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CYP1A1 and *CYP2D6* polymorphism and risk of lung cancer in a North Indian population

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This case–control study was conducted to examine the association between the *CYP1A1* and *CYP2D6* genotypes and lung cancer risk among North Indians. The estimated relative risk for lung cancer associated with the *CYP1A1 Val/Val* allele was 2.68, and was four-fold when cases with small cell lung cancer (SCLC) were considered alone. With regard to the metabolism of debrisoquine, no poor metabolizers were found amongst the subjects. The odds ratio of risk with the heterozygous extensive metabolizer (*HEM*) genotype was 1.5. However, in the presence of at least a single copy of the variant *CYP1A1 MspI* allele and the *CYP2D6 HEM* genotype, the risk was two-fold for squamous cell carcinoma (SQCC). When the *CYP1A1 Val/Val* and *CYP2D6 HEM* genotypes were taken together, the risk for SCLC was four-fold. Stratified analysis indicated an interaction between bidi smoking and variant *CYP1A1* genotypes on the risk for SQCC and SCLC. Heavy smokers (Brinkman index > 400) with *Val/Val* genotypes were at a very high risk of developing lung cancer (odds ratio 29.30, 95% confidence interval 2.42–355, $p = 0.008$). Heavy smokers with *CYP1A1 MspI* (*CYP1A1*1/2A* or *CYP1A1*2A/*2A*) genotype had a seven-fold risk for SCLC compared with non-smokers. This study is the first to be carried out on a North Indian population, and, although small, suggests that *CYP1A1* and *CYP2D6* polymorphisms might have a role in determining the risk for lung cancer and should be investigated further.

Keywords: *CYP2D6*, *CYP1A1*, odds ratio, lung cancer, polymorphism, genotypes.

Introduction

Lung cancer is the leading cause of death due to cancer worldwide, and tobacco use accounts for 85–90% of lung cancer cases. Although only about 1–2% of all cancers are currently attributable to mutations in a single gene, the role of genetic susceptibility is still important, especially with regard to gene–environment interactions (Strauss 1997).

Genetic polymorphisms are known for enzymes involved in the activation of tobacco-related carcinogens such as polycyclic aromatic hydrocarbons (PAHs), nitrosamines and arylamines. PAHs are activated by CYP1A1, a phase I drug-metabolizing enzyme, into reactive forms that produce DNA adducts, and the reactive metabolites of PAHs are detoxified by glutathione-*S*-transferase phase II enzymes. A rare mutation at the *MspI* site in the *CYP1A1* gene has been reported to be associated with an increased risk of lung cancer, particularly squamous cell

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carcinoma (Nerbert 1991, Hayashi *et al*. 1992). The *CYP1A1 Ile/Val* mutation in the haem-binding region results in a two-fold increase in microsomal enzyme activity and is in complete linkage disequilibrium in Caucasians with the *CYP1A1 MspI* mutation. Significant ethnic differences in the frequency of homozygous *CYP1A1 MspI* alleles (*CYP1A1*2A/*2A*) have been observed, and both the *MspI* and *Val* alleles are rarer in Caucasian than in Japanese populations (Crofts *et al*. 1993, Kiyohara *et al*. 1998).

CYP2D6 codes for the phase I enzyme debrisoquine-4-hydroxylase, which is involved in the metabolic activation of many carcinogens related to lung cancer (Daly *et al*. 1993). *CYP2D6* polymorphism can be determined by the administration of debrisoquine and subsequent analysis of the urinary metabolic ratio (MR) (Wilkinson *et al*. 1989). According to MR values, individuals can be classified as extensive metabolizers (EMs), heterozygous extensive metabolizers (HEMs) or poor metabolizers (PMs). There is a considerable ethnic variation in the frequency of the PM genotype. In Caucasians, the incidence of the PM genotype appears to be around 7%, whereas in Chinese and Japanese it is less than 1% (Gao and Zhang 1999, Agundez *et al*. 2001). A characteristic G > A transition mutation at the junction of exon 3 and intron 4, which leads to an early termination codon and defective mRNA, accounts for approximately 80% of the mutant alleles (Febbo *et al*. 1998). Some epidemiological studies have provided support that lung cancer risk is increased in EM individuals, whereas others have not (Stucker *et al*. 1995).

Little is known about the impact of *CYP1A1* and *CYP2D6* polymorphisms on the risk of lung cancer in the North Indian population. In India bidi (a type of Indian cigarette) is the main mode of smoking, followed by cigarettes, hookahs and cigars. In view of the prevalence of bidi smoking and lung cancer in India, and the lack of data on the second biggest population in the world, a case–control study is warranted to evaluate the role of *CYP1A1* and *CYP2D6* genes as genetic modifiers in the aetiology of lung cancer. The present study reports the impact of genetic polymorphisms of *CYP1A1* and *CYP2D6* genes alone and together on the susceptibility to lung cancer in the North Indian population.

Materials and methods

Study subjects

This case–control study included 100 patients suffering from lung cancer and 76 healthy controls selected from community centres, educational institutes and employee groups. The controls selected in this study were matched by region to the lung cancer cases. In order to achieve an approximate balance of age, ethnicity and gender between the cases and controls, we sampled potential controls based on the distribution of these factors amongst the cases. Effort was made to obtain cases and controls of the same ethnicity. Lung cancer cases were recruited from patients undergoing bronchoscopy at the Department of Pulmonary Medicine, Post Graduate Institute of Medical Education and Research and from the Department of Chest and Infectious Diseases, Government Medical College, Chandigarh, India. Informed consent was obtained from all the cases and controls before taking a 4 ml sample of blood. At recruitment, each participant was personally interviewed to obtain detailed information about smoking, dietary habits, alcohol consumption and demographic characteristics. Smoking history, including smoking dosage (number of bidis smoked), duration, ages at beginning and cessation of smoking habits and type of brand was obtained. The smoking dose was assessed using the Brinkman index (BI), which was calculated by multiplying the number of bidis smoked per day by the number of years smoked. Individuals with a smoking index > 400 were classified as heavy smokers and those with an index < 400 as light smokers. All the lung cancer cases were histologically confirmed by a pathologist. The particular

subjects selected in this study represented five of the eight regions that comprise the northern plains of India, namely the states of Himachal Pradesh, Haryana, Uttar Pradesh, Punjab and Chandigarh.

CYP1A1 genotyping

Genomic DNA was isolated from peripheral blood samples from cases and controls according to the protocol of Field *et al*. (1999). *CYP1A1* genotyping for *MspI* and *Ile/Val* polymorphism was analysed using polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP) and allele-specific PCR methods (Sivaraman *et al*. 1994). The primers for the *MspI* RFLP were P79 (5′-AAG AGG TGT AGC CGC TGC ACT) and P80 (5′-TAG GAG TCT CTC ATG CCT), which amplify a 335 bp fragment. Briefly, genomic DNA was amplified in 1 × reaction buffer (Sigma, St Louis, Missouri, USA) with 2 mM $MgCl_2$, 100 μg bovine serum albumin (BSA), 200 μm deoxynucleotide triphosphates (dNTPs), 1.5 U Taq (MBI, Fermentas, Lithuania) and 0.5 μM of each primer. Initial denaturation was performed at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C, annealing at 56°C and extension at 72°C for 1 min each, and a final extension at 72°C for 5 min in a thermal cycler (Minicycler, MJ Research, Waltham, MA, USA). The PCR products were digested with 15 units of MspI restriction enzyme (Sigma) at 37°C for 3 h, and then subjected to electrophoresis in 2.5% agarose gel (USB, Cleveland, USA) and stained with ethidium bromide. The wild-type allele *CYP1A1*1/*1* revealed a single band of 335 bp, the variant mutant allele *CYP1A1*2A/*2A* resulted in two fragments of 206 and 129 bp, whereas the heterozygous allele *CYP1A1*1/*2A* showed three bands of 335, 206 and 129 bp.

The *Ile/Val* polymorphism at exon 7 of the *CYP1A1* gene was assessed by allele-specific PCR. For this, genomic DNA was amplified with primers 1A (5′-GAA AGG CTG GGT CCA TCT), 2A (5′-AAG ACC TCC CAG CGG GCA AT), and 2G (5′-AAG ACC TCC CAG GCA AC) (primer 1A was used in conjunction with primers 2A and 2G in two allele-specific PCR reactions) in the presence of 1 × reaction buffer with 2 mM $MgCl_2$, 100 μg BSA, 200 μm dNTPs and 1.5 U Taq. Initial denaturation was performed at 95°C for 5 min, followed by 25 cycles of denaturation at 95°C, annealing at 63°C and extension at 72°C for 1 min each, and a final extension at 72°C for 5 min in a thermal cycler. The PCR products were analysed in 2% agarose gel and stained with ethidium bromide. PCR analysis resulted in a 322 bp fragment with the classification wild allele (*Ile/Ile*), heterozygote (*Ile/Val*) and mutant (*Val/Val*). *Ile/Val* polymorphism analysis was repeated in duplicate.

PCR-RFLP analysis of CYP2D6 gene polymorphism

A PCR-RFLP assay was used for the detection of the G1934A mutation (resulting in the PM allele); *CYP2D6*4* is at the junction of intron 3/exon 4 of the *CYP2D6* gene. Amplification of genomic DNA was carried out in a reaction volume of 50 μl containing 0.5 μM of primers D1 (5′-GCT TCG CAA CCA CTC CCG-3′) and D2 (5′-AAA TCC TGC TCT TCC GAG GC-3′), 200 μM dNTP, 100 μg BSA, 1 × PCR buffer, 1.5 U Taq and 1.5 mM $MgCl_2$. Initial denaturation was performed at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C, annealing at 57°C and extension at 72°C for 1 min each, and a final extension at 72°C for 4 min in a thermal cycler. The PCR products were digested with 10 units of *BstN1* restriction enzyme (New England Biolabs, Beverly, MA, USA) at 65°C for 16 h and subjected to electrophoresis in 2.5% agarose gel prepared in Tirs–acetic acid–ethylene diamine tetra-acetic acid (TAE) buffer along with a DNA marker and electrophoresed at 60 V. The wild-type allele (EM) was identified by the presence of 230 and 104 bp alleles. The G1934A mutation did not have a restriction site for *BstN1* and only produced an undigested 334 bp fragment, and was designated as mutant (PM), whereas the heterozygous genotypes (HEM) showed three fragments of 334, 230 and 104 bp.

Statistical analysis

SPSS and Epical 2000 (version 1.02) were used for the statistical analyses. The odds ratio (OR), 95% confidence interval (95% CI) and p values were calculated. The OR was adjusted for age, sex and smoking using logistic regression analysis.

Results

The average ($\pm$SD) age was 55.5 ± 11.3 years for the cases and 50.9 ± 8.1 for the controls. No significant difference in the gender distribution was observed between the two groups. Although an effort was made to obtain a frequency match on smoking status between cases and controls, there were more smokers in the cancer group compared with the controls (86% and 77.6%, respectively).

Moreover, the cancer cases had a higher percentage of heavy smokers (BI > 400) than controls (83.7% of cases compared with 72.9% of controls).

Details of the *CYP1A1* and *CYP2D6* polymorphisms found are given in Table 1. Among the cases and the controls, the frequency of the *MspI* variant allele (*CYP1A1*1/*2A*) was 45% and 38.2%, respectively; thus the lung cancer group had a higher frequency of the *MspI* allele.

The frequency of the *Ile/Ile* allele was 4% and 10.5% among lung cancer cases and controls, respectively, whereas that of the *Val/Val* allele was 29% and 19.7%, respectively. The frequency of the *Val/Val* allele was higher in SCLC (50%) than in SQCC (22.5%).

For the *CYP2D6* genotypes, the frequency of the heterozygous carriers (*HEM*) was 25% and 18.42% in cases and controls, respectively, whereas that of the homozygous genotype (*EM*) was 75% and 81.5%, respectively (Table 1). In this study there were no subjects with the homozygous mutant genotype (*PM*). The frequency of *HEM* was higher in SQCC (26.7%) than in SCLC (20.8%).

Table 2 presents the ORs and 95% CIs for all the lung cancer cases and for the specific histological cell types with respect to the *CYP1A1* and the *CYP2D6* genotypes. As the frequency of the *CYP1A1*2A/*2A* genotype was low, the heterozygous *CYP1A1*1/*2A* and mutant *CYP1A1*2A/*2A* alleles were taken as a single genotype. No significant association, however, was found between *MspI* genotype and lung cancer. The presence of at least one copy of the *MspI* variant allele (*CYP1A1*1/*2A or CYP1A1*2A/*2A)* gave on OR of 1.30 (95% CI 0.65–2.62, $p = 0.284$) for SQCC.

For the *CYP1A1 (Ile/Val)* genotypes, the presence of both the mutant alleles (*Val/Val)* was associated with a 2.5-fold elevated risk for lung cancer (OR 2.68, 95% CI 0.64–11.27, $p = 0.0451$). There was a strong association between the *Val/Val* genotype and SCLC (OR 4.06, 95% CI 1.53–10.83, $p = 0.008$). The presence of at least one copy of the *MspI* variant (*CYP1A1*1/*2A* or *CYP1A1*2A/*2A*) and the *Val/Val* allele of the *CYP1A1* genotype was associated with an OR of 2.85 (95% CI 0.22–2.33) for lung cancer (data not shown). The OR for SCLC with this genotype was 3.24 (95% CI 0.87–12.08), compared with 1.86 for SQCC (95% CI 0.61–5.67).

Amongst those with lung cancer, 67% were carriers of *Ile/Val*; of these, 37% had the *CYP1A1*1/*1* genotype, 28% the *CYP1A1*1/*2A* genotype and 2% the *CYP1A1*2A/*2A* genotype. Amongst the controls, however, 53 (69.7%) were carriers of *Ile/Val*, of which 19 (25%) were linked to *CYP1A1*1/*2A* and five (6.09%) to *CYP1A1*2A/*2A*. Among the 15 (10.97%) control carriers of the *Val/Val* allele, only seven (9.2%) were linked to *CYP1A1*1/*1* and the remaining were non-linked. Overall, 55% of the lung cancer cases revealed genetic linkage with both the *CYP1A1* genotypes.

For the *CYP2D6* genotypes, individuals carrying a single copy of the variant allele (*HEM*) were at a 1.5-fold increased risk for lung cancer (OR 1.48, 95% CI 0.71–3.08). The association was stronger for SQCC (OR 1.62, 95% CI 0.74–3.54), than for SCLC (OR 1.17, 95% CI 0.37–3.66).

Table 3 shows the combined *CYP1A1 MspI* and *CYP2D6* genotypes. The *MspI* variant allele (*CYP1A1*1/*2A* or *CYP1A1*2A/*2A*) along with the *CYP2D6 HEM*

Table 1. Numbers of lung cancer patients and controls with *CYP1A1* and *CYP2D6* genotypes.

	CYP1A1 MspI variant						*CYP1A1 Ile/Val* variant						*CYP2D6* variant					
	*CYP1A1*1/*1*		*CYP1A1*1/*2A*		*CYP1A1*2/*2A*		*Ile/Ile*		*Ile/Val*		*Val/Val*		*EM*		*HEM*		*PM*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Controls	42	55.3	29	38.2	5	6.6	8	10.5	53	69.7	15	19.7	62	81.5	14	18.4	—	—
Lung cancer	49	49	45	45	6	6	4	4	67	67	29	29	75	75	25	25	—	—
SQCC	32	45	36	50.8	3	4.22	4	5.6	51	71.8	16	22.5	52	73.2	19	26.7	—	—
SCLC	14	58.3	8	33.3	2	8.33	—	—	12	50	12	50	19	79.16	5	20.8	—	—
Others	3	75	1	25	—	—	—	—	4	100	—	—	5	100	—	—	—	—

Table 2. Lung cancer risk as indicated by OR values in relation to the *CYP1A1* and *CYP2D6* genotypes in 100 cases and 76 controls.

	All			SQCC ($n = 71$)			SCLC ($n = 24$)		
	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value
***CYP1A1 MspI* variant**									
*CYP1A1*1/*1*	1.0			1.0			1.0		
*CYP1A1*1/*2A*	1.6	0.60–2.22		1.3	0.67–2.86		0.79	0.28–2.22	
*CYP1A1*2A/*2A*	1.17	0.32–4.23		0.85	0.18–4.05		1.27	0.22–7.52	
*CYP1A1*1/*2A* or *CYP1A1*2A/*2A*	1.16	0.62–2.15	0.501	1.30	0.65–2.62	0.284	0.86	0.33–2.25	0.977
***CYP1A1 Ile/Val* variant**									
Ile/Ile	1.0			1.0			1.0		
Ile/Val	2.29	0.64–11.27		1.52	0.41–5.62		—		
Val/Val	2.68	0.64–11.27	0.0045	1.01	0.54–2.61	0.831	4.06	1.53–10.83	0.008
***CYP2D6* variant**									
EM	1.0			1.0			1.0		
HEM	1.48	0.71–3.08		1.02	0.74–3.554		1.17	0.37–3.68	

ORs were adjusted for age, gender and smoking habits.

Table 3. Lung cancer risk as indicated by OR values in relation to combined *CYP2D6* and *CYP1A1 MspI* genotypes.

Combined genotypes		All			SQCC			SCLC		
CYP1A1 MspI variant	*CYP2D6* variant	No. of cases/ controls	OR	95% CI	No. of cases/ controls	OR	95% CI	No. of cases/ controls	OR	95% CI
*CYP1A1*1/*1*	*EM*	38/35	1.0		23/35	1.0		12/35	1.0	
*CYP1A1*1/*2A* or *CYP1A1*2A/*2A*	*EM*	37/27	1.26	0.64–2.48	29/27	1.63	0.78–3.43	6/27	0.65	0.22–1.95
*CYP1A1*1/*1*	*HEM*	11/7	1.45	0.5–4.15	9/7	1.96	0.64–5.99	2/7	0.83	0.15–4.57
*CYP1A1*1/*2A* or *CYP1A1*2A/*2A*	*HEM*	14/7	1.84	0.67–5.09	10/7	2.17	0.72–6.53	4/7	1.67	0.41–6.71

ORs were adjusted for age, gender and smoking habits.

Table 4. Lung cancer risk as indicated by OR values in relation to combined *CYP2D6* and *CYP1A1 Ile/Val* genotypes.

Combined genotypes		All			SQCC			SCLC		
CYP1A1 Ile/Val variant	*CYP2D6* variant	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI
Ile/Val	*EM*	56/47	1.0		43/47	1.0		9/47	1.0	
Val/Val	*EM*	19/15	1.06	0.49–2.32	9/15	0.66	0.2–1.65	9/15	3.13	1.05–9.33
Ile/Val	*HEM*	15/10	1.26	0.52–3.06	12/10	1.31	0.5–13.34	3/10	1.57	0.36–6.84
Val/Val	*HEM*	10/4	2.10	0.62–7.13	7/4	1.91	0.52–6.99	3/4	3.92	0.75–20.5

ORs were adjusted for age, gender and smoking habits.

genotype had an OR of 1.84 (95% CI 0.67–5.09). Such a genotype had a two-fold elevated risk for SQCC (OR 2.17, 95% CI 0.72–6.53).

The impact of the combined genotypes of *CYP1A1 Ile/Val* and *CYP2D6* in relation to lung cancer and histology was also analysed (Table 4). The presence of at least one copy of the *CYP2D6* variant allele and the *Val/Val* allele of the *CYP1A1* gene was associated with a two-fold increased risk of lung cancer (OR 2.10, 95% CI 0.62–7.13).

Individuals who were heavy smokers and carried the *CYP1A1 Val/Val* genotypes were at a five-fold increased risk for lung cancer compared with light (BI < 400) and non-smokers, with an OR of 3.75 (95% CI 1.06–13.26) (Table 5). This gene–smoking association, although strong, was not seen with the *CYP1A1 CYP1A1*1/*2A* or *CYP1A1*2A/*2A* genotype, where both light and heavy smokers had the same attributable risk of lung cancer (OR 1.55, 95% CI 0.36–6.66 and OR 1.74, 95% CI 0.48–6.35, respectively). However, compared with non-smokers, there was a 3.5-fold increase in lung cancer in both the heavy and the light smoker groups. Heavy smokers (BI > 400) with *Val/Val* genotype had a 1.5-fold risk over non-smokers of developing SQCC (OR 1.54, 95% CI 0.37–6.43) (data not shown). In the presence of a single copy of the variant *MspI* allele, no significant effect between tobacco exposure and risk for SQCC was observed. Similarly, heavy smokers with the *Val/Val* genotype had a very high risk for developing SCLC, with an OR of 29.30 (95% CI 2.42–355, $p = 0.008$) (data not shown). A single copy of the variant *MspI* allele was associated with a seven-fold elevated risk for SCLC (OR 7.15, 95% CI 0.56–90.90) compared with non-smokers. Heavy smokers with the *CYP2D6 HEM* genotype had an approximately five-fold increased risk of developing lung cancer (OR 4.72, 95% CI 0.86–26.0, $p = 0.15$).

Discussion

This study investigated the prevalence of genetic polymorphisms in the *CYP1A1* and *CYP2D6* genes and their association with lung cancer risk in a North Indian population. It was found that the *CYP1A1 Val/Val* genotype is associated with an increased risk of lung cancer with an OR of 2.68. The risk for SCLC with this genotype was four-fold. This observation is in concordance with Sugimura *et al.* (1998), who also reported an elevated risk for SCLC with the *Val/Val* genotype. The proportion of individuals with *Ile/Val* polymorphism varies in different ethnic groups; the frequency of the *Val/Val* genotype is considerably lower in Caucasians than in Japanese (Okada *et al*. 1994). In the present study there was a high frequency of both the *Val/Val* and the *Ile/Val* genotypes in the population investigated. Our results for frequency of *Ile/Val* are not in agreement with the Hardy–Weinberg equilibrium equation. Hong *et al.* (1998) also observed a high frequency of the *Ile/Val* genotype in a Korean population, but the frequency of the *Val/Val* genotype was low. Again, their results for the *Ile/Val* polymorphism did not obey the Hardy–Weinberg equilibrium equation. In an Indian population with oral cancer, a high frequency of both the heterozygous and the mutant genotypes has been reported (Sreelekha *et al*. 2001). A high frequency of the *Ile/Val* and *Val/Val*

Table 5. Interaction of *CYP1A1 Ile/Val,* CYP1A1 *MspI* and *CYP2D6* genotypes and smoking on the overall risk of lung cancer.

	Non-smoker		Light smoker		Heavy smoker	
Genotype	OR	95% CI	OR	95% CI	OR	95% CI
***CYP1A1 Ile/Val* variant**						
Ile/Ile/ or Val/Val	1.0		1.41	0.44–4.59	1.17	0.44–3.07
Val/Val	0.64	0.02–7.2	0.77	0.18–3.31	3.75⋆	1.06–13.26
***CYP1A1 MspI* variant**						
*CYP1A1*1/*1*	1.0		0.61	0.14–2.68	1.09	0.29–4.06
CYP1A1*1/*2A* or *CYP1A1*2A/*2A	0.53	0.10–2.74	1.55	0.36–6.66	1.74	0.48–6.35
***CYP2D6* variant**						
EM	1.0		0.84	0.26–2.68	1.62	0.65–4.06
HEM	1.0		2.22	0.28–17.63	4.72	0.86–26.04

ORs were adjusted for age, gender and smoking habits.
⋆ $p = 0.048$.

genotypes compared with controls has been reported in Chilean lung cancer cases (Quinones *et al*. 2001). However, as far we can ascertain, this is the first study reporting a high representation of the *Val*/*Val* genotype both in cases and controls (29% and 19.7%, respectively) in an Indian population.

An association between the *CYP1A1 MspI* genotype and an increased risk of SQCC has been observed in Japanese, Caucasian and Hawaiian populations (Kawajiri *et al*. 1990, Le Marchand *et al*. 1998), but this was not apparent in our study, as the OR for this category of patients was 1.30.

When the combined effects of both the *CYP1A1* polymorphisms on lung cancer risk was analysed, an elevated risk among with those with *Val*/*Val* and variant *MspI* alleles (*CYP1A1*1*/**2A* or *CYP1A1*2A*/**2A*) was apparent. The risk was more pronounced for SCLC than for SQCC. In Japanese studies, the association was clearly stronger for SQCC (Nakachi *et al*. 1991, 1995). In contrast, studies on *CYP1A1* and lung cancer conducted in Caucasians have mostly been inconsistent, with some early studies finding no association (Tefre *et al*. 1991, Hirvonen *et al*. 1992, Shields *et al*. 1993) and more recent ones reporting an increased risk with the variant alleles (Xu *et al*. 1996). The prevalence of *MspI* genotype is increased in lung cancer as a consequence of linkage with *Ile*/*Val* genotypes (Cascorbi *et al*. 1996).

Zhang *et al*. (1996) reported that the isoleucine to valine substitution alone does not increase the enzymatic activity, and suggested that the *Ile*/*Val* genotype in linkage with *MspI* genotype might result in increased inducibility and increased risk of lung cancer. The functional effects of the *CYP1A1* polymorphisms have been investigated, with contradictory results. This implies that these polymorphisms may cause higher concentrations of enzyme *in vivo,* due to enhanced inducibility and increased stability. Thus it might be possible that, in the Indian population, the presence of a single variant *MspI* allele in combination with a homozygous mutant allele *(Val*/*Val)* might increase the metabolic activation of pro-carcinogens to carcinogens. Hence, *CYP1A1* polymorphisms may play an important role in lung cancer risk in South East Asian populations, including those from India.

For the *CYP2D6* gene, no PMs were observed. The frequency of *PM* genotype Caucasians has been reported to be 7%. It is 9.5% in Swedish (Rannug *et al*. 1995), 7.1% in Italian (Shaw *et al*. 1998), 5.4% in French (Laforest *et al*. 2000), 3.7% in German (Roots *et al*. 1992) and 3.6% in British (Wolf *et al*. 1992) populations. In Japanese and Chinese populations, the frequency of *PM* is less than 1% (Gao and Zhang 1999). It is thought that the *PM* genotype may not be a susceptibility risk factor for lung cancer in the present population as it is absent. The heterozygous alleles (*HEM*) are more represented in this population and have an OR of 1.62 for SQCC.

The presence of a single copy of the variant *MspI* allele (*CYP1A1*1*/**2A* or *CYP1A1*2A*/**2A*) and the *HEM* genotype of *CYP2D6* had an OR of 1.8 for lung cancer. The *Val*/*Val* and *HEM* genotypes have a two-fold increased risk of lung cancer overall; this risk was four-fold when only SCLC was taken into account.

Bidi is the most prevalent form of smoking in India. Manufactured in India, bidis consist of tobacco wrapped in a tendu or temburni leaf, and, unlike cigarettes, they are not filtered. Being a cruder form of tobacco, they have a higher

concentration of tar and nicotine and thus are more carcinogenic than cigarettes. The present data clearly indicates that the heavy smokers (BI > 400) with a *Val*/*Val* genotype have a five-fold risk of lung cancer over light smokers (BI < 400). These data are consistent with the study of Song *et al.* (2001), who found a strong increased risk of lung cancer in heavy smokers with the *Val*/*Val* genotype. Other reports, however, have demonstrated a strong association with lung cancer for light smokers (Nakachi *et al*. 1993); this risk factor was found to be more highly associated with SCLC than with SQCC.

Conclusions

To summarize, the present study, the first to be carried out on an ethnic North Indian population, indicates that, apart from the *Ile*/*Val* polymorphism, the polymorphic genes *CYP1A1 MspI* and *CYP2D6* have no significant association with lung cancer when analysed as a single genotype, but when these genotypes are combined (e.g. both the *CYP1A1* polymorphic alleles) an increased risk of lung cancer, especially SCLC and SQCC, is apparent. The combined genotype of susceptible *CYP1A1* and *CYP2D6* genes revealed a higher risk than that ascribed to a single susceptible gene, with the association being strongest for *CYP1A1* variant alleles and the *CYP2D6 HEM* gene. These data provide additional evidence that these polymorphic genes are an important determinant in the susceptibility to bidi smoking-induced lung carcinogenesis. They may also support the hypothesis that susceptibility to certain cancers may depend on ethnic-specific gene polymorphisms. As India has a rich diversity of ethnic populations with different life styles, this study provides an impetus to conduct larger prospective studies involving different ethnic Indian populations in order to identify particular subpopulations at higher risk for lung cancer. The inconsistency between our results and other Asian populations, such as Japanese, Chinese, Korean and Taiwanese, may be explained by different environmental factors.

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