

## RESEARCH ARTICLE

# Associations of *CYP1A1*, *GSTM1* and *GSTT1* Polymorphisms with Lung Cancer Susceptibility in a Northern Indian Population

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### Abstract

**Background:** Susceptibility to lung cancer has been shown to be modulated by inheritance of polymorphic genes encoding cytochrome P450 1A1 (*CYP1A1*) and glutathione S transferases (*GSTM1* and *GSTT1*), which are involved in the bioactivation and detoxification of environmental toxins. This might be a factor in the variation in lung cancer incidence with ethnicity. **Materials and Methods:** We conducted a case-control study of 218 northern Indian lung cancer patients along with 238 healthy controls, to assess any association between *CYP1A1*, *GSTM1* and *GSTT1* polymorphisms, either separately or in combination, with the likelihood of development of Lung cancer in our population. **Results:** We observed a significant difference in the *GSTT1* null deletion frequency in this population when compared with other populations (OR=1.87, 95% CI: 1.25-2.80-0.73, P=0.002). However, *GSTM1* null genotype was found associated with lung cancer in the non-smoking subgroup. (P=0.170). **Conclusions:** Our study showed the *GSTT1* null polymorphism to be associated with smoking-induced lung cancer and the *GSTM1* null polymorphism to have a link with non-smoking related lung cancer.

**Keywords:** Lung cancer - genetic polymorphism - *CYP1A1* - *GSTM1* - *GSTT1* - odds ratio - smoking

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### Introduction

Carcinoma of the lung is the most common cancer and the most frequent cause of death in the patients with cancer around the world (Bethesda, 2001). Environmental carcinogens such as active and passive smoking, air pollution and environmental exposures have strong influences on individual factors (Perera, 1998). In humans, there are several genetic polymorphisms of the enzymes involved in metabolic activation and detoxification of pulmonary carcinogens. Interindividual differences in ability to activate and detoxify carcinogens are expected to affect the risk of developing lung cancer (Raunio et al., 1995). Cytochrome P-450s, cytochrome P450 1A1 (*CYP1A1*), glutathione S-transferase M1 (*GSTM1*), and (*GSTT1*) phase II detoxifying enzymes are involved in the formation and elimination of carcinogens, have been extensively studied as possible modulators of risk for lung cancer that could explain varying susceptibilities to the disease (Taningher et al., 1999).

*CYP1A1* gene is involved in the activation step in the metabolism of polycyclic aromatic hydrocarbons (PAHs),

such as those found in tobacco smoke, converting them to carcinogens (Gonzalez, 1990). Glutathione transferases (GSTs) comprise a multigene family encoding enzymes that catalyse the conjugation of glutathione to a wide variety of compounds with an electrophilic centre (Hayes and Pulford, 1995). *GSTM1* is involved in the detoxification of tobacco-related carcinogens, such as epoxides and hydroxylated metabolites of benzo ( $\alpha$ )-pyrene (Ketterer et al., 1992), whereas *GSTT1* is involved in the biotransformation of several low molecular weight toxins such as ethylene oxides, butadiene, etc. (Guengerich et al., 1995), which are constituents of tobacco smoke.

It is likely that several genetic polymorphisms cooperate in increasing individual risk. There may be specific genotypes or genotype combinations that greatly increase the risk of developing lung cancer. In view of the prevalence of tobacco smoking, and the incidence of lung cancer in India, we investigated the distribution and susceptibility of *CYP1A1*, *GSTM1*, and *GSTT1* gene polymorphism in lung Cancer and healthy controls in Northern Indian Population or to determine whether any of the polymorphisms confer an increased risk.

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## Materials and Methods

The study group included 456 (218 Lung cancer cases and 238 healthy controls) were recruited from the Department of Pulmonary Medicine, King George's Medical University, (Erstwhile C.S.M. Medical University), Lucknow, India. Eligible cases included all patients with newly diagnosed Lung cancer presented between April 2007 and December 2009. All cases were either newly diagnosed or previously treated patients. All cancer cases were assessed by histological examination. During the study period, we included 218 Lung cancer cases (189 males and 29 females) and 238 healthy controls (191 males and 47 females). Ethical approval was obtained from the institutional Ethical Committee of the King George's Medical University, Lucknow, India. Controls of the same geographic origin were selected from individuals who attended the outpatient department.

### Inclusion/exclusion criteria for case/control

A questionnaire was completed by both patient and control groups to provide relevant information regarding the risk factors for Lung cancer. The information collected included socio-demographic characteristics such as gender, age, lifetime occupational history (including exposure to known carcinogens), area of origin, family history of cancer among first degree relatives, smoking status, which included smoking duration and pack years smoked, medication history and pre-existence of respiratory or lung diseases. In order for the age and gender distributions of controls to match those of Lung cancer patients, most of the controls were age matched and the majority were males. Controls were also interviewed and asked about histories of cancer, occupation and smoking habits. Smoking information included past and/or present smoking status, amount smoked and duration of smoking. Smoking status of the subjects was calculated as the average tobacco consumption expressed in pack years. Pack years were computed as the number of cigarettes smoked per day multiplied by the duration of smoking in years.

### Sample collection

Blood samples were collected from study subjects after obtaining their written informed consent. Peripheral blood (2 ml) collected from patients and all controls and was stored at -80°C until use.

### Blood collection and DNA extraction

EDTA-buffered whole blood (5 ml) was drawn for subsequent DNA extraction by standard salting-out method (Miller et al., 1988).

### Genotype analysis

*GSTM1* and *GSTT1* null allele were determined by using multiplex polymerase chain reaction (PCR) with the *CYP1A1* gene as an internal positive control (Setiawan et al., 2000). Briefly, a 215-bp region between exons 4 and 5 of the *GSTM1* gene and 480-bp products for were amplified along with a 312-bp size product of *CYP1A1*.

The PCR products were electrophoresed on a 2% agarose gel. The absence of 480 and 215 bp bands indicated homozygous null genotypes of *GSTM1* and *GSTT1*, respectively.

*CYP1A1* -6235 T>C polymorphism involves the substitution of CTGG to CCGG in the MspI site at base 264 from the additional polyadenylation signal in the 3' flanking region. The region of interest was amplified by PCR using the primer sequences described by Kawajiri et al. (1990). *CYP1A1* T and C alleles were determined by the presence or absence of the MspI restriction site through different band patterns on 2% agarose gel. The wild-type genotype (*CYP1A1*/TT) showed a single band of 360 bp. The variant genotype (*CYP1A1*/CC) resulted in two fragments of 220 and 140 bp, whereas the heterozygous genotype (*CYP1A1*/TC) showed three bands of 360, 220, and 140 bp.

### Statistical methods

Variables selected from the data set were age, gender, smoking status (non smokers, ex-smokers, and smokers), pack years of smoking, and polymorphisms in the *CYP1A1*, *GSTM1* and *GSTT1* genes. We estimated the study specific odds ratios (OR) of Lung cancer for each polymorphism using binary logistic regression modeling with 95% confidence intervals (CIs), and the difference in genotype prevalence and association between case and control group were assessed and adjusted for age, gender and smoking status.

To determine whether the genotype frequencies were significantly different between the patient and control population, a probability of  $P < 0.05$  was considered. Age, gender, smoking status and pack years were included as covariates as well as all the possible genotypes studied. *GSTM1* and *GSTT1* polymorphism was dichotomized into null genotype and wild type, while *CYP1A1* MspI polymorphism was categorized into homozygous wild type and variant allele-containing genotypes.

Besides the main effect of *CYP1A1*, *GSTM1* and *GSTT1* polymorphism on Lung cancer. Wild type of *CYP1A1* and non-null genotypes of *GSTM1* and *GSTT1* were used as reference groups to assess the combined effects of the two genes. To evaluate the possible interaction between genetic polymorphisms and smoking, a group of subjects with non-null genotype and no current smoking habits was used as a reference group.

## Results

Mean age of healthy subjects (controls) and lung cancer patients was  $56.15 \pm 7.84$  and  $56.14 \pm 11.91$  years, respectively (t test p value=ns). Lung cancer was highly prevalent in males (189 out of 218; 86.7%) than in females (29 out of 218; 13.3%). In patients with Lung cancer most of the cases were with squamous cell carcinoma (54.1%). Regarding smoking habit, 58.7% were smokers, 9.6% ex-smokers and 31.7% non-smokers among lung cancer patients with mean pack years of  $13.95 \pm 7.93$  (years); in controls 72.3% were non-smoker, 13.4% ex-smoker and 14.3% smokers with mean pack year of  $10.5 \pm 5.62$  (years).

## Association with susceptibility to lung cancer

There were no consistent patterns of elevated risk associated with the *GSTM1* null genotype, but the frequency of the

*GSTT1* null genotype was 24.4% in controls and 37.6% in Lung cancer and showed significant association (OR=1.87, 95%CI=1.25–2.80, P=0.002). However no significant association for lung cancer was found for *CYP1A1* 6235T>C polymorphism (TT, TC, and CC) in Lung cancer patients (64.2%, 31.7%, 4.1%) vs healthy controls (55.0%, 37.8%, 7.1%).

**Table 1. Demography Characteristic of Lung Cancer Patients and Healthy Controls**

	Controls n=238	Cases n=218
Mean Age± SD (Year)	56.15±7.84	56.14±11.91
Sex		
Male*	191 (80.3%)	189 (86.7%)
Female	47 (19.7%)	29 (13.3%)
Smoking History		
Smoker	34 (14.3%)	128 (58.7%)
Ex-smoker	32 (13.4%)	21 (9.6%)
Non-smoker	172 (72.3%)	69 (31.7%)
Pack Year	10.53±5.62	13.95±7.93
Histopathology		
Squamous cell		118 (54.1%)
Adenocarcinoma		65 (29.2%)
Mixed cell		27 (12.4%)
Small cell		8 (3.7%)

\* OR (95%CI) p value=0.078

**Table 2. Distribution of Genotype/allele Frequency of *GSTT1*, *GSTM1* and *CYP1A1* -6235 T>C (rs4646903) in Association with Lung Cancer**

	Control n=238	Cases n=218	OR (95%CI)	p value
<i>GSTT1</i>				
Present	180 (75.6%)	136 (62.4%)	1 Reference	
Null	58 (24.4%)	82 (37.6%)	1.87(1.25-2.80)	0.002
<i>GSTM1</i>				
Present	148 (62.2%)	134 (61.5%)	1 Reference	
Null	90 (37.8%)	84 (38.5%)	1.03 (0.71-1.51)	0.875
<i>CYP1A1</i> -6235 T>C (rs4646903)				
TT	131 (55.0%)	140 (64.2%)	1	
TC	90 (37.8%)	69 (31.7%)	0.69 (0.29-1.64)	0.400
CC	17 (7.1%)	9 (4.1%)	0.50 (0.21-1.15)	0.100
Allele				
T	355 (74.6%)	349 (80.0%)	1	
C	121 (25.4%)	87 (20.0%)	1.39 (1.02-1.90)	0.040

\* OR (95%CI) p value=0.078

**Table 3. Association of *GSTT1* and *GSTM1* Gene Polymorphism with Smoking**

	Control n=238	Case n=218	OR (95%CI)	p value
Non-Smoker				
<i>GSTT1</i>	N=170	N=69		
Present	127 (74.7%)	51 (73.9%)	1	
Null	43 (25.3%)	18 (26.1%)	0.96 (0.51-1.82)	0.899
Smoker				
<i>GSTT1</i>	N=68	N=149		
Present	53 (77.9%)	85 (57.0%)	1	
Null	15 (22.1%)	64 (43.0%)	0.38 (0.19-0.72)	0.004
Non-Smoker				
<i>GSTM1</i>	N=170	N=69		
Present	106 (62.4%)	32 (46.4%)	1	
Null	64 (37.6%)	37 (53.6%)	0.52 (0.30-0.92)	0.024
Smoker				
<i>GSTM1</i>	N=68	N=149		
Present	42 (61.8%)	102 (68.5%)	1	
Null	26 (38.2%)	47 (31.5%)	1.34 (0.74-2.45)	0.334

**Table 4. Association of *CYP1A1* -6235 T>C (rs 4646903) Gene Polymorphism with Smoking**

	Control n=238	Cases n=218	OR (95%CI)	p value
Non- Smoker (N=170) (N=69)				
<i>CYP1A1</i> -6235 T>C (rs 4646903)				
TT	90 (50.9%)	45 (65.2%)	1	
TC	66 (38.8%)	23 (33.3%)	0.22 (0.50-0.96)	0.044
CC	14 (8.2%)	1 (1.4%)	0.15 (0.35-0.66)	0.012
Smoker (N=68) (N=149)				
<i>CYP1A1</i> -6235 T>C (rs4646903)				
TT	41 (60.3%)	95 (63.8%)	1	
TC	24 (35.3%)	46 (30.9%)	1.15 (0.42-3.14)	0.791
CC	3 (4.4%)	8 (5.4%)	0.94 (0.35-2.50)	0.898

## Gene environment interaction

On analyzing the interaction of genotypes with smoking, *GSTT1* null genotype was found significantly associated with lung cancer patients who smoked (OR=0.38, 95%CI=0.19-0.72, P=0.004), whereas *GSTM1* null genotype were significant associated with lung cancer patients who were non smoker (OR=0.52; 95%CI=0.30-0.90 P=0.024).

## Discussion

Various form of *CYP1A1*, *GSTM1* and *GSTT1* gene have risk for development of Lung cancer in various population (Garte, 2001). The levels of expression and catalytic activities of cytochrome p450 and *GSTM1* and *GSTT1* enzymes in lungs, and their metabolic balance, may be an important determinant host factor underlying Lung cancer. In this study, we evaluate the effect of *GSTT1*, M1 and *CYP1A1* Msp1 gene polymorphisms in Northern Indian Lung cancer patients and controls.

*GSTM1* and *GSTT1* members of the glutathione S-transferase multigene family are candidate cancer susceptibility genes because of their ability to regulate the conjugation of carcinogenic compounds to excretable hydrophilic metabolites. Individuals who are carriers of homozygous deletions in the *GSTM1* and *GSTT1* genes may have an impaired ability to eliminate carcinogenic compounds metabolically and may therefore be at an increased cancer risk. The frequencies of homozygous *GSTM1* and *GSTT1* deletion carriers are surprisingly high in most human populations, and noticeable differences between ethnic groups exist (Nelson et al., 1995). *GSTM1* null genotype was present in about 50% of Caucasians, 33% of African Americans and 45% of Japanese (Persson et al., 1999; Roy et al., 2001; Chan-Yeung et al., 2004) and various studies have shown an increased Lung cancer risk for *GSTM1* null genotype independent of ethnic background (Chen et al., 1996; Gao and Zhang, 1999). *GSTT1* null genotype was present in 64% of Chinese, 60% of Koreans, 28% of Caucasians and 22% of African Americans (Nelson et al., 1995).

In our study, frequency of *GSTT1* null genotype in Lung cancer patients is higher than healthy controls (37.6% vs 24.4%); while *GSTM1* null genotype is similar to healthy controls (38.5% vs 37.8%). *GSTT1* null genotype was significantly associated with lung cancer patients (p- value 0.001) but *GSTM1* null genotype was

not associated with it. This is consistent with some other similar findings in African Americans population (Taioli et al., 1998; Sorensen et al., 2004), but conflicts with certain other reports in China (Lan et al., 2000). Although intra ethnic as well as inter ethnic differences exist in the Indian population, the prevalence of in *GSTM1* null genotype and *GSTT1* null genotype in our population is comparable with that found in other Indian studies (Mishra et al., 2004; Naveen et al., 2004).

*CYP1A1* gene is important for the activation of pre carcinogens (Ingelman-Sundberg et al., 2001). It is located in the 3' flanking region of the *CYP1A1* gene, which is originally found to be associated with Lung cancer in Asians (Kawajiri et al., 1990). In our study, there is a lower frequency of *CYP1A1* genotype (TT, TC, and CC) in Lung cancer patients (64.2%, 31.7%, 4.1%) vs healthy controls (55.0%, 37.8%, 7.1%) no significant association was found, which is contradictive for other study (Sobti et al., 2004; Sreeja et al., 2005).

Tobacco smoke contains numerous carcinogens, including PAHs such as benzo[ $\alpha$ ]pyrene (B[ $\alpha$ ]P), which may play an important role in lung carcinogenesis and exposures in experimental animals have shown to induce squamous cell carcinoma (Deutsch-Wenzel, 1983). Deletions at one or both of GST loci and, with consequent, less detoxification of xenobiotic toxic substances, an individual may become susceptible to diseases produced by toxic substances present in the environment; hence, this positive association raises the possibility that the two enzymes are working in tandem rather than in a complementary way.

While cigarette smoking is the main attributable factor for lung cancer, environmental pollution and asbestos are considered the other risk factors. However, these risk factors cannot explain all Lung cancer cases and there is a substantial body of epidemiological evidence linking occupational exposures to dusts, gases/vapours, and fumes to chronic airflow obstruction, with a substantial population attributable risk (15-20%) in non-smokers as well (Meldrum, 2005). In our study, *GSTT1* null genotype was significantly associated with Lung cancer in smoker and *GSTM1* null genotype was significantly associated with Lung cancer in non-smoker patients; *CYP1A1* homozygote TC/CC genotype showed a protective role with non-smoker Lung cancer patient.

In conclusion, the limitation of this study is small sample sizes and the fact that only a few genes involved in the detoxification of smoke products were studied. In conclusion, we found association of *GSTT1* null polymorphism with Lung cancer in a northern India population. Moreover, we also found association between *GSTT1* null with smoker and *GSTM1* null in non smokers with Lung cancer.

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## References

- Bethesda M (2001). SEER Cancer Statistics Review. National Institutes of Health 1973-1998.
- Chan-Yeung M, Tan-Un KC, Ip MS, et al (2004). Lung cancer susceptibility and polymorphisms of glutathione-S-transferase genes in Hong Kong. *Lung Cancer*, **45**, 155-60.
- Chen H, Sandler DP, Taylor JA, et al (1996). Increased risk for myelodysplastic syndromes in individuals with glutathione transferase theta 1 (*GSTT1*) gene defect. *Lancet*, **347**, 295-7.
- Deutsch-Wenzel RP, Brune H, Grimmer G, Dettbarn G, Misfeld J (1983). Experimental studies in rat lungs on the carcinogenicity and dose-response relationships of eight frequently occurring environmental polycyclic aromatic hydrocarbons. *J Natl Cancer Inst*, **71**, 539-44.
- Gao Y, Zhang Q (1999). Polymorphisms of the *GSTM1* and *CYP2D6* genes associated with susceptibility to lung cancer in Chinese. *Mutat Res*, **444**, 441-9.
- Garte S, Boffetta P, Caporaso N, Vineis P (2001). Metabolic gene allele nomenclature. *Cancer Epidemiol Biomarkers Prev*, **10**, 1305-6.
- Gonzalez FJ (1990). Molecular genetics of the P-450 superfamily. *Pharmacol Ther*, **45**, 1-38.
- Guengerich FP, Thier R, Persmark M, et al (1995). Conjugation of carcinogens by theta class glutathione s-transferases: mechanisms and relevance to variations in human risk. *Pharmacogenetics*, **5**, 103-7.
- Hayes JD, Pulford DJ (1995). The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol*, **30**, 445-600.
- Ingelman-Sundberg M, Oscarson M, Daly AK, Garte S, Nebert DW (2001). Human cytochrome P-450 (CYP) genes: a web page for the nomenclature of alleles. *Cancer Epidemiol Biomarkers Prev*, **10**, 1307-8.
- Kawajiri K, Nakachi K, Imai K, et al (1990). Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P450IA1 gene. *FEBS Lett*, **263**, 131-3.
- Ketterer B, Harris JM, Talaska G, et al (1992). The human glutathione S-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. *Environ Health Perspect*, **98**, 87-94.
- Lan Q, He X, Costa DJ, et al (2000). Indoor coal combustion emissions, *GSTM1* and *GSTT1* genotypes, and lung cancer risk: a case-control study in Xuan Wei, China. *Cancer Epidemiol Biomarkers Prev*, **9**, 605-8.
- Meldrum M, Rawbone R, Curran AD, Fishwick D (2005). The role of occupation in the development of chronic obstructive pulmonary disease (COPD). *Occup Environ Med*, **62**, 212-4.
- Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*, **16**, 1215.
- Mishra DK, Kumar A, Srivastava DS, Mittal RD (2004). Allelic variation of *GSTT1*, *GSTM1* and *GSTP1* genes in North Indian population. *Asian Pac J Cancer Prev*, **5**, 362-5.
- Naveen AT, Adithan C, Padmaja N, et al (2004). Glutathione S-transferase M1 and T1 null genotype distribution in South Indians. *Eur J Clin Pharmacol*, **60**, 403-6.
- Nelson HH, Wiencke JK, Christiani DC, et al (1995). Ethnic differences in the prevalence of the homozygous deleted genotype of glutathione S-transferase theta. *Carcinogenesis*, **16**, 1243-5.
- Perera FP (1998). Molecular epidemiology of environmental carcinogenesis. *Recent Results Cancer Res*, **154**, 39-46.
- Persson I, Johansson I, Lou YC, et al (1999). Genetic

- polymorphism of xenobiotic metabolizing enzymes among Chinese lung cancer patients. *Int J Cancer*, **81**, 325-9.
- Raunio H, Husgafvel-Pursiainen K, Anttila S, et al (1995). Diagnosis of polymorphisms in carcinogen-activating and inactivating enzymes and cancer susceptibility-a review. *Gene*, **159**, 113-21.
- Roy B, Majumder PP, Dey B, et al (2001). Ethnic differences in distributions of *GSTM1* and *GSTT1* homozygous "null" genotypes in India. *Hum Biol*, **73**, 443-50.
- Setiawan VW, Zhang ZF, Yu GP, et al (2000). *GSTT1* and *GSTM1* null genotypes and the risk of gastric cancer: a case-control study in a Chinese population. *Cancer Epidemiol Biomarkers Prev*, **9**, 73-80.
- Sobti RC, Sharma S, Joshi A, Jindal SK, Janmeja A (2004). Genetic polymorphism of the *CYP1A1*, *CYP2E1*, *GSTM1* and *GSTT1* genes and lung cancer susceptibility in a north indian population. *Mol Cell Biochem*, **266**, 1-9.
- Sørensen M, Autrup H, Tjønneland A, et al (2004). Glutathione S-transferase T1 null-genotype is associated with an increased risk of lung cancer. *Int J Cancer*, **110**, 219-24.
- Sreeja L, Syamala V, Hariharan S, et al (2005). Possible risk modification by *CYP1A1*, *GSTM1* and *GSTT1* gene polymorphisms in lung cancer susceptibility in a South Indian population. *J Hum Genet*, **50**, 618-27.
- Taioli E, Ford J, Trachman J, et al (1998). Lung cancer risk and *CYP1A1* genotype in African Americans. *Carcinogenesis*, **19**, 813-7.
- Taningher M, Malacarne D, Izzotti A, Ugolini D, Parodi S (1999). Drug metabolism polymorphisms as modulators of cancer susceptibility. *Mutat Res*, **436**, 227-61.