Polymorphisms of Cytochrome P450 2E1 Gene and Gastric Cancer Risk: A Case Control Study from West Bengal, India

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Abstract

Background: RsaI/PstI and DraI polymorphisms of cytochrome P450 2E1 (CYP2E1) gene are regarded as the most common polymorphisms associated with gastric cancer (GC). Very few data of these polymorphisms have been reported from India with regard to GC risk. We evaluated RsaI: -C1053T (rs2031920), PstI: -G1295C (rs3813867) site in the 5' flanking region, DraI: T7668A (rs6413432) site in intron 6, -G71T rs6413420 and G4768A or V179I rs6413419 polymorphisms of CYP2E1 with GC risk in the population of West Bengal, India.

Methods: We enrolled 105 GC patients (cases) and corresponding sex, age and ethnicity matched normal 108 individuals (controls) for the study. Genotyping for rs6413419, rs6413420, rs6413432, rs2031920, rs3813867 of CYP2E1 was performed using DNA sequencing and RFLP analysis.

Results and conclusion: Our results suggest that the difference between genotype frequencies of rs3813867 and rs2031920, although not statistically significant but the allele frequencies of rs3813867 (OR=2.29, 95% CI=1.01-5.18, p=0.042) and rs2031920 (OR=2.29, 95% CI=1.01-5.18, p=0.042) showed significant difference between GC and control individuals. This when pooled with smoking augmented GC risk by nearly 4-fold. Therefore, a larger population is needed to be screened to confirm the association of these studied SNPs with GC in India.

Keywords Gastric cancer; Cytochrome P4502E1; SNPs

Introduction

Gastric cancer (GC) is one of the most common cancers of the gastrointestinal (GI) tract. It accounts for nearly 8% of the total cancer cases and results in 10% of the overall cancer related deaths. More than 70% of new cases of GC are reported to occur in the developing countries [1]. Although the exact mechanisms of gastric carcinogenesis are still unknown, yet it has been hypothesized to be caused by the interplay of both environmental and genetic factors which differs among different ethnic groups, gender, age and habitual behaviours [2-5]. Earlier epidemiological studies showed the role of smoking, drinking and H pylori infection towards the development of GC [6,7]. However, only a small section of the people exposed to these environmental factors develops GC in the long run, which is indicative of the fact that host genetic factors may also play critical role in gastric carcinogenesis. Polymorphisms of several genes associated with GC risk have been identified worldwide. Of them, cytochrome P4502E1 (CYP2E1) gene encoding the metabolizing enzyme CYP2E1 has been of much importance. It is responsible for the metabolic activation of various carcinogens, such as benzene, vinyl chloride and N-dimethylnitrosamines and hence related to cancer threat [8-10]. The activity of this ethanol-inducible enzyme varies with several single nucleotide polymorphisms (SNPs) in the gene encoding this enzyme. For instance, RsaI/PstI polymorphisms, which are in complete linkage disequilibrium (LD), in the 5'-flanking promoter region of CYP2E1 are

reported to affect its transcriptional activation [11]. However, the results are controversial. In contrast, the DraI polymorphisms have been found to be associated with only enhanced transcription [12]. RsaI/PstI and DraI polymorphisms are regarded as the most common and influential polymorphisms in CYP2E1 [12] and have been studied with various cancer risks [13-17]. But how the genetic variations of CYP2E1 can elevate the GC risk remains elusive. Moreover, to the best of our knowledge till date, very few data of this gene with regard to GC risk has been reported from India. Thus, our study was aimed to find out the association of CYP2E1 polymorphisms (rs6413419, rs6413420, rs6413432, rs2031920, rs3813867) with GC risk in the population of West Bengal, India.

Subjects and Methods

Patients and control samples

One hundred and five GC cases were recruited from the Department of Surgery, Institute of Post Graduate Medical Education and Research (IPGME and R), Kolkata, West Bengal, India from December 1, 2012 to April 30, 2015. All the individuals enrolled in our study were Bengali. Patients who were newly diagnosed with GC without any other chronic disease and histopathologically confirmed were included. According to histology, the gradations of tumour tissue samples were done using Lauren's classification [18]. One hundred and eight age, sex and ethnicity matched healthy control subjects who neither had cancer nor any familial history of neoplasms were selected

from the same geographical region and socioeconomic status. Their non-cancer status was confirmed by medical examinations including radiographic examinations. Ethical approval for this study was given by the institutional ethics committee of Institute of Post Graduate Medical Education and Research (IPGME and R), Kolkata, West Bengal, India. A signed informed consent was taken from each of the participants. Each individual participating in the study was interviewed for their life style, socio-demographic characteristics, family history of cancer or any other chronic diseases, habitual behaviors like smoking, drinking and dietary habits and physical activity.

Body mass index (BMI)

Height and weight were measured in both patients and controls wearing light clothing and no shoes, to the nearest 0.1 cm and 0.1 Kg, respectively. BMI was calculated with the standard formula: $BMI=Weight (kg)/Height (m)^2$.

Blood collection and DNA extraction

Whole blood (~5 mL) samples were taken from all subjects and collected in separate tubes (contain EDTA, 0.5 M). Genomic DNA was extracted using the DNA extraction kit (QIAamp Blood Kit, QIAGEN, Hilden, Germany) according to the manufacture's instruction.

PCR analysis and direct sequencing

Five SNPS (rs6413419, rs6413420, rs6413432, rs2031920, rs3813867) of CYP2E1 gene were amplified using specific primers by polymerase chain reaction (PCR). The primers used for each of the amplifications were described in Table 1. PCR amplification was done in a 30 μl volume containing 100 ng of DNA, 0.5 μM of each primer, 0.2 mM of deoxyribonucleotide triphosphate mix, (Invitrogen Carlsbad, CA, USA), 1.5 mM magnesium chloride, 1x buffer and 2.5 Unit Taq Polymerase (Invitrogen). PCR conditions were as follows: denaturation at 94°C for 3 min followed by 44 cycles of denaturation for 30 s, annealing at 58°C-60°C for 30 s, extension at 72°C for 45 s and final extension at 72°C for 5 min. PCR products were subjected to direct DNA sequencing. For rs6413419 and rs6413420, forward and reverse strand sequencing was carried out using the big dye terminator kit (Applied Biosystems, Foster City, CA, USA) on an automated DNA capillary sequencer (Model 3700; Applied Biosystems).

SNPs (rs number)	Sequence (5' 3') F: Forward R: Reverse	Digestion pieces (restriction enzyme)	Amplicon Length
rs6413419	F: CTCAAACAGGTTCAGACATTCAC R: GTTGATGACTGATGAAAGAGGAAAG		347 bp
rs6413420	F: AACATGTTCCTGGATGTGTTTG R: ATGATGGGAAGCGGGAAAG		421 bp
rs6413432	F: AGGCTCGTCAGTTCCTGAAAG R: TTCCATGGTTCCCCTAGTGC	Allele T: 867 Allele A:559, 308 (Dra1)	867 bp
rs3813867 rs2031920	F:CCAGTCGAGTCTACATTGTCA R:TTCATTCTGTCTTCTAACTGG	Allele C: 413 Allele G: 114, 299 (PstI) Allele T: 413 Allele C: 353,60 (RsaI)	413 bp

Table 1: Primer sequences and restriction enzymes used for detection of CYP2E1 gene polymorphisms.

RFLP analysis

PCR amplified products were digested using DraI (rs6413432), PstI (rs3813867) and RsaI (rs2031920) enzymes according to the manufacturer's instructions (New England Biolabs Inc.). Fragments were separated and analyzed by 2.5% agarose gel electrophoresis. Samples of five randomly selected subjects were analyzed twice by direct DNA sequencing to assess the consistency of the genotyping protocol.

Statistical analysis

For each SNP, the genotypic data were analyzed by using multivariate logistic regression model. The demographic variables and life style habits (smoking and alcohol consumption) between cases and controls were compared by t-tests (for continues variables) and chi-square tests (for categorical variables). Hardy-Weinberg equilibrium of each SNP was examined using a $χ^2$ test. Next, unconditional logistic regression model was used to evaluate the risk of GC with regard to smoking status, alcohol consumption and BMI. All the tests were done using GraphPad InStat software (GraphPad InStat software, San Diego, CA) and SNPassoc version 1.81 software (Catalan Institute of Oncology, Barcelona, Spain). Linkage disequilibrium (LD) pattern was analyzed using Haploview 4.2.

Results

Characteristics of study participants

The study was performed with 105 GC patients (78% males and 22% females) having mean age of 55.43 ± 10.86 years (range 22–80 years). There was a male predominance of GC frequency compared to females. Based on the anatomical location, 98% of the patients had non-cardia and only 2% had cardia type of GC. Histology revealed the sample population to be of 49% intestinal, 23% diffuse and 28 % indeterminate type. Smoking (OR=2.45, 95% CI=1.41-4.26, p=0.001) and alcohol consumption (OR=2.77, 95% CI=1.52-5.06, p=0.001) varied significantly between cases and controls where both were found to independently increase the risk of GC. Only 20% of the cases were *H. pylori* positive and familial cluster for GC occurrence was not observed in our study Table 2.

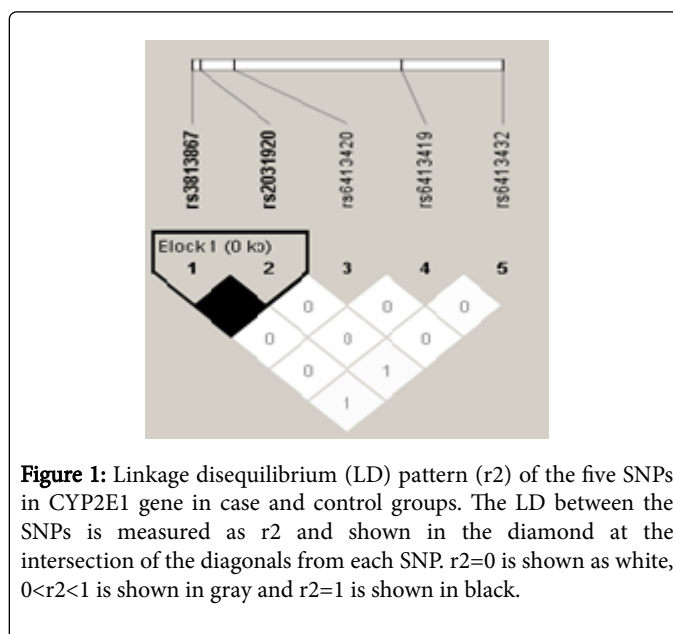| Characteristics	Control (n=108)	Case (n=105)	Odds ratio (95% CI)	p value
*Age (years ± SD)	53.64 ± 7.88	55.43 ± 10.86		0.169
Sex				
Male	89	82		
Female	19	23		0.429
*BMI (kg/m^2)	23.28 ± 1.97	20.55 ± 2.75		<0.001
Anatomical location				
Cardia	-	3		
Non-cardia	-	102		
Histological subtypes of tumour				
Intestinal	-	52		
Diffuse	-	24		
Indeterminate	-	29		
Alcohol consumption				
No	85	60		
Ever	23	45	2.77 (1.52-5.06)	0.001
Cigarette/bidi smoking				
No	66	41		
Ever	42	64	2.45 (1.41-4.26)	0.001

Table 2: Clinical characteristics of gastric cancer patients and controls, *At diagnosis.

Out of the 5 SNPs studied, rs3813867 and rs2031920 were found to be strongly in LD Figure 1, as has been reported in other studies of the world population.

There were no significant differences between GC and controls in the distribution of either allelic or genotype frequencies of rs6413419, rs6413420 and rs6413432 Table 3.

However, only the allele frequencies of rs3813867 (PstI) (OR=2.29, 95% CI=1.01-5.18, p=0.042) and rs2031920 (RsaI) (OR=2.29, 95% CI=1.01-5.18, p=0.042) showed significant difference between GC and control individuals Table 3 and Figure 2.

A stratification analysis was conducted to evaluate the effects of interaction of PstI/RsaI with the risk of GC according to smoking status, alcohol consumption status and BMI. The difference between genotype frequencies of rs3813867 and rs2031920, although not statistically significant, yet individuals having C and T alleles respectively who were smokers had nearly 4-fold risk of GC (OR=3.98, 95% CI=1.07-14.73) Table 4.
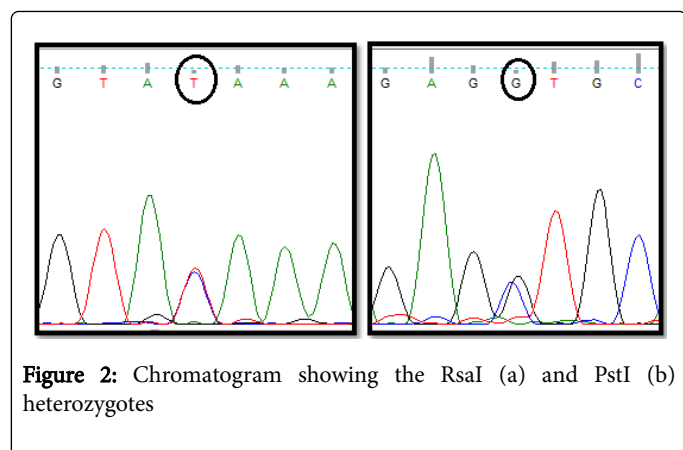

Figure 1: Linkage disequilibrium (LD) pattern (r2) of the five SNPs in CYP2E1 gene in case and control groups. The LD between the SNPs is measured as r2 and shown in the diamond at the intersection of the diagonals from each SNP. r2=0 is shown as white, 0<r2<1 is shown in gray and r2=1 is shown in black.

Figure 2: Chromatogram showing the RsaI (a) and PstI (b) heterozygotes

Genotype	Controls (n-108) n (%)	Cases (n-105) n (%)	OR* (95% CI)	p-value
CYP2E1 rs6413419 GG	106 (98.1)	102 (97.1)	1.00	
GA	2 (1.9)	3 (2.9)	1.56 [0.26-9.52]	0.628
AA	0 (0)	0 (0)	1.00	1.00
G allele	99%	99%	1.00 [0.15-6.75]	
A allele	1%	1%		
CYP2E1 rs6413420 GG	102 (94.4)	97 (92.4)	1.00	0.741
	6 (5.6)	8 (7.6)	1.40 [0.47-4.19]	0.73

Genotype	Controls	Cases	OR	p-value
GT TT G allele T allele	0 (0) 97% 3%	0 (0) 96% 4%	-- 1.00 1.40 [0.49-4.01]	
CYP2E1CYP2E1 rs6413432 TT TA AA TT TA+AA T allele A allele	78 (72.2) 30 (27.8) 0 (0) 78 (72.2) 30 (27.8) 87% 13%	72 (68.5) 32 (30.5) 1 (1) 72 (68.5) 33 (31.5) 84% 16%	1 1.16 [0.64-2.09] -- 1 1.27 [0.71-2.38] 1 1.30 [0.74-2.29]	0.743 0.602 0.436
CYP2E1 rs3813867 GG GC CC G allele C allele	99 (91.6) 9 (8.4) 0 (0) 96% 4%	87 (82.8) 18 (17.2) 0 (0) 91% 9%	1.00 2.28 [0.97-5.33] -- 1.00 2.29 [1.01-5.18]	0.053 0.042
CYP2E1 rs2031920 CC CT TT C allele T allele	99 (91.6) 9 (8.4) 0 (0) 96% 4%	87 (82.8) 18 (17.2) 0 (0) 91% 9%	1.00 2.28 [0.97-5.33] -- 1.00 2.29 [1.01-5.18]	0.053 0.042

Table 3: Genotype and allele frequencies of CYP2E1 and association with gastric cancer risk, *Odds ratio were adjusted for age, sex, BMI, alcohol and smoking status.

	Exposure	Status	Genotypes	Control (n-108)	Case (n-105)	OR (95% CI)*	P value
rs3813867(PstI)	Smoking	Non-smoker	GG GC	60 6	38 3	Reference: 0.79 (0.19-3.35)	0.748
		Smoker	GG GC	39 3	49 15	Reference: 3.98 (1.07-14.73)	0.019
	Alcohol	Non-alcoholic	GG GC	80 5	59 3	Reference: 0.86 (0.20-3.76)	0.844
		Alcoholic	GG GC	19 4	28 15	Reference: 2.54 (0.73-8.86)	0.135
	Smoking+Alcohol	Both non-smoker and non-alcoholic	GG GC	6 24	35 2	Reference: 1.83 (0.25-13.55)	0.549
		Both smoker and alcoholic	GG GC	19 4	25 11	Reference: 2.09 (0.58-7.60)	0.257
	BMI	<22	GG GC	23 4	53 14	Reference:	0.587
rs2031920 RsaI	Smoking	Non-smoker	CC CT	6 60	38 3	Reference: 0.79 (0.19 - 3.35)	0.748
		Smoker	CC CT	39 3	49 15	Reference: 3.98 (1.07 - 14.73)	0.019
	Alcohol	Non-alcoholic	CC CT	80 5	59 3	Reference: 0.86 (0.20-3.76)	0.844
		Alcoholic	CC CT	19 4	28 15	Reference: 2.54 (0.73-8.86)	0.135

Smoking+Alcohol	Both non-smoker and non-alcoholic	CC CT	64 2	35 2	Reference: (0.25-13.55)	1.83 0.549
	Both smoker and alcoholic	CC CT	19 4	25 11	Reference: (0.58-7.60)	2.09 0.257
BMI	<22	CC CT	23 4	53 14	Reference: (0.22-14.79)	1.79 0.587

Table 4: Interaction between CYP2E1 rs3813867, rs2031920 polymorphisms and smoking and alcohol consumption in gastric cancer, *Odds ratio were adjusted for age, sex, BMI, alcohol and smoking status.

Discussion

The genes that encode the CYP family of enzymes are highly polymorphic and thus lead to inter individual variation in enzyme activity. The activation of various carcinogens like polycyclic aromatic hydrocarbons, nitrosamines and heterocyclic amines involves the active role of these enzymes. An increased risk of GC associated with CYP2E1 polymorphisms that can modify the effects of environmental factors have now been confirmed in many populations throughout the world i.e. Korean [19,20], Chinese [21] and Taiwanese [22]. This enzyme also reduces oxygen molecule to highly active form, which is carcinogenic, and may lead to cancer development [23]. Previous research showed that CYP2E1 gene polymorphisms may be a cause of development of GC [24-29]. The most widely studied SNPs of CYP2E1 are RsaI: -C1053T (rs2031920), PstI -G1295C (rs3813867) site in the 5' flanking region, DraI: T7668A (rs6413432) site in intron 6, -G 71T rs6413420 and G4768A or V179I rs6413419 [30-33]. The present study was aimed to find out the association between these five most studied SNPs of CYP2E1 and GC incidence in the population of West Bengal, India. We screened 105 GC patients and 108 normal individuals for the study. RsaI/PstI and DraI polymorphisms are regarded as the most frequent and powerful polymorphisms in CYP2E1 that are considered to enhance transcription [12]. Our results suggest that the difference between genotype frequencies of rs3813867 and rs2031920, although not statistically significant but the allele frequencies of rs3813867 (OR=2.29, 95% CI=1.01-5.18, p=0.042) and rs2031920 (OR=2.29, 95% CI=1.01-5.18, p=0.042) showed significant difference between GC and control individuals. It was also found to interact with smoking thereby increasing the risk of GC by nearly 4-fold. However, a study in India from the north eastern state of Mizoram found no association of RsaI polymorphism with GC risk [33]. We found no significant association of SNPs rs6413419 and rs6413420 with GC in our study. Similar results were also observed for oral cancer susceptibility in South Indians [12]. Interestingly, in their study, V179I rs6413419 locus was monomorphic which was different from our finding where we obtained heterozygotes in both case and control population. In another study, CYP2E1 polymorphism and a history of habitual drinking were shown to increase the risk of intestinal-type GC [34]. In India, the incidence of GC varies across different registries. A higher incidence has been reported in the South compared to the North. The highest rate of GC cases is reported from the North Eastern state of Mizoram [35]. But the same being comparatively low in the state of West Bengal, the sample size of this study was limited. Therefore, a larger population is needed to be screened to validate these findings.

Conclusion

We conducted the first study of association between CYP2E1 polymorphisms and the risk of GC from West Bengal, India. Our results suggest that the difference between genotype frequencies of rs3813867 and rs2031920, although not statistically significant but the allele frequencies of rs3813867 and rs2031920 showed significant difference between GC and control individuals. This when combined with smoking increased the risk of GC by nearly 4-fold. However, study with larger sample may contribute to a better understanding towards the association of GC with the studied SNPs.

Conflict of Interest

We have no conflict of interest to declare.

Acknowledgement

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