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RESEARCH ARTICLE

Association of genetic variants of xenobiotic and estrogen metabolism pathway (*CYP1A1* and *CYP1B1*) with gallbladder cancer susceptibility

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Abstract Gallbladder carcinoma is a highly aggressive cancer with female predominance. Interindividual differences in the effectiveness of the activation/detoxification of environmental carcinogens and endogenous estrogens may play a crucial role in cancer susceptibility. The present study included 410 patients with carcinoma of the gallbladder (GBC) and 230 healthy subjects. This study examined association of *CYP1A1-MspI*, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu with GBC susceptibility. *CYP1A1-MspI* [CC] and *CYP1A1*-Ile462Val [iso/val] genotypes were found to be significantly associated with GBC (p=0.006 and p=0.03, respectively), as compared to healthy controls, while *CYP1B1*-Val432Leu was not associated with GBC. The *CYP1A1* haplotype [C-val] showed a significant association with GBC (p=0.006). On stratification based on gender, the *CYP1A1-MspI* [CC] genotype showed an increased risk of GBC in females (p=0.018). In case-only analysis, tobacco users with *CYP1A1-MspI* [CT] genotypes were at a higher risk of GBC (p=0.008). Subdividing the GBC patients on the basis of gallstone status, the *CYP1A1* haplotype [C-val] imparted a higher risk in patients without stones when compared to controls (p= 0.001). The results remained significant even after applying Bonferroni correction. Multivariate analysis revealed an increased risk of *CYP1A1* iso/val and val/val genotypes in GBC patients having BMI >25 (p=0.021). The *CYP1A1* polymorphisms may confer increased risk of GBC, probably due to impaired xenobiotic or hormone metabolism through a gallstone-independent pathway.

Keywords Genetic susceptibility · Polymorphism · Case-control association study

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Introduction

Carcinoma of the gallbladder (GBC) is the most widespread biliary tract malignancy across the world with one of the highest incidence and mortality rates in northern India (21.5/100,000) [1, 2]. Being a multifaceted disease, numerous genetic variants along with diverse environmental and nutritional factors may act together possibly as disease modifiers. The identification of these risk sets of genetic variants will aid in understanding the pathobiology of the disease.

Epidemiologic studies provide evidence about an individual's susceptibility to cancer modulation by both genetic as well as environmental factors. Xenobiotic-metabolizing enzymes act as the first line of defense against environmental

carcinogens, and most of the cancers arise as a consequence of contact to these carcinogens [3]. The genetic differences in the effectiveness of the activation/detoxification of carcinogens play a vital role in an individual's susceptibility towards cancer.

The human cytochrome P450 1A1 (*CYP1A1*) gene, which encodes aryl hydrocarbon hydroxylase, is a phase I enzyme and participates in the metabolism of xenobiotics, i.e., activation of tobacco-related procarcinogens, such as polycyclic aromatic hydrocarbons, nitrosamines, and aromatic amines [4], and a small number of endogenous substrates such as estrogens [5, 6]. Among the different reactions catalyzed by *CYP1A1*, hydroxylation at a vacant position of an aromatic ring is a well-thought-out hallmark for the initiation of carcinogenesis, through the formation of highly reactive conversion products that can cause oncogenic mutations in experimental animals and humans [7, 8].

A functional genetic variant of *CYP1A1* (Ile462Val, rs1048943) polymorphism is located in exon 7 in the heme-binding region of the *CYP1A1* gene, near the active site of the enzyme [9]. Another variant, T>C transition (rs4646903), so-called *MspI*, polymorphism is located in the 3′ noncoding region [10]. Both the polymorphisms are found to contribute towards increased enzymatic activity of CYP1A1 [11]. CYP1B1 is the main cytochrome P450 responsible for the extrahepatic 4-hydroxylation [12]. In the *CYP1B1* gene, a nonsynonymous polymorphism, Val432Leu, is caused by a substitution of valine to leucine at codon 432 in exon 3 and is also linked to a higher catalytic activity [13]. Several studies have shown *CYP1A1*-Ile462Val (rs1048943), *CYP1A1-MspI* (rs4646903), and *CYP1B1*-Val432Leu (rs1056836) to be associated with various cancers, including hormone-related cancers [14, 15]. Previously, we had reported *CYP1A1-MspI* (rs4646903) polymorphism to be associated with tobacco-related risk of GBC but in a smaller sample size [15].

Given the potential role of these genes in various cancers, the present study was planned to evaluate the functional genetic variants in *CYP1A1* and *CYP1B1* on the risk of gallbladder carcinoma in a large cohort from North India.

Material and methods

Study subjects

The current case-control study included 640 subjects comprising 410 consecutive newly diagnosed GB cancer patients (fine needle aspiration cytology and histopathologically proven), recruited from the clinics of the Department of Surgical Oncology, King George Medical University (KGMU), Lucknow, and the Department of Surgical Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, and 230 healthy controls belonging to same ethnicity. Staging of cancer was documented according to the AJCC/UICC staging [16]. The healthy controls were recruited from unrelated individuals of general population. Inclusion criteria for controls were absence of prior history of cancer, precancerous lesions, and absence of gallstones proven by ultrasonography and were frequency-matched to cancer cases on age, gender, and ethnicity. After obtaining informed consent, the subjects were personally interviewed for information on food habits, occupation, and tobacco usage. Ethics approval for the work was granted by the local ethics committee of the institute. The study protocol was approved by the institutional ethical committees of both the institutes, and the authors followed the norms of the World Medical Association's Declaration of Helsinki. All the participants gave written informed consent for the study.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting-out method. The polymorphisms were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. As a negative control, PCR mix without DNA sample was used to ensure a contamination-free PCR product. Polymorphisms in *CYP1A1* and *CYP1B1* were determined by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described [17]. The digested PCR fragments were separated on polyacrylamide gel, stained with ethidium bromide, and observed under ultraviolet light. Genotyping was performed without knowledge of the case or control status. Ten percent of samples for each genotype were sequenced which showed 100 % concordance. Among control subjects, genotype frequencies for each SNP were examined for deviation from the Hardy-Weinberg equilibrium (HWE) using the χ^2 test.

Statistical analysis

The sample size was calculated using QUANTO 1.1, using minor allele frequency data from HapMap (http://hapmap.ncbi.nlm.nih.gov/). Gene-gene and gene-environment interactions were estimated by the logistic regression model, which included an interaction term as well as variables for genotypes (*CYP1A1* and *CYP1B1*) and potential confounders (age and gender). Statistical analysis was done using SPSS statistical analysis software, version 16.0 (SPSS, Chicago, IL, USA). Statistical analysis of the haplotype estimation and linkage disequilibrium was conducted using the SNPstats software (http://bioinfo.iconcologia.net/snpstats/start.htm). For multiple comparisons, Bonferroni correction was applied. The p-corrected value, i.e., $p<0.025$, was taken as significant for subgroup analysis on gender stratification and gallstone status stratification.

Results

Characteristic profile of the study subjects

Among 410 GBC cases and 230 controls, the mean age was 52.32±10.6 and 43.87±11.57 years, respectively. Genomic control method ruled out the possibility of population stratification in our study. Most of the GBC patients were in advanced stages of cancer (stage III and stage IV). In GBC cases, 25 (6 %) had stage II adenocarcinomas, 180 (44.0 %) stage III, and 205 (50 %) stage IV. Among GBC, 31 % of the cases were tobacco users and 37 % of the cases had early age of onset, i.e., <50 years. Gallstones were present in 53 % of GBC. Characteristics of GBC patients and age- and sex-matched controls are given in supplementary Table S1. Several other characteristics of GBC patients are given in supplementary Table S2.

Distribution of studied polymorphisms in controls

The distribution of *CYP1A1-MspI* (rs4646903), *CYP1A1*-Ile462Val (rs1048943), and *CYP1B1*-Val432Leu (rs1056836) polymorphism is shown in Table 1. The observed genotype frequencies of all the studied polymorphisms in controls were in accordance with the Hardy-Weinberg equilibrium ($p>0.05$).

Table 1 Overall frequency distribution of *CYP1A1-MspI*, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu polymorphisms in GBC and healthy subjects

Genotype	Controls *n* (%)	GBC *n* (%)	OR (95 % CI)	*p* value
CYP1A1-MspI genotypes/alleles (age- and sex-adjusted)				
TT	142 (61.7)	215 (52.4)	1 (reference)	–
TC	77 (33.5)	160 (39.0)	1.45 (0.94–2.25)	0.091
CC	11 (4.8)	35 (8.5)	*3.4 (1.41–8.19)*	*0.006*
T	361 (78)	590 (72)	1 (reference)	–
C	99 (22)	230 (28)	*1.67 (1.19–2.35)*	*0.003*
CYP1A1-Ile462Val genotypes/alleles (age- and sex-adjusted)				
Ile/Ile	179 (77.8)	272 (66.3)	1 (reference)	–
Ile/Val	50 (21.7)	134 (32.7)	*1.6 (1.04–2.65)*	*0.032*
Val/Val	1 (0.4)	4 (1.0)	2.08 (0.13–32.7)	0.603
Ile	408 (89)	678 (83)	1 (reference)	–
Val	52 (11)	142 (17)	*1.55 (1.01–2.37)*	*0.041*
CYP1B1-Val432Leu genotypes/alleles (age- and sex-adjusted)				
Leu/Leu	180 (78.3)	310 (75.6)	1 (reference)	–
Leu/Val	49 (21.3)	97 (23.7)	1.20 (0.73–1.95)	0.461
Val/Val	1 (0.4)	3 (0.7)	1.18 (0.06–20.3)	0.906
Leu	409 (89)	717 (87)	1 (reference)	–
Val	51 (11)	103 (13)	1.173 (0.75–1.83)	0.484

Significant values are given in italics

GBC carcinoma of the gallbladder, *OR* odds ratio, *CI* confidence interval

Association of *CYP1A1-MspI*, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu polymorphisms with gallbladder cancer

Table 1 shows the risk of gallbladder cancer in relation to each of the SNPs of *CYP1A1-MspI*, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu. On comparing the genotype frequency of *CYP1A1-MspI* in GBC patients with that of controls, the homozygous variants [CC] and [C] allele showed a statistically significant risk with GBC ($p=0.006$; odds ratio (OR)=3.4; $p=0.003$; OR=1.67). Similarly, the *CYP1A1*-Ile462Val heterozygous variant [iso/val] genotype and [val] allele also showed a statistically significant risk of developing GBC ($p=0.03$; OR=1.6; $p=0.04$; OR=1.5). On the contrary, no significant difference was observed in the distribution of *CYP1B1*-Val432Leu polymorphism in any groups both at genotype and allele levels.

Haplotype analysis of *CYP1A1-MspI* and *CYP1A1*-Ile462Val in case and control groups

The analysis revealed that the haplotype [C-val] of *CYP1A1* was significantly associated with GBC risk ($p=0.006$; OR=2.5). Global haplotype analysis indicated a significant difference between cases and controls based on the distribution pattern of the four haplotypes ($p=0.012$) (Table 2).

Association of *CYP1A1-MspI*, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu polymorphisms with gallbladder cancer on stratification on gender

Table 3 shows the risk of gallbladder cancer in relation to each of the SNPs of *CYP1A1*-Ile462Val, *CYP1A1-MspI*, and *CYP1B1*-Val432Leu on the basis of gender. On comparing the genotype frequency distribution of *CYP1A1-MspI* in GBC female patients with that in female controls, the homozygous

Table 2 Frequency distribution of haplotypes for *CYP1A1-MspI* and *CYP1A1*-Ile462Val polymorphisms in GBC patients and controls

Gallbladder cancer patients and controls				
Haplotypes	Frequency		*p* value	OR* (95 % CI)
	HC (230; %)	GBC (410; %)		
T, iso	0.7305	0.6535	–	1.00
C, iso	0.1564	0.1733	0.1	1.46 (0.93–2.31)
C, val	0.0588	0.1072	*0.0065*	*2.59 (1.31–5.11)*
T, val	0.0543	0.066	0.89	1.05 (0.49–2.25)

Significant values are given in italics

GBC carcinoma of the gallbladder, *HC* healthy controls, *OR* odds ratio, *CI* confidence interval

*$p=0.012$, global haplotype association

Table 3 Frequency distribution of *CYP1A1-MspI*, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu polymorphisms after subdividing on the basis of gender in GBC patients and controls

Genotype/allele	Male				Female			
	Frequency, *n* (%)		*p* value	OR (95 % CI)	Frequency, *n* (%)		*p* value	OR (95 % CI)
	Control (69)	GBC (122)			Control (161)	GBC (285)		
CYP1A1-MspI genotypes/alleles (age- and sex-adjusted)								
TT	41 (59.4)	61 (50)	–	1 (reference)	101 (62.7)	154 (53.5)	–	1 (reference)
TC	23 (33.3)	48 (39.3)	0.151	1.9 (0.7–4.6)	54 (33.5)	112 (38.9)	0.25	1.3 (0.8–2.2)
CC	5 (7.2)	13 (10.7)	0.130	2.9 (0.7–11.5)	6 (3.7)	22 (7.6)	*0.01*[a]	*4.0* (*1.2–12.6*)
T	105 (76.1)	170 (69.7)	–	1 (reference)	256 (79.5)	420 (72.9)	–	1 (reference)
C	33 (23.9)	74 (30.3)	*0.04*	*1.9* (*1.0–3.6*)	66 (20.5)	156 (27.1)	*0.02*[a]	*1.5* (*1.0–2.3*)
CYP1A1-Ile462Val genotypes/alleles (age- and sex-adjusted)								
Ile/Ile	54 (78.3)	77 (63.1)	–	1 (reference)	125 (77.6)	195 (67.7)	–	1 (reference)
Ile/Val	15 (21.7)	44 (36.1)	0.119	2.02 (0.8–4.9)	35 (21.7)	90 (31.3)	0.12	1.5 (0.8–2.6)
Val/Val	0 (0)	1 (0.8)	NA	NA	1 (0.6)	3 (1.0)	0.62	1.9 (0.13–30)
Ile	123 (89.1)	198 (81.1)	–	1 (reference)	285 (88.5)	480 (83.6)	–	1 (reference)
Val	15 (10.9)	146 (18.9)	0.159	1.78 (0.7–3.9)	37 (11.5)	96 (16.7)	0.13	1.4 (0.8–2.4)
CYP1B1-Val432Leu genotypes/alleles (age- and sex-adjusted)								
Leu/Leu	53 (76.8)	85 (69.7)	–	1 (reference)	127 (78.9)	225 (78.1)	–	1 (reference)
Leu/Val	16 (23.2)	35 (28.7)	0.24	1.7 (0.6–4.2)	33 (20.5)	62 (21.5)	0.89	1.0 (0.5–1.8)
Val/Val	0 (0)	2 (1.6)	NA	NA	1 (0.6)	1 (0.3)	0.69	0.4 (0.01–19)
Leu	122 (88.4)	205 (84.0)	–	1 (reference)	287 (89.1)	512 (88.9)	–	1 (reference)
Val	16 (11.6)	39 (16.0)	0.232	1.63 (0.7–3.6)	35 (10.9)	64 (11.1)	0.98	1.0 (0.5–1.7)

Significant values are given in italics

GBC carcinoma of the gallbladder, *OR* odds ratio, *CI* confidence interval, *NA* not available

[a] Results remained significant after Bonferroni correction

variants [CC] and [C] allele showed a statistically significant risk in female patients (p=0.006; OR=4.0; p=0.02; OR=1.5) and the risk was borderline in the case of males at the allelic level (p=0.04; OR=1.9). The results were significant after Bonferroni correction in the female group. On the contrary, no significant differences were observed in the distribution of *CYP1A1*-Ile462Val and *CYP1B1*-Val432Leu polymorphisms in any groups both at genotype and allele levels, for developing GBC.

Modulation of risk in the presence or absence of gallstones in GBC patients with *CYP1A1* polymorphisms and risk haplotype

Since gallstones were present in 53 % of GBC patients, the cancer cases were stratified on the basis of gallstones. The homozygous variants [CC] and [C] allele of *CYP1A1-MspI* showed a statistically significant risk in patients without stones (p=0.001; OR=4.7; p=0.001; OR=1.9) as compared to healthy controls. Similarly, the *CYP1A1*-Ile462Val heterozygous variant [iso/val] genotype and [val] allele showed a statistically significant risk for GBC among patients without stones (p=0.01; OR=1.9; p=0.01; OR=1.7). On the contrary, no significant differences were observed in the distribution of *CYP1B1*-Val432Leu polymorphism in any groups both at genotype and allele levels (Table 4). The haplotype [C-val] of *CYP1A1* was significantly associated with GBC risk in patients without stones (p=0.001; OR=3.37). The results remained significant after Bonferroni correction. Global haplotype analysis indicated significant differences between cases and controls based on the distribution pattern of the four haplotypes (p=0.0027). On the contrary, no significant differences were observed in the distribution of the four haplotypes in patients with gallstones as shown in Table 5.

CYP1A1-MspI, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu polymorphisms and interaction with tobacco usage, age of onset, diet, and BMI

Because tobacco is a leading risk factor for most cancers, we also stratified our data by tobacco usage in a case-only analysis to explore the modulation of risk for GBC. Genotypes TC and C allele of *CYP1A1-MspI* were significantly associated with GBC risk in tobacco users (p=0.008; OR=1.91;

Table 4 Frequency distribution of *CYP1A1-MspI*, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu polymorphisms after subdividing on the basis of gallstone status

Genotype/allele	GBC (no stone)				GBC (stone)			
	Frequency, *n* (%)		*p* value	OR (95 % CI)	Frequency, *n* (%)		*p* value	OR (95 % CI)
	Control (230)	GBC (185)			Control (230)	GBC (206)		
CYP1A1-MspI genotypes/alleles (age- and sex-adjusted)								
TT	142 (61.7)	100 (49)	–	1 (reference)	142 (61.7)	115 (55.8)	–	1 (reference)
TC	77 (33.5)	80 (39.2)	0.067	1.6 (0.9–2.6)	77 (33.5)	80 (38.8)	0.20	1.4 (0.8–4.2)
CC	11 (4.8)	24 (11.8)	*0.001*[a]	*4.7 (1.8–12)*	11 (4.8)	11 (5.3)	0.21	2.1 (0.6–7)
T	361 (78.5)	280 (68.6)	–	1 (reference)	361 (78.5)	310 (75.2)	–	1 (reference)
C	99 (21.5)	128 (31.4)	*0.001*[a]	*1.9 (1.3–2.8)*	99 (21.5)	102 (24.2)	0.10	1.4 (0.9–2.1)
CYP1A1-Ile462Val genotypes/alleles (age- and sex-adjusted)								
Ile/Ile	179 (77.8)	130 (63.7)	–	1 (reference)	179 (77.8)	142 (68.9)	–	1 (reference)
Ile/Val	50 (21.7)	71 (34.8)	*0.015*[a]	*1.9 (1.1–3.2)*	50 (21.7)	63 (30.6)	0.16	1.5 (0.8–2.6)
Val/Val	1 (0.4)	3 (1.5)	0.422	3.3 (0.7–66)	1 (0.4)	1 (0.5)	0.71	1.9 (0.05–79)
Ile	408 (88.7)	331 (81.13)	–	1 (reference)	408 (88.7)	347 (84.2)	–	1 (reference)
Val	52 (11.3)	77 (18.87)	*0.018*[a]	*1.7 (1.1–2.8)*	52 (11.3)	65 (15.7)	0.389	1.25 (0.7–2.1)
CYP1B1-Val432Leu genotypes/alleles (age- and sex-adjusted)								
Leu/Leu	180 (78.3)	151 (74)	–	1 (reference)	180 (78.3)	159 (77.2)	–	1 (reference)
Leu/Val	49 (21.3)	50 (24.5)	0.47	1.2 (0.7–2.1)	49 (21.3)	47 (22.8)	0.339	1.3 (0.7–2.5)
Val/Val	1 (0.4)	3 (1.5)	0.58	2.1 (0.1–37)	1 (0.4)	0 (0)	NA	NA
Leu	409 (89)	352 (86.27)	–	1 (reference)	409 (89)	365 (88.5)	–	1 (reference)
Val	51 (11)	56 (13.73)	0.25	1.1 (0.9–1.3)	51 (11)	47 (11.4)	1.0	1.0 (0.8–1.2)

Significant values are given in italics

GBC carcinoma of the gallbladder, *OR* odds ratio, *CI* confidence interval, *NA* not available

[a] Results remained significant after Bonferroni correction

p=0.004; OR=1.6) (Table 6), whereas no associations were observed in the other two SNPs.

We also stratified our GBC patients by age of onset, early (<50) versus late (>50), in a case-only analysis to explore the modulation of GBC risk. However, none of the genotypes of the studied polymorphisms showed a statistically significant association with GBC (data not shown). *CYP1A1-MspI* polymorphism was associated with intake of nonvegetarian diet (OR=9.1; p=0.05) (supplementary Table S3). Multivariate analysis revealed an increased risk of the iso/val and val/val genotypes of *CYP1A1* with obesity (OR=6.4; p=0.021). There were no additional

Table 5 *CYP1A1-MspI* and *CYP1A1*-Ile462Val haplotype analyses in gallbladder cancer patients with stones and without stones

Haplotype analysis of *CYP1A1* in gallbladder cancer without stone and controls*					Haplotype analysis of *CYP1A1* in gallbladder cancer with stone and controls**			
Haplotypes	Frequency				Frequency			
	HC (230)	GBC (185)	*p* value	OR[a] (95 % CI)	HC (230)	GBC (206)	*p* value	OR[a] (95 % CI)
T, iso	0.730	0.635	–	1.00	0.730	0.669	–	1.00
C, iso	0.156	0.175	0.07	1.65 (0.96–2.82)	0.156	0.172	0.23	1.39 (0.82–2.36)
C, val	0.058	0.138	*0.001*[a]	*3.37 (1.59–7.15)*	0.054	0.082	0.4	1.43 (0.62–3.31)
T, val	0.054	0.050	0.91	1.05 (0.43–2.59)	0.059	0.075	0.17	1.70 (0.80–3.62)

Significant values are given in italics

GBC carcinoma of the gallbladder, *HC* healthy controls, *OR* odds ratio, *CI* confidence interval

*p=0.0027, global haplotype association; **p=0.3, global haplotype association

[a] Results remained significant after Bonferroni correction

Table 6 Risk of *CYP1A1-MspI*, *CYP1A1*-Ile462Val, and *CYP1B1*-Val432Leu genotypes and tobacco usage in GBC patients (case-only analysis)

Genotype/allele	Nontobacco user (276), *n* (%)	Tobacco user (124), *n* (%)	*p* value	OR (95 % CI)
CYP1A1-MspI polymorphism				
TT	159 (57.6)	52 (41.9)	1 (reference)	–
TC	98 (35.5)	58 (46.8)	*0.008*	*1.91 (1.1–3.0)*
CC	19 (6.9)	14 (11.3)	*0.051*	2.22 (0.9–4.9)
T	416 (75.36)	162 (65.32)	1 (reference)	–
C	136 (24.64)	86 (34.68)	0.004	1.65 (1.1–2.3)
CYP1A1-Ile462Val polymorphism				
Ile/Ile	187 (67.8)	80 (64.5)	1 (reference)	–
Ile/Val	86 (31.2)	44 (35.5)	0.575	1.14 (0.71–1.83)
Val/Val	3 (1.1)	0 (0)	NA	NA
Ile	460 (83.3)	204 (82.3)	1 (reference)	-
Val	92 (16.7)	44 (17.7)	0.877	1.03 (0.6–1.56)
CYP1B1-Val432Leu polymorphism				
Leu/Leu	215 (77.9)	87 (70.2)	1 (reference)	–
Leu/Val	60 (21.7)	35 (28.2)	0.265	1.33 (0.8–2.2)
Val/Val	1 (0.4)	2 (1.6)	0.349	3.35 (0.2–42)
Leu	490 (88.8)	209 (84.3)	1 (reference)	–
Val	62 (11.2)	39 (15.7)	0.191	1.35 (0.8–2.1)

Significant values are given in italics

GBC carcinoma of the gallbladder, *OR* odds ratio, *CI* confidence interval, *NA* not available

associations between multiparity, earlier age at menarche, higher age at menopause, etc. (data not shown).

Discussion

CYP genes code for major families of cytosolic and endoplasmic reticulum enzymes which catalyze the activation and detoxification of reactive electrophilic compounds including most of the environmental carcinogens (e.g., benzo[a]pyrene). CYP1A1 is a phase I enzyme that regulates the metabolic activation of majority of procarcinogens present in tobacco smoke, for example, aromatic amines and PAHs [4], and the enzyme is also involved in hydroxylation of estrogens [5, 6]. In this case-control study of gallbladder cancer patients, we investigated associations of functionally relevant genetic variants in two genes (*CYP1A1*, *CYP1B1*) encoding key proteins of the xenobiotic metabolic pathway with GBC risk. Our results showed *CYP1A1-MspI* and *CYP1A1*-Ile462Val (rs1048943) genetic variations to be significantly associated with predisposition to GBC, but no association was found for *CYP1B1*-Val432Leu polymorphism in conferring risk for GBC. Subgroup analysis revealed association of *CYP1A1-MspI* polymorphism with overall female cancer patients as well as GBC patients without stones.

In previous studies, *CYP1A1*-Ile462Val polymorphism was reported to be associated with an increased risk of GBC in patients from Chile [18], Hungary [19], and Japan [20]. However, in a study by Park et al. from China, *CYP1A1*-Ile462Val was negatively associated with gallbladder cancer, whereas *CYP1A1* IVS1+606G>T (rs2606345) conferred an increased risk of GBC [21]. *CYP1A1* polymorphisms have also been reported to be conferring risk of several cancers such as cervical [22], gastric [23], breast [24, 25], and ovarian [26] cancers. These conflicting reports might be due to environmental factors and ethnicity differences. It is also possible that *CYP1A1* polymorphisms are in linkage disequilibrium with other functional variations which can influence the results in different populations.

There is only one study on *CYP1B1* Leu432Val in gallbladder cancer till now with null association [21]. We also found no association of this polymorphism with GBC risk. Recent meta-analysis studies showed inconsistent association of the *CYP1B1* Leu432Val polymorphism with endometrial [27], colorectal [28], lung [29], and breast [30] cancers.

Gallbladder cancer is often associated with cholelithiasis and cholecystitis [31, 32], and 53 % of patients in the present study had associated gallstones and the rest 47 % patients were without gallstones. On stratification of GBC patients on the basis of presence/absence of stones, variant genotypes of *CYP1A1-MspI* and *CYP1A1*-Ile462Val polymorphisms were associated with an increased risk of GBC in the patient group without stones. Haplotype analysis also showed an increased risk to GBC in carriers of the risk haplotype [C-val] and in the patient group without stones on subgroup analysis. Previous results of our lab also showed association of *CYP1A1-MspI* polymorphism with GBC patients without stones. Similarly, Park et al. [21] also found no association of *CYP1A1* polymorphisms with gallstone disease in the Chinese population.

This might be due to the fact that only a small fraction of patients with cholelithiasis (1–3 %) develop GBC [33], and approximately 53.0 % of patients with GBC in our study were free of gallstones. It is believed that gallbladder cancer involves two separate pathways, one involving gallstones and the other which is gallstone independent. Gallstone-independent effect may be due to alteration in xenobiotic metabolism by CYP1A1 genetic variants. Ours as well as the results of Park et al. [21] suggest that the *CYP1A1*-related risk of GBC may not involve gallstones as an intermediary step. There are some studies which indicate that people with porcelain gallbladder have a high risk of developing gallbladder cancer [34]. However, in our study, none of our patients were found to have associated porcelain gallbladder; therefore, modulation of GBC risk due to porcelain gallbladder was not studied.

CYP1A1 has been shown to be involved in the activation of tobacco-related N-nitrosamines, [35] which provoke cancer in experimental animals. The procarcinogens require activation by hydroxylation at α-position carbon of the N-nitroso group, a reaction catalyzed by CYPs [36]. The *CYP1A1*-Ile462Val (rs1048943) and *CYP1A1-MspI* (rs4646903) are associated with the elevated enzyme activity [11, 37]. The *CYP1A1*-Ile462Val occurs in the heme-binding motif of the protein, and benzo[a]pyrene (constituent of cigarette smoke and tobacco) metabolism studies have reported an almost twofold increase in variant protein (valine) enzyme activity than that of the wild-type (isoleucine) protein [38]. Ile/Val variant encoding the DNA of *CYP1A1* when transfected into a yeast cell expression system, benzo[a]pyrene mutagenic potency, and aromatic hydrocarbon hydroxylase activity were increased [38]. In the present study, *CYP1A1-MspI* (rs4646903) polymorphism is associated with an increased risk of GBC in tobacco users in case-only analysis. Previous results of our lab in a smaller sample size also showed an increased risk of tobacco-related risk of gallbladder cancer in the North Indian population [15]. Hence, genetic differences in activation/detoxification of carcinogens may be playing an essential role in individual susceptibility towards GBC due to increased carcinogenic intermediates. *CYP1A1-MspI* polymorphism has also been shown to be associated with head and neck cancer susceptibility in North Indians [39]. A recent study by Lourenco et al. reported association of *CYP1A1-MspI* polymorphism with tobacco as well as alcohol intake in conferring risk of head and neck squamous cell carcinoma [40].

In addition to the effect of *CYP1A1* on xenobiotic metabolism, the encoded enzyme is involved in estrogen metabolism. Results from functional studies suggested that both the val variant of *CYP1A1*-Ile462Val and the C variant of *CYP1A1* may be associated with increased catalytic activity towards estrogens [11, 41], i.e., the presence of these variants may well result in increased production of catechol estrogens and estrogen quinones. Estrogen quinones can react directly with DNA and form both stable adducts and depurinating adducts that can generate mutations [42–44]. These estrogen-derived DNA adducts have been identified in human breast tissue [45]. The elevated conversion of estradiol to 2-OH-E2 has been detected in several tissues, including biliary epithelium [46], and has been linked with biliary tract cancers by causing decreased gallbladder motility, resulting in increased inflammation and infection in the biliary tract [47, 48]. GBC is predominately detected in females and associated with obesity and multiple pregnancies, conditions associated to higher levels of estrogens. It suggests that endogenous estrogens may be involved in disease pathogenesis by altering bile acid composition and gallbladder motility. This study found very significant associations of *CYP1A1-MspI* polymorphism in female cancer patients.

It is well known that the total risk of gallbladder disease increases with increasing body mass index probably because circulating levels of endogenous estradiol are higher in overweight and obese women. Our results of multivariate analysis showed significant associations of *CYP1A1*-Ile462Val in GBC cases with obesity (BMI >25 kg/m^2). Park et al. also found that *CYP1A1* IVS1+606-associated risk of GBC was more pronounced among subjects having BMI >23 kg/m^2 [21]. A possible reason may be that obese subjects tend to have lower levels of sex hormone-binding globulin (SHBG) and hence increased levels of bioavailable estradiol [49, 50]. We did not find additional associations between multiparity, earlier age at menarche, higher age at menopause, etc. with GBC risk. Therefore, genes related to estrogen metabolism may also play an important role in gallbladder carcinogenesis. The association of GBC risk in nonvegetarian diets may also be related to *CYP1A1-MspI* polymorphism. However, this observation requires validation in a larger sample size.

This study has several strengths and limitations. All controls were under Hardy-Weinberg equilibrium; all patients were histopathologically confirmed, age-matched, and gender ethnicity-matched subjects; and strict quality control for genotyping was followed. The sample size in the present study is sufficient to yield 80 % power. Bonferroni correction was applied in subgroup analysis. However, like the majority of association studies, there is a need to replicate this study in other populations for definitive associations with gallbladder carcinogenesis.

Conclusion

Our study showed that *CYP1A1* polymorphisms may confer increased risk of gallbladder cancer, through impaired xenobiotic or hormone metabolism in a gallstone-independent pathway.

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Conflicts of interest None

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