0.70-1.63)	0.74
		A1	320 (80.0)	329 (82.3)	Reference	
		A2	80 (20.0)	71 (17.7)	1.15 (0.81-1.65)	0.41
p.R213R	AA		200 (100.0)	200 (100.0)	-	-
(rs1800372)	AG		0 (0.00)	0 (0.00)	-	-
	GG		0 (0.00)	(0.00)	-	-
		A	400 (100.0)	400 (100.0)	-	-
		G	(0.00)	(0.00)	-	-
r.13494g>a	GG		124 (62.0)	135 (67.5)	Reference	
(rs1625895)	GA		66 (33.0)	56 (28.0)	1.28 (0.83-1.98)	0.25
	AA		10 (05.0)	9 (4.5)	1.21 (0.48-3.08)	0.90
	GA+AA		76 (38.0)	65 (32.5)	1.27 (0.84-1.92)	0.24
		G	314 (78.5)	326 (81.5)	Reference	
		A	86 (21.5)	74 (18.5)	1.20 (0.85-1.70)	0.28

*n- Number of subjects, Figures in parentheses represents frequency of each genotype and allele; OR- Odds ratio; CI- confidence interval. Statistically significant p-values ($p<0.05$) are indicated in bold

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 the Chi-square ($\chi 2$) test. This test was used to demonstrate the significant difference of genotype and allele frequencies between the breast cancer patients and normal controls. The odds ratios (OR) with 95% confidence interval (CI) were estimated to calculate the relative risk of breast cancer susceptibility for each genotype and allele. A value of $p<0.05$ was considered statistically significant. All the statistical values were calculated using SPSS (Version 16, SPSS Inc, Chicago, IL).

Results

General characteristics of study subjects

This case-control study consisted of 200 sporadic breast cancer patients (197 females and 3 males) and 200 gender and age matched unrelated healthy control individuals (197 females and 3 males). The mean age was 49.4±11.9 years (Range 25-85 years) for the cases and 47.1±12.5 years (Range 24-80 years) for the controls. Breast cancer incidence was higher among individuals more than 40 years of age (80%) compared to those less than 40 years (20%). Out of 200 patients, 22 had stage I, 110 had stage II, 53 had stage III and 15 had stage IV tumors.

Association between TP53 polymorphisms and breast cancer risk

The genotyping results of the five polymorphisms of TP53 (p.P47S, p.R72P, PIN3 Ins16bp, p.R213R and r.13494g>a) are presented in Table 2. The observed genotypes frequencies of two polymorphisms (p.R72P and r.13494g>a) were in HWE ($p>0.05$). In PIN3 Ins16bp polymorphism, we observed deviation from HWE in patients ($p<0.05$) which could be attributed to selection bias. For p.P47S polymorphism, we observed the PP genotype in 99.5% of the patients and PS genotype in only 1 patient. All the controls had the wild type PP genotype.

The frequencies of RR, RP and PP genotype of p.R72P was found to be 23.5% vs 33.5%, 51.5% vs 45.5% and 25% vs 21% in patients and controls respectively. Heterozygous (RP) genotype was increased in breast cancer patients as compared to controls (51.5 vs 45.5%) and showed 1.61 folds significantly increased risk for breast cancer (OR=1.61, 95% CI, 1.01-2.58, p=0.04). Carrier of P allele (RP+PP) also demonstrated 1.64 folds increased risk for breast cancer (OR=1.64, 95% CI, 1.06-2.54; p= 0.02). The frequencies of R and P allele were 49.3 vs 56.3% and 50.7 vs 43.7% in patients and controls respectively.

In breast cancer patients the frequencies of A1A1, A1A2 and A2A2 genotypes of PIN3 Ins16bp polymorphism was 67%, 26% and 7% respectively whereas in controls the genotype frequencies were 68.5%, 27.5% and 4% respectively. No significant difference was observed in the genotype and allele frequency between the breast cancer patients and controls. Carriers of A2 allele (A1A2+A2A2) were higher in patients as compared to the controls but the results were not statistically significant (p=0.74).

For p.R213R (c.639A>G), none of the breast cancer patients and controls analyzed exhibited A to G nucleotide substitution at position 639, and all individuals had homozygous wild type genotype. The frequencies of GG, GA and AA genotypes of TP53 r.13494g>a polymorphism were 62 vs 67.5%, 33 vs 28% and 5 vs 4.5% in patients and controls respectively. There was no significant difference between genotype and allele frequency in the breast cancer patients and controls.

To study the association between breast cancer and possible combinations of the TP53 polymorphisms, we performed genotype-genotype combination analysis

Table 3. Interaction between p.R72P, PIN3 Ins16bp and r.13494g>a Polymorphisms in Breast Cancer Patients and Healthy Controls

Combination	No. of patients n (%)	No. of controls n (%)	OR (95% CI)	p-value
p.R72P-PIN3 Ins16bp				
RR-A1A1	46 (23.0)	60 (30.0)	Reference	
RR-A1A2	1 (0.5)	7 (3.5)	0.19 (0.02-1.57)	0.14
RP-A1A1	71 (35.5)	56 (28.0)	1.65 (0.98-2.78)	**0.05**
RP-A1A2	30 (15.0)	33 (16.5)	1.18 (0.63-2.22)	0.60
RP-A2A2	2 (1.0)	1 (0.5)	NC	NC
PP-A1A1	17 (8.5)	21 (10.5)	1.06 (0.50-2.23)	0.89
PP-A1A2	21 (10.5)	15 (7.5)	1.82 (0.85-3.93)	0.13
PP-A2A2	12 (6.0)	7 (3.5)	2.24 (0.81-6.13)	0.11
PIN3 Ins16bp-r.13494g>a				
A1A1-GG	118 (59.0)	124 (62.0)	Reference	
A1A2-GG	6 (3.0)	11 (5.5)	0.57 (0.20-0.60)	0.28
A1A1-AG	16 (8.0)	13 (6.5)	1.29 (0.60-2.80)	0.51
A1A2-AG	41 (20.5)	41 (20.5)	1.05 (0.64-1.73)	0.84
A2A2-AG	9 (4.5)	2 (1.0)	NC	NC
A1A2-AA	5 (2.5)	3 (1.5)	NC	NC
A2A2-AA	5 (2.5)	6 (3.0)	0.87 (0.26-2.95)	0.82
p.R72P-r.13494g>a				
RR-GG	44 (22.0)	62 (31.0)	Reference	
RR-AG	1 (0.5)	4 (2.0)	NC	NC
RR-AA	0 (0.0)	1 (0.5)	NC	NC
RP-GG	66 (33.0)	54 (27.0)	1.72 (1.01-2.92)	**0.04**
RP-AG	37 (18.5)	37 (18.5)	1.41 (0.77-2.56)	0.26
RP-AA	2 (1.0)	0 (0.0)	NC	NC
PP-GG	14 (7.0)	19 (9.5)	1.04 (0.47-2.29)	0.92
p.R72P- PIN3 Ins16bp - r.13494g>a				
RR-A1A1-GG	45 (22.5)	59 (29.5)	Reference	
RP-A1A2-AG	24 (12.0)	26 (13.0)	1.21 (0.61-2.38)	0.58
RP-A1A1-GG	60 (30.0)	47 (23.5)	1.67 (0.97-2.88)	0.06
PP-A1A2-AG	17 (8.5)	12 (6.0)	1.85 (0.80-4.27)	0.14
PP-A1A1-GG	13 (6.5)	18 (9.0)	0.95 (0.42-2.13)	0.89
PP-A1A2-AA	3 (1.5)	2 (1.0)	-	-
RP-A1A2-AA	2 (1.0)	-	-	-
PP-A2A2-AG	7 (3.5)	1 (0.5)	-	-
PP-A1A1-AG	4 (2.0)	2 (1.0)	-	-
RP-A1A1-AG	11 (5.5)	10 (5.0)	1.44 (0.56-3.69)	0.44*
PP-A2A2-AA	5 (2.5)	6 (3.0)	-	-
RR-A1A1-AG	1 (0.5)	1 (0.5)	-	-
RP-A1A2-GG	4 (2.0)	-	-	-
PP-A1A2-GG	1 (0.5)	1 (0.5)	-	-
RP-A2A2-AG	2 (1.0)	1 (0.5)	-	-
RP-A1A2-GG	1 (0.5)	7 (3.5)	-	-
RR-A1A2-AG	-	3 (1.5)	-	-
RR-A1A2-AA	-	1 (0.5)	-	-
RR-A1A2-GG	-	3 (1.5)	-	-

*n- Number of subjects, Figures in parentheses represents frequency of each genotype and allele; OR- Odds ratio; CI- confidence interval. Statistically significant p-values ($p<0.05$) are indicated in bold

of three (p.R72P, PIN3 Ins16bp and r.13494g>a) polymorphisms (Table 3). We observed that interaction between p.R72P and PIN3 Ins16bp polymorphism (RP-A1A1) showed significant risk of breast cancer (OR=1.65, 95%CI: 0.98-2.78, p=0.05). The genotype combination RP-GG of p.R72P and r.13494g>a polymorphism showed 1.72 folds risk for breast cancer (OR=1.72, 95%CI: 1.01-2.92, p=0.04). Analysis of genotype combinations of p.R72P, PIN3 Ins16bp and r.13494g>a polymorphisms of TP53 showed marginally significant risk for breast cancer in individuals with RP-A1A1-GG genotype combination (OR=1.67, 95%CI: 0.97-2.88, p=0.06) (Table 3). The associations between the p.R72P, PIN3 Ins16bp, r.13494g>a polymorphisms and the risk of breast cancer were further examined with stratification on age at onset, menopausal status and clinical stage. No significant association was observed (data not shown).

Discussion

Breast cancer is a multifactorial disease resulting from the interaction between genetic and environmental factors (Pern et al., 2012). It has been demonstrated that P53 protein regulate activity of key effectors of cellular processes including DNA repair, cell cycle arrest, senescence and apoptosis (Levine, 1997; Riley et al., 2008). Functional inactivation of TP53 pathway is thought to affect TP53 signalling and further alter cancer risk (Moll and Schramm, 1998; Robles et al., 2002). TP53 is one of the major tumor suppressor genes which carry out essential functions in preservation of genome integrity (Costa et al., 2008). Over the last few years, several studies were conducted to investigate the association between individual TP53 polymorphism and breast cancer risk in different populations, however their results are ambiguous. In this hospital based study, we investigated the association between five potentially functional polymorphisms of TP53 (p.P47S, p.R72P, PIN3 Ins16bp, p.R213R and r.13494g>a) and risk of breast cancer in North Indian Punjabi population.

In the present study, only one patient out of 200 was heterozygous for p.P47S polymorphism. This observation was in contrast to the previous report, showing a significant association between the mutant S47 phenotype and cancer risk (Felley-Bosco et al., 1993). S47 allele was also found to be associated with increased risk of developing colorectal cancer in South Indian population (Singamsetty et al., 2014). It has been reported that the S47 phenotype has a decreased capacity to induce apoptosis, to transactivate two (p53AIP1 and PUMA) p53 target genes, and to bind to MAPK1 protein as compared with the wild-type P47 phenotype (Li et al., 2005; Murphy, 2006). No association of p.P47S polymorphism was observed in Kuwaiti (Alawadi et al., 2011) and Saudi (Al-Qasem et al., 2012) breast cancer patients. Association of p.P47S polymorphism was also not observed in bladder (Santos et al., 2009), gliomas (Pinto et al., 2008), urinary bladder (Jaiswal et al., 2011), colorectal cancer (Sameer et al., 2010) and primary open angle glaucoma (Daugherty et al., 2009). The low frequency of p.P47S polymorphism in our population also indicates that p.P47S polymorphism is not implicated in breast cancer.

In the present study, we observed significant association of RP genotype and Pro allele with increased breast cancer risk. Similarly, RP genotype has been reported to be associated with increased breast cancer risk in Iranian population (Boroujeni et al., 2013). Association of Pro allele with increased risk of breast cancer has been reported in Swedish (Sjalander et al., 1996), American (Weston et al., 1997), German (Wang-Gohrke et al., 1998, 2002), Russian (Suspitsin et al., 2003), Japanese (Huang et al., 2003; Noma et al., 2004), Slovakian (Franekova et al., 2007), Turkish (Akkiprik et al., 2009), Iranian (Kazemi et al., 2009), Kashmiri (Sayeed et al., 2010), Arabic (Alawadi et al., 2011), Austrian (Proestling et al., 2012) and Spanish (Rodrigues et al., 2013) population. Individuals with PP genotype had an increased risk of developing a cancer over their lifetimes compared to individuals with RR genotype (van Heemst et al., 2005).

No significant difference was observed in the frequency of RR genotype between the patients (23.5%) and controls (33.5%) in the present study. Similarly, no association between TP53 codon 72 variants and breast cancer risk was observed in Japanese (Kawajiri et al., 1993), Pakistani (Khaliq et al., 2000), Tunisian (Mabrouk et al., 2003), Finnish (Tommiska et al., 2005), Iranian (Khadang et al., 2007), Brazilian (Vieira et al., 2008) and South Indian populations (Suresh et al., 2011; Vijayaraman et al., 2012). Contrary to our results, association between RR genotype and breast cancer risk have been reported in Turkish (Buyru et al., 2003), Italian (Bonafe et al., 2003), Jewish (Ohayon et al., 2005), Greek (Papadakis et al., 2000; Kalemi et al., 2005), Brazilian (Damin et al., 2006; Aoki et al., 2009) and Chinese (Ma et al., 2006) population.

RR genotype has been described as a potential risk factor, while the RP as a protection factor against breast cancer among Saudi women (Al-Qasem et al., 2012). The R72 variant is more susceptible than P72 variant to degradation induced by human papillomavirus E6 protein, which may result in an increased susceptibility to human papillomavirus induced tumors in homozygous R72 individuals (Storey et al., 1998). Recently, a meta-analysis on twenty Indian case control studies with total of 3,258 cancer cases and 4,260 healthy controls did not find any significant association of p.R72P polymorphism with cancer risk (Mandal et al., 2014).

The R72 variant has been reported to be a more potent inhibitor of chemotherapy-induced apoptosis than the P72 variant (Bergamaschi et al., 2003). Patients, homozygous for R72 allele, with breast, lung or head and neck cancers have been shown to survive and respond better to chemotherapy and radiotherapy (Sullivan et al., 2004; Nelson et al., 2005; Tommiska et al., 2005; Xu et al., 2005).

It has been reported that intronic variants may affect gene regulation through aberrant splicing or through disruption of critical DNA-protein interactions (Hillebrandt et al., 1996). In the present study PIN3 Ins16bp polymorphism was not found to be associated with the risk of developing breast cancer. Similar to our results, no association of PIN3 Ins16bp polymorphism

with risk of breast cancer was observed in Turkish (Akkiprik et al., 2009), Arabian (Alawadi et al., 2011) and Iranian (Pouladi et al., 2014) patients. In the present study higher frequency of A2A2 genotype was observed in the patients (7%) as compared to the controls (4%) but the results were not statistically significant. The A2A2 genotype has been reported to be associated with increased breast cancer risk in several studies (Weston et al., 1997; Wang-Gohrke et al., 2002, Costa et al., 2008; Guleria et al., 2012). On the contrary, six fold increased risk for breast cancer in individuals with A1A1 genotype has been reported in Slovakian population (Franekova et al., 2007). In the present study, carriers of A2 allele (A1A2+A2A2) were higher in patients as compared to the controls but the results were not statistically significant (p=0.74). Association of A1A2 genotype with breast cancer risk has been reported in Iranian women (Faghani et al., 2011). A recent meta-analysis showed significant association of 16bp duplication polymorphism of TP53 with an increased risk of breast cancer (Wu et al., 2013). Though, no association of PIN3 Ins16bp polymorphism with increased breast cancer risk has been reported (De Vecchi et al., 2008; Hrstka et al., 2009; Trifa et al., 2010), the breast cancer patients with A2A2 genotype of PIN3 polymorphism were reported to have better survival when treated with anthracycline based chemotherapy (Bisof et al., 2012).

For p.R213R (c.639A>G), all subjects had homozygous wild type genotype. Thus, there was no association of p.R213R polymorphism with breast cancer similar to reports in ovarian (Mazars et al., 1992), Barretts esophagus patients (Pilger et al., 2007) and different tumors (Iihan et al., 1995).

In the present study, no association of r.13494g>a polymorphism was observed with the risk of developing breast cancer. Similar findings were documented in Caucasians (Mavridou et al., 1998), Turkish (Akkiprik et al., 2009) and Spanish (Rodrigues et al., 2013) breast cancer patients. In contrast to our results, association of r.13494g>a polymorphism with the risk of developing breast (Peller et al., 1995), ovarian (Wang-Gohrke et al., 1999) and colon (Peller et al., 1995) cancer has been reported. Functional analysis using an in vitro cell survival assay demonstrated that lymphoblastoid cell lines derived from patients with the r.13494g>a variant exhibited a reduced level of apoptosis after chemotherapy and prolonged cell survival following DNA damage (Lehman et al., 2000).

We observed that PR-A1A1 genotype combination of p.R72P and PIN3 Ins16bp and RP-GG combination of p.R72P and r.13494g>a polymorphism showed significant risk of breast cancer (OR=1.65, 95%CI: 0.98-2.78, p=0.05; OR=1.72, 95%CI: 1.01-2.92, p=0.04). The RP-A1A1-GG genotype combination of p.R72P and PIN3 Ins16bp and r.13494g>a polymorphism showed marginally significant risk for breast cancer in the study population (OR=1.67, 95%CI: 0.97-2.88, p=0.06). Significant association of PIN3 Ins16bp and r.13494g>a polymorphisms with lymph node metastases and tumor aggressiveness has been previously documented in breast cancer patients (Hrstka et al., 2009). The difference in TP53 polymorphisms and cancer susceptibility in different countries indicates that additional factors like environmental factors, lifestyle and other genetic modifiers may modulate cancer susceptibility associated with these polymorphisms.

We observed that 80% of breast cancer patients were of age>40 years. In Asia, 84% of breast cancers are diagnosed from the age of 40 onwards (Ferlay et al., 2012). The interaction of p.R72P and PIN3 Ins16bp polymorphisms had been observed even in smaller sample size reported earlier (Guleria et al., 2012). In the current study, the role of p.R72P in breast cancer susceptibility was confirmed along with the PIN3 Ins16bp and r.13494g>a as it conferred increased risk. Women subjects of current study were exposed to agricultural chemicals, generally overweight/obese and the major tumor suppressor gene TP53 showing almost wild type genotypes. Wild-type p53 plays a crucial role in maintaining genomic stability by allowing the repair of damaged DNA through induction of a transient G1 arrest or eliminating the damaged cells by triggering apoptosis (Nigro et al., 1989). In light of increasing incidence of breast cancer being reported from the region further studies exploring the interaction of TP53 polymorphisms with other genes involved in DNA repair pathways would be beneficial in deriving more accurate risk markers.

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