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Genetic polymorphisms in *GSTM1*, *GSTT1*, *GSTP1*, *GSTM3* and the susceptibility to gallbladder cancer in North India

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Abstract

The glutathione *S*-transferase (GSTs) are polymorphic supergene family of detoxification enzymes that are involved in the metabolism of numerous potential carcinogens. Several allelic variants of polymorphic GSTs show impaired enzyme activity and are suspected to increase the susceptibility to various cancers. To find out the association of GST variants with risk of gallbladder cancer, the distribution of polymorphisms in the GST family of genes (*GSTT1*, *GSTM1*, *GSTP1*, and *GSTM3*) were studied in 106 cancer patients and 201 healthy controls. Genotypes were analysed by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (RFLP). The frequencies of *GSTM1* null and *GSTM3*BB* genotypes did not differ between patients and controls. The overall frequency of *GSTT1* null was lower in cases as compared with controls ($p = 0.003$, Odds ratio (OR) = 0.2, 95% confidence interval (CI), 0.1–0.6). After sex stratification, the *GSTT1* null frequency was reduced only in female patients ($p = 0.008$, OR = 0.2, 95% CI = 0.1–0.6). However, the *GSTP1*, *ile/val* genotype and the *val* allele were significantly higher in cases than controls ($p = 0.013$, OR = 1.9, 95% CI = 1.1–3.1; $p = 0.027$, OR = 1.5, 95% CI = 1.0–2.1), respectively. To study gene–gene interactions, a combined risk of gallbladder cancer due to *ile/val* or *val/val* were calculated in combination with null alleles of *GSTM1* and *GSTT1* or the *B allele of *GSTM3*, but there was no enhancement of risk. Gallstones were present in 57.5% of patients with gallbladder cancer, but there were no significant differences between allelic/genotype frequencies of the studied *GST* genes polymorphisms between patients with or without gallstones. To best of our knowledge, this is the first paper showing *ile/val* genotypes and *val* allele of *GSTP1* to be associated with higher risk of gallbladder cancer.

Keywords: *Gallbladder cancer, glutathione* S*-transferase, gallstone, genetic polymorphism, xenobiotics.*

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Introduction

Carcinoma of the gallbladder is the most common malignant lesion of the biliary tract and the fifth most common among malignant neoplasms of the digestive tract (Nagorney and McPherson 1988, Misra et al. 1997). It is a highly fatal disease with a

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poor prognosis. Epidemiological studies have revealed a wide geographical, ethnic and cultural variation in the incidence of gallbladder cancer. The carcinoma affects women two-to-six times more commonly than men. The incidence rate varies greatly in different parts of the world (Misra et al. 2003).

Gallbladder cancer is multifactorial and associated risk factor identified so far including cholelithiosis, obesity, reproductive factors, chronic infection and environmental exposure to specific chemicals (Lazcano-Ponce et al. 2001). Such geographic variation may be related to different genetic and environmental factors including dietary patterns (Coleman et al. 1993). Molecular epidemiological studies have now provided evidence that individual's susceptibility to cancer is modulated by both genetic and environmental factors. Inherited differences in the effectiveness of the activation/detoxification of carcinogens play a crucial role in host susceptibility. Thus, there is an urgent need to know the host genetic markers that could predispose an individual to cancer.

Glutathione *S*-transferases (GSTs) are a super family of dimeric phase II metabolic enzymes. The multi-gene family consists of four major genes, *GSTA* (alpha), *GSTT1* (theta), *GSTM1* (mu), and *GSTP1* (pi), and they play an active role in the detoxification and elimination of carcinogens. GST enzymes catalyse the conjugation of toxic and carcinogenic electrophilic molecules with glutathione and thereby protect cellular macromolecules from damage (Boyer and Kenney 1985). The genes for four members of the GST family, namely *GSTM1*, *GSTT1*, *GSTP1* and *GSTM3*, display polymorphisms that have been associated with increased risk for certain cancers (Bell et al. 1993, McWilliams et al. 1995, Yengi et al. 1996). *GSTM1* is expressed in hepatocytes and at a lower level in biliary epithelial cells in approximately 50% of individuals due to genetic polymorphism (DeWaziers et al. 1990). In human liver, *GSTP1* is essentially absent from normal hepatocytes, but it is expressed in a high amount in biliary epithelium including the gallbladder (Hayes et al. 1989). The *GSTM1* null and *GSTT1* null alleles represent homozygous deletions of *GSTM1* and *GSTT1* genes and result in the absence of enzymatic activity (Strange and Fryer 1999). The *GSTM3* gene has two alleles identified so far: *GSTM3*A* and *GSTM3*B*, of which the latter has a 3 base pair deletion in intron 6, known as a recognition motif for the YY1 transcription factor. The *GSTM3*B* allele has increased transcription potential that results in an enhanced detoxification activity of *GSTM3*-encoded protein (Yengi et al. 1996). There are several studies that indicate that *GSTM1* effects may be modulated by the *GSTM3* genotype (Shiy et al. 1997). *GSTP1* is a major isoform that can eliminate DNA oxidative products of thymidine or uracil propenal (Berhane et al. 1994). A 313 A > G transition in *GSTP1* gives rise to the *ile*/*val* polymorphism, which confers reduced enzyme activity (Ali-Osman et al. 1997). The liver is the major site of xenobiotic metabolism, but the biliary epithelium lining has a large surface area over which bile, which is initially secreted by hepatocytes and transported to intestine, is modified. However, the contribution of biliary epithelium cells to the biotransformation of xenobiotics remains poorly defined (Degott et al. 1992). There is a possibility that the altered biotransformation of xenobiotics including carcinogens may contribute towards a susceptibility to gallbladder cancer. Therefore, the present authors have explored the potential relationships between the genotypes of four GST enzymes and the risk of gallbladder cancer.

Materials and methods

Subjects

The present case control study comprised 106 consecutive cases of proven gallbladder cancer from the Department of Gastroenterology and Gastro-surgery of Sanjay Gandhi Post Graduate institute of Medical Sciences (SGPGIMS), Lucknow, UP, India. The clinical profile of patients was based on hospital investigations. The staging of cancer was documented according to the American Joint Committee on Cancer (Misra et al. 2003). A total of 201 controls were recruited from staff of SGPGIMS and the unrelated persons visiting the hospital for minor medical or surgical problems. The inclusion criteria for the controls were the absence of a prior history of cancer, pre-cancerous lesions, asthma, coronary artery disease, or diabetes mellitus. After obtaining informed consent, all individuals were personally interviewed for information on their ethnicity, food habits, occupation, drinking and tobacco usage.

Drinkers were defined as habitual drinkers, occasional drinkers and non-drinkers. Tobacco usage in any form such as smoking cigarette, bidi (leaf-rolled unrefined tobacco) or chewing (non-smoking tobacco) was recorded. Lifetime tobacco exposure was measured in terms of chewing-years and pack-years. The majority of the female patients were housewives; and the male patients were not engaged in any hazardous occupations. The study was approved by the Ethical Committee of the authors' Institute.

Genotyping

A total of 5 ml blood was collected in sterile ethylenediamine tetra-acetic acid (EDTA) vials from all subjects. DNA was extracted using a salting out method (Miller et al. 1988). Polymorphisms at *GSTM1*, *GSTM3*, *GSTT1* and *GSTP1* gene loci were determined using the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

GSTM1 *and* GSTT1. Homozygous null deletion polymorphisms in *GSTM1* and *GSTT1* genes were determined by multiplex PCR using specific primers (Setiawan et al. 2000) and the *CYP1A1* gene as an internal control. A total of 100 ng DNA as a template with 10 pmol of each primer and 1.5 units *Taq* DNA polymerase (Invitrogen, Carslbad, CA, USA) was used in a total volume of 25 µl. The annealing temperature was 58°C; PCR was carried out for 34 cycles. The PCR products were separated on 2% agarose gel.

GSTP1. The presence of the *GSTP1* codon 105 polymorphism was screened by PCR-RFLP analysis (Toruner et al. 2001). A total of 100 ng DNA was used as a template with 10 pmol of each primer and 1.5 units Taq DNA polymerase in a total volume of 25 µl. The annealing temperature was 60°C; 35 cycles were carried out for PCR. The 176-bp PCR product was digested with *Alw261* (Fermentas Inc., Maryland, USA) overnight at 37°C and electrophoresed on 10% polyacrylamide gel. The *GSTP1* (*ile*/*ile*) genotype corresponded to a 176-bp band; the *GSTP1* (*ile*/*val*) genotype showed 176-, 95- and 81-bp bands; and the *val*/*val* genotype bands corresponded to 81 and 95 bp.

GSTM3. The presence of the *GSTM3* polymorphism was screened by PCR amplification of intron 6 (Loktionov et al. 2001). A total of 50 ng DNA was used as a template with 10 pmol of each primer and 1.5 units Taq DNA polymerase in a total volume of 25 μl. The annealing temperature was 61°C; 30 cycles were carried out. The *GSTM*A* and *GSTM*B* alleles were detected as 79- and 76-bp DNA bands on 20% polyacrylamide gel.

Quality control

Twenty per cent of samples from both patients and controls were re-genotyped by other laboratory personnel and no discrepancy in genotyping was noticed.

Statistical analysis

Differences in genotype prevalence between the case and control groups were assessed by the Chi-square test. A $p < 0.05$ was considered as being statistically significant. A Student's *t*-test was employed to compare the lifetime tobacco exposure in cases and controls. Age- and sex-adjusted odds ratio (OR) and 95% confidence interval (CI) associated with the putative at-risk GST genotypes were calculated by unconditional logistic regression analysis. A test for the Hardy–Weinberg equilibrium for *GSTP1* and *GSTM3* was conducted by comparing observed and expected genotype frequencies using Chi-square analysis. All analyses were performed using the SPSS statistical analysis software, version 11.5 (SPSS, Chicago, IL, USA).

Results

The mean age was 52 ± 11.2 years for cases and 52.6 ± 9.6 for controls. The distributions of age and sex among these two groups of subjects were approximately similar. The symptomatic gallstones were present in 57.5% of patients. The percentage of patients with gallstones was higher in females (63%) than in males (53%). Tobacco usage either by smoking or chewing was present in 16% of patients and in 21% of controls. Among cigarettes smokers and tobacco chewers, the lifetime exposer level was not significant between cases and controls. The alcohol drinkers were 5.4% in patients and 8.5% in controls. Most of the patients were in advance stages of cancer (stages 3 and 4) and due to less of a difference between stages 3 and 4, the genotypic association was not calculated. A total of 21% patients presented with cholangitis and 11% with cholecystitis (Table I).

The frequency distribution of various *GST* genotypes in cases of gallbladder cancer and controls is presented in Table II. The genotype distributions for *GSTP1* and *GST M3* in controls were in agreement with the Hardy–Weinberg equilibrium. The frequency of the *GSTM1* null genotype was not different in gallbladder cancer patients than in controls. However, the *GSTT1* null was lower in cases as compared with controls ($p = 0.003$). After segregating data in males and females, the *GSTT1* null frequency was reduced only in female patients ($p = 0.008$), whereas no difference in *GSTM1* null and *GSTT1* null was observed in males (Table II).

Highly significant differences were observed in *GSTP1*, where the *ile/val* genotype frequency was significantly higher in cases than in controls ($p = 0.013$, OR = 1.7, 95% CI = 1.0–2.8) with a concomitant decrease in the *GSTP1 ile/ile* genotype. In addition, at the allele level, the *val* frequency was also higher in gallbladder cancer patients.

Table I. Characteristic of gallbladder cancer patients and controls.

Variables	Cases, *n* (%)	Controls, *n* (%)
Total	106	201
Female	72 (70)	118 (58.7)
Age ± SD	52 ± 11.2	52.6 ± 9.6
Stages:		
0, I	none	
II	5 (5.3)	
III	36 (37.9)	
IV	54 (56.8)	
Gallstone present	61 (57.5)	
Multiple stones (more than two)	20 (18.9)	
Cholangitis	23 (21.5)	
Cholecystitis	12 (11.2)	
Exclusive smoking habit	6 (5.6)	13 (6.4)
Exclusive chewing habit	7 (6.6)	23 (11.4)
Mixed habits (smoking and chewing)	4 (3.7)	7 (3.4)
Life time tobacco smoke (mean of smoking dose ± SD), PY#	10.4 ± 7.6	12.7 ± 5.8
Life time tobacco chewing (mean of smoking dose ± SD), CY*	177.2 ± 92.6	186.2 ± 50.7
Drinking habit:		
Habitual	2 (1.8)	7 (3.5)
Occasional	4 (3.6)	10 (5)

*Chewing-year (CY) = frequency of tobacco chewing per day multiplied by the duration of the habit in years.
#Pack-year (PY) = number of packs of tobacco smoke (one pack = 20 cigarette or 40 bidi; one cigarette = two bidi) per day multiplied by the duration of the habit in years.
Drinking was divided into habitual (consume daily) and occasional (fewer than two drinks per week, usually small doses.

Table II. Frequency distribution and age- and sex-adjusted OR and CI of *GSTT1*, *GSTM1*, *GSTP1*, and *GSTM3* genotypes and alleles in gallbladder cancer patients and controls.

Genotype/allele	Cases, *n* (%)	Controls, *n* (%)	*p*	Age adjusted OR (95% CI)
GSTM1 null	46 (43.4)	78 (38.8)	0.356	1.2 (0.7–2.0)
GSTT1 null	7 (6.6)	42 (20.9)	0.003	0.2 (0.1–0.6)
GSTP1				
ile/val	54 (50.9)	76 (37.8)	0.013	1.9 (1.1–3.1)
val/val	10 (9.4)	13 (6.5)	0.350	2.1 (0.8–5.1)
val	74* (34.0)	102* (25.6)	0.027	1.5 (1.0–2.1)
GSTM3				
A/B	18 (17.0)	29 (14.4)	0.560	1.1 (0.6–2.0)
B/B	0	4 (2.0)		
B	18* (8.5)	37* (10.0)	0.527	0.8 (0.4–1.4)

*Chromosomes number.
The presence of the *GST T1*, *M1* and *ile/ile*, *A/A* genotypes was taken as a reference group for risk analysis. To analyse the risk with *GSTP1 val* and *GSTM3B* allele, the *GSTP1 ile* and *GSTM3A* allele were taken as a reference group.

The frequencies of the *GSTM3*, both at the genotype and the allele levels, were similar in cases and controls. Even after sex-based segregation, no differences were observed in male and female patients as compared with controls (Table III).

Gallstones were present in 57.5% of patients with gallbladder cancer and it was the single most important risk factor for gallbladder cancer. To find out if any differences were present in *GST* gene polymorphisms in gallbladder cancer patients with stones as compared with patients without stones, a case-only analysis was carried out for polymorphisms in all four *GST* genes. We did not observe any significant frequency differences in the *GSTMI*, *GSTT1*, and *GSTP1* polymorphisms between cases with and without gallstones. The *AB* genotype and *B* allele of *GSTM3* showed a slightly higher risk (OR = 1.7 and 1.6, respectively) in stone patients, but they were not significant (Table IV). The numbers of cases using tobacco or alcohol in the present study were too low to find out the interaction of these environmental factors in a modulation of a risk for gallbladder cancer.

In order to look for gene–gene interactions, various combinations of genotypes were examined (Table V). The *GSTP1 ile/val* and *val* allele posed up to a twofold risk of gallbladder cancer. The combination of *GSTP1 ile/val* or *val/val* genotype with the *GSTM1* null genotype ($p = 0.003$, OR = 1.8, 95% CI = 1.0–3.8) or *GSTM3*AB* or **BB* homozygous genotypes ($p = 0.025$, OR = 1.8, 95% CI = 1.0–3.0) were associated with gallbladder cancer. However, no statistical significant effects were seen in the gene–gene interaction in the presence or absence of gallstones (data not shown).

Discussion

Gallbladder cancer is a very common malignant lesion of the biliary tract in North India and multiple risk factors including carcinogens have been proposed. The wide geographical and ethnic variation in the incidence of gallbladder cancer suggests that there are major genetic and environmental influences on its development. Most of the carcinogens are lipophilic and require conversion into water-soluble hydrophilic compounds for easy removal from the body through the excretory system. This conversion or detoxification of carcinogens is achieved by the addition of glutathione to the carcinogenic compounds by several enzymes of the GST family of phase II detoxification enzymes. The glutathione *S*-transferases are expressed from a multigene family, and extensive genetic polymorphisms have been reported. Keeping this view in mind, the present study was undertaken to assess the possible effects of individual gene polymorphism within the GST family on gallbladder cancer risk.

Ethnic differences in the prevalence of *GSTM1* null, *GSTT1* null and *GSTM3* genotypes have been reported (Cotton et al. 2000, Geisler and Olshan 2000, Buch et al. 2002). In various populations of the world, homozygous deletion of *GSTT1* has been reported in 10–65% of healthy individuals, whereas the *GSTM1* null genotype is prevalent in 20–50% of individuals. In our control group, polymorphic frequencies of the four genes belonging to the GST family were within the range reported from other studies from India (Sikdar et al. 2001, Buch et al. 2002, Srivastava et al. 2005).

In the case control study, the frequency of *GSTM1* null was not significantly different in gallbladder cancer patients as compared with controls, but the *GSTT1* null genotype was significantly lower in cases. Various studies have revealed a positive association between *GSTT1* and *GSTM1* null genotypes and an increased risk for skin, lung, stomach, and bladder, and prostate and colorectal cancers

Table III. Frequency distribution and age- and sex-adjusted OR and CI of *GSTT1*, *GSTM1*, *GSTP1*, and *GSTM3* genotypes and alleles in gallbladder cancer patients and controls after sex stratification.

	Male				Female			
Genotype/allele	Cases, *n* (%)	Controls, *n* (%)	*p*	Age-adjusted OR (95% CI)	Cases, *n* (%)	Controls, *n* (%)	*p*	Age-adjusted OR (95% CI)
GSTM1 null	14 (42.4)	34 (41.0)	0.509	1.3 (0.5–3.2)	32 (43.8)	44 (37.3)	0.524	1.2 (0.6 –2.2)
GSTT1 null	4 (12.1)	20 (24.1)	0.242	0.4 (0.1–1.6)	3 (4.1)	22 (18.6)	0.008	0.2 (0.1–0.6)
GSTP1								
ile/val	16 (48.5)	30 (36.1)	0.284	1.6 (0.6–3.9)	38 (52.1)	46 (39.0)	0.091	1.7 (0.9–3.3)
val/val	2 (6.1)	6 (7.2)	0.627	1.5 (0.2–9.2)	8 (11.0)	7 (5.9)	0.076	2.7 (0.8–8.4)
val	20* (69.7)	42* (25.3)	0.331	1.3 (1.0–1.1)	54* (64.4)	60* (25.8)	0.072	1.5 (0.9–2.3)
GSTM3								
A/B	6 (9.1)	10 (12.0)	0.408	1.6 (0.5–5.1)	12 (16.4)	19 (16.1)	0.640	0.8 (0.3–1.8)
B/B	0 (0)	1 (1.2)			0 (0)	3 (2.5)		
B	6* (18.2)	12* (92.2)	0.602	1.3 (0.4–3.8)	12* (8.2)	25* (11.4)	0.280	0.6 (0.3–1.3)

*Chromosomes number.
The presence of the *GST T1*, *M1* and *ile/ile*, *A/A* genotypes was taken as a reference group for risk analysis.
To analyse the risk with *GSTP1 val* and *GSTM3B* allele, the *GSTP1 ile* and *GSTM3A* alleles were taken as a reference group.

Table IV. Frequency distribution and age- and sex-adjusted OR and 95% CI of *GSTT1*, *GSTM1*, *GSTP1*, and *GSTM3* genotypes and allele analysis in cases with a status of gallstones.

	Stone present ($n=61$)	Stone absent ($n=45$)		
Genotype/allele	*n* (%)	*n* (%)	*p*	Age-adjusted OR (95% CI)
GSTM1 null	27 (44.3)	19 (42.2)	0.860	1.1 (0.4–2.3)
GSTT1 null	3 (4.9)	4 (8.9)	0.491	0.6 (0.1–2.8)
GSTP1				
ile/val	31 (50.8)	23 (51.1)	0.962	1.1 (0.4–2.2)
val/val	4 (6.6)	6 (13.3)	0.200	0.4 (0.1–1.6)
val	39* (31.1)	35* (37.8)	0.292	0.7 (0.4–1.3)
GSTM3				
A/B	12 (19.7)	6 (13.3)	0.327	1.7 (0.5–5.1)
B/B				
B	12* (9.8)	6* (6.7)	0.351	1.6 (0.5–4.6)

*Chromosomes number.
The presence of the *GST T1*, *M1* and *Ile/Ile*, *A/A* genotypes was taken as a reference group for risk analysis.
To analyse the risk with *GSTP1 val* and *GSTM3B* allele, the *GSTP1 ile* and *GSTM3A* alleles were taken as a reference group.

(Cotton et al. 2000, Setiawan et al. 2000, Gao et al. 2002, Srivastava et al. 2005). The *GSTM1* null genotype has also been shown to be a risk factor for the development of oral cancer among Indian tobacco chewers and smokers (Buch et al. 2002). Although *GSTM1* is known to be expressed in gallbladder epithelium in low levels, its exact contribution in the detoxification pathway is unknown. The present study suggests that polymorphism of *GSTM1* does not significantly contribute towards a risk of gallbladder cancer. The *GSTT1* null genotype especially is associated with a greater risk of colorectal, gastric cancers and bladder cancers (Stoehlmacher et al. 2002, Pallib et al. 2005, Srivastava et al. 2005). On the other hand, the present observation suggests a protective role of the *GSTT1* null genotype in gallbladder cancer. Studies of *GSTT1* polymorphism have also observed a significant under-representation of the null genotype of *GSTT1* in squamous cell carcinoma of the lung and in hepatocellular carcinoma (Bian et al. 2000, Risch et al. 2001). The protective effect of *GSTT1* null may imply that certain procarcinogens can be activated to carcinogens by the *GSTT1* present. In animal studies, it was found that the presence of *GSTT1* could activate dichloromethane into a mutagen, inducing lung and liver cancer (Pemble et al. 1994).

Table V. Age-adjusted OR and 95% CI for a combination of two putative *GST* genotypes and their association with gallbladder cancer.

GST status		OR (95% CI)
GSTM1 null	*GSTT1* null	0.9 (0.6–1.6)
GSTM1 null	*GSTP1 ile/val* or *val/val*	1.8 (1.0–3.1)
GSTM1 null	*GSTM3 A/B* or *B/B*	1.2 (0.7–1.9)
GSTP1 ile/val or *val/val*	*GSTM3 A/B* or *B/B*	1.8 (1.0–3.0)
GSTP1 ile/val or *val/val*	*GSTT1* null	1.4 (0.9–2.4)
GSTT1 null	*GSTM3 A/B* or *B/B*	0.5 (0.3–0.9)

The presence of the *GSTM1*, *GSTT1*, *GSTP1* (*Ile/Ile*) and *GSTM3* (*A/A*) genotypes was taken as the reference group.

In the present study, the protective effect of *GSTT1* was limited to females. It may be possible that sex hormones and the *GSTT1* genotype present may modulate risk factors that affect the levels of putative procarcinogens that can be biotransformed to carcinogen by *GSTT1*.

The present study did not observe any significant changes in the frequency of both alleles as well as genotypes of *GSTM3* in gallbladder cancer. Some studies have reported a significant risk of upper aerodigestive tract cancers in individuals with a B allele of *GSTM3*. The presence of *GSTM3*B* has been associated with a risk of basal cell skin and laryngeal cancer, but it has a protective effect for oral cancers. On the other hand, the *GSTM3 AA* genotype was found to be risk factor for developing oral cancer from leukoplakia in a dose-related tobacco usage study (Sikdar et al. 2001). However, the data suggest that the *GSTM3* polymorphism does not modulate gallbladder cancer risk probably because vice habits, particularly tobacco usage, were limited in our cohort of gallbladder cancer patients.

GSTP1 is an important GST isoform that is widely expressed in normal human epithelial tissue and highly expressed in malignant transformation (Harrison et al. 1990). The present study shows that *GSTP1 ile/val* and *val/val* genotypes are over-represented in gallbladder cancer as compared with controls. It also tried to find out whether environmental factors such as tobacco and alcohol have any effect on the modulation of gallbladder cancer risk by *GSTP1* polymorphism, but no statistically significant conclusion was possible because of a low number of patients with exposure to these agents. The *val/val* genotype has been reported to confer a high risk for bladder, testicular and prostate cancers. In humans, several polymorphisms are known in *GSTP1*, but a 313 A > G substitution creates the *ile/val* polymorphism at codon 105 that leads to expression of enzyme with reduced activity. It has been demonstrated that *GSTP1* with 105 Val possess significantly decreased activity against PAH and 1-chloro-2,4-dinitrobenzene (Ji et al. 1997, Watson et al. 1998). *In vitro*, cDNA expression studies also suggest that Ile105Val substitution reduces enzyme activity (Palmisano et al. 2000). Individuals with heterozygote alleles were reported to exhibit intermediate activity (Watson et al. 1998). From molecular modelling studies, the lower enzyme activity has been attributed to the conversion of the amino acid in the hydrophobic binding site for electrophilic substrate and thus it affects substrate binding (Zimnaik et al. 1994). The *GSTP1* 105Val variant has been associated with hypermethylation of the promoter regions of a cyclin-dependent kinase inhibitor, *P16ink4a*, a tumour suppressor gene, as well as *MGMT* (*O-6-methylguanine-DNA methyltransferase*), a DNA repair gene (Gilliland et al. 2002), thus resulting in an increased risk of developing non-small-cell lung cancer (Palmisano et al. 2000).

The gene–gene interaction in the risk alleles of the *GST* family has been observed to modulate the risk in various cancers. Because *ile/val* substitution was a major risk allele in gallbladder cancer, the present paper analysed the joint risk due to the presence of *GSTP1 ile/val* with the presence of null alleles of *GSTT1*, *GSTM1* or the *B* allele of *GSTM3*. However, the combined risk was not significantly different from *GSTP1 ile/val* alone. Therefore, it appears that the risk of gallbladder cancer susceptibility due to *ile/val* polymorphism is not significantly modulated by other members of the GST family. However, it may be added that due to a small sample size, the gene–gene interaction study is statistically underpowered to provide a clear picture of the interactions.

The presence of long-standing gallstones is a major risk factor for gallbladder cancer (Lazcano-Ponce et al. 2001). In the present study, gallstones were also present in 57.5% of patients. The present authors carried out a case-only analysis to ascertain if there was any association between *GST* gene polymorphism in patients with gallstones compared with those who did not harbour any stones. However, they did not find any significant differences in the frequency distribution of various alleles belonging to all four genes for *GST*. It appears that gallbladder cancer risk due to *GST* gene polymorphisms is independent of the presence of gallstones.

To the best of our knowledge, this is the first report of the association of *GST* gene polymorphisms with the risk of gallbladder cancer. Like other cancers, gallbladder cancer is also believed to results from complex interactions between genetic and environmental factors. Low-penetrance gene polymorphisms in glutathione *S*-transferases may be partly responsible for an individual's susceptibility in the presence of appropriate environment insults. The present study was carried out in a limited number of cases and it would be desirable to undertake large-scale molecular epidemiological studies both to confirm the association as well as to investigate other genes of xenobiotics metabolism.

In summary, the present results indicate that the *ile*/*val* and *val*/*val* genotypes of the *GSTP1* could be associated with an increased risk of gallbladder cancer, although the mechanism behind it is still unclear.

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