RESEARCH ARTICLE

Analysis of TP53 Polymorphisms in North Indian Sporadic Esophageal Cancer Patients

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Abstract

Background: To investigate the relationship of five TP53 polymorphisms (p.P47S, p.R72P, PIN3 ins16bp, p.R213R and r.13494g>a) with the esophageal cancer (EC) risk in North Indians. Materials and Methods: Genotyping of p.P47S, p.R72P, PIN3 ins16bp, p.R213R and r.13494g>a polymorphisms of TP53 in 136 sporadic EC patients and 136 controls using polymerase chain reaction and PCR-RFLP. Results: The frequencies of genotype RR, RP and PP of p.R72P polymorphism were 16.91 *vs* 26.47%, 58.82 *vs* 49.27% and 24.27 *vs* 24.27% among patients and controls respectively. We observed significantly increased frequency of RP genotype in cases as compared to controls (OR=1.87, 95% CI, 1.01-3.46, p=0.05). The frequencies of genotype A1A1, A1A2 and A2A2 of PIN3 ins16bp polymorphism were 69.12 *vs* 70.59%, 27.20 *vs* 25% and 3.68 *vs* 4.41% among patients and controls. There was no significant difference among genotype and allele distribution between patients and controls. The frequencies of genotype GG, GA and AA of r.13494g>a polymorphism were 62.50 *vs* 64.70%, 34.56 *vs* 30.15% and 2.94 *vs* 5.15% among patients and controls respectively. No significant difference between genotype and allele frequency was observed in the patients and controls. For p.P47S and p.R213R polymorphisms, all the cases and controls had homozygous wild type genotype. The RP-A1A1-GG genotype combination shows significant risk for EC (OR=2.01, 95%CI: 1.01-3.99, p=0.05). Conclusions: Among the five TP53 polymorphisms investigated, only p.R72P polymorphism may contributes to EC susceptibility.

Keywords: Esophageal cancer - TP53 - polymorphism

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Introduction

Among human cancers, esophageal cancer (EC) appears to be a complex multistep process with multifunctional etiologies in which environmental, geographical and genetic factors have been attributed to play critical roles in the development of cancer (Tsigris et al., 2007). Worldwide, EC ranks eighth in cancer incidence and sixth in cancer mortality (Ferlay et al., 2010). The geographical regions with higher risk of esophageal cancer, which extend from Turkey through countries such as Iran, Mongolia, Kazakhstan and the Taihang mountain range in North-central China are collectively called "Asian esophageal cancer belt" (Mao et al., 2011). The estimated number of esophageal cancer cases in India for the year 2015 and 2020 are 42,184 and 42,513 respectively (Takiar et al., 2010). In India, high incidence of esophageal cancer has been reported in states of Jammu and Kashmir, Assam and Karnataka (Ali et al., 2011).

Genetic variants in genes controlling DNA repair and cell proliferation have been proven to be important in determining individual susceptibility to the occurrence of common cancers (Hunt et al., 2013). Tumor suppressor genes mediate cellular response to genotoxic insults through its effects on gene transcription, DNA synthesis and repair, genomic stability and apoptosis (Vogelstein and Kinzler, 1992). TP53 (OMIM 191170) "the guardian of the genome" is mostly inactivated in sporadic human tumors resulting in inactivation of a wide range of anti-proliferative responses regulating cell cycle progression, apoptosis, autophagy, differentiation, senescence, DNA repair and oxidative metabolism (Levine, 1997; Hainaut and Hollstein, 2000; Petitjean et al., 2007; Levine and Oren, 2009). TP53 is located on 17p13.1, comprises 11 exons and encodes a 53kDa phosphoprotein made by 393 amino acids forming five highly conserved regions and four functional domains (Harris and Hollstein, 1993). The p53 protein has also other biological functions like senescence, angiogenesis, cellular differentiation and immune response (Suzuki and Matsubara, 2011).

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The TP53 pathway is well known for maintaining genomic integrity and preventing cells from undergoing oncogenic transformation (Hamroun et al., 2006). Genetic polymorphisms can contribute to differences between individuals in susceptibility to various cancers by affecting the regulation of gene expression (Wade et al., 2013). Analysis of polymorphisms in a variety of genes has revealed a correlation between specific allele variants and cancer predisposition (Rogler et al., 2011). The loss of p53 function also mediates resistance to chemotherapy induced apoptosis, which is often associated with poor clinical outcome (Owen et al., 1997).

In TP53 gene, polymorphisms have been identified in both coding and non-coding regions (Murphy, 2006; Bojesen and Nordestgaard, 2008; Costa et al., 2008; Whibley et al., 2009). About 200 genetic polymorphisms have been identified in TP53 (http://p53.iarc.fr), many of which show geographic and population variations, but their effects on cancer risk appear to be inconsistent across studies (Whibley et al., 2009; Stacey et al., 2011).

The p.R72P and p.P47S are functionally important polymorphisms of TP53. The p.P47S is present in the N-terminal domain of TP53 that leads to non-synonymous amino acid substitution from Proline to Serine. The p.P47S polymorphism has been investigated in various cancers including breast (Alawadi et al., 2011), colorectal (Sameer et al., 2010), gliomas (Pinto et al., 2008), bladder (Santos et al., 2011), brain (Almeida et al., 2009) and urinary bladder (Jaiswal et al., 2011) cancer. Significant association of S47 has been reported in Caucasian lung cancer (Felley-Bosco et al., 1993) and South Indian colorectal cancer (Singamsetty et al., 2014) patients. So far there is no published report on p.P47S polymorphism in EC.

The p.R72P is non-conservative change of the arginine to proline located within the prolin-rich domain of TP53 which is the critical site for apoptosis signaling (Sakamuro et al., 1997). The two isoforms of this polymorphism (R72 and P72) differ in their biochemical and biological properties and behave differently (Thomas et al., 1999). The R72 form has been reported to induce apoptosis more effectively than P72 form (Dumont et al., 2003). Several studies have focused on the association between TP53 p.R72P polymorphism and esophageal cancer susceptibility. However, contradictory data is available, where few studies reported an association while several studies found no association. Positive association between p.R72P polymorphism and EC have previously reported in European and Asian (Kawaguchi et al., 2000), Chinese (Lee et al., 2000; Li et al., 2002; Lu et al., 2004; He et al., 2005; Hong et al., 2005; Cai et al., 2006; Shao et al., 2008; Yang et al., 2008; Ma et al., 2012; Yang et al., 2013), South African (Vos et al., 2003), German (Pantelis et al., 2007), Caucasian (Cescon et al., 2009; Renouf et al., 2013) and Korean (Piao et al., 2011) population. Still other studies which have failed to demonstrate any association between codon 72 variants of TP53 and EC cancer risk have been in Chinese (Guimaraes et al., 2001; Hu et al., 2003), Japanese (Hamajima et al., 2002) and Caucasian (Liu et al., 2010) population.

A rare polymorphism p.R213R localized in exon 6 resulted from alteration of CGA to CGG at codon 213. Though, published studies substantially lack information on p.R213R polymorphism in EC. No association of p.R213R polymorphism with cancer risk has been observed in Brazilian Barretts esophagus patients (Pilger et al., 2007).

Intronic polymorphisms of TP53 gene may influence coding-region sequence alterations that results in increase of a deleterious phenotype (Malkinson and You, 1994). PIN3 Ins16bp polymorphism (rs17878362) a 16 base pair duplication in intron 3 has been implicated in regulation of gene expression and DNA protein interactions (Mattick 1994, 2004).

Several case-control studies have reported an increased risk of various cancer types associated with PIN3 Ins16bp polymorphism, 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). To date, there are three reports examining the association between PIN3 ins16bp polymorphism and the risk for EC. Two of these reports found a positive association between A2A2 genotype and EC (Vos et al., 2003; Malik et al., 2011), whereas one study from North India failed to find an association (Umar et al., 2012).

The r.13494g>a is a rare polymorphism in the intron 6 of TP53 resulting from G > A transition at 61bp downstream of exon 6. The r.13494g>a has been studied in various cancers including ovarian (Wang-Gohrke et al., 1999; Yair et al., 2000), head and neck (Mitra et al., 2003; Chen et al., 2007), esophageal (Pilger et al., 2007), breast (Peller et al., 1995; Sjalander et al., 1996; Weston et al., 1997; Akkiprik et al., 2009; Singh et al., 2008; Hrstka et al., 2009), cervical (Mitra et al., 2004), colorectal (Mammano et al., 2009), lung (Biros et al., 2001; Wang et al., 2007), prostate cancer (Mittal et al., 2011) and yielded inconsistent results for association.

The previous published studies showed that these polymorphisms vary in different ethnic and population groups. In Punjab state in North West part of India, Population Based Cancer Registry (PBCR) has reported prevalence of cancer as 90 patients per one lakh of population with maximum incidence varying in different districts, i.e. in Mutktsar (136.3 per lakh), Mansa (134.8 per lakh), Bathinda (125.8 per lakh) to lower incidence in Taran Taran district (40.9 per lakh). Amritsar district has highest incidence of cancer (81.2 per lakh) in Majha region of Punjab. (http://www.downtoearth.org.in/content/punjab-cancer-capital-india). In Amritsar city, the third largest city of Punjab state, increased incidence of esophageal cancer is being reported (personal Communication, SGRD Rotary Cancer Hospital, Vallah, Sri Amritsar). There is no published report on TP53 polymorphisms in the esophageal cancer patients from this region. The present case-control study was conducted to investigate the relationship of five TP53 polymorphisms (p.P47S, p.R72P, PIN3 ins16bp, p.R213R and r.13494g>a) with the esophageal cancer risk in North Indian sporadic esophageal cancer patients. The identification of susceptibility factors that predispose individuals to esophageal cancer will give further insight

into the etiology of this cancer and provide targets for the future development of therapeutic approaches.

Materials and Methods

Clinical evaluation and selection of subjects

This study was approved by the ethical committee of Guru Nanak Dev University, Amritsar, Punjab, India. The patients were recruited from Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Amritsar, Punjab. Patients included were those who had no prior history of any cancer and had not undergone chemotherapy, radiotherapy or blood transfusion. The controls were age and gender matched unrelated healthy individuals from the same geographical region as that of patients. The individuals who had family history for any type of cancer or chronic diseases or on regular medication were not included in the study. Relevant information including self reported personal history and disease history of each subject was recorded on a pre-tested structured questionnaire by interview and from medical records. After informed consent, 5ml venous blood was collected from each subject.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leucocytes using standard phenol chloroform method (Adeli and Ogbonna, 1990). The DNA fragment harboring p.P47S, p.R72P, PIN3, p.R213R and r.13494g>a polymorphisms were amplified using the published primer sequences (Table 1). Amplification was performed in 15µl reaction volume containing 0.4µl of dNTPs, 6pmoles of each oligonucleotide primer and 0.9U of Taq DNA polymerase (Bangalore GeNei). A negative control without DNA template was included in each reaction. Details of reaction conditions have been mentioned in Table 1. The amplified PCR products were analyzed on ethidium bromide stained agarose gel. The PCR products were digested with appropriate restriction enzyme (Table 1) using the manufacturer instructions (New England Biolabs, Beverly, MA), followed by agarose gel electrophoresis. The genotype were categorized as wild type, heterozygous and homozygous variant based on band sizes as mentioned in Table 1 and Figures 1-4. Genotyping was performed without knowledge of case/control status to ensure quality control.

Statistical analysis

Continuous variables were analyzed by t-test and presented as means ± standard deviation (SD). Categorical variables were presented as percentages and were compared by chi-square test. Hardy Weinberg equilibrium (HWE) was tested by comparing the observed to expected genotype frequencies in controls using a χ^2 test. The odds ratios (ORs), 95%CI ranges and corresponding p-values were calculated using the Web-Assotest program (http://www.ekstroem.com) for measuring the association between different genotypes and esophageal

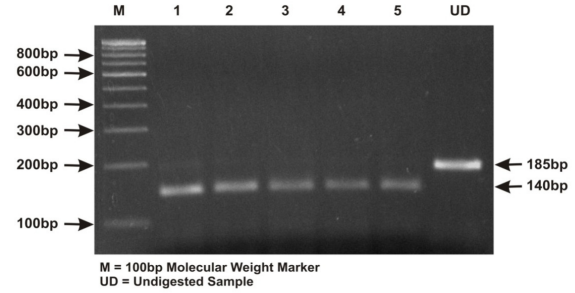

Figure 1. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of p.P47S Polymorphism of TP53. Lane 1-5=Wild type homozygous genotype (PP)

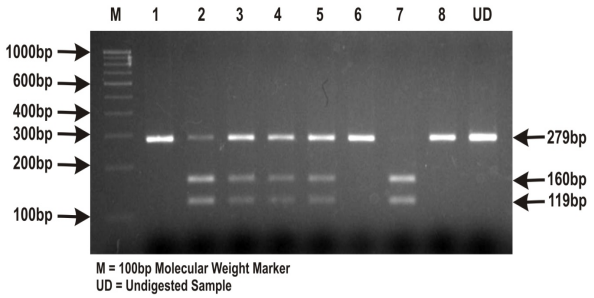

Figure 2. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of p.R72P Polymorphism of TP53. Lane 1, 6 and 8=PP genotype; Lane 2-5=Heterozygous genotype (RP); Lane 7=RR genotype

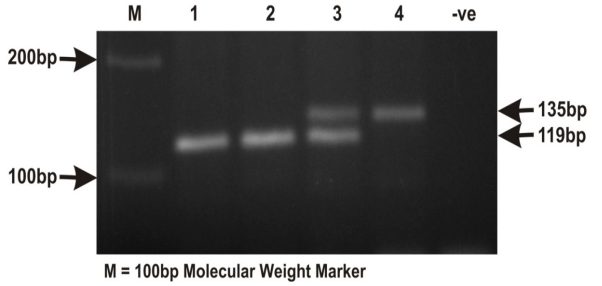

Figure 3. A photograph of Gel Demonstrating the Different Genotypes of PIN3 Polymorphism of TP53. Lane 1 and 2=A1A1 genotype, Lane 3=A1A2 genotype; Lane 4=A2A2 genotype

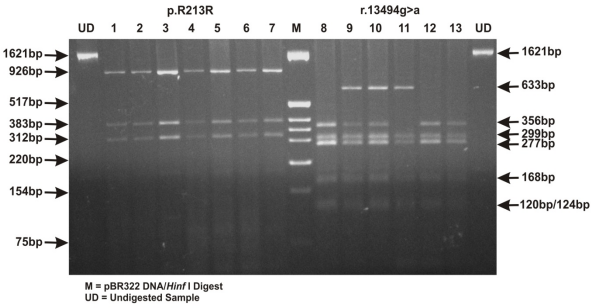

Figure 4. Restriction Digestion of PCR Products Demonstrating the Patterns of Digestion in Different Genotypes of p.R213R and r.13494g>a Polymorphisms of TP53. Lane 1-7=Wild type homozygous genotype (AA). Lane 8, 12 and 13=GG genotype; Lane 9 and 10=GA genotype; Lane 11=AA genotype

cancer risk. The ORs, 95%CI ranges and p-value for genotypic combinations were calculated using online Vassar Stats Calculator (http://www.faculty.vassar.edu/lowry/VassarStats.html). The cut off p-value adopted for statistical analysis was 0.05.

Results

Characteristics of subjects

A total of 136 sporadic esophageal cancer patients (47 males and 89 females) and 136 age and gender matched unrelated healthy individuals (47 males and 89 females) were analyzed in this study. The mean age of patients and controls was 55.34±12.54 years and 53.08±12.27 years respectively. There were 111 rural and 25 urban cases. EC incidence was higher among the individuals more than 50 years of age as compared to those less than 50 years. When the incidence was compared between males and females, a preponderance of EC was observed among females (65%).

Genotypic frequencies of TP53 polymorphisms and esophageal cancer risk

The genotype and allele frequencies of TP53 polymorphisms in esophageal cancer patients and controls individuals are shown in Table 2. The observed genotypes frequencies of two polymorphisms (PIN3 ins16bp and r.13494g>a) were in HWE (p>0.05). In p.R72P polymorphism, we observed deviation from HWE in patients (p<0.05). The frequencies of genotype RR, RP and PP of p.R72P polymorphism were 16.91 *vs* 26.47%, 58.82 *vs* 49.27% and 24.27 *vs* 24.27% among patients and controls respectively. We observed significantly increased frequency of RP genotype in cases as compared to controls

Table 1. Detail of TP53 Polymorphisms and Reaction Conditions Used for Screening

Variant (SNP No.)	Location	Screening Method	PCR product size (bp)	Annealing Temperature, MgCl2	Restriction enzyme	Restriction digestion patterns for different Alleles	Primers References
p.P47S (rs1800371)	Exon 4	PCR-RFLP	201/185*	59°C, 1mM	MspI	S allele-201/185 P allele-156/140 and 45	Pinto et al., 2008
p.R72P (rs1042522)	Exon 4	PCR-RFLP	279	59°C, 1mM	BstUI	P allele 279 R allele-160 and 119	Kazemi et al., 2009
PIN3 (rs17878362)	Intron 3	PCR	-	61°C, 1mM	-	A1 allele-119 A2 allele-135	Costa et al., 2008
p.R213R (rs1800372)	Exon 6	PCR-RFLP	1621	59°C, 1.5mM	TaqI	A allele-312, 383 and 926 G allele-695 and 926	Pilger et al., 2008
r.13494g>a (rs1625895)	Intron 6	PCR-RFLP	1621	59°C, 1.5mM	MspI	G allele-356, 277, 277, 299, 168, 124 and120 A allele - 633, 299, 277, 168, 124 and 120	Pilger et al., 2008

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

Table 2. Genotype and Allele Frequencies of TP53 Polymorphisms in Esophageal Cancer Patients and Healthy Controls

Variant (SNP No.)	Genotype	Allele	Patients n(%)	Controls n(%)	OR (95% CI)	p value
p.P47S	PP		136(100)	136(100)		
(rs1800371)	PS		-	-		
	SS		-	-		
		P	272(100)	272(100)		
		S	-	-		
p.R72P	RR		23(16.91)	36(26.47)	1(Reference)	
(rs1042522)	RP		80(58.82)	67(49.26)	1.87(1.01-3.46)	**0.05**
	PP		33(24.27)	33(24.27)	1.57(0.77-3.19)	0.22
		R	126(46.32)	139(51.10)	1(Reference)	
		P	146(53.68)	133(48.90)	1.21(0.87-1.70)	0.27
PIN3Ins16bp	A1A1		94(69.12)	96(70.59)	1(Reference)	
(rs17878362)	A1A2		37(27.20)	34(25.00)	1.11(0.64-1.92)	0.7
	A2A2		5(3.68)	6(4.41)	0.85(0.25-2.88)	0.79
		A1	225(82.72)	226(83.09)	1(Reference)	
		A2	47(17.28)	46(16.91)	1.03(0.66-1.60)	0.92
p.R213R	AA		136(100)	136(100)	136(100)	
(rs1800372)	AG		-	-		
	GG		-	-		
		A	272(100)	272(100)		
		G	-	-		
r.13494g>a	GG		85(62.50)	88(64.70)	1(Reference)	
(rs1625895)	GA		47(34.56)	41(30.15)	1.19(0.71-1.98)	0.51
	AA		4(2.94)	7(5.15)	0.59(0.17-2.09)	0.41
		G	217(79.78)	217(79.78)	1(Reference)	
		A	55(20.22)	55(20.22)	1.00(0.66-1.52)	1

Statistically significant p values (p<0.05) are displayed in bold; OR- Odds ratio; CI- confidence interval

and revealed 1.87 folds increased risk for EC (OR=1.87, 95%CI, 1.01-3.46, p=0.05. The frequencies of R and P allele were 46.32 vs 51.10% and 53.68 vs 48.90% in patients and controls respectively.

The frequencies of genotype A1A1, A1A2 and A2A2 of TP53 PIN3 ins16bp were 69.12 *vs* 70.59%, 27.20 *vs* 25% and 3.68 *vs* 4.41% among patients and controls. No significant difference was found among genotype and allele distribution of PIN3 ins16bp polymorphism between patients and controls. The frequencies of genotype GG, GA and AA of TP53 r.13494g>a were 62.50 *vs* 64.70%, 34.56 *vs* 30.15% and 2.94 *vs* 5.15% among patients and controls respectively. There was no significant difference between genotype and allele frequency in the EC patients and controls. For p.P47S and p.R213R polymorphisms, all the cases and controls had homozygous wild type genotype.

We also evaluated all possible interactions of p.R72P with PIN3 ins16bp, p.R72P with r.13494g>a and PIN3 ins16bp with r.13494g>a polymorphisms. It was observed that RP-A1A1 combination of p.R72P and PIN3 ins16bp polymorphism showed significant risk for EC (OR=1.93, 95%CI: 0.99-3.73, p=0.05). The genotype combination RP-GG of p.R72P and r.13494g>a polymorphism showed 1.95 folds risk for EC (OR=1.95, 95%CI: 0.99-3.83, p=0.05). Analysis of genotype combinations of p.R72P, PIN3 ins16bp and r.13494g>a polymorphisms of TP53 showed significant risk for EC in individuals with RP-A1A1-GG genotype combination (OR=2.01, 95%CI: 1.01-3.99, p=0.05).

We stratified our study subjects according to gender, age, smoking, alcohol consumption etc. to investigate the relationship of the studied polymorphisms with EC susceptibility risk. We found that RP genotype of p.R72P was significantly associated with risk for EC in rural (p=0.05) as well as patients with age >50 years (p=0.02).

Discussion

The pathogenesis of esophageal cancer has not been fully elucidated till date. Despite the advances in surgical techniques and treatments, the prognosis of EC remains poor, since the disease is usually diagnosed at an advanced stage. TP53 is a central mediator of responses to DNA damage and cellular stress and would therefore be expected to play major roles in determining not only

Table 3. Summary of Published Studies on Five TP53 Polymorphisms in Esophageal Cancer in Different Populations

Polymorphism	Population	Screening Method	Patients	Controls	Inference	Reference
p.P47S	North Indian	PCR-RFLP	136	136	No association	Present study
PIN3 16bp Ins	North Indian	PCR	136	136	No association	Present study
	South African	PCR-SSCP DNA sequencing	73	115	↑ risk with A2A2 genotype	Vos et al., 2003
	North Indian	PCR	135	195	↑ risk with A2A2 genotype	Malik et al., 2011
	North Indian	PCR	255	255	No association	Umar et al., 2012
p.R72P	North Indian	PCR-RFLP	136	136	↑ risk with RP genotype	Present study
	European and Asian	Sequencing	75	-	↑ risk with RR genotype	Kawaguchi et al., 2000
	Chinese	PCR-RFLP	90	254	↑ risk with PP genotype	Lee et al., 2000
	Chinese	PCR-RFLP	32	57	No association	Guimaraes et al., 2001
	Japanese	PCR	102	241	No association	Hamajima et al., 2002
	Chinese	Allele Specific PCR	62	50	↑ risk with RR genotype and HPV infection	Li et al., 2002
	Chinese	PCR-SSCP DNA sequencing	120	232	No association	Hu et al., 2003
	South African	PCR-SSCP DNA sequencing	73	115	↑ risk with R allele	Vos et al., 2003
	Chinese	PCR-RFLP	104	-	↑ risk with RR genotype and HPV infection	Lu et al., 2004
	Chinese	PCR-RFLP	110	-	↑ risk with RR genotype and HPV infection	He et al., 2005
	Chinese	PCR-RFLP	758	1420	↑ risk with PP genotype	Hong et al., 2005
	Chinese	PCR-RFLP	204	389	↑ risk with PP genotype	Cai et al., 2006
	German	PCR DNA sequencing	53	-	↑ risk with R allele and HPV infection	Pantelis et al., 2007
	Chinese	PCR-RFLP	673	694	↑ risk with PP genotype	Shao et al., 2008
	Chinese	DNA sequencing	435	550	↑ risk with RR genotype and HPV infection	Yang et al., 2008
	Caucasian	TaqMan assay	371	-	↑ risk with PP genotype	Cescon et al., 2009
	Caucasian	TaqMan assay	312	454	No association	Liu et al., 2010
	Korean	Real Time PCR	340	1700	↑ risk with P allele	Piao et al., 2011
	Chinese	PCR-RFLP	226	226	↑ risk with RP and PP genotype	Ma et al., 2012
	Caucasian	PCR-RFLP	358	-	↑ risk with PP genotype	Renouf et al., 2013
	Chinese	TaqMan assay	307	311	↑ risk with RR and RP genotype	Yang et al., 2013
r.13494g.>a	North Indian	PCR-RFLP	136	136	No association	Present study
	Brazilian	PCR-RFLP	45	100	No association	Pilger et al., 2007
p.R213R	North Indian	PCR-RFLP	136	136	No association	Present study
	Brazilian	PCR-RFLP	35	100	No association	Pilger et al., 2007

the level of tumor aggressiveness but also of chemo sensitivity and radio sensitivity (Vogelstein et al., 2000). Mutations and polymorphisms in TP53 are associated with different types of cancer and other diseases. Considering the previous reports assessing the TP53 polymorphisms in esophageal cancer with inconsistent results (Table 3), we had hypothesized that five polymorphisms in the TP53 might be associated with susceptibility to esophageal cancer in Punjab, North West India.

In the present study, all the cases and controls had homozygous wild type genotype for p.P47S polymorphism. This result is in agreement with the some earlier reported studies where no association of p.P47S polymorphism was observed in breast cancer (Alawadi et al., 2011; Al-Qasem et al., 2012), bladder cancer (Santos et al., 2011), gliomas (Pinto et al., 2008), urinary bladder cancer (Jaiswal et al., 2011), colorectal cancer (Sameer et al., 2010) and primary open angle glaucoma patients (Daugherty et al., 2009). On the contrary, significant association of S47 has been reported in Caucasian lung cancer (Felley-Bosco et al., 1993), Brazilian brain tumor (Almeida et al., 2009) and South Indian colorectal cancer (Singamsetty et al., 2014) patients. It has been reported that the S47 phenotype has a decreased capacity to induce apoptosis, to transactivate two p53 target genes (p53AIP1 and PUMA), and to bind to MAPK1 protein as compared with the wild-type P47 phenotype (Li et al., 2005; Murphy, 2006).

In p.R72P polymorphism, we observed deviation from HWE in patients ($p<0.05$) which may be due to genetic reasons including non-random mating, the alleles reflecting recent mutations that have not reached equilibrium, as well as methodological reasons (Mitchell et al., 2003; Hosking et al., 2004). In the present study, RP genotype of p.R72P polymorphism was significantly associated with increased risk of esophageal cancer as compared to RR and PP genotypes (OR=1.87; 95%CI=1.01-3.46; p=0.05). Association of RP genotype with increased cancer risk has previously been documented in Chinese esophageal squamous cell carcinoma patients (Yang et al., 2013). We observed higher frequency of RR genotype in controls as compared to patients. The impact of p.R72P polymorphism of TP53 in development of esophageal cancer is still controversial. RR genotype of p.R72P polymorphism has been reported to be associated with increased risk for esophageal cancer in European and Asian (Kawaguchi et al., 2002), Chinese patients (Li et al., 2002; Lu et al., 2004; He et al., 2005; Yang et al., 2008; Yang et al., 2013). Association of R72 allele with increased risk of EC has also been reported in South African (Vos et al., 2003), and German (Pantelis et al., 2007) patients. A positive association of the R72 allele with p53 overexpression has been reported in esophageal cancer tissue (Lee et al., 2006). A meta-analysis of nine case-control studies involving a total of 2,114 EC cases and 3,431 controls revealed that TP53 R72 allele is a protective factor for EC (Jiang et al., 2011).

The frequency of PP genotype was same in patients as well as in controls in the current study. The distribution of the TP53 p.R72P polymorphism varies by ethnic group. Asians have been reported to express the P allele, whereas Caucasians express the R allele (Siddique et al., 2005). PP genotype has been described as risk genotype for EC in Chinese (Lee et al., 2000; Hong et al., 2005, Cai et al., 2006, Shao et al., 2008) and Caucasians (Cescon et al., 2009; Renouf et al., 2013). Association of P72 allele with increased risk of EC has also been reported in Korean patients (Piao et al., 2011). Significant association of PP genotype of p.R72P polymorphism has been reported with increased risk for adenocarcinoma in Chinese non-smoker females (Ren et al., 2013) and cervical cancer in Indians (Zhou et al., 2012). p.R72P polymorphism has also been reported to be associated with risk of cervical cancer development in Human papillomavirus (HPV) 16 infected women (Chansaenroj et al., 2013). In esophageal cancer, HPV may have a role only in the presence of altered promoter methylation of Aurora A and tobacco use (Mohiuddin et al., 2013).

The R72 variant has been reported to be a more potent inhibitor of chemotherapy-induced apoptosis than the corresponding P72 variant (Bergamaschi et al., 2003). Patients, homozygote 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). The RP genotype has been associated with better tumor response to neoadjuvant chemotherapy in lung cancer patients (Gervas et al., 2009). These p53-mediated responses are crucial both in reducing cancer frequency in vertebrates and in mediating the response of commonly used cancer therapies (Johnstone et al., 2002; Lozano and Zambetti 2005; Haupt and Haupt 2006).

Polymorphisms in the non-coding region of TP53 could also play an important role in the regulation of gene expression. It has been reported that PIN ins16bp polymorphism may have an impact on the levels and alternative splicing of TP53 mRNA (Gemignani et al., 2004). In the present study, no association of PIN3 polymorphism with EC risk was observed. This was consistent with a study conducted in other states of North India which revealed no association of PIN3 polymorphism with EC risk (Umar et al., 2012). However, A2A2 genotype of PIN3 polymorphism has been reported to be associated with increased risk of esophageal cancer in Kashmiri North Indian (Malik et al., 2011) and South African (Vos et al., 2003) patients.

For p.R213R (c.639A>G), all subjects had homozygous wild type genotype. No association of p.R213R polymorphism has been reported in ovarian (Mazars et al., 1992) and Barretts esophagus (Pilger et al., 2007) patients.

In the present study, no association of r.13494g>a polymorphism was observed with the risk of developing EC. Similarly, no association of r.13494g>a polymorphism has been reported in Brazilian Barretts esophagus patients (Pilger et al., 2007). Apart from esophageal cancer, no association of this polymorphism was documented in Caucasians breast (Mavridou et al., 1998), Israeli ovarian (Yair et al., 2000) and Indian cervical (Mitra et al., 2005) cancer patients. In contrast, 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) lung (Biros et al., 2001; Wang et al.,

2007; Cherdyntseva et al ., 2010), prostrate (Mittal et al., 2011) cancer has also 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 EC (OR=1.93, 95%CI: 0.99-3.73, p=0.05; OR=1.95, 95%CI: 0.99-3.83, p=0.05). The RP-A1A1-GG genotype combination of p.R72P and PIN3 Ins16bp and r.13494g>a polymorphism showed significant risk for EC in the current study (OR=2.01, 95%CI: 1.01-3.99, p=0.05).

In the present study, 44.12% of the patients were underweight. A low body mass index reportedly increases the risk for esophageal squamous cell carcinoma (Ryan et al., 2006). Leanness has been associated with increased risk for squamous cell carcinoma of esophagus in North Americans (Gallus et al., 2001; Smith et al., 2008).

We observed that 60.29% of EC patients were of age>50 years. It has been reported that people between the age of 50 and 70 years have a greater risk of developing esophageal cancer with 3-4 times higher risk in men as compared to women (Steyerberg et al., 2007; Ferlay et al., 2010; Mao et al., 2011) though in the present study more females were affected compared to males. Ageing is associated with the ability to maintain and repair somatic cells (Kirkwood et al., 2000). It has been proposed that age associated tissue dysfunction is caused by the accumulation of molecular and cellular damage (Hasty et al., 2003). In patients, 13.97% subjects were smokers, 29.41% were alcoholic while 11.77% were smokers and alcoholic. Several studies investigating the effects of smoking and alcohol intake on the risk of esophageal cancer have demonstrated that long duration, high consumption and the interaction of these habits may increase the risk of EC (Franceschi et al., 1990; Launoy et al., 1997; Zambon et al., 2000; Castellsague et al., 2004; Lee et al., 2005; Muwonge et al., 2008; Chen et al., 2010; Wu et al., 2011).

About 81.62% of patients were from rural background with agriculture as family occupation and 50.74% of them were exposed to pesticides and other agriculture chemicals. Many pesticides are carcinogenic in nature and may increase the risk of cancer through a variety of mechanisms including genotoxicity, tumor promotion, hormonal action and immunotoxicity. In a study from Brazil, it has been reported that the agricultural workers were at a higher risk for esophageal cancer as compared to non-agricultural workers (Meyer et al., 2011).

In conclusion, our data suggest that among the five TP53 polymorphisms investigated, only p.R72P polymorphism and the RP-A1A1-GG genotype combination may contributes to EC susceptibility. The discrepancy among results of the present and previous studies may be due to ethnic and geographic differences, environmental factors and life style among various populations. Further comprehensive studies on other genes of various pathways involved in esophageal cancer along with TP53 are needed to elucidate accurate EC risk in Punjab, North-West India.

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