RESEARCH ARTICLE

CYP2E1 Genetic Polymorphism with Dietary, Tobacco, Alcohol Habits, *H. pylori* Infection Status and Susceptibility to Stomach Cancer in Mizoram, India

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Abstract

Background: The incidence of stomach cancer in India is highest in the state of Mizoram. In this population based matched case-control study, we evaluated the relationship between *CYP450 2E1 RsaI* polymorphism and risk of stomach cancer taking into considering various important dietary habits along with tobacco, alcohol consumption and *H. pylori* infection status. Materials and Methods: A total of 105 histologically confirmed stomach cancer cases and 210 matched healthy population controls were recruited. *CYP2E1 RsaI* genotypes were determined by PCR-RFLP and *H. pylori* infection status by ELISA. Information on various dietary, tobacco and alcohol habits was recorded in a standard questionnaire. Results: Our study revealed no significant association between the *CYP2E1 RsaI* polymorphism and overall risk of stomach cancer in Mizoram. However, we observed a non-significant protective effect of the variant allele (A) of *CYP2E1* against stomach cancer. Tobacco smokers carrying C/C genotype have three times more risk of stomach cancer, as compared to non-smokers carrying C/C genotype. Both *Meiziol* and cigarette current and past smokers who smoked for more than 10 times per day and carrying the (C/C) genotype are more prone to develop stomach cancer. Smoke dried fish and preserved meat (smoked/sun dried) consumers carrying C/C genotype possesses higher risk of stomach cancer. No significant association between *H. pylori* infection and *CYP2E1 RsaI* polymorphism in terms of stomach cancer was observed. Conclusions: Although no direct association between the *CYP2E1 RsaI* polymorphism and stomach cancer was observed, relations with different tobacco and dietary risk habits in terms of developing stomach cancer exist in this high risk population of north-eastern part of India. Further in-depth study recruiting larger population is required to shed more light on this important problem.

Keywords: Stomach cancer - CYP 450 2E1 *RsaI* polymorphism - risk habits - Mizoram - India

Asian Pac J Cancer Prev, **15 (20)**, 8815-8822

Introduction

CThe 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 105 populations respectively (NCRP, 2010). The age-adjusted rates for only the Aizawl district of the Mizoram state are 55.4 and 24.4 per 105 populations in males and females respectively (NCRP, 2010). Stomach cancer is a multistep process involving association 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). Numerous studies have shown that consumption of alcohol, tobacco and different food habits are important risk factors of stomach cancer in addition to *Helicobacter pylori* infections (Correa, 1992; Russo et al., 2001; García-G et al., 2012). In earlier studies on risk factors of gastric cancer in Mizoram, it was found that peculiar food habits in this region might be associated with the high prevalence of stomach cancer in Mizoram along with tobacco use and other factors (Phukan et al., 2005; 2006). 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). Subsequently, we reported the effect of genetic polymorphism of glutathione S-transferases M1 and T1 and codon 72 polymorphism of p53 gene and risk of stomach cancer in Mizoram taking

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into account dietary and tobacco related habits (Malakar et al., 2012, 2014). In this study, we report the effect of *CYP2E1* gene polymorphism taking into consideration tobacco use, alcohol consumption, dietary habits, and *Helicobacter pylori* status on risk of gastric cancer in the state of Mizoram.

Cytochrome P450 2E1, a member of the cytochrome P450 mixed-function oxidase system, is involved in the metabolism of xenobiotics in the body. In human, the *CYP2E1* enzyme is encoded by the *CYP2E1* gene (Kolble et al., 1993). The cytochrome P450 proteins are mono-oxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. The enzyme metabolizes both endogenous substrates, such as ethanol, acetone, and acetal, as well as exogenous substrates including benzene, carbon tetrachloride, ethylene glycol, and nitrosamines which are pre-mutagens found in cigarette smoke (RefSeq, 2008). N-Nitrosamines present in tobacco and diet are well-recognized as carcinogens involved in cancer development in various sites, including the oesophagus and stomach (IARC, 1993; 2000). Functional *CYP2E1* gene polymorphisms might therefore impact on susceptibility for cancers, for which a role is suspected for etiological agents such as N nitrosamines (Gao et al., 2002). *CYP2E1* is strongly suspected of candidates for gastric cancer susceptibility markers interacting with the environmental chemicals (Camus et al., 1993) and possibly generating DNA-damaging agent(s). Molecular cloning and the discovery of polymorphism in the regulatory region of the *CYP2E1* gene has tempted many investigators to test the hypothesis that the *CYP2E1* genotype is related to its metabolic power, and in turn, to individual susceptibility to human cancer. The results of such studies, however, have not been consistent and ethnic difference has been reported (Kato et al., 1992).

In this population based matched case-control study, we have evaluated the relationship between the *CYP2E1* gene polymorphism and stomach cancer risk in a high incidence area considering various important dietary habits along with tobacco & alcohol habits and *H. pylori* infection status simultaneously for the first time in Mizoram state in the north eastern part of India.

Materials and Methods

Study subjects

This population based matched case-control study was 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 -80ºC 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), 400µM 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'-TCGTCAGTTCCTGAAAGCAGG-3' and 5'-

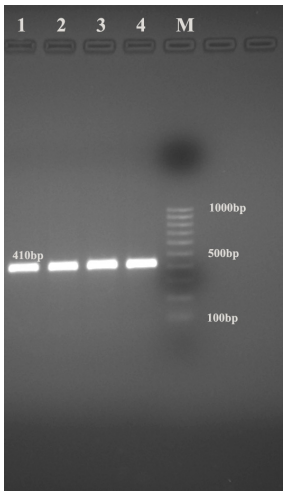

Figure 1. Agarose Gel Stained with EtBr Showing 410 bp *CYP2E1* Gene PCR Product. Lane M=100 bp DNA ladder; Lane 1, 2, 3, 4 =*CYP2E1* gene PCR product

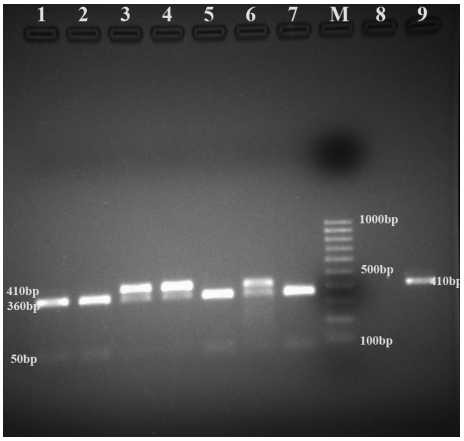

Figure 2. PCR-RFLP Product in Agarose Gel Stained with EtBr Showing *CYP2E1* Gene *RsaI* Polymorphism. Lane M =100 bp DNA ladder; Lane 1, 2, 5, 7 = Homozygous C/C genotype; Lane 3, 4, 6 = Heterozygous C/A genotype; Lane 9 = Homozygous A/A genotype; Lane 8 = Blank

GAGCTCTGATGCAAGTATCGCA-3' which produced a 410 base-pair band (Kato et al., 1994; Nishimoto et al., 2000). The PCR amplifications were carried out in Applied Biosystems Thermal Cycler. The PCR cycles were 94°C for 4 min, followed by 45 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min with a final extension at 72°C for 7 min. After confirmation of the amplified product of the expected size of 410 bp on agarose gel (Figure 1), the PCR products were digested with 5 units of restriction enzyme *RsaI* (Fermentus product) at 37°C for 8 hours. The digested products were electrophoresed through a 2.5% Agarose gel and stained with ethidium bromide and documented in Gel-Doc. The *RsaI*-site-homozygously-absent individuals have a single 410-bp band (A allele homozygous, A/A), whereas *RsaI*-site homozygote have two smaller sized band viz., 360-bp and 50-bp (homozygous type C/C genotype) (Figure 2). Heterozygous cases have three bands viz., 410-bp, 360-bp and 50-bp (heterozygous genotype C/A (Figure 2).

*Detection of **Helicobacter pylori** infection*

Indirect IgG ELISA (BIO-RAD) was used to detect antibodies against *Helicobacter pylori* in serum of both cases and controls.

Statistical analysis

Univariate and multiple logistic regressions were used 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 $p \leq 0.05$ (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 *CYP2E1* polymorphism and stomach cancer risk. For this, taking (C/C) genotype as reference, the (C/A) and (A/A) genotype risk on stomach cancer was determined.

The dominant (C/C *vs*. C/A + A/A) and recessive (A/A *vs*. C/C + C/A) effects of the variant (A/A) allele were also determined. For interactions study, tests were performed by making possible combinations for each *CYP2E1 RsaI* 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

The *CYP2E1* genotype distribution in all the subjects were 275 (C/C) (87.3%), 39 (C/A) (12.4%) and 1 (A/A) (0.3%). The frequencies of (C/C), (C/A) and (A/A) genotypes were 93 (88.5%), 11 (10.5%) and 1 (1.0%) in stomach cancer cases and 182 (86.7%), 28 (13.3%) and 0 (0%) in controls respectively. The merged frequencies of (C/A or A/A) genotypes in cases and controls are 12 (11.4%) and 28 (13.3%) respectively.

Table-1 shows the frequency distribution of *CYP2E1 RsaI* genotypes and estimated ORs for gastric cancer. There were very few patients in the homozygous variant allele (A/A) group, and, therefore, by combining the prevalence of heterozygotes (C/A) and homozygotes (A/A) for the variant genotype group (C/A or A/A), the comparison was made. Taking genotype C/C as reference, the ORs for stomach cancer of heterozygous genotype (C/A) and grouped genotype (C/A or A/A) has been

Table 1. Association of *CYP2E1* Gene *RsaI* Polymorphism with Tobacco and Alcohol Habits and Risk of Stomach Cancer in Mizoram

Interactions	Cases, n (%)	Controls, n (%)	Univariate OR[#] (95% CI)	p-value	Multivariate OR (95% CI)	p-value
CYP2E1 genotype[a]						
C/C	93 (88.5)	182 (86.7)	1.0 (ref)		1.0 (ref)	
C/A	11 (10.5)	28 (13.3)	0.77 (0.37-1.61)	0.48	0.62[a] (0.29-1.35)	0.23
A/A	1 (1.0)	0 (0.0)	not done		not done	
C/A or A/A	12 (11.5)	28 (13.3)	0.84 (0.41-1.73)	0.63	0.66[a] (0.31-1.41)	0.29
Tobacco smoking[b]						
(C/C) X Non-Smoker	20 (19.0)	77 (36.7)	1.0 (ref)		1.0 (ref)	
(C/C) X Smoker	73 (69.5)	105 (50.0)	2.92 (1.60-5.35)	<0.01	3.11[b] (1.67-5.81)	<0.01
(C/A or A/A) X Non-Smoker	8 (3.8)	1 (1.0)	0.42 (0.05-3.49)	0.42	0.28[b] (0.03-2.51)	0.26
(C/A or A/A) X Smoker	20 (9.5)	11 (10.5)	2.24 (0.94-5.35)	0.07	2.07[b] (0.85-5.05)	0.11
Tuibur habit[c]						
(C/C)X Non-User	75 (71.4)	157 (74.8)	1.0 (ref)		1.0 (ref)	
(C/C) X User	18 (17.1)	25 (11.9)	1.59 (0.79-3.22)	0.2	1.78[c] (0.85-3.71)	0.12
(C/A or A/A) X Non-User	6 (5.7)	20 (9.5)	0.63 (0.25-1.60)	0.33	0.50[c] (0.19-1.35)	0.17
(C/A or A/A) X User	6 (5.7)	8 (3.8)	1.59 (0.55-4.63)	0.4	1.36[c] (0.45-4.12)	0.59
Alcohol habit[d]						
(C/C)X Non-Drinker	51 (48.6)	109 (51.9)	1.0 (ref)		1.0 (ref)	
(C/C) X Drinker	42 (40.0)	73 (34.8)	1.24 (0.66-2.35)	0.51	0.93[d] (0.47-1.84)	0.82
(C/A or A/A) X Non-Drinker	8 (7.6)	12 (5.7)	1.40 (0.56-3.50)	0.47	0.92[d] (0.34-2.51)	0.87
(C/A or A/A) X Drinker	4 (3.8)	16 (7.6)	0.57 (0.17-1.93)	0.37	0.32[d] (0.09-1.18)	0.09

OR[#]: Univariate odds ratio matched for age, sex and ethnicity; OR[a]: Adjusted for tobacco smoking habit, tuibur habit and alcohol habit; OR[b]: Adjusted for tuibur habit and alcohol habit; OR[c]: Adjusted for tobacco smoking habit and alcohol habit; OR[d]: Adjusted for tobacco smoking habit and tuibur habit

shown. The multivariate adjusted OR for C/A genotype was 0.62 (95%CI 0.29-1.35) and for grouped genotype (C/A or A/A) was 0.66 (95%CI 0.31-1.41).

Table-1 also shows risk of stomach cancer based on *CYP2E1 RsaI* polymorphism taking into consideration tobacco use and alcohol consumption. Taking non-smoker C/C genotype group as reference, the tobacco smokers belonging to C/C genotype demonstrated to be significantly associated with three (3) times higher risk of stomach cancer in multiple logistic regression analysis (OR, 3.11; 95%CI, 1.67-5.81). The combined genotype group (C/A or A/A) among the tobacco smokers also demonstrated increased risk of stomach cancer, although it remains insignificant (OR, 2.07; 95%CI, 0.85-5.05). Similarly, a non-significant increased risk of stomach cancer was observed among the tuibur users carrying C/C genotype. Alcohol users did not show any significant effect of *CYP2E1 RsaI* polymorphism on stomach cancer.

Detailed analysis showing the association of *CYP2E1* genotypes with tobacco use and risk of stomach cancer has been presented in Table-2. Past smokers carrying both (C/C) and (C/A or A/A) genotype reveals increased risk of stomach cancer (OR, 3.16; 95%CI, 1.50-6.67 and OR, 7.17; 95%CI, 2.14-24.07 respectively) as compared to the non-smokers carrying (C/C) genotype. In case of the current smokers, the persons carrying (C/C) genotypes are at three times higher risk of stomach cancer (OR, 3.32; 95%CI, 1.69-6.54) than the non-smokers with (C/C) genotypes. Among different types of smoking practices, people carrying (C/C) genotype who smokes both *Meiziol* and cigarettes together showed highest association of the risk of stomach cancer (OR, 15.54; 95%CI, 4.50-53.66), followed by people belonging to the combined genotype (C/A or A/A) who smokes both cigarettes and *Meiziol* (OR, 13.17; 95%CI, 1.22-142.15), as compared to the non-smokers carrying (C/C) genotype. Among *Meiziol* smokers, people who carry (C/C) genotypes demonstrated three times higher risk of stomach cancer (OR, 3.75; 95%CI, 1.90-7.41) than the (C/C) genotype carrying non-smokers. Persons who smoke more than 10 times per day and carrying the (C/C) genotype showed four times significantly increased risk of stomach cancer (OR, 4.60; 95%CI, 2.30-9.17) than the non-smokers carrying (C/C) genotype. People who smoked for more than 30 years, carrying (C/C) genotype demonstrated more risk of suffering from stomach cancer (OR, 2.99; 95%CI, 1.51-5.90), than people who smoked for less than 30 years and carrying (C/C) genotype (OR, 2.09; 95%CI, 0.64-6.80), taking the non-smokers belonging to (C/C) genotype carriers as reference group. Persons carrying (C/C) genotype, who started smoking before attaining 15 years of age, are two times more prone to develop stomach cancer (OR, 4.12; 95%CI, 1.93-8.78) than those people carrying (C/C) genotype who started smoking after crossing 15 years of their age (OR, 2.37; 95%CI, 1.25-4.50). Persons belonging to the (C/C) genotype who had stopped smoking recently or within last 10 years are at significantly higher risk of stomach cancer (OR, 3.89;

Table 2. Association of *CYP2E1* Gene Polymorphism with Tobacco Smoking Habits and Risk of Stomach Cancer

Interactions	Cases, n (%)	Controls, n (%)	Univariate OR# (95% CI)	p-value
Smoking Status				
(C/C)X Non-Smoker	20 (19.0)	76 (36.2)	1.0 (ref)	
(C/A or A/A) X Non-Smoker	1 (1.0)	8 (3.8)	0.40 (0.05-3.33)	0.39
(C/C) X Past Smoker	26 (24.8)	39 (18.6)	3.16 (1.50-6.67)	<0.01
(C/A or A/A) X Past Smoker	8 (7.6)	6 (2.9)	7.17 (2.14-24.07)	<0.01
(C/C) X Current Smoker	47 (44.8)	67 (31.9)	3.32 (1.69-6.54)	<0.01
(C/A or A/A) X Current Smoker	3 (2.9)	14 (6.7)	0.74 (0.20-2.76)	0.65
Smoking Types				
(C/C)X Cigarette Smoker	10 (9.5)	46 (21.9)	0.75 (0.29-1.93)	0.55
(C/A or A/A) X Cigarette Smoker	1 (1.0)	7 (3.3)	0.47 (0.05-4.18)	0.5
(C/C) X *Meiziol* Smoker	48 (45.7)	55 (26.2)	3.75 (1.90-7.41)	<0.01
(C/A or A/A) X *Meiziol* Smoker	7 (6.7)	12 (5.7)	2.18 (0.74-6.39)	0.16
(C/C) X (Cigarette + *Meiziol*) Smoker	15 (14.3)	5 (2.4)	15.54 (4.50-53.66)	0
(C/A or A/A) X (Cigarette + *Meiziol*) Smoker	3 (2.9)	1 (0.5)	13.17 (1.22-142.15)	0.03
Smoking Frequency/ Day				
(C/C)X (≤10)	14 (13.3)	44 (21.0)	1.39 (0.63-3.07)	0.42
(C/A or A/A) X (≤10)	3 (2.9)	8 (3.8)	1.29 (0.32-5.23)	0.72
(C/C) X (>10)	59 (56.2)	62 (29.5)	4.60 (2.30-9.17)	0
(C/A or A/A) X (>10)	8 (7.6)	12 (5.7)	2.86 (0.96-8.45)	0.58
Smoking Duration (in Years)				
(C/C)X (≤30)	38 (36.2)	59 (28.1)	2.78 (1.36-5.69)	<0.01
(C/A or A/A) X (≤30)	6 (5.7)	11 (5.2)	2.36 (0.75-7.45)	0.14
(C/C) X (>30)	35 (33.3)	47 (22.4)	2.99 (1.51-5.90)	<0.01
(C/A or A/A) X (>30)	5 (4.8)	9 (4.3)	2.09 (0.64-6.80)	0.22
Smoking age started (in years)				
(C/C)X (≤15)	26 (24.8)	25 (11.9)	4.12 (1.93-8.78)	<0.01
(C/A or A/A) X (≤15)	1 (1.0)	5 (2.4)	0.95 (0.10-8.73)	0.96
(C/C) X (>15)	47 (44.8)	81 (38.6)	2.37 (1.25-4.50)	<0.01
(C/A or A/A) X (>15)	10 (9.5)	15 (7.1)	2.56 (1.01-6.47)	0.04
Year since stopped smoking				
(C/C)X (<10)	60 (57.1)	68 (32.4)	3.89 (2.03-7.46)	0
(C/A or A/A) X (<10)	8 (7.6)	14 (6.7)	2.49 (0.90-6.86)	0.08
(C/C) X (≥10)	13 (12.4)	38 (18.1)	1.30 (0.56-3.03)	0.54
(C/A or A/A) X (≥10)	3 (2.9)	6 (2.9)	2.29 (0.52-10.20)	0.28

OR#: Univariate odds ratio matched for age, sex and ethnicity

Table 3. Association of *CYP2E1* Gene *RsaI* Polymorphism with Various Uncommon Food Habits and Risk of Stomach Cancer in Mizoram

Interactions	Cases, n (%)	Controls, n (%)	Univariate OR[#] (95% CI)	p-value	Multivariate OR (95% CI)	p-value
Smoke dried fish taking habit[a]						
(C/C)X Non-Consumer	6 (5.7)	58 (27.6)	1.0 (ref)		1.0 (ref)	
(C/C) X Consumer	87 (82.9)	124 (59.0)	7.44 (3.03-18.32)	0	5.85[a] (2.28-15.02)	<0.01
(C/A or A/A) X Non-Consumer	2 (1.9)	9 (4.3)	1.76 (0.26-12.01)	0.56	2.58[a] (0.40-16.61)	0.32
(C/A or A/A) X Consumer	10 (9.5)	19 (9.0)	4.95 (1.64-14.96)	<0.01	3.86[a] (1.20-12.46)	0.02
Preserved meat (smoked/sun dried) consuming habit[b]						
(C/C)X Non-Consumer	6 (5.7)	38 (18.1)	1.0 (ref)		1.0 (ref)	
(C/C) X Consumer	87 (82.9)	144 (68.6)	4.74 (1.76-12.74)	<0.01	3.08[b] (1.10-8.68)	0.03
(C/A or A/A) X Non-Consumer	1 (1.0)	6 (2.9)	1.09 (0.10-11.61)	0.943	1.55[b] (0.13-19.17)	0.73
(C/A or A/A) X Consumer	11 (10.5)	22 (10.5)	4.05 (1.25-13.13)	0.01	2.39[b] (0.68-8.42)	0.18
Fermented pork fat (sa-um)[#] taking habit[c]						
(C/C)X Non-Consumer	9 (8.6)	29 (13.8)	1.0 (ref)		1.0 (ref)	
(C/C) X Consumer	84 (80.0)	153 (72.9)	1.86 (0.80-4.32)	0.15	1.12[c] (0.36-3.43)	0.85
(C/A or A/A) X Non-Consumer	1 (1.0)	6 (2.9)	0.59 (0.06-5.69)	0.65	0.44[c] (0.04-4.78)	0.5
(C/A or A/A) X Consumer	11 (10.5)	22 (10.5)	1.73 (0.60-5.03)	0.31	1.02[c] (0.26-3.99)	0.98
Preserved bamboo shoot taking habit[d]						
(C/C)X Non-Consumer	7 (6.7)	22 (10.5)	1.0 (ref)		1.0 (ref)	
(C/C) X Consumer	86 (81.9)	160 (76.2)	1.68 (0.69-4.12)	0.26	1.77[d] (0.64-4.86)	0.27
(C/A or A/A) X Non-Consumer	1 (1.0)	4 (1.9)	0.84 (0.08-8.96)	0.89	1.23[d] (0.08-18.34)	0.88
(C/A or A/A) X Consumer	11 (10.5)	24 (11.4)	1.46 (0.49-4.35)	0.5	1.41[d] (0.41-4.82)	0.59
Fermented soya-bean (bekang)[¥] taking habit[e]						
(C/C)X Non-Consumer	9 (8.6)	32 (15.2)	1.0 (ref)		1.0 (ref)	
(C/C) X Consumer	84 (80.0)	150 (71.4)	2.05 (0.92-4.60)	0.08	1.29[e] (0.45-3.68)	0.63
(C/A or A/A) X Non-Consumer	1 (1.0)	7 (3.3)	0.50 (0.06-4.50)	0.54	0.43[e] (0.04-4.62)	0.49
(C/A or A/A) X Consumer	11 (10.5)	21 (10.0)	1.90 (0.69-5.26)	0.21	1.19[e] (0.34-4.20)	0.79

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

95%CI, 2.03-7.46), than those people carrying (C/C) genotype, who quits smoking habit 10 years ago (OR, 1.30; 95%CI, 0.56-3.03).

Table-3 demonstrated risk of gastric cancer based on *CYP2E1 RsaI* polymorphism taking into consideration various uncommon food habits practiced routinely in Mizoram state. Among these, the smoke dried fish consumers carrying C/C genotype demonstrated to be significantly associated with five (5) times higher risk of stomach cancer (OR, 5.85; 95%CI, 2.28-15.02), in comparison with the non-consumers of the smoked dried fish carrying C/C genotype in a multiple logistic regression model after adjusting the confounding variables like preserved meat, Sa-um, bamboo shoot & bekang consuming habit. The smoke dried fish consumers belonging to the combined genotype group of (C/A or A/A) also showed three times higher risk of stomach cancer, as compared to the non-consumers belonging to the C/C genotype (OR, 3.86; 95%CI, 1.20-12.46). Similarly, the preserved meat (smoked or sun dried) consumers carrying the C/C genotype showed three times significantly higher risk of stomach cancer (OR, 3.08; 95%CI, 1.10-8.68), as compared to the non-consumers of preserved meat carrying C/C genotype. Preserved meat consumers belonging to the combined group of genotype (C/A or A/A) showed four times significantly increased risk of stomach cancer in the univariate analysis, but failed to attain the statistical significance in the adjusted multivariate model. In our study of examining the relationship between *CYP2E1 RsaI* polymorphism and fermented pork fat (Sa-um), preserved bamboo shoot & fermented soyabean (Bekang) consuming habits did not show any significant increase of the risk of stomach cancer in Mizoram state of India. However, a non-significant trend of increasing risk was observed among the C/C genotype carriers practicing these uncommon food habits, as compared to the C/C genotype carriers among non-consumers of these foods.

We have also performed the stratified analysis to check the association of *CYP2E1* gene *RsaI* polymorphism and risk of stomach cancer in terms of sex, age & *H. pylori* infection status. In gender stratified analysis, the combined genotype group (C/A or A/A) showed a protective effect on males against stomach cancer, although it was not significant (OR, 0.62; 95%CI, 0.26-1.48), as compared to the males carrying (C/C) genotype, but in females it was not found as protective. Similarly, in age stratified analysis, individuals of≤60 years of age belonging to the genotype group (C/A or A/A) demonstrated a non-significant protective effect against stomach cancer (OR, 0.46; 95%CI, 0.15-1.42), whereas in older people (>60 years), it was not protective. A non-significant reduced risk of stomach cancer was observed among *H. pylori* positive individuals belonging to the combined genotype group (C/A or A/A) (OR, 0.61; 95%CI, 0.25-1.52), as compared to the *H. pylori* positive individuals belonging to the genotype (C/C). In contrast, among *H. pylori* negative individuals belonging to the genotype (C/A or A/A), a non-significant increased risk of stomach cancer (OR, 2.25; 95%CI, 0.60-8.50) was observed, as compared to the *H. pylori* negative (C/C) genotype individuals.

The stomach cancer cases and the controls were found to be within the Hardy-Weinberg Equilibrium (HWE). For the study subjects, the genotype distribution for the HWE assumption with allele frequencies are 0.06 (cases) and 0.07 (controls) for A alleles and 0.94 (cases) and 0.93 (controls) for C alleles. For dominant model of inheritance, no significant increase in risk of stomach cancer in terms

of *CYP2E1 RsaI* genotypes was observed. However, for recessive model of inheritance, due to lack of sample in the homozygous variant allele group, the test could not be performed. A non-significant protective effect of the variant allele (A) of *CYP2E1* against stomach cancer in Mizoram was observed in our study.

Discussion

The people of Mizoram have very peculiar lifestyle and dietary habits as compared to other parts of the country, as they consumes many uncommon foods which includes smoked dried salted meat and fish, soda (alkali) & Saum (the traditional fermented food) etc. (Phukon et al., 2006). The extensive intake of various traditional tobacco products by the Mizo people is also a common practice. All these habits lead this population to a substantial exposure to various environmental and dietary risks of different types of cancers including stomach cancer (Phukon et al., 2005).

Although many studies around the world have been carried out so far to examine the association of *CYP2E1* polymorphism and various cancer risk including stomach cancer, the results are not consistent and ethnic differences has been reported (Wang et al., 2010; Ye et al., 2010; Balaji et al., 2011; Shahriary et al., 2012; Tian et al., 2012; Lakkakula et al., 2013). Few studies revealed a positive association between *CYP2E1 RsaI* polymorphism and stomach cancer risk (Wu et al., 2002; Boccia et al., 2007). Some other studies found no risk association of *CYP2E1* polymorphism and stomach cancer (Kato et al., 1995; Nishimoto et al., 2000; Park et al., 2003; Darazy et al., 2011; Zhuo et al., 2012). We have undertaken this study, as there is no report available so far from this region, which examines the association of *CYP2E1* polymorphism and stomach cancer risk considering various dietary, tobacco and alcohol habits, enabling also the study of the relationship between environment and *CYP2E1* gene *RsaI* polymorphism in terms of stomach cancer risk in Mizoram.

Our study revealed no association between the *CYP2E1 RsaI* polymorphism and overall risk of stomach cancer in Mizoram. However, we observed a non-significant protective effect of the variant allele (A) of *CYP2E1* against stomach cancer in our study. Earlier, many studies reported differently about the association of *CYP2E1* gene polymorphism and stomach cancer risk. Kato et al., (1995) did not find any association between *CYP2E1 RsaI* genotype and stomach cancer. Tsukino et al., (2002); Park et al., (2003) and Darazy et al., (2011) also found no such association. In another study, Kato et al., (2011) reported that *CYP2E1 RsaI* polymorphisms do not influence the development of primary stomach cancer, but may do so in specific conditions, such as the remnant stomach after gastrectomy. In a meta-analysis, Zhuo et al., (2012) did not find any significant associations of *CYP2E1 RsaI*/*PstI* polymorphisms with the gastric cancer risk. Masuda et al., (2007) reported that gastric cancer in patients younger than 40 years is closely associated with *H. pylori* infection, but not with genetic polymorphism of *CYP2E1*. However, Nishimoto et al., (2000) suggests that the *CYP2E1 RsaI* variant genotype is associated with a reduced risk of upper gastrointestinal tract cancers. Wu et al., (2002) found an association of gastric cancer with *CYP2E1 RsaI*, but not with *CYP2E1 DraI*. Earlier, in a meta-analysis, Boccia et al., (2007) concluded that *CYP2E1-PstI*/*RsaI* polymorphism may be a risk factor for gastric cancer in Asian populations.

A non-significant protective effect was observed for the heterozygous genotype (C/A) and grouped genotype (C/A or A/A) for stomach cancer in Mizoram, as compared to the homozygous genotype group (C/C). For *CYP2E1 RsaI* polymorphism, various studies reported differently across the globe about the associations of homozygote wild genotype, heterozygote and homozygote variant genotype and stomach cancer. Feng et al., (2012) revealed that for *CYP2E1 PstI*/*RsaI* polymorphism, C2C2 homozygotes and C2 carriers were associated with an increased risk of gastric cancer when compared with C1C1 homozygotes. Earlier Malik et al., (2009) found that *CYP2E1* C1C2 genotypes modulate the risk of gastric cancer in Kashmiri population of India. Gonzalez et al., (2004) reported that the polymorphism *CYP2E1 PstI* could be associated with a reduced risk of having gastric cancer in Costa Rican population.

Most of the earlier studies revealed inconsistent findings about associations between the *CYP2E1* polymorphism and various cancer risks. According to few studies, increased risk of oral (Bouchardy et al., 2000), pharyngeal (Bouchardy et al., 2000), oesophageal (Lin et al., 1998; Tan et al., 2000), colorectal (Saeed et al., 2013), liver (Yu et al., 1995), bladder (Deng et al., 2014) and lung (Wu et al., 1997; Le Marchand et al., 1998) cancers were observed in the common genotype or alleles (Changming et al., 2002). Increased risk of oral (Hung et al., 1997), nasopharyngeal (Hildesheim et al., 1997) and liver (Ladero et al., 1996) cancers were observed in rare genotype or allele carriers in another few studies. However, many other studies did not find any significant association between the *CYP2E1* polymorphisms and cancer risks of the oral cavity and pharynx (Matthias et al., 1998), oesophagus (Morita et al., 1997), stomach (Kato et al., 1995; Nishimoto et al., 2000), lung (Kato et al., 1992; Hirvonen et al., 1993; London et al., 1993; Persson et al., 1993), and bladder (Anwar et al., 1996).

Our study revealed that tobacco smokers belonging to C/C genotype are at three times higher risk of stomach cancer as compared to the non-smoker C/C genotype group. The combined genotype group (C/A or A/A) among the tobacco smokers also demonstrated increased risk, although it remains insignificant. Both current and past *Meiziol* & cigarette together smokers who smoke for more than 10 times per day and carrying the (C/C) genotype demonstrated significantly increased risk of stomach cancer in our study. Zhuo et al., (2012) in a meta-analysis reported that *CYP2E1 RsaI*/*PstI* polymorphisms may modify the susceptibility to gastric cancer among individuals who have a smoking history.

We have reported for the first time from this part of India about the association between *CYP2E1 RsaI* polymorphism and important dietary habits, where we revealed that smoke dried fish and preserved meat

(smoked/sun dried) consumers of Mizoram, who carries the C/C genotype have significantly high risk of developing stomach cancer. Smoke-drying and preservation leads to formation of N-nitroso compounds which are animal carcinogens and possible human carcinogens (Correa et al., 1992). These smoke dried and sun dried salted popular food items consumed extensively in Mizoram, which contains more nitrates and nitrites and they have significant association with *CYP2E1 RsaI* polymorphism which may leads to formation of gastritis to gastric cancer.

In conclusion, the *CYP2E1 RsaI* genotype distribution we have found among Mizo community as 87.3% (C/C), 12.4% (C/A) and 0.3% (A/A). Our study demonstrated no association between the *CYP2E1 RsaI* polymorphism and overall risk of stomach cancer in Mizoram. However, we observed a non-significant protective effect of the variant allele (A) of *CYP2E1* against stomach cancer in our study. Tobacco smokers carrying C/C genotype have three times higher risk of stomach cancer, as compared to non-smokers carrying C/C. Both current and past *Meiziol* & cigarette smokers who smoked for more than 10 times per day and carrying the (C/C) genotype had significantly increased risk of stomach cancer. Smoke dried fish and preserved meat (smoked/sun dried) consumers of Mizoram, who carries the C/C genotype have higher risk of developing stomach cancer. No significant association between *H. pylori* infection and *CYP2E1 RsaI* polymorphism in terms of stomach cancer was detected. Substantial relationship between *CYP2E1 RsaI* polymorphisms and different tobacco & dietary risk habits for developing stomach cancer are exist in this high risk population of north-eastern part of India. Further elaborate study including large population is required to validate these preliminary findings.

Acknowledgements

Authors sincerely acknowledge the Indian Council of Medical Research (ICMR), Department of Health Research, Government of India for funding this study vide letter No. 79/2/RMRC/NE/2009-NCD-III dated 30th March, 2009. M. Malakar is grateful to ICMR, New Delhi for granting ICMR-Senior Research Fellowship (IRIS ID: 2008-06430).

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