RESEARCH ARTICLE

p53 Codon 72 Polymorphism Interactions with Dietary and Tobacco Related Habits and Risk of Stomach Cancer in Mizoram, India

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Abstract

Background: This study was carried out to investigate the interaction of p53 codon 72 polymorphism, dietary and tobacco habits with reference to risk of stomach cancer in Mizoram, India. A total of 105 histologically confirmed stomach cancer cases and 210 age, sex and ethnicity matched healthy population controls were included in this study. Materials and Methods: The p53 codon 72 polymorphism was detected by PCR-RFLP and sequencing. *H. pylori* infection status was determined by ELISA. Information on various dietary and tobacco related habits was recorded with a standard questionnaire. Results: This study revealed that overall, the Pro/Pro genotype was significantly associated with a higher risk of stomach cancer (OR, 2.54; 95%CI, 1.01-6.40) as compared to the Arg/Arg genotype. In gender stratified analysis, the Pro/Pro genotype showed higher risk (OR, 7.50; 95%CI, 1.20-47.0) than the Arg/Arg genotype among females. Similarly, the Pro/Pro genotype demonstrated higher risk of stomach cancer (OR, 6.30; 95%CI, 1.41-28.2) among older people (>60 years). However, no such associations were observed in males and in individuals <60 years of age. Smoke dried fish and preserved meat (smoke dried/sun dried) consumers were at increased risk of stomach cancer (OR, 4.85; 95%CI, 1.91-12.3 and OR, 4.22; 95%CI, 1.46-12.2 respectively) as compared to non-consumers. Significant gene-environment interactions exist in terms of p53 codon 72 polymorphism and stomach cancer in Mizoram. Tobacco smokers with Pro/Pro and Arg/Pro genotypes were at higher risk of stomach cancer (OR, 16.2; 95%CI, 1.72-153.4 and OR, 9.45; 95%CI, 1.09-81.7 respectively) than the non-smokers Arg/Arg genotype carriers. The combination of tuibur user and Arg/Pro genotype also demonstrated an elevated risk association (OR, 4.76; 95%CI, 1.40-16.21). Conclusions: In conclusion, this study revealed that p53 codon 72 polymorphism and dietary and tobacco habit interactions influence stomach cancer development in Mizoram, India.

Keywords: Stomach cancer - p53 codon 72 polymorphism - risk habits - gene-environment interactions - Mizoram, India

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Introduction

The incidence of stomach cancer in India is highest in the state of Mizoram. The age-adjusted rates (AAR) for males and females are 42.9 and 20.5 per 10^5 populations respectively (NCRP., 2010). The age-adjusted rates for only the Aizawl district of the Mizoram state are 55.4 and 24.4 per 10^5 populations in males and females respectively (NCRP., 2010). Stomach cancer is a multistep process involving interactions of genetic and environmental factors. Many studies revealed that tobacco, alcohol, different food habit, infection etc. promote the occurrence of most of the cancers including stomach cancer worldwide. Diet has been associated as a co-factor in the progression from gastritis to gastric cancer; accordingly the incidence of stomach cancer varies around the world depending on dietary patterns (Ward et al., 1999). The lifestyle and dietary habit of the people of Mizoram are different from other parts of the country, as they consumes many uncommon foods which includes smoke and sun dried salted meat and fish, soda (alkali), traditional fermented food etc (Phukan et al., 2006).

Although, all the molecular events leading to gastric cancer are still not completely known, there are now sufficient reports available to suggest that the functional inactivation of p53 gene through allelic loss and point

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mutations play an important role (Renault et al., 1993; Tamura et al., 1999; Karim et al., 2009). Several studies have suggested that a mutant allele promotes transformation by inactivating normal p53 function in a dominant-negative fashion (Finlay et al., 1989). The 20 kb sized p53 tumour suppressor gene located on the short arm of the chromosome 17 at 17p13 contains 11 exons (Dhingra et al., 1996). Out of these, the exon-4 codon 72 polymorphism (rs1042522) has been seen more common, which results in the substitution of arginine (CGC) by proline (CCC) in the trans-activating domain (Matlashewski et al., 1987; Shepherd et al., 2000). These changes in its amino acid sequence can modify the ability of p53 to bind to response elements in target genes, altering recognition motifs for post-translational modifications or may compromise the p53 stability and interactions with other proteins (Walker et al., 1996; Thomas et al., 1999; Xiang et al., 2012) which may lead to tumour progression and a poor prognosis (Katkoori et al., 2009). The frequencies of allelic variants at this codon not only differ among different ethnic groups (Beckman et al., 1994), they also lead to racial differences of cancer susceptibility including stomach cancer (Birgander et al., 1996).

In this population based matched case-control study, we have evaluated the relationship between the p53 codon 72 polymorphism and stomach cancer risk in a high incidence area considering various dietary habits along with tobacco and alcohol habits and *H. pylori* infection simultaneously for the first time in Mizoram.

Materials and Methods

Study subjects

This study was a population based matched case-control study executed from 2009-2012. All cases and matched controls were ethnic Mizos of the Mizoram state. All cases (n=105) were newly diagnosed and histopathologically confirmed stomach cancer patients who consented to participate in this study and were recruited from Aizawl civil hospital and other private clinics of Mizoram. The patients with severe clinical symptoms, patients with recurrent cancer or too old to be interviewed and who refused to be interviewed were excluded from this study. Two age (±5 years), sex and ethnicity matched population based healthy neighbourhood controls (n=210) were selected for each case. Socio-demographic information and other risk habits like dietary habits of meat, fish and other foods, tobacco intake, alcohol consumption etc. were collected from cases and controls by face-to-face interviews and information gathered was recorded in a pre-designed questionnaire.

5-10 ml of peripheral whole blood was collected from each of the study subjects in EDTA-containing vials and stored at -80˚C until analysed. All participants were given an explanation of nature of the study and informed and written consent was obtained from all the cases and the controls. The institutional ethical committee of the Regional Medical Research Centre, N. E. Region, Dibrugarh approved this study.

DNA extraction and genotyping

Extraction and purification of high molecular weight genomic DNA was carried out with Quiagen DNeasy(R) Blood kit. A PCR reaction mixture of 25μl volume was prepared containing 12.5μl of Promega GoTaq(R) Hot Start Master Mix, 2X (GoTaq DNA polymerase, 2X GoTaq Reaction Buffer, pH 8.5, 400 µM of each dNTP and 3 mM $MgCl_2$), 5 pmol of each primer and 200 ng of template DNA. Primer sequences for PCR amplification were 5'-TTGCCGTCCCAAGCAATGGATGA-3' and 5'-TCTGGGAAGGGACAGAAGATGAC-3' which produced a 199 base-pair band (Ara et al., 1990; Hiyama et al., 2002). The PCR amplifications were carried out in Applied Biosystems Thermal Cycler. The PCR cycles were 94˚C for 10 min followed by 40 cycles of 94˚C for 30 sec, 55˚C for 1 min and 72˚C for 1 min with a final extension at 72˚C for 10 min. After confirmation of the amplified product of the expected size of 199 bp on agarose gel, the PCR products were digested with 5 units of restriction enzyme BstUI (New England Biolabs, Baverly, MA) at 60˚C for 16 hours. The digested products were electrophoresed through a 2.5% Agarose gel and stained with ethidium bromide. The Pro/Pro genotype is not cleaved by BstUI at codon 72 and has a single uncut band of 199 base pairs. The Arg/Arg genotype yields two small fragments of 113 bp and 86 bp. The heterozygote Arg/Pro genotype has three fragments of 199, 113 and 86 bp size. The RFLP results were confirmed by sequencing 10% of the randomly selected samples from both cases and controls in an automated DNA Sequencer (ABI Prism 3130 xl Genetic Analyser, CA, USA) (Figure 2).

Statistical analysis

Univariate and multiple logistic regressions were used

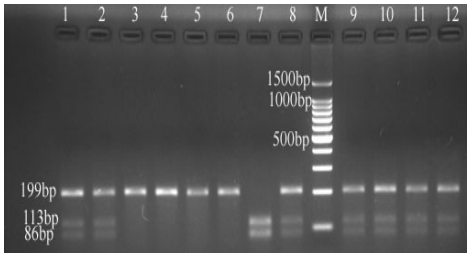

Figure 1. Agarose Gel Stained with Ethidium Bromide Showing p53 Codon 72 Polymorphism. Lane M=100 bp DNA ladder; Lane 1, 2, 8, 9, 10, 11, 12=Heterozygote *Arg/Pro* genotype; Lane 3, 4, 5, 6=Homozygote *Pro/Pro* genotype; Lane 7=Homozygote wild type *Arg/Arg* genotype

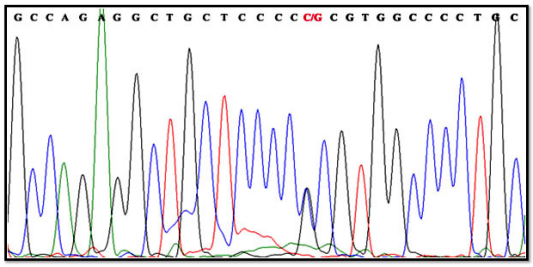

Figure 2. Representative Heterozygote *Arg/Pro* Genotype of p53 Codon 72 Polymorphism Confirmed by Sequencing. The **Red Letter** represents the polymorphic site

for data analysis. The conditional maximum likelihood method (Breslow et al., 1980) was used to estimate the parameters of regression model, because of the matched study design and significance was taken at p≤0.05 (two tailed). Initially, the univariate analysis was carried out. The crude measure of association between single putative risk factor and stomach cancer was expressed as odds ratio (OR) and its 95% confidence interval (95%CI) was calculated from the standard error (SE) of the regression co-efficient. To control for the confounding variables such as smoking, tuibur habit, alcohol consumption, different dietary habits like fish, meat consumption etc., the data were analysed by conditional multiple logistic regression. Odds ratio (OR) with 95%CIs were used to assess the strength of association between the p53 Arg72Pro polymorphism and stomach cancer risk. For this, taking Arg/Arg homozygote as reference, the Arg/Pro and Pro/Pro genotype risk on stomach cancer was examined. The dominant (Arg/Arg *vs* Arg/Pro+Pro/Pro) and recessive (Pro/Pro *vs* Arg/Arg+Arg/Pro) effects of the variant Pro/Pro allele (Zhang et al., 2010; Francisco et al., 2011; He et al., 2011; Weng et al., 2012) were also determined. For interactions study, tests were performed by making possible combinations for each p53 genotype with all the considered co-variables, and the univariate crude OR and multivariate adjusted OR were calculated. The Hardy-Weinberg Equilibrium (HWE) was used to test for linkage disequilibrium. The statistical packages used for the analysis are Epi-Info version-7 (CDC, Atlanta) and SPSS version 17.0 (SPSS Inc. Chicago, USA).

Results

Table 1 summarizes the demographic characteristics

Table 1. Demographic Characteristics and p53 Codon 72 Genotypes Distribution in Stomach Cancer Cases and Controls

Variables		Categories		Cases, n (%)		Controls, n (%)	
Sex		Male		81 (77.1)		162 (77.1)	
		Female		24 (22.9)		48 (22.9)	
		Total		105		210	
Age (years)		≤60		57 (54.3)		102 (48.6)	
		>60		48 (45.7)		108 (51.4)	
Characteristics of Study Subjects		Arg/Arg no. (%)		Arg/Pro no. (%)		Pro/Pro no. (%)	
Stomach cancer patients		11 (10.5)		56 (53.3)		38 (36.2)	
Control		36 (17.1)		110 (52.4)		64 (30.5)	
Total		47 (14.9)		166 (52.7)		102 (32.4)	
		Case	Control	Case	Control	Case	Control
Sex	Male (total =243 nos.)	9 (11.1)	27 (16.7)	44 (54.3)	77 (47.5)	28 (34.6)	58 (35.8)
	Female (total=72 nos.)	2 (8.3)	9 (18.8)	12 (50.0)	33 (68.8)	10 (41.7)	6 (12.5)
Age (year)	≤60 (total=159 nos.)	8 (14.0)	19 (18.6)	31 (54.4)	50 (49.0)	18 (31.6)	33 (32.4)
	>60 (total=156 nos.)	3 (6.2)	17 (15.7)	25 (52.1)	60 (55.6)	20 (41.7)	31 (28.7)

Table 2. p53 Codon 72 Genotypes, Tobacco & Different Dietary Habits and Risk of Stomach Cancer in Mizoram, India

Characteristics	Categories	Cases, n (%)	Controls, n (%)	Univariate OR* (95 % CI)	p-value	Multivariate Adjusted OR (95 % CI)	p value
p53 Genotype	Arg/Arg	11 (10.5)	36 (17.1)	1.0 (ref)		1.0 (ref)	
	Arg/Pro	56 (53.3)	110 (52.4)	1.71 (0.81-3.63)	0.16	1.88[a] (0.78-4.52)	0.16
	Pro/Pro	38 (36.2)	64 (30.5)	2.04 (0.90-4.62)	0.08	2.54[a] (1.01-6.40)	0.04
Tobacco Smoking	Non-Smoker	21 (20.0)	85 (40.5)	1.0 (ref)		1.0 (ref)	
	Smoker	84 (80.0)	125 (59.5)	2.90 (1.63-5.17)	<0.01	2.89[b] (1.47-5.67)	<0.01
Tuibur habit	Non-User	81 (77.1)	177 (84.3)	1.0 (ref)		1.0 (ref)	
	User	24 (22.9)	33 (15.7)	1.64 (0.89-3.03)	0.11	2.68[c] (1.27-5.66)	<0.01
Smoke dried fish taking habit	No	8 (7.6)	67 (31.9)	1.0 (ref)		1.0 (ref)	
	Yes	97 (92.4)	143 (68.1)	6.39 (2.82-14.47)	<0.01	4.85[d] (1.91-12.31)	<0.01
Preserved meat (smoked/sun dried)	No	5 (5.7)	44 (21.0)	1.0 (ref)		1.0 (ref)	
consuming habit	Yes	99 (94.3)	166 (79.0)	5.52 (2.09-14.62)	<0.01	4.22[e] (1.46-12.21)	<0.01
Fermented pork fat (sa-um)[#]	No	8 (7.6)	35 (16.7)	1.0 (ref)		1.0 (ref)	
taking habit	Yes	97 (92.4)	175 (83.3)	2.48 (1.10-5.60)	0.03	1.84[f] (0.54-6.28)	0.33
Preserved bamboo shoot taking habit	No	7 (6.9)	26 (12.4)	1.0 (ref)		1.0 (ref)	
	Yes	98 (93.3)	184 (87.6)	1.96 (0.83-4.66)	0.13	1.85[g] (0.65-5.31)	0.25
Fermented soya-bean (bekang)	No	10 (9.5)	39 (18.6)	1.0 (ref)		1.0 (ref)	
[¥]taking habit	Yes	95 (90.5)	171 (81.4)	2.22 (1.04-4.74)	0.04	1.03[h] (0.35-3.08)	0.96

*OR: Univariate odds ratio matched for age, sex and ethnicity; OR[a]: Adjusted for tobacco smoking, tuibur, smoked fish, preserved meat, sa-um, bamboo shoot & bekang consuming habit; OR[b]: Adjusted for p53 codon 72 genotypes, tuibur, smoked fish, preserved meat, sa-um, bamboo shoot & bekang consuming habit; OR[c]: Adjusted for p53 codon 72 genotypes, tobacco smoking, smoked fish, preserved meat, sa-um, bamboo shoot & bekang consuming habit; OR[d]: Adjusted for p53 codon 72 genotypes, tobacco smoking, preserved meat, tuibur, sa-um, bamboo shoot & bekang consuming habit; OR[e]: Adjusted for p53 codon 72 genotypes, tobacco smoking, tuibur, smoked fish, sa-um, bamboo shoot & bekang consuming habit; OR[f]: Adjusted for p53 codon 72 genotypes, tobacco smoking, tuibur, smoked fish, preserved meat, bamboo shoot & bekang consuming habit; OR[g]: Adjusted for p53 codon 72 genotypes, tobacco smoking, tuibur, smoked fish, preserved meat, sa-um & bekang consuming habit; OR[h]: Adjusted for p53 codon 72 genotypes, tobacco smoking, tuibur, smoked fish, preserved meat, sa-um & bamboo shoots consuming habit; [#]Sa-um: Fermented pork fat; [¥]Bekang: Fermented Soya bean; Allele probabilities for stomach cancer cases: Arg: 0.37; Pro: 0.63; p-value for HWE: 0.14; Allele probabilities for stomach cancer Controls: Arg: 0.43; Pro: 0.57; p-value for HWE: 0.33

and distribution of p53 codon 72 genotypes in cases and controls. The genotype distribution of all the subjects were 47 Arg/Arg (14.9%), 166 Arg/Pro (52.7%) and 102 Pro/Pro (32.4%). The frequencies of Arg/Arg, Arg/Pro and Pro/Pro genotypes were 10.5%, 53.3% and 36.2% in stomach cancer cases and 17.1%, 52.4% and 30.5% in controls (Table 1). The Pro/Pro genotype demonstrated to be significantly associated with two times higher risk of stomach cancer in multiple logistic regression analysis (OR, 2.54; 95%CI 1.01-6.40) (Table 2). Tobacco smokers showed significantly increased risk of stomach cancer (OR, 2.89; 95%CI, 1.47-5.67) as already reported earlier (Phukan et al., 2005; Malakar et al., 2012). Among different dietary habits tested, consumption of smoke dried fish and preserved meat (smoke dried/ sun dried) appeared to be associated with high risk of stomach cancer (OR, 4.85; 95%CI, 1.91-12.3 and OR, 4.22; 95%CI, 1.46-12.2, respectively) in Mizoram (Table 2). The fermented pork fat (sa-um) and the fermented soyabean (bekang) consuming habits appeared to increase the risk of stomach cancer in univariate analysis (OR, 2.48; 95%CI 1.10-5.60 and OR, 2.22; 95%CI 1.04-4.74, respectively), but not in

Table 3. Interactions between p53 codon 72 Genotypes & different Risk Habits on Risk of Stomach Cancer in Mizoram

Interactions	Cases, n (%)	Controls, n (%)	Univariate OR# (95% CI)	p value	Multivariate OR# (95% CI)	p value
Tobacco smoking[a]						
Arg/Arg X Non-Smoker	1 (1.0)	14 (6.7)	1.0 (ref)		1.0 (ref)	
Arg/Arg X Smoker	10 (9.5)	22 (10.5)	7.10 (0.82-61.6)	0.07	6.58 (0.69-62.4)	0.1
Arg/Pro X Non-Smoker	13 (12.4)	47 (22.4)	4.10 (0.49-34.2)	0.19	4.45 (0.49-40.5)	0.18
Arg/Pro X Smoker	43 (41.0)	63 (30.0)	10.38 (1.32-81.8)	0.03	9.45 (1.09-81.7)	0.04
Pro/Pro X Non-Smoker	7 (6.7)	24 (11.4)	4.36 (0.47-40.1)	0.19	3.85 (0.38-39.2)	0.25
Pro/Pro X Smoker	31 (29.5)	40 (19.0)	13.79 (1.64-115.9)	0.02	16.26 (1.72-153.4)	0.01
Tuibur habit[b]						
Arg/Arg X Non-User	10 (9.5)	29 (13.8)	1.0 (ref)		1.0 (ref)	
Arg/Arg X User	1 (1.0)	7 (3.3)	0.48 (0.05-4.47)	0.51	0.24 (0.01-4.02)	0.32
Arg/Pro X Non-User	41 (39.0)	96 (45.7)	1.34 (0.59-3.05)	0.48	1.20 (0.47-3.09)	0.7
Arg/Pro X User	15 (14.3)	14 (6.7)	3.31 (1.15-9.52)	0.03	4.67 (1.35-16.2)	0.01
Pro/Pro X Non-User	30 (28.6)	52 (24.8)	1.84 (0.76-4.47)	0.17	1.70 (0.62-4.65)	0.29
Pro/Pro X User	8 (7.6)	12 (5.7)	2.38 (0.68-8.29)	0.17	4.04 (0.91-17.9)	0.07
Smoke dried fish taking habit[c]						
Arg/Arg X Non-User	1 (1.0)	11 (5.2)	1.0 (ref)		1.0 (ref)	
Arg/Arg X User	10 (9.5)	25 (11.9)	4.40 (0.50-38.3)	0.18	3.07 (0.27-34.9)	0.37
Arg/Pro X Non-User	3 (2.9)	32 (15.2)	0.80 (0.07-9.77)	0.85	0.58 (0.04-9.05)	0.69
Arg/Pro X User	53 (50.5)	78 (37.1)	9.07 (1.10-74.8)	0.04	6.55 (0.64-67.4)	0.11
Pro/Pro X Non-User	4 (3.8)	24 (11.4)	2.10 (0.20-22.0)	0.54	2.56 (0.19-34.3)	0.48
Pro/Pro X User	34 (32.4)	40 (19.0)	10.69 (1.24-91.8)	0.03	7.84 (0.72-85.7)	0.09
Preserved meat (smoked/sun dried) consuming habit[d]						
Arg/Arg X Non-User	1 (1.0)	5 (2.4)	1.0 (ref)		1.0 (ref)	
Arg/Arg X User	10 (9.5)	31 (14.8)	3.53 (0.27-46.4)	0.34	1.17 (0.08-17.8)	0.91
Arg/Pro X Non-User	4 (3.8)	23 (11.0)	1.63 (0.13-20.3)	0.7	1.00 (0.07-13.7)	0.99
Arg/Pro X User	52 (49.5)	87 (41.4)	6.40 (0.52-79.2)	0.15	2.21 (0.16-30.9)	0.56
Pro/Pro X Non-User	2 (1.9)	16 (7.6)	1.22 (0.07-21.3)	0.89	0.52 (0.02-11.0)	0.67
Pro/Pro X User	36 (34.3)	48 (22.9)	7.95 (0.63-100.0)	0.11	3.40 (0.25-46.8)	0.36
Fermented pork fat (sa-um)[#]taking habit[e]						
Arg/Arg X Non-User	1 (1.0)	10 (4.8)	1.0 (ref)		1.0 (ref)	
Arg/Arg X User	10 (9.5)	26 (12.4)	6.48 (0.55-76.0)	0.14	10.04 (0.28-362.4)	0.21
Arg/Pro X Non-User	2 (1.9)	12 (5.7)	2.08 (0.17-26.0)	0.57	6.95 (0.26-187.7)	0.25
Arg/Pro X User	54 (51.4)	98 (46.7)	9.14 (0.85-98.5)	0.07	16.11 (0.49-532.0)	0.12
Pro/Pro X Non-User	6 (5.7)	13 (6.2)	8.02 (0.61-105.1)	0.11	14.76 (0.44-490.5)	0.13
Pro/Pro X User	32 (30.5)	51 (24.3)	10.86 (0.97-120.4)	0.06	20.89 (0.61-717.5)	0.09
Preserved bamboo shoot taking habit[f]						
Arg/Arg X Non-User	2 (1.9)	8 (3.8)	1.0 (ref)		1.0 (ref)	
Arg/Arg X User	9 (8.6)	28 (13.3)	1.23 (0.22-6.90)	0.81	0.59 (0.08-4.43)	0.6
Arg/Pro X Non-User	2 (1.9)	9 (4.3)	0.89 (0.10-7.63)	0.92	0.72 (0.06-8.71)	0.8
Arg/Pro X User	54 (51.4)	101 (48.1)	2.07 (0.43-9.98)	0.36	1.28 (0.20-8.19)	0.79
Pro/Pro X Non-User	3 (2.9)	9 (4.3)	1.42 (0.20-10.4)	0.73	0.55 (0.05-6.37)	0.63
Pro/Pro X User	35 (33.3)	55 (26.2)	2.48 (0.51-12.1)	0.26	1.86 (0.30-11.6)	0.51
Fermented soya-bean (bekang)[¥]taking habit[g]						
Arg/Arg X Non-User	1 (1.0)	11 (5.2)	1.0 (ref)		1.0 (ref)	
Arg/Arg X User	10 (9.5)	25 (11.9)	4.84 (0.54-43.6)	0.16	4.29 (0.18-101.2)	0.37
Arg/Pro X Non-User	4 (3.8)	16 (7.6)	3.07 (0.30-31.2)	0.34	7.32 (0.28-192.3)	0.23
Arg/Pro X User	52 (49.5)	94 (44.8)	6.61 (0.82-53.5)	0.07	6.85 (0.32-148.6)	0.22
Pro/Pro X Non-User	5 (4.8)	12 (5.7)	4.97 (0.50-49.8)	0.17	11.92 (0.44-325.1)	0.14
Pro/Pro X User	33 (31.4)	52 (24.8)	7.73 (0.93-64.4)	0.06	9.10 (0.41-201.9)	0.16

[a]OR* : Adjusted for tuibur habit, smoked fish, preserved meat, sa-um, bamboo shoot and bekang consuming habit; [b]OR*: Adjusted for tobacco smoking, smoked fish, preserved meat, sa-um, bamboo shoot and bekang consuming habit; [c]OR*: Adjusted for tobacco smoking, tuibur habit, preserved meat, sa-um, bamboo shoot and bekang consuming habit; [d]OR*: Adjusted for tobacco smoking, tuibur habit, smoked fish, sa-um, bamboo shoot and bekang consuming habit; [e]OR*: Adjusted for tobacco smoking, tuibur habit, smoked fish, preserved meat, bamboo shoot and bekang consuming habit; [f]OR*: Adjusted for tobacco smoking, tuibur habit, smoked fish, preserved meat, sa-um and bekang consuming habit; [g]OR*: Adjusted for tobacco smoking, tuibur habit, smoked fish, preserved meat, sa-um and bamboo shoot consuming habit; [#]:Sa-um: Fermented pork fat; [¥]:Bekang: Fermented Soya bean; OR[#]:Univariate odds ratio matched for age, sex and ethnicity.

multivariate model (Table 2).

Interaction analysis of p53 codon 72 genotypes with risk habits (Table 3) revealed that tobacco smokers are at higher risk of stomach cancer than the non-smokers in all the three genotypes, viz., Arg/Arg, Arg/Pro and Pro/Pro. The significant highest risk association was seen with the combinations of smokers with Pro/Pro genotype (OR, 16.2; 95%CI 1.72-153.4) followed by smokers with Arg/Pro genotype (OR, 9.45; 95%CI 1.09-81.7) as compared to the non-smokers carrying Arg/Arg genotype (Table 3). In case of interactions with tuibur habit, the Arg/Pro genotype demonstrated 4 fold increased risk of stomach cancer (OR, 4.67; 95%CI 1.35-16.2). Smoke dried fish consumers belonging to Pro/Pro genotype and Arg/Pro genotypes had significantly increased risk (OR, 10.6; 95%CI 1.01-91.8 and OR, 9.07; 95%CI 1.10-74.8, respectively) than the Arg/Arg genotype in the univariate analysis (Table 3). The combinations with other dietary habits and p53 codon 72 genotypes did not show any significant interactions in multivariate adjusted model. However, the Pro/Pro genotype among consumers in all the tested dietary habits demonstrated a tendency of increased risk than the other two genotypes, i.e., Arg/pro and Arg/Arg.

In gender stratified analysis, the Pro/Pro genotype showed highly significant association (OR, 7.50; 95%CI, 1.20-47.0) with stomach cancer than the Arg/Arg genotype among females, but this association was not significant in male subjects (Table 4). Similarly, in age stratified analysis, the Pro/Pro genotype demonstrated higher risk of stomach cancer (OR, 6.30; 95%CI, 1.41-28.2) as compared to the Arg/Arg genotype in the older people (>60 years). However, no such associations were observed in individuals <60 years of age. No significant risk association was observed between p53 codon 72 genotypes and stomach cancer among *H. pylori* positive & negative individuals (Table 4). The stomach cancer cases and the controls were found to be within the Hardy-Weinberg Equilibrium (HWE) (Table 2). For the study subjects, the genotype distribution for the HWE assumption with allele frequencies are 0.37 (cases) and 0.43 (controls) for Arg alleles and 0.63 (cases) and 0.57 (controls) for Pro alleles. For dominant and recessive models of inheritance, no significant increase in risk of stomach cancer in terms of p53 codon 72 genotypes were observed (Table 5).

Table 4. Stratified Analysis of p53 codon 72 Genotypes & Risk of Stomach Cancer in Terms of Sex, Age and *H. pylori* Status

Genotypes /Groups	Cases, n (%)	Controls, n (%)	OR (95 % CI)	p-value
Among males				
Arg/Arg	9 (11.1)	27 (16.7)	1.0 (ref)	
Arg/Pro	44 (54.3)	77 (47.5)	1.71 (0.74-3.97)	0.21
Pro/Pro	28 (34.6)	58 (35.8)	1.45 (0.60-3.49)	0.41
Among females				
Arg/Arg	2 (8.3)	9 (18.8)	1.0 (ref)	
Arg/Pro	12 (50.0)	33 (68.8)	1.64 (0.31-8.68)	0.56
Pro/Pro	10 (41.7)	6 (12.5)	7.50 (1.20-47.0)	0.03
Among individuals of ≤60 years age				
Arg/Arg	8 (14.0)	19 (18.6)	1.0 (ref)	
Arg/Pro	31 (54.4)	50 (49.0)	1.47 (0.58-3.7)	0.42
Pro/Pro	18 (31.6)	33 (32.4)	1.30 (0.47-3.54)	0.61
Among individuals of >60 years age				
Arg/Arg	3 (6.2)	17 (15.7)	1.0 (ref)	
Arg/Pro	25 (52.1)	60 (55.6)	2.75 (0.71-10.6)	0.14
Pro/Pro	20 (41.7)	31 (28.7)	6.30 (1.41-28.2)	0.02
Among *H pylori* negative individuals				
Arg/Arg	3 (10.3)	7 (11.9)	1.0 (ref)	
Arg/Pro	15 (51.8)	34 (57.6)	1.03 (0.23-4.53)	1
Pro/Pro	11 (37.9)	18 (30.5)	1.43 (0.30-7.00)	0.65
Among *H pylori* positive individuals				
Arg/Arg	8 (11.0)	27 (20.0)	1.0 (ref)	
Arg/Pro	40 (54.8)	66 (48.9)	2.41 (0.84-6.93)	0.1
Pro/Pro	25 (34.2)	42 (31.1)	2.34 (0.72-7.60)	0.16

Table 5. Logistic Regression Model for Determining Effect of p53 Codon 72 Polymorphism on Stomach Cancer

Associations	Case/Control n (%)	OR (95% CI)	p value
Dominant Model			
Arg/Arg	11 (10.5)/36 (17.1)	1.0 (ref)	
Arg/Pro & Pro/Pro	94 (89.5)/174 (82.9)	1.77 (0.86-3.63)	0.12
Recessive Model			
Arg/Arg & Arg/Pro	67 (63.8)/146 (69.5)	1.0 (ref)	
Pro/Pro	38 (36.2)/64 (30.5)	1.29 (0.79-2.12)	0.31

Discussion

The Mizoram state has the highest rate of stomach cancer incidence in India (Rao et al., 1998; Phukan et al., 2004), where only a few studies have been carried out so far to detect the genetic (Ihsan et al., 2011; Malakar et al., 2012) and environmental risk factors associated with stomach cancer. Our study is the first population based matched case-control study to investigate p53 codon 72 polymorphism of stomach cancer in Mizoram state of India, which also considers gene-environment interactions along with dietary habits and *H. pylori* infection status.

Our study reveals high Pro allele frequency in both cases and controls. A meta-analysis (Xiang et al., 2012) reveals a wide variation in the Pro allele frequencies in control groups of different ethnic and geographical regions ranging from 0.25 to 0.54 (Wu et al., 2004; De Feo et al., 2009). Our study also shows that the Pro/Pro genotype is distributed more in cases than in controls. However, this distribution of Pro/Pro genotype is quite high in comparison to most of the ethnic groups of Indian studies, although Sameer et al., (2010) reported higher distribution (45.4%) of Pro/Pro genotype in colorectal cancer cases in Kashmir. Four studies from China (Mu et al., 2005; Cai et al., 2006; Yang et al., 2007; Shao et al., 2008) also reported higher distribution of homozygous Pro genotypes in stomach and oesophageal cancer cases ranging from 26.4% to 36.3%. Meta-analysis of Zhou et al., (2007) reported a significant lower frequency of Arg allele in Asian gastric cancer patients. In our study, it was found that the Pro/Pro genotype carriers are at two time's higher risk of stomach cancer than the Arg/Arg genotype carriers in Mizoram. The stratified analysis performed in our study revealed high risk associations of the Pro/Pro genotypes and stomach cancer among the females and the older age people. A recent meta-analysis also

provides evidence that the Pro allele increases the risk of gastric cancer in Asians (Xiang et al., 2012). Gao et al., (2009) demonstrated that Pro/Pro genotype was associated with increased risk of diffuse type gastric cancer among Asians, but decreased risk of intestinal gastric cancer among Caucasians. Several independent studies reported that homozygous individuals with Pro are more likely to develop gastric cancer (Hiyama et al., 2002; Sul et al., 2006), lung cancer (Birgander et al., 1996; Wang et al., 1999), breast cancer (Sjalander et al., 1996; Papadakis et al., 2000), bladder cancer (Pandith et al., 2010). However, these results are not consistent, as many studies reported differently. Zang et al., (2003) reported that Arg/Arg genotype is associated with high risk of gastric cancer. Ke-Xiang et al., (2012) in a Chinese population showed that Arg/Pro+Pro/Pro genotypes increased risk of gastric cancer. No significant association between p53 alleles and gastrointestinal cancers was reported by Mojtahedi et al., (2010).

In our previous two reports (Phukan et al., 2005; Malakar et al., 2012), we have reported that tobacco smoking is a significant risk factor for stomach cancer in Mizoram, which is consistent with several cohort and case-control studies (Hansson et al., 1994; Tredaniel et al., 1997; Russo et al., 2001; Garcia-Gonzalez et al., 2012), where they reported it from different localities across the globe. Although tuibur independently fails to attain the statistical significance in the univariate model in our current study, it found to be modulating the risk of stomach cancer when practiced with other dietary and tobacco related risk habits as reported in our previous study (Malakar et al., 2012).

Apart from tobacco related habits, we have also assessed the risk of some important dietary habits in terms of stomach cancer in our study. Out of these, the smoke dried fish and preserved meat (smoke dried / sun dried) taking habits appeared to be highly associated risk factors for stomach cancer in Mizoram, which is consistent with our previous report (Phukan et al., 2006). Smoke-drying and preservation leads to formation of N-nitroso compounds which are animal and human carcinogens (Correa et al., 1992). Earlier, many epidemiological studies from India (Siddiqi et al., 1992; Rao et al., 2002; Phukan et al., 2006; Sumathi et al., 2009) have reported positive association of dietary items containing substantial amount of N-nitroso compounds with stomach cancer.

The gene environment interaction study between p53 codon 72 genotypes and important risk habits showed that combinations of tobacco smokers with Pro/Pro and Arg/Pro genotypes are at higher risk of stomach cancer than the non-smokers carrying Arg/Arg genotype. Tuibur users belonging to Arg/Pro genotype demonstrated increased risk of stomach cancer. The Pro/Pro genotype among consumers in all the tested dietary habits showed a tendency of increased risk association of stomach cancer than the other two genotypes, i.e., Arg/pro and Arg/Arg. Thus the results of our study demonstrate the existence of significant gene-environment interactions between p53 codon 72 polymorphisms and different dietary and tobacco habits on the increased risk of stomach cancer.

In conclusion, our study revealed that individuals belonging to Pro/Pro genotype of p53 codon 72 had increased risk of stomach cancer, particularly among the females and older people in Mizoram, India. Significant gene–environment interactions between p53 codon 72 polymorphisms and different tobacco and dietary risk habits of stomach cancer were also evident in this high risk population of Mizoram. Future studies with larger population and including investigations of important gene-gene and gene-environment interactions may provide a more conclusive picture.

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