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CYP7A1 (−204 *A>C;* rs3808607 and −469 *T>C;* rs3824260) promoter polymorphisms and risk of gallbladder cancer in North Indian population

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Abstract

Cholesterol 7-α hydroxylase (*CYP7A1*), which is a rate-limiting enzyme for cholesterol catabolism and bile acid synthesis, may affect cholesterol homeostasis and result in gallstone formation that is a major risk factor for gallbladder cancer (GBC) pathogenesis. Genetic variations in *CYP7A1* may influence its expression and thus may affect the risk of gallstone disease and GBC. We aimed to study the association of 2 promoter polymorphisms of *CYP7A1* (−204 *A>C* [rs3808607] and −469 *T>C* [rs3824260]) in gallstone and GBC susceptibility in North Indian population. The study included 185 GBC patients, 195 symptomatic gallstone patients, and 200 healthy controls. Genotyping for both polymorphisms was done by polymerase chain reaction–restriction fragment length polymorphism method. Although the *CC* genotype of *CYP7A1* −204 *A>C* was not significantly associated with gallstone disease ($P = .083$, odds ratio [OR] = 1.69, 95% confidence interval [CI] = 0.9-3.0), it was conferring higher risk for GBC ($P = .018$, OR = 2.05, 95% CI = 1.1-3.7). However, *CYP7A1* −469 *T>C* was not associated with gallstone disease and GBC risk in our population. After subgroup stratifications on the basis of sex and gallstone status, *CC* genotype and variant allele of *CYP7A1* −204 *A>C* imparted higher risk for GBC in women ($P = .003$, OR = 3.30, 95% CI = 1.5-7.2) and patients without gallstones ($P = .045$, OR = 1.91, 95% CI = 1.2-3.6). Haplotype analysis of the 2 polymorphisms showed that *C,T* ($P = .045$, OR = 1.84, 95% CI = 1.0-3.3) and *C,C* ($P = .0001$, OR = 3.10, 95% CI = 1.6-6.0) haplotypes had elevated risk of GBC predisposition. *CYP7A1* −469 *T>C* is not associated with gallstone disease or GBC risk. Although *CYP7A1* −204 *A>C* might play a modest role in gallstone susceptibility, it is an independent risk factor for GBC in North Indian population. Underlying mechanism for GBC susceptibility by *CYP7A1* (−204 *A>C* and −469 *T>C*) haplotype appears to be independent of gallstone pathway and is believed to involve genotoxicity resulting from subnormal bile acid production.

1. Introduction

Liver plays a central role in regulation and maintenance of whole-body sterol balance. Conversion of cholesterol into bile acids in liver, along with secretion of cholesterol into bile, is quantitatively the major pathway for eliminating cholesterol from the body. Cholesterol 7-α hydroxylase (CYP7A1, EC 1.14.13.17), encoded by *CYP7A1*, is the first and rate-limiting step in the classic bile acid synthesis pathway. Activity of CYP7A1 (a cytochrome P-450 enzyme) is regulated by bile acids, cholesterol, and hormones [1].

Increased expression of *CYP7A1* messenger RNA in the presence of cholesterol, along with its down-regulation in the presence of bile acid, has been observed in in vitro and animal models [2,3]. Pharmacologic manipulation of *CYP7A1* expression by bile acid–binding resins, in family-based analysis, has also emphasized the role of bile acids and cholesterol in the transcriptional regulation of *CYP7A1* [4,5]. Recently, studies have focused on the role of *CYP7A1* in gallstone disease; however, the role of *CYP7A1* in patients with gallstones remains controversial [6].

Various genetic variations have been reported in *CYP7A1* gene. It is assumed that 2 common promoter polymorphisms may affect CYP7A1 activity, presumably by influencing the rate of transcription of *CYP7A1* gene.

Conflict of interest: none.

Institutional approval: The study was approved by the appropriate committee of the institution, and informed consent was obtained from all the subjects.

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The −204 *A>C* polymorphism has been widely studied and associated with subclinical atherosclerosis [7], low-density lipoprotein cholesterol level [8], gallstone disease [9], etc. However, studies exploring the role of −469 *T>C* polymorphism showed no association of this genetic variant with plasma low-density lipoprotein cholesterol concentration [10,11]. Defect in CYP7A1 has been suggested to play a pathogenic role in cholesterol gallstone disease [12,13]. In North Indian population, the incidence of gallbladder cancer (GBC) is very high (4.5 per 100 000 for men and 10.1 per 100 000 for women). Etiology of GBC is complex; and associated risk factors identified so far include cholelithiasis, obesity, reproductive factors, chronic infection, environmental exposure to specific chemicals, and genetic factors [14]. There is a close pathophysiologic correlation between GBC and gallstone disease, and more than 50% of GBC patients are associated with gallstones [15]. Therefore, *CYP7A1* may also be a major candidate gene for GBC susceptibility [16].

Considering the pivotal role of *CYP7A1* in maintaining cholesterol homeostasis, we hypothesized that genetic variants of *CYP7A1* may play a significant role in conferring interindividual variations in GBC risk. This study aimed to explore the role of genetic variants in *CYP7A1* (−204 *A>C* [rs3808607] and −469*T>C* [rs3824260]) in conferring genetic susceptibility for gallbladder disease in North Indian population that has one of the highest incidence of both gallstone disease as well as GBC.

2. Material and methods

2.1. Patients

The present case-control study comprised 185 cases of GBC patients (fine needle–aspirated cell cytology and histopathologically proven), 195 cholesterol gallstone patients (positive for gallstone in USG examination) undergoing cholecystectomy, and 200 healthy controls. Both the GBC and gallstone patients were recruited from the patients attending the clinics of Department of Gastroenterology and Gastrosurgery of Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP India, from March 2005 to November 2008. Staging of cancer was documented according to American Joint Committee on Cancer [17,18]. The subjects included in the present study were the same as in our previous study, and successively diagnosed cases were also included in this study [16]. Control subjects were ultrasonically proven gallstone-free healthy adults without any history of cancer. These subjects were randomly selected from the general population and were frequency-matched to GBC and gallstone cases on age and sex. The study was approved by the local ethics committee of the institute. Informed consent was obtained from all the subjects, and blood samples from the subjects were collected in EDTA vials and stored at −70°C.

2.2. Genotyping

Laboratory personnel were blinded to the case-control status of the subjects. The genomic DNA was extracted from peripheral blood using the standard salting-out method [19]. Genotyping of *CYP7A1* −204 *A>C* (rs3808607) and −469 *T>C* (rs3824260) polymorphisms was done by using polymerase chain reaction (PCR)–restriction fragment length polymorphism.

The target sequence containing −204 *A>C* polymorphic site was amplified using PCR procedure using primer sequences described by Wang et al [5]. Genotyping for this polymorphism was done as reported in our previous study [16].

The primer set used to amplify the *CYP7A1* −469 *T>C* polymorphic site was as described by Abrahamsson et al [20]. Genetic variation *T>C* at −469 position creates the site for *Tai*I enzyme. The PCR product was digested with 5 units of *Tai*I restriction enzyme (Fermentas, Glen Burnie, MD) for 6 hours at 65°C. The digested products were separated on 2.5% agarose gel.

The presence of C nucleotide at the polymorphic site creates restriction site for *Tai*I enzyme; and 2 bands of 481 and 93 base pairs were obtained upon digestion, whereas PCR product from *T* allele remained undigested (574 base pairs).

To improve the genotyping quality and validation, 20% of samples were regenotyped by other laboratory personnel; and results were reproducible with no discrepancy in genotyping. Genotyping of 10% of samples was confirmed by DNA sequencing.

2.3. Subgroup stratifications

To explore the sex-specific effect of these polymorphisms, analysis was done after stratification of all the subjects according to sex and gallstone status.

2.4. Statistical analysis

The sample size of the present study was calculated using QUANTO 1.1 program (hydra.usc.edu/gxe), considering the minor allele frequency of the single nucleotide polymorphisms analyzed. Desired power of our study was set at 80% (probability of not making type II error). Descriptive statistics of patients and controls were presented as mean and SDs for continuous measures, whereas frequencies and percentages were used for categorical measures. The χ^2 goodness-of-fit test was used for any deviation from Hardy-Weinberg equilibrium in controls. Differences in genotype and allele frequencies between study groups were estimated by χ^2 test. Gallbladder cancer risk in relation to *CYP7A1* genotypes was estimated by using unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). The ORs were adjusted for confounding factors such as age and sex. All statistical analyses were performed using SPSS software version 11.5

(SPSS, Chicago, IL), tests of statistical significance were 2-sided, and differences were taken as significant when P value was < .05. Statistical analysis of the haplotypes estimation and linkage disequilibrium was conducted using the Arlequin software version 2.00 by expectation-maximization algorithm [21]. The false-positive report probability for statistically significant observations was estimated using the methods described by Wacholder et al [22].

3. Results

Demographic characteristics of GBC patient and controls are summarized in Table 1. The mean age was not significantly different in GBC patients, gallstone patients, and controls. Gallstones were present in 51.4% of GBC patients, and most of the GBC patients were in advanced stages of cancer (stage III and stage IV). All cancer patients were incident cases, and none of the controls had family history of cancer. It was evident from the interview that all the cancer patients and controls belonged to same ethnic population, that is, North Indians.

3.1. Distribution of genotypes of CYP7A1 −204 A>C and −469 T>C polymorphisms in controls

In our studied population, distribution of genotypes of −204 *A>C* and −469 *T>C* polymorphisms was in Hardy-Weinberg equilibrium in controls (P > .05). In *CYP7A1* −204 *A>C* polymorphism, control genotype frequencies of *AA*, *AC*, and *CC* were 35%, 50.5%, and 14.5%, respectively. The distributions of *TT, TC*, and *CC* genotypes in controls for −469 *T>C* polymorphisms were 23.0%, 51.5%, and 26.0%, respectively (Tables 2 and 3).

3.2. Gallbladder cancer cases and controls

On comparing the frequency distribution of genotypes of *CYP7A1* −204 *A>C* polymorphism, there was an increase in the frequency distribution of *CC* genotype in GBC group as compared with controls (14.5% and 23.8%, respectively), conferring significantly high risk for GBC (P = .018, OR = 2.05, 95% CI = 1.1-3.7) (Table 2). At the allele level also, there was a significantly increased risk for GBC in the presence of *C* allele (P = .022, OR = 1.39, 95% CI = 1.1-1.8). The dominant genetic model for this polymorphism was not associated with GBC risk (Table 2).

On comparing the frequency distribution of genotypes and alleles of *CYP7A1* −469*T>C* polymorphism, no significant difference in distribution was observed between

Table 1
Characteristic profile of controls and GBC patients

Variables	GBC	GS	Controls
	n (%)	n (%)	n (%)
Total	185	195	200
Female	117 (63.2)	131 (67.2)	125 (62.5)
Male	68 (36.8)	64 (32.8)	75 (37.5)
Age ± SD	53.39 ± 10.95	49.44 ± 12.36	52.81 ± 10.77
Stages	0, I	None	
	II	11 (5.9)	
	IV	83 (44.9)	
	III	91 (49.2)	
Gallstone present	95 (51.4)	195 (100)	None
Gallstone absent	90 (48.6)	None	200 (100)

Comparison of mean age ± SD years: GBC vs controls: P = .934; GBC vs gallstone patients: P = .096: gallstone patients vs controls: P = .096. GS indicates gallstone patients.

Table 2
Genotypes and alleles frequencies for the *CYP7A1* −204 *A>C (rs3808607)* polymorphism in GBC, GS, and healthy controls

	GBC vs HC			
Genotypes/ alleles	Frequency (%)		P value	OR (95% CI)
	GBC (185) n (%)	HC (200) n (%)		
AA	52 (28.1)	70 (35.0)	–	1 (Reference)
AC	89 (48.1)	101 (50.5)	.476	1.18 (0.7-1.8)
CC	44 (23.8)	29 (14.5)	**.018**	2.05 (1.1-3.7)
A	193 (52.2)	241 (60.2)	–	1 (Reference)
C	177 (47.8)	159 (39.8)	**.022**	1.39 (1.1-1.8)
AC + CC	133 (71.9)	130 (65.0)	.151	1.37 (0.8-2.1)
	GS vs HC			
	GS (195) n (%)	HC (200) n (%)		
AA	62 (31.8)	70 (35)	–	60 (32.4)
AC	91 (46.7)	101 (50.5)	.739	1.09 (0.6-1.7)
CC	42 (21.5)	29 (14.5)	.083	1.69 (0.9-3.0)
A	215 (55.1)	241 (60.2)	–	–
C	175 (44.9)	159 (39.8)	.114	1.26 (0.9-1.6)
AC + CC	132 (67.7)	130 (65.0)	.427	1.18 (0.7-1.8)

Significant values are given in bold. HC indicates healthy controls.

Table 3
Genotypes and alleles frequencies of *CYP7A1* −469*T>C (rs3824260)* polymorphism in GBC, GS, and HC

	GBC vs HC			
Genotypes/ alleles	Frequency (%)		P value	OR (95% CI)
	GBC (185)	HC (200)		
	n (%)	n (%)		
TT	45 (24.3)	46 (23.0)	–	1 (Reference)
TC	96 (51.9)	102 (51.0)	.899	0.96 (0.5-1.5)
CC	44 (23.8)	52 (26.0)	.896	0.88 (0.4-1.5)
T	186 (50.3)	194 (48.5)	–	1 (Reference)
C	184 (49.7)	206 (51.5)	.686	0.94 (0.7-1.2)
TC + CC	140 (75.7)	154 (77.0)	.792	0.93 (0.5-1.5)
	GS vs HC			
	GS (195) n (%)	HC (200) n (%)		
TT	38 (19.5)	46 (23.0)	–	1 (Reference)
TC	103 (52.8)	102 (51.0)	.600	1.14 (0.6-1.9)
CC	54 (27.7)	52 (26.0)	.646	1.14 (0.6-2.0)
T	179 (45.9)	194 (48.5)	–	1 (Reference)
C	211 (54.1)	206 (51.5)	.565	1.08 (0.8-1.4)
TC + CC	157 (80.5)	154 (77.0)	.583	1.15 (0.7-1.8)

Table 4
Frequency distribution of haplotypes of *CYP7A1* −204 *A>C* (rs3808607) and *CYP7A1* −469*T>C* (rs3824260) polymorphisms in GBC, GS, and HC

GBC and HC				
Haplotypes	Frequency		*P* value	OR (95% CI)
	GBC	Controls		
A,T	0.3051	0.4238	–	1.00
A,C	0.2862	0.3063	.38	1.27 (0.7-2.1)
C,T	0.2165	0.1787	**.045**	1.84 (1.0-3.3)
C,C	0.1922	0.0912	**.0001**	3.10 (1.6-6.0)
Global haplotype association *P* value = .0093				

GS and HC				
	GS	HC		
A,T	0.3055	0.4238	–	1.00
A,C	0.2001	0.3063	.99	1.00 (0.6-1.5)
C,T	0.2594	0.1787	.14	1.22 (1.3-3.6)
C,C	0.2351	0.0912	.065	1.41 (1.9-5.9)
Global haplotype association *P* value < .0001				

Significant values are given in bold.

GBC cases and controls. Carrier analysis also showed no significant difference in distribution of genotypes (Table 3).

Haplotypes were constructed for *CYP7A1* −204 *A>C* and −469 *T>C*; and results were computed for GBC, gallstone patients, and healthy controls. The *A,T* haplotype was the most common among all the 3 groups. On comparing the frequency distribution of haplotypes in GBC patients with that of controls, the *C,T* and *C,C* haplotype frequencies were significantly higher in GBC group and imposed higher risk for the disease (P = .045, OR = 1.84, 95% CI = 1.0-3.3 and P = .0001, OR = 3.10, 95% CI = 1.6-6.0, respectively) (Table 4).

3.3. Gallstone patients and controls

On comparing the frequency distribution of genotypes and alleles of *CYP7A1* −204 *A>C* polymorphism, frequency of *CC* genotype was higher in gallstone patients as compared with controls (21.5% vs 14.5%); but the difference was not statistically significant (P = .083) (Table 2). On comparing the frequency distribution of genotypes and alleles of *CYP7A1* −469 *T>C* polymorphism between gallstone cases and controls, no significant difference in their distribution was observed (Table 3).

Haplotype analysis of *CYP7A1* −204 *A>C* and *CYP7A1* −469*T>C* locus also showed no significant difference in the distribution of any of the haplotypes in gallstone patients in comparison to controls (Table 4).

3.4. Effect of gallstone status

Gallbladder cancer patients were stratified on the basis of presence or absence of gallstones. The GBC patients without gallstone (absence of gallstones) were compared with healthy controls, and the cancer patients with gallstones were compared with gallstone patients.

This analyses suggested that for *CYP7A1* −204 *A>C* polymorphism, GBC patients without stones had a significantly (P = .045) increased frequency of *CC* genotype as compared with controls; and this difference conferred high risk (OR = 1.91, 95% CI = 1.2-3.6) for GBC (Table 5). However, *CYP7A1* −469*T>C* polymorphism showed no significant difference in the distribution of any of the genotypes and alleles in GBC patients with or without gallstones (data not shown).

Haplotype frequency was also compared after subdividing GBC patients on the basis of gallstone status, and results for *C,T* haplotype were similar to those obtained at genotype level in *CYP7A1* −204 *A>C* (Table 6).

3.5. Effect of sex

After stratification of all the subjects according to sex, it was observed that significantly increased risk due to presence of *CYP7A1* −204 *A>C*, *CC* genotype was confined

Table 5
Frequency distribution of *CYP7A1* −204 *A>C* (rs3808607) polymorphism genotypes after subgroup stratifications

Host characteristic	Control/GBC	OR (95% CI) *P* value	Gallstone/GBC	OR (95% CI) *P* value
Gallstone	n = 200/90	Gallstone absent	n = 195/95	Gallstone present
AA	70/27	Reference	62/25	Reference
AC	101/41	1.02 (0.5-1.8) P = .944	91/48	1.27 (0.7-2.3) P = .416
CC	29/22	1.91 (1.2-3.6) ***P* = .045**	42/22	1.29 (0.6-2.6) P = .471
A	241/95	Reference	215/98	Reference
C	159/85	1.33 (0.9-1.9) P = .113	175/92	1.22 (0.8-1.7) P = .259
AC + CC	130/63	1.15 (0.50-2.64) P = .753	132/70	1.31 (0.7-2.2) P = .338
Sex	n = 75/68	Male	n = 125/117	Female
AA	17/21	Reference	53/31	Reference
AC	43/31	0.58 (0.2-1.2) P = .186	58/58	1.64 (0.9-2.9) P = .092
CC	15/16	0.84 (0.3-2.1) P = .722	14/28	3.30 (1.5-7.2) ***P* = .003**
A	77/73	Reference	164/120	Reference
C	73/63	0.89 (0.5-1.4) P = .655	86/114	1.77 (1.2-2.5) ***P* = .002**
AC + CC	58/47	0.65 (0.3-1.3) P = .265	72/86	1.96 (1.1-3.3) ***P* = .015**

Significant values are given in bold.

Table 6
Comparison of frequency distribution of haplotypes of *CYP7A1* −204*A>C* (rs3808607) and *CYP7A1* −469*T>C* (rs3824260) polymorphism in GBC patients having stone with gallstone patients and GBC patients without stone with controls

Haplotypes	GBC with stone				GBC without stone			
	GBC	GS	*P* value	OR (95% CI)	GBC	Controls	*P* value	OR (95% CI)
A,T	0.3029	0.3055	–	1.00	0.3011	.4238	–	1.00
A,C	0.2129	0.2001	.41	1.32 (0.6-2.5)	0.24	0.3063	.63	1.21 (0.5-2.6)
C,T	0.3239	0.2594	.058	1.85 (1.0-3.3)	0.2267	0.1787	.12	2.00 (0.8-4.7)
C,C	01603	0.2351	.51	0.78 (0.3-1.6)	0.2323	0.0912	**.001**	4.24 (1.7-10.0)

Significant value is given in bold.

to female GBC patients (*P* = .003, OR = 3.30, 95% CI = 1.5-7.2). At the allele level also, the significantly increased risk was confined to female GBC patients (*P* = .002, OR = 1.77, 95% CI = 1.2-2.5). Similarly, carrier analysis (dominant model) also showed an increased risk for GBC in women (*P* = .015, OR = 1.96, 95% CI = 1.1-3.3) (Tables 5 and 7).

Even after stratification of subjects on the basis of sex, there was no significant difference in the distribution of genotypes and alleles of *CYP7A1* −469 *T>C* polymorphism in GBC patients and controls (data not shown).

4. Discussion

CYP7A1 enzyme (EC 1.14.13.17) is found exclusively in the liver, enhancing the significance of this gene as a target to study the molecular mechanisms implicated in hepatic-specific gene expression [23].

We observed that *CC* genotype of *CYP7A1* −204 *A>C* (rs3808607) polymorphism was conferring marginal risk (statistically insignificant) for gallstone disease, which emphasizes that *CYP7A1* gene may affect gallstone pathogenesis but that its role is inconsequential. On the other hand, carriers of variant allele of *CYP7A1* −204 *A>C* polymorphism had significantly higher risk for GBC. The increased risk was persistent in the presence of *CC* genotype, *C* allele and also in the dominant model of carrier analysis. The *CYP7A1* −469 *T>C* (rs3824260) polymorphism also resides in the promoter region; it was hypothesized that this genetic variation may also play an important role in transcription efficiency, but this polymorphism was not found to be associated with GBC risk or gallstone susceptibility. Subgroup stratifications also showed no association of −469 *T>C* with GBC susceptibility in any of the subgroups. Haplotype analysis of the *CYP7A1* locus revealed that the presence of *C,C* haplotype conferred very high risk for GBC, whereas there was a moderate risk for GBC in the presence of *C,T* haplotype. Based on OR, it appears that −469 *T>C* polymorphism does not contribute individually to the risk of GBC, but further enhances the GBC risk resulting from *CYP7A1* −204 *A>C* polymorphism. In our study, female subjects carrying *CYP7A1* −204 *C* allele were at significantly higher risk for GBC development. Erickson et al [24] also showed that *CYP7A1* deficiency follows a sex-specific trend, affecting women to a larger extent [25]. It is possible that this polymorphism may affect the expression of some female hormone response elements resulting in increased GBC susceptibility in women.

Table 7
False-positive report probability of results of *CYP7A1* −204 *A>C* (rs3808607) polymorphism

Genotype/ allele	OR (95% CI)	Power[a]	Reported *P*	Prior probability			
				.1	.05	.01	.001
GBC vs HC							
Genotypes (AA reference)							
CC	2.05 (1.1-3.7)	0.474	.018	**.255**	**.419**	.790	.974
C	1.39 (1.1-1.8)	0.998	.022	**.165**	**.295**	.686	.957
GBC without gallstone vs HC							
CC	1.91 (1.2-3.6)	0.560	.045	**.413**	.598	.886	.987
GBC vs HC (female)							
CC	3.30 (1.5-7.2)	0.110	.003	**.197**	**.341**	.730	.965
C	1.77 (1.2-2.5)	0.800	.002	**.022**	**.045**	**.198**	.714
AC + CC	1.96 (1.1-3.3)	0.570	.015	**.192**	**.333**	.723	.963

Bold-faced values indicate the false-positive report probability (≤0.5) for the most likely prior probability.

[a] Estimation of statistical power to detect an OR of 2.0 with an α level equal to the observed *P* value.

As *CYP7A1* is involved in cholesterol homeostasis, it was hypothesized that risk of the genetic variants on GBC might be influenced through effects on gallstone formation. However, after segregation of GBC patients on the basis of gallstone status, frequency distribution of haplotypes in GBC patients without gallstone and its comparison with controls showed very high risk for GBC. World over, 2 main pathways of GBC pathogenesis have been identified. In the first pathway, the cancer is commonly associated with gallstones and chronic inflammation of the gallbladder. In the second pathway, GBC is not accompanied by gallstones. The main mechanism by which decreased expression of *CYP7A1* may affect GBC susceptibility appears to be mediated by subnormal bile acid production. Decreased bile acid production, which will also reduce the bile flow, may result in accumulation of free radicals and other toxic products as a result of lipid peroxidation [26]. In the course of cellular xenobiotic and normal metabolic processes, lipid oxides, diene conjugates, and other lipid peroxidation

products together with carcinogens are produced in liver, most of which in normal course are washed away from liver through bile. Their accumulation in gallbladder may initiate primary and secondary free radical reactions that may induce neoplasia [26]. This notion is further emphasized by the presence of elevated levels of lipid peroxidation (free radical oxidation) products in North Indian GBC patients [27]. Out of various lipid peroxides identified in biological system, the 4-hydroxyalkenals and in particular 4-hydroxynonenal, which is one of the major products of liver microsomal lipid peroxidation, have shown high genotoxic effect and hence neoplastic potential [28,29].

The mechanism by which these genetic variations regulate the basal transcriptional level of the *CYP7A1* gene is not very clear. *CYP7A1* gene obtained from nuclei of transcriptionally active HepG2 cells have shown that between −50 and −200, a region of hypersensitivity is present that was absent in transcriptionally inactive HeLa cell nuclei and in free DNA [30]. In addition, several cell-specific enhancer elements between nt −432 and −220 in *CYP7A1* promoter are present, which are transcriptionally regulated, partially by hepatocyte nuclear factor–3. Of this regulatory stretch, deletion of the segment from −213 to −91 has shown an approximately 40% reduction in promoter activity [30,31].

In our previous study, we suggested that the crucial location of −204 *A>C* polymorphism in *CYP7A1* gene promoter might be responsible for conferring interindividual susceptibility to GBC. This notion is further strengthened in this study by studying haplotype analysis. As observed in rat models, the altered gallbladder bile composition to lithogenic profile due to CYP7A1 deficiency may impair fatty acid metabolism and decrease hepatic canalicular bile acid transport [24]. Similarly, we can speculate an identical mechanism in humans that results in increased GBC susceptibility.

In summary, to the best of our knowledge, this is the first report exploring the role of haplotypes of *CYP7A1* −204 *A>C* and −469 *T>C* polymorphisms in conferring susceptibility to GBC. As the association studies are population specific, the observation requires validation in other high- and low-risk populations.

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