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Combined effect of *GSTM1*, *GSTT1* and *GSTP1* polymorphisms on histological subtypes of lung cancer

R. C. SOBTI[1], PUSHPINDER KAUR[1], SATINDER KAUR[1], A. K. JANMEJA[2], S. K. JINDAL[3], J. KISHAN[4], & SARA RAIMONDI[5]

[1]*Department of Biotechnology, Panjab University, Chandigarh 160014, India,* [2]*Department of Chest and Infectious Diseases, Government Medical College Hospital, Chandigarh 160032, India,* [3]*Department of Pulmonary Medicine, PGIMER, Chandigarh 160012, India,* [4]*TB and Chest Disease Hospital, Government Medical College Patiala, Punjab, India and* [5]*Fondazione Policlinico IRCCS, Milan, Italy*

Abstract

Genetic polymorphisms are natural genetic variations in the gene sequence that occur at a frequency of >1% in the population. This genetic variability (polymorphisms) can be a factor in cancer risk. The functional polymorphisms in GST genes play an important role in susceptibility to lung cancer. In our previous study, we reported that the combination of certain genotypes of *GSTM1*, *GSTT1* and *CYP1A1* is associated with lung cancer. The study has been extended to investigate the potential role of polymorphism in *GSTP1* alone or in combination with the status of *GSTM1* and *GSTT1* genes in the likelihood of development of lung cancer. A total of 302 subjects (151 cases and 151 controls) were evaluated. Using a case–control design, individuals were genotyped for GSTs using multiplex polymerase chain reaction and restriction fragment length polymorphism techniques. The data obtained were analyzed using multiple logistic regression. The combined 'at risk' genotypes of *GSTM1* null and *GSTT1* null in comparison with 'wild-type' genotypes seems to be associated with a greater risk of lung cancer, but the results are not significant (odds ratio (OR) 2.0, 95% confidence interval (CI) 0.68–5.96) and for squamous cell carcinoma (SqCC) it was 1.6-fold (OR 1.6, 95% CI 0.49–5.68). In summary, our case–control study of lung cancer revealed that the effect of these polymorphisms is not very marked for different genotypic combinations of *GSTP1, GSTM1* and *GSTT1* in the context of developing lung cancer in a north Indian population. However, the increased risk was limited to SqCC, and was not found for other histological subtypes. Further analyses on this topic are needed.

Keywords: *Genetic polymorphism, GSTs, lung cancer risk*

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Introduction

Most common cancers such as those of lung, larynx, mouth, oesophagus, bladder and kidney result from the interaction between genetic and environmental factors (Bennet et al. 1999). Among the various factors, cigarette smoking plays an important role in

Correspondence: Professor R. C. Sobti, Department of Biotechnology, Panjab University, Chandigarh 160014, India. Tel: +91-172-2534087 (laboratory); +91-09417044523 (mobile). Fax: +91-172-2541409 (office). E-mail: rcsobti@pu.ac.in

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the aetiology of lung cancer. It is the most common cause of cancer-related deaths in both men and women. Lung cancer can be divided into two major histological groups – small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) (Pass et al. 1996). Although studies evaluating the role of genetic polymorphisms in lung cancer risk have generally grouped all NSCLC together, histologically NSCLC includes the three most common subtypes – squamous cell carcinoma (SqCC), large cell carcinoma and adenocarcinoma (AC) (Ginsberg et al. 1997). Controversy still exists as to whether the different histological subtypes are different manifestations of the same disease process or different processes shared by some distinct risk factors. In order to demonstrate the important effect of environmental factors on the risk of lung cancer, a search for gene susceptibility was carried out.

Numerous polymorphisms occur in the genes encoding glutathione S-transferases (GSTs) (Mannervik 1985, Hayes & Pulford 1995). Among the GST enzymes, GSTM1, GSTT1 and GSTP1 play a major role in the detoxification of metabolites of carcinogens in tobacco smoke (Vos & Blanderen 1990). GSTM1 is expressed at high levels in the liver, but has also been found in several tissues including the lung (Mace et al. 1998, Strange et al. 1999). GSTM1 is known to detoxify arene oxides, including the ultimate carcinogenic form of benzo[a]pyrene (BP), BP-diol epoxide, where it is not involved in the detoxification of aromatic amines. The GSTT1 enzyme is expressed at high levels in the liver and in many extrahepatic tissues including the lung (Juronen et al. 1996). The *GSTT1* null phenotype (*GSTT1**0/*0) is a result of a deletion of the entire *GSTT1* gene (Pemble et al. 1994). GSTP1 is widely expressed in different human epithelial tissues (Terrier et al. 1990) and is the major GST in human lungs (Antilla et al. 1993). The polymorphism of *GSTP1* is associated with an increased risk of lung, bladder and testicular cancers (Harries et al. 1997, Watson et al. 1998). The *GSTP1* gene is polymorphic with respect to a single base change in exons 5 and 6, which results in an amino acid change from Ile/Val and Ala/Val, respectively, leading to reduced enzyme activity (Board et al. 1989, Johansson et al. 1998). The enzyme activity is affected by substitution at position 105, which is located in a hydrophobic substrate-binding site. Individuals with the 105 Val allele seem to have a higher risk of developing cancer than those with the 105 Ile allele, as the former allele has a higher catalytic efficiency than the latter for carcinogenic aromatic epoxides (Sundberg et al. 1998). Many studies have examined the association between *GSTP1* polymorphisms and risk of lung cancer, but no statistically significant associations have been obtained (Mironova et al. 1998, Kihara et al. 1999, To-Figueras et al. 2001, Lewis et al. 2002, Wang et al. 2003). *GSTM1* has been studied with respect to polymorphisms and cancer risk. It detoxifies active metabolites of polycyclic aromatic hydrocarbons (PAHs) (Hayes et al. 1995). *GSTT1* is also known to be involved in the detoxification of several environmental carcinogens such as 1,3-butadiene and ethylene oxide in tobacco smoke and ambient air (Landi 2000) and it has been documented that *GSTM1* and *GSTT1* genes are missing in some individuals. The association of null genotypes of *GSTM1* and *GSTT1* has been widely studied, but results with respect to their association with risk of lung cancer are contradictory (Seideigard et al. 1990, D'Errico et al. 1996, Ryberg et al. 1997, LeMarchand et al. 1998, Mironova et al. 1998, Kiyohara et al. 2000, Stucker et al. 2002).

Individuals who have more than one risk-associated polymorphism may have a greater risk of developing lung cancer. Some studies have reported that the

combination of *GSTP1* (Val/Val) and *GSTM1* null genotypes is associated with a higher risk of lung cancer (Kihara et al. 1999, Mironova et al. 1998, Wang et al. 2003). In our earlier study, we reported that the combination of *GSTM1*, *GSTT1* with *CYP1A1* genotypes promotes a higher risk of lung cancer (Sobti et al. 2004). Another study, however, has found that lung cancer risk is not associated with *GSTT1* and *GSTP1* (Lewis et al. 2002).

We have extended our investigations for checking the potential role of *GSTP1* gene polymorphisms alone and in combination with *GSTM1* and *GSTT1* genes on susceptibility to lung cancer in a north Indian population.

Materials and methods

Sample collection

Histologically confirmed incident adult patients with lung cancer ($n = 151$) reviewed by a pathologist were recruited from three medical institutes – the Post Graduate Institute of Medical Education and Research (PGIMER), the Government Medical College Hospital (GMCH) both at Chandigarh, India and the TB and Chest Diseases Hospital of Government Medical College, Patiala (Punjab, India) of north India. None of the patients had received radiation or chemotherapy. The relevant demographic and epidemiological information including detailed diet and smoking information along with informed consent was obtained through a questionnaire. The controls ($n = 151$) were also hospital based and were free of benign and malignant tumours both at the time of, and prior to, diagnosis, but were admitted for minor pulmonary complaints such as respiratory infection and bronchitis. Peripheral blood samples were obtained from all subjects and DNA was extracted using the SDS/proteinase K and phenol-chloroform method (http://www.genome.ou.edu/protocols_book/protocols_partIII.html).

Histological data were available for 143 cases, whereas no such information could be obtained for the remaining eight cases (non-classified). The tumours were not defined, as they were so advanced that histological diagnosis was not relevant to the palliative treatment given. This study was approved by the human subjects ethical committees of all the involved institutions.

Genotyping of GSTM1 *and* GSTT1 *genes*

The *GSTM1* and *GSTT1* genetic polymorphisms were evaluated using multiplex polymerase chain reaction (PCR) techniques as already described (Arand et al. 1996). The primer sequences (Sigma Aldrich, St Louis, MO, USA) are shown in Table I.

Table I. The primer sequences for *GSTM1* and *GSTT1* and control albumin.

Genes	Primer sequence	Size (bp)
GSTM1	(F)–GAACTCCCTGAAAAGCTAAAGC (R)–GTTGGGCTCAAATATACGGTGG	215
GSTT1	(F)–TTCCTTACTGGTCCTCACATCTC (R)–TCACCGGATCATGGCCAGCA	480
Albumin	(F)–GCCCTCTGCTAACAAGTCCTAC (R)–GCCCTAAAAAGAAAATCGCCAATC	350

Each PCR reaction mixture (50 μl) contained 10 pmol of each primer, 2.5 mM of $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate (dNTPs), 1 unit of Taq polymerase, 100 ng of genomic DNA and 5% DMSO. Amplification was performed with initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C, annealing at 64°C and extension at 72°C for 1 min each and a final extension at 72°C for 7 min. DNA fragments of expected size were amplified using primers specific for *GSTT1* and *GSTM1* genes, respectively (Figure 1). The albumin gene was used as internal standard. To detect the pattern of genes, the amplified DNA fragments were electrophoresed through a 2% agarose gel and observed under UV light after staining with ethidium bromide.

The *GSTP1* gene in exon 5 at codon 105 was analyzed by the method of Kihara et al. (1999). The DNA was amplified with the *GSTP1* primers (F) – GTAGTTTGCCCAAGGTCAAG, (R) – AGCCACCTGAGGGGTAAG (Sigma Aldrich). PCR reaction mixture (50 μl) was prepared as described above except for the addition of DMSO. The cycling parameters included initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min 30 s, and extension at 72°C for 1 min 30 s. The final polymerization step was carried out at 72°C for 7 min to complete the elongation process. PCR products were digested with 2 units of restriction enzyme Alw 261 (MBI Fermentas, USA) overnight at 37°C. The digestion distinguishes between the restriction sites on the Ile and Val alleles (ACa/gTCT), where small characters a/g represent the polymorphic site.

PCR–restriction fragment length polymorphism (RFLP) patterns resulted in a band of 113 bp in all samples, which represented a control cut for confirmation of proper digestion. In the wild-type *GSTP1* (Ile/Ile), bands of 329 and 113 bp were generated,

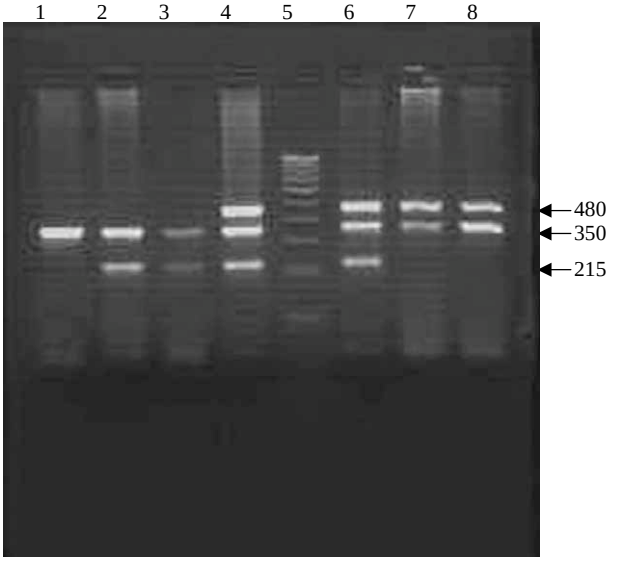

Figure 1. Polymerase chain reaction amplified product of *GSTM1T1*. Lane 1, *GSTM1T1* null; lanes 2 and 3, *GSTM1* (present) (215 bp), *GSTT1* (null); lanes 4 and 6, *GSTM1* (present) (215 bp), *GSTT1* (present) (480bp); lanes 7 and 8 *GSTT1* (present) (480 bp), *GSTM1* (null); lane 5, 100 bp DNA marker.

whereas in the homozygous mutant type *GSTP1* (Val/Val), the bands of 216, 113 and 107 bp were produced. In heterozygous mutant type *GSTP1* (Ile/Val), all the four bands were present (Figure 2).

Statistical analysis

Age, gender, smoking status and genotypes of *GSTM1*, *GSTT1* and *GSTP1* genes were tabulated for cases and controls. To determine any difference between cases and controls according to age, number of cigarettes smoked per day, pack-years and gender, *t*-tests and χ^2 tests were performed. Cases were further categorized into various histological subtypes, in order to find out any specific association of genetic polymorphisms according to histological type.

The association between polymorphisms in GST genes and risk of lung cancer was estimated by computing odds ratios (ORs) and 95% confidence interval (CI) using a multivariate logistic regression analysis which included several potential confounding variables (e.g. age, gender and smoking status – smokers and people who had never smoked, separately). Subjects were considered as non-smokers if they had never smoked as much as one cigarette per day for a year, while the smokers were those who had smoked cigarettes over their lifetime. Under the hypothesis that *GSTM1*, *GSTT1* and *GSTP1* genotypes may be differentially associated with histological subtypes, the ORs for each polymorphism and for the combinations between *GSTP1* and either of the GST types (*GSTM1* or *GSTT1*) were tested in the models also for SCLC, NSCLC, SqCC and AC. Statistical analysis was performed using SAS version 8.

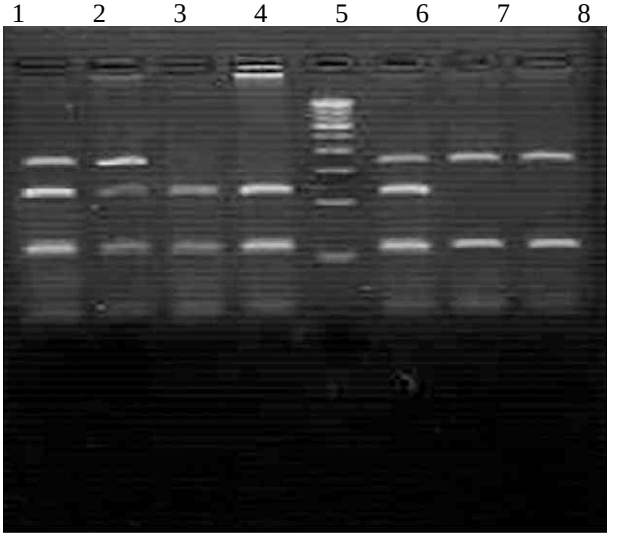

Figure 2. Restriction fragment length polymorphism analysis of *GSTP1*. Lanes 1, 2 and 6, heterozygous mutant (329, 216, 113 and 107 bp); lanes 3 and 4, homozygous mutant (216, 113 and 107 bp); lanes 7 and 8, homozygous wild (329 and 113 bp); lane 5, 100 bp DNA marker.

Results

Baseline characteristics

Demographic variables such as age, gender, and smoking status have been summarized in Table II, according to the case/control status. The average age was calculated as 56.9 ± 10.4 years for cases and 56.4 ± 11.1 years for controls ($p = 0.67$). Most of the study participants were smokers with the higher percentage of these among cases (81.4%) than controls (74.1%). Moreover, the cancer cases had a higher value of pack years (64.1 ± 50.8) than controls (37.9 ± 29.2) ($p < 0.0001$), while the average number of cigarettes smoked per day was 14.4 ± 12.2 for cases and 8.7 ± 8.8 for controls ($p < 0.0001$). The frequencies of males and females in the case and control groups were comparable ($p = 0.27$).

Distribution of GSTM1, GSTT1 *and* GSTP1 *polymorphism in north Indian population*

The frequency of *GSTM1* and *GSTT1* null genotypes was higher in cases (41.7% and 17.8%, respectively) compared with the controls (35% and 13.2%, respectively). The frequencies of the three genotypes of *GSTP1* were as follows: Ile/Ile 51.6%, Ile/Val 45% and Val/Val 3.3% in cases and Ile/Ile 41%, Ile/Val 55.0% and Val/Val 4.0% in controls. Table II also presents the association of polymorphisms in individual GST genes with lung cancer. The *GSTM1* (null) genotype was not found to be a risk factor for developing lung carcinoma compared with the controls. But the *GSTT1* (null) genotype was found to be a marginal risk (OR 1.4, 95% CI 0.74–2.62). It seems that *GSTP1* (Ile/Val) could be a protective factor for the development of lung cancer, even if the association is borderline (OR 0.63, 95% CI 0.40–1.01).

Table II. Descriptive characteristics of the study population.

Variables	Cases	Control	OR (95% CI)
Sample size, *n*	151	151	
Age (years), mean ± SD	56.9 ± 10.4	56.4 ± 11.1	
Gender, *n* (%)			
Male	130 (86.0)	123 (81.4)	
Female	21 (13.9)	28 (18.5)	
Smoking status, *n* (%)			
Non-smoker[a]	28 (18.5)	39 (25.8)	1 (ref.)
Smokers[b]	123 (81.4)	112 (74.1)	1.48 (0.85–2.56)
GSTM1 (%)			
Present	88	98	1 (ref.)
Null	63	53	1.30 (0.81–2.08)
GSTT1 (%)			
Present	124	131	1 (ref.)
Null	27	20	1.39 (0.74–2.62)
GSTP1 (%)			
Ile/Ile	78	62	1 (ref.)
Ile/Val	68	83	0.63 (0.40–1.01)
Val/Val	5	6	0.64 (0.19–2.22)

OR, odds ratio adjusted for age and gender; CI, confidence interval; ref., reference.
[a]Non-smokers (never smoked as much as one cigarette per day for a year); [b]smokers (smoked cigarettes over their lifetime).

Distribution of GST polymorphism in histological subtypes

Further, the variables were categorized for lung cancer histological subtypes as SCLC, NSCLC, SqCC, AC and NC. Among 151 cases in the present study, 63.5% were identified to have SqCC, 15.8% SCLC, 7.9% NSCLC, 7.2% AC and 5.2% NC (Table III).

When genotypes of GSTs were considered with the risk to individual subtypes, a 2.4-fold increased risk in AC was found with the *GSTM1* gene, although the result was not significant. Patients with NSCLC also showed an OR higher than 1.00 (1.77, 95% CI 0.54–5.84). A borderline increased risk was observed (OR 3.76, 95% CI 0.99–14.28) for NSCLC that presented a deletion in the *GSTT1* gene. The individuals with *GSTP1* (Ile/Val) were at marginal increased risk of AC subtype (OR 1.30, 95% CI 0.36–4.66) compared with controls. No significant correlation was observed between polymorphisms in GSTs and their association with smoking and drinking habits.

Gene–gene interactions

GSTM1–GSTT1 genotypes. Individuals having both *GSTM1* and *GSTT1* null genotypes had an increased risk for lung cancer (OR 2.0, 95% CI 0.68–5.96) while for SqCC it was 1.6-fold (OR 1.6, 95% CI 0.49–5.68) (Table IV).

GSTM1–GSTP1 genotypes. The ORs and 95% CI were also calculated for lung cancer risk in relation to both *GSTM1* and *GSTP1* genotypes and there was a weak association in all the *GSTM1* and *GSTP1* genotypes (Table V). Individuals with *GSTM1* null and *GSTP1* (Ile/Ile) showed increased risk towards NSCLC (OR 2.5, 95% CI 0.45–14.05) and AC (OR 1.7, 95% CI 0.26–11.64). Similarly, with the *GSTM1* (null) and *GSTP1* (Ile/Val/Val/Val) combined genotype, an association was observed with AC (OR 2.4, 95% CI 0.47–12.34) and NSCLC (OR 1.8, 95% CI 0.33–10.46) development.

Table III. Odds ratio[a] (95% confidence interval) for *GSTM1*, *GSTT1* and *GSTP1* polymorphisms stratified according with histological subtypes.

Gene polymorphism	SqCC ($n=96$) (63.5%)	SCLC ($n=24$) (15.8%)	NSCLC ($n=12$) (7.9%)	AC ($n=11$) (7.2%)
GSTM1				
Present	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Null	1.13 (0.66–1.93)	1.29 (0.53–3.15)	1.77 (0.54–5.84)	2.41 (0.69–8.41)
GSTT1				
Present	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Null	1.19 (0.57–2.48)	0.86 (0.23–3.21)	3.76 (0.99–14.28)	1.35 (0.27–6.85)
GSTP1				
Ile/Ile	1 (ref.)	1 (ref.)	1 (ref.)	1 (ref.)
Ile/Val	0.59 (0.35–1.00)	0.36 (0.14–0.92)	1.07 (0.32–3.61)	1.30 (0.36–4.66)
Val/Val	0.59 (0.14–2.50)	0.58 (0.06–5.34)	–	–

SqCC, squamous cell carcinoma; SCLC, small cell lung carcinoma; NSCLC, non-small cell lung carcinoma; AC, adenocarcinoma; ref., reference.

[a]Odds ratio adjusted for age, gender and smoking status.

Table IV. Odds ratios[a] (OR) for the combined effect of *GSTM1* and *GSTT1* genotype and risk of lung cancer.

GSTM1	*GSTT1*	All OR	SqCC	SCLC	NSCLC	AC
Present	Present	1.0 (ref.) (73/85)	1 (51/85)	1 (10/85)	1 (4/85)	1 (4/85)
Null	Null	2.0 (0.68–5.96) (12/7)	1.6 (0.49–5.68) (7/7)	1.0 (0.15–6.81) (1/7)	4.9 (1.05–23.36) (2/7)	2.7 (0.35–22.03) (1/7)
Present	Null	1.3 (0.56–3.24) (15/13)	1.0 (0.36–2.88) (8/13)	1.0 (0.27–4.35) (2/13)	2.9 (0.59–13.21) (2/13)	1.6 (0.19–13.21) (1/13)
Null	Present	1.3 (0.75–2.21) (51/46)	1.0 (0.59–2.01) (30/46)	1.4 (0.49–3.86) (9/46)	1.7 (0.47–6.81) (4/46)	2.1 (0.61–7.76) (5/46)

SqCC, squamous cell carcinoma; SCLC, small cell lung carcinoma; NSCLC, non-small cell lung carcinoma; AC, adenocarcinoma; ref., reference.
[a]OR adjusted for age, gender and smoking status.

GSTT1–GSTP1 genotypes. The impact of combinations of genotypes of *GSTT1* and *GSTP1* on the risk of lung cancer was also evaluated. No significant correlation was observed between polymorphisms in *GSTT1* and *GSTP1* and their association with lung cancer risk (Table VI). When these data were dissected according to histological subtypes, the combination of *GSTM1* (null) and *GSTP1* (Ile/Ile) genotypes revealed 2.1-fold (95% CI 0.77–6.05) increased risk for SCLC, 2.5-fold (95% CI 0.34–19.25) for NSCLC and 3.3-fold (95% CI 0.41–27.27) for AC. In NSCLC, the combination of *GSTT1* (null) with *GSTP1* combined (Ile/Val/Val/Val) genotype showed a 2.5-fold increased risk (95% CI 0.63–10.32). The risk came down to 1.5-fold with *GSTM1* (present) and *GSTP1* combined (Ile/Val/Val/Val).

Discussion

The most common form of human genetic variation is the single nucleotide polymorphism. Genetic polymorphism may contribute to individual susceptibility to cancer, but the molecular mechanism behind this is still not clear. In this case–control study, we are reporting for the first time the data on the association of *GSTP1* with *GSTM1* and *GSTT1* in lung cancer as a whole and in various histological subtypes in a

Table V. Odds ratios (OR) for the combined effect of *GSTM1* and *GSTP1 g*enotype and risk of lung cancer.

GSTM1	*GSTP1*	All OR	SqCC	SCLC	NSCLC	AC
Present	Ile/Ile	1.0 (ref.) (50/40)	1 (34/40)	1 (9/40)	1 (2/40)	1 (2/40)
Null	Ile/Ile	1.0 (0.48–2.17) (28/22)	0.9 (0.39–2.13) (17/22)	1.2 (0.33–4.41) (6/22)	2.5 (0.45–14.05) (3/22)	1.7 (0.26–11.64) (2/22)
Null	Ile/Val/ Val/Val	0.9 (0.45–1.80) (35/31)	0.7 (0.34–1.67) (20/31)	0.6 (0.21–1.86) (4/31)	1.8 (0.33–10.46) (3/31)	2.4 (0.47–12.34) (4/31)
Present	Ile/Val/ Val/Val	0.7 (0.52–0.97) $p < 0.04$ (38/58)	0.5 (0.25–1.03) (25/58)	0.4 (0.15–1.21) (5/58)	1.3 (0.26–7.07) (4/58)	1.0 (0.18–5.92) (3/58)

SqCC, squamous cell carcinoma; SCLC, small cell lung carcinoma; NSCLC, non-small cell lung carcinoma; AC, adenocarcinoma; ref., reference.
[a]OR adjusted for age, gender and smoking status. Significant p-value < 0.05.

Table VI. Odds ratios (OR)[a] for the combined effect of *GSTT1* and *GSTP1* genotype and risk of lung cancer.

GSTT1	*GSTP1*	All OR	SqCC	SCLC	NSCLC	AC
Present	Ile/Ile	1.0 (ref.) (66/57)	1 (45/57)	1 (12/57)	1 (66/57)	1 (3/57)
Null	Ile/Ile	1.3 (0.93–1.86) (12/5)	1.2 (0.69–2.21) (6/5)	2.1 (0.77–6.05) (3/5)	2.5 (0.34–19.25) (1/5)	3.3 (0.41–21.27) (1/5)
Present	Ile/Val/ Val/Val	0.6 (0.40–1.14) (58/74)	0.6 (0.34–1.12) (36/74)	0.5 (0.21–1.60) (9/74)	0.7 (0.20–3.00) (4/74)	1.5 (0.39–5.76) (6/74)
Null	Ile/Val/ Val/Val	0.8 (0.36–2.06) (15/15)	0.7 (0.28–2.06) (9/15)	– –	2.5 (0.63–10.32) (3/15)	1.2 (0.14–11.22) (1/15)

SqCC, squamous cell carcinoma; SCLC, small cell lung carcinoma; NSCLC, non-small cell lung carcinoma; AC, adenocarcinoma; ref., reference.
[a]OR adjusted for age, gender and smoking status.

north Indian population. In our lung cancer samples, there were no cases of the large cell subtype. All other lung histological subtypes were included in the main association analysis of overall lung cancer risk, but the number of NSCLC ($n = 12$) and adenocarcinoma ($n = 11$) was too small. It was found that there is an association of *GSTM1* and *GSTT1* null genotypes with an increased risk of SqCC with lung cancer in general.

The prevalence of GST genotypes differs within India. The genotype frequencies in our control group were similar to those reported by other authors in the north Indian population (Mishra et al. 2004). In south India, 30.4% of the population lacked the *GSTM1* gene and 16.8% lacked the *GSTT1* gene. The highest frequency of *GSTM1* null was observed in Karnataka (36.4%), while Andhra Pradesh had the lowest frequency of *GSTM1* and *GSTT1* combined null genotype (1.7%) (Naveen et al. 2004). In a study carried out in a north Indian population on the prostate (Mittal et al. 2004, Srivastava et al. 2005), cervix (Sharma et al. 2004) and oral cancer (Sreelekha et al. 2001), no significant association between the *GSTM1* and *GSTT1* null genotypes was found. Similarly for *GSTP1*, a non-significant association was observed by Mittal et al. (2005) in bladder cancer in a north Indian population. But Srivastava et al. (2004) found a significant association with the null alleles of *GSTM1* and *GSTT1* genotypes.

Many studies have tried to establish links between polymorphic expression of different GSTs and risk of lung cancer in different ethnic groups (Seideigard et al. 1990, Ryberg et al. 1997, Harries et al. 1997, Mironova et al. 1998, Kihara et al. 1999), but the results are conflicting (Lewis et al. 2002, Wang et al. 2003). Interindividual variability in GST enzyme activity is believed to confer differences in susceptibility to cancers with major environmental determinants such as lung cancer (Kato et al. 1995, Rebbeck 1997, Hirvonen et al. 1999). The inconsistency in results might be due to polymorphism frequency differences across different ethnicities. All the three genes *GSTP1*, *GSTM1* and *GSTT1* play an important role in the cellular defence system (Antilla et al. 1995, Mainwaring et al. 1996, Lewis et al. 2002, Wang et al. 2003). *GSTP1* is widely expressed in normal human epithelial tissues and has been shown to be overexpressed in lung cancer (Terrier et al. 1990, Antilla et al. 1993). Two genetic polymorphisms are known for *GSTP1*. These are Ile-105-Val,

resulting from an A > G transition at base 1578, and Ala-114-Val, resulting from a C > T transition at base 2293. Two studies have examined the frequency of the Ile-105-Val *GSTP1* and Ala-114-Val *GSTP1* polymorphism in different cancers (Harries et al. 1997, Watson et al. 1998). Epidemiological and functional studies on the polymorphism of *GSTP1* suggest that the Ile and Val alleles confer increased susceptibilities to different types of cancer (Sundberg et al. 1998).

In our study, an association between the polymorphisms of GSTs was observed in individuals with SqCC. It is possible that individuals with *GSTM1* and *GSTT1* null genotypes are more susceptible to carcinogenic substrates. In these a close relationship between carcinogen exposure and histological subtypes has been evaluated. Several studies have associated *GSTP1* polymorphism at codon 105 with *GSTM1* and *GSTT1* with susceptibility to SCLC and AC (Alexandrie et al. 1994, Garcia-Closas et al. 1997, LeMarchand et al. 1998, Ford et al. 2000). Studies on different populations have also indicated that separate *GSTM1, GSTT1* and *GSTP1* polymorphisms are not statistically related to lung cancer, but there is a strong association between the combined *GSTM1, GSTT1* and *GSTP1* genotypes which interact to increase the risk of lung cancer (Table VII).

Investigations on the association between *GSTP1* polymorphism and lung cancer have shown that the *GSTP1* Val genotype is not significantly associated with lung cancer risk (Hayashi et al. 1992, Alexandrie et al. 1995, Kihara et al. 1995, Garcia-Closas et al. 1997, Ryberg et al. 1997, Watson et al. 1998, Mironova et al. 1998, Kihara et al. 1999, Ford et al. 2000, To-Figueras et al. 2001, Dialyna et al. 2003). However, Stucker et al. (2002) reported that genotypes deficient for *GSTMI* and *GSTP1* are important risk modifiers for lung cancer. Cote et al. (2005) observed that specific combinations of GST polymorphism increased the risk of early-onset lung cancer. On the other hand, a meta-analysis carried out by Zheng et al. (2006) on 130 genetic association studies provides the most comprehensive assessment in relation to lung cancer of five GST gene polymorphisms which indicates that the risk of lung cancer is not strongly associated with I105V and A114V polymorphisms in the *GSTP1* gene or with the *GSTM3* intron 6 polymorphism. Similarly, Skuladottir et al. (2005) carried out a pooled study on lung cancer patients in Norway and Denmark

Table VII. Studies in different populations on *GSTM1, GSTT1* and *GSTP1* polymorphisms.

	Population	Significantly associated/ not significantly associated	Reference
GSTM1/GSTT1			
Null/null	Greece	Significantly associated	Dialyna et al. 2003
Null/null	Japanese	Significantly associated	Kiyohara et al. 2000, Hayashi et al. 1992, Kihara et al. 1995
GSTM1/GSTP1			
Null/Val/Val	Chinese	Significantly associated	Wang et al. 2003
Null/Val/Val	Japanese	Significantly associated	Alexandrie et al. 1994
GSTM1			
Null	Chinese	Not significantly associated	Wang et al. 2003
GSTP1			
Val/Val	Chinese	Not significantly associated	Wang et al. 2003
Smoking+GSTs	Chinese	Not significantly associated	Wang et al. 2003

populations on xenobiotic gene polymorphism and found a non-significant association with lung cancer. As in studies on Chinese and German populations (Miller et al. 2002, Wang et al. 2003), we have also not observed a significant effect of *GSTP1* (Ile/Val or Val/Val) genotype on lung cancer. The combination of *GSTP1* (Ile/Val or Val/Val) polymorphism with *GSTM1* and *GSTT1* null does not seem to be associated with increased risk of lung cancer. In our study, the control population consisted of a group of patients with a range of different diagnoses. GST polymorphism may be associated with altered risk in these conditions so that the risk in the case population may be biased; controls with no history of lung disease would be more suitable, but more difficult to recruit from a bronchoscopy clinic. After adjustment for age, gender and smoking status, our results still agree with the published studies on north Indian populations and other ethnic groups worldwide. Besides this, our study has several important strengths such as: (1) both cases and controls underwent the same diagnostic procedure, (2) the controls and cases were drawn from same base (ethnic) population, and (3) interviewing was blind to case status.

We have conducted this case–control study to evaluate the association between this polymorphism with specific histological subtypes and risk of lung cancer. The data thus obtained confirm results from other studies that Ile/Val or Val/ Val genotypes of *GSTP1* polymorphism in combination with both *GSTM1* and *GSTT1* null genotypes do not have a marked tendency to develop lung cancer but show an increased risk for SqCC. Further investigations need to be performed with more samples on a histological basis.

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