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Association of functionally important polymorphisms in cytochrome P4501B1 with lung cancer

Parag P. Shah[a], Arvind P. Singh[a], Madhu Singh[a], Neeraj Mathur[a], Bhartendu. N. Mishra[b], Mohan C. Pant[c], Devendra Parmar[a,*]

[a] Developmental Toxicology Division, Indian Institute of Toxicology Research (Formerly Industrial Toxicology Research Centre), P.O. Box 80, M.G. Marg, Lucknow 226001, India
[b] Department of Biotechnology, IET, Sitapur Road, Lucknow 226021, India
[c] Department of Radiotherapy, King George's Medical University, Shahmina Road, Lucknow 226001, India

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ABSTRACT

In the present study, genotype and haplotype frequencies of four polymorphisms of cytochrome P450 1B1 (*CYP1B1*) that cause amino acid changes (Arg-Gly at codon 48, Ala-Ser at codon 119, Leu-Val at 432 and Asn-Ser at codon 453) were studied in 200 patients suffering from lung cancer and equal number of controls. A significant difference was observed for the distribution of variant genotypes of *CYP1B1*Arg48Gly and Ala119Ser polymorphisms (*CYP1B1*2*) in cases when compared to the controls. No significant difference was observed for the distribution of variant genotypes of *CYP1B1*Leu432Val (*CYP1B1*3*) and *CYP1B1*Asn453Ser (*CYP1B1*4*) polymorphism. When the four SNPs were analyzed using a haplotype approach, SNPs at codon 48 (Arg48Gly) and codon 119 (Ala119Ser) exhibited complete linkage disequilibrium (LD) in all the cases and controls. Significant differences in the distribution of the three haplotypes (G-T-C-A, G-T-G-A and G-T-C-G) were observed in the cases when compared to controls. Tobacco use in the form of smoking as well as chewing was found to significantly increase the risk of lung cancer in patients by interacting with *CYP1B1*Ala119Ser genotypes demonstrating the role of gene–environment interaction in lung cancer. Further, the risk of lung cancer increased several fold in the patients carrying the genotype combinations of *CYP1B1*Ala119Ser and *CYP1B1*Leu432Val with *GSTM1*, a phase II enzyme suggesting the importance of gene–gene interactions in enhancing the susceptibility to lung cancer.

1. Introduction

Lung cancer represents the most common malignancy and has the highest mortality rate among all cancers [1]. Epidemiological studies have demonstrated tobacco smoking as well as environmental tobacco smoke in non-tobacco users as the major risk factor in the development of lung cancer [2,3]. However, only 10–15% of lifelong smokers develop lung cancer indicating that genetic factors may play an important role in determining the susceptibility to lung cancer [4–6]. The functional polymorphisms for both phase I and phase II drug metabolizing enzymes involved in the metabolic activation/detoxification of tobacco carcinogens has been extensively studied as a possible modulator of risk for lung cancer and could explain varying susceptibility to disease [7,8].

Cytochrome P4501B1 (*CYP1B1*), a conserved member of the cytochrome P450 (*CYP*) superfamily, is involved in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs) including benzo(*a*)pyrene and dimethylbenz(*a*)anthracene (DMBA), but with a product distribution that is distinct from *CYP1A1* [9,10]. Endobronchial mucosal biopsies taken from active cigarette smokers have shown that tobacco-induced expression of *CYP1B1* varies among individuals [11]. In humans, *CYP1B1* is genetically polymorphic and more than 50 single nucleotide polymorphisms (SNPs) have been reported so far, of which certain deleterious mutations are associated with primary congenital glaucoma [12,13]. Of the most common SNPs of *CYP1B1* gene, four have been reported to result in amino acid substitutions including Arg by Gly at codon 48(*CYP1B1*2*), Ala by Ser at codon 119 (*CYP1B1*2*), Leu by Val at codon 432 (*CYP1B1*3*) and Asn by Ser at codon 453 (*CYP1B1*4*). Shimada et al. [14] reported higher catalytic activity for Val432 variants than the Leu432 variants of the enzyme suggesting that polymorphisms in the human *CYP1B1* gene, especially those at codon 432 may contribute to differential susceptibility towards PAH and tobacco induced cancers.

CYP1B1 has been shown to be expressed in human lung and appear in significantly higher levels in the peripheral leukocytes of lung cancer patients. Multivariate analysis confirmed that there were more subjects displaying *CYP1A1* mRNA expres-

* Corresponding author. Tel.: +91 522 2627586x261; fax: +91 522 2628227/21547.
E-mail address: parmar_devendra@hotmail.com (D. Parmar).

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sion in tumor than non-tumor tissue [11,15]. Although *CYP1B1* is supposed to play an important role in lung cancer, only few studies have evaluated possible association between genetic polymorphisms in *CYP1B1* and lung cancer. No association was reported between lung cancer and *CYP1B1* polymorphism in case-cohort studies [15–17] while Wenzlaff et al. [18] showed that *CYP1B1*Leu432Val polymorphism was significantly associated with increased susceptibility to lung cancer susceptibility among cases who have never smoked. *CYP1B1*Leu432Val polymorphism was also found to modulate the individual's susceptibility towards lung cancer among smokers in Chinese population [19].

Since not much information is available on the possible role of SNPs in *CYP1B1* with susceptibility to lung cancer in Indian population, a case–control study was designed to investigate the association of functionally important SNPs in *CYP1B1* with susceptibility to lung cancer. To identify the involvement of gene–environment interaction in lung cancer, the interaction of *CYP1B1* genotypes and susceptibility to lung cancer was also studied in cigarette smokers and alcohol users. As tobacco intake in the form of tobacco chewing is prevalent in India, interaction of *CYP1B1* genotypes with tobacco chewing in influencing susceptibility to lung cancer was also studied. To further understand the importance of gene–gene interaction, attempts were made to study the interaction of *CYP1B1* genotypes with the glutathione *S*-transferase variants (*GSTM1* null), the important phase II detoxification enzyme and their association with lung cancer risk.

2. Material and methods

A case–control study was conducted at Chhatrapati Shahuji Maharaj Medical University (Formerly King George's Medical University, KGMU), Lucknow, India to study the association of polymorphism in *CYP1B1* with lung cancer. The study was carried out in accordance with the guidelines laid down by the Human Ethical Committee of KGMU, Lucknow and Industrial Toxicology Research Centre (ITRC), Lucknow after taking their approval. Male patients ($n = 200$) suffering from lung cancer and visiting the OPD facility of Radiation Oncology Unit, Radiotherapy Department, KGMU were included in this study. All cases were diagnosed with squamous cell carcinoma of lung by cytological, imaging and histopathological examinations. The control group included 200 healthy men from the same geographical location (Northern India) and the same ethnicity. The volunteers (both controls and patients) were selected at random during the same period. All were informed about the study and their consent was taken prior to the study. The controls and patients were asked to fill up the detailed questionnaire regarding their family history, medical history, life style habits such as frequency of smoking/tobacco chewing/alcohol intake per day, etc. The individuals having regular smoking habits and smoking index (S.I.) (cigarettes/day × 365 days) of 730 or more were classified as smokers [7]. Likewise, cumulative exposure of alcohol drinking was derived by multiplying the total yearly consumption of alcohol (in l/year) by the duration of habitual alcohol drinking (in years). Those who had cumulative exposure of about 90 l of alcohol were considered as regular alcohol users in our study [20]. Similarly, smokeless tobacco dose was estimated as 'chewing year' (i.e. CY = frequency of tobacco chewed-kept/day × duration of year). Those who had CY index of 365 or more were considered as tobacco chewers [21].

2.1. DNA isolation and determination of CYP1B1 and GSTM1 genotypes

500 μl blood samples were collected into citrate containing tubes from each patient and control. DNA was isolated from whole blood with the QIAamp DNA mini kit (Qiagen, CA) essentially following the manufacturer's protocol.

PCR-RFLP assay was used for identifying four polymorphisms in *CYP1B1* gene. The method of Tang et al. [22] was followed for genotyping the Leu432Val polymorphism of *CYP1B1*, while for genotyping Arg48Gly, Ala119Ser and Asn453Ser polymorphisms, the PCR reactions were carried out as described by Sutter et al. [23]. PCR products were digested with the respective restriction enzymes as described earlier [22,23] and subsequently resolved by 2.0% agarose gel electrophoresis. *GSTM1* genotypes were determined by the method of Zhong et al. [24]. For quality control, randomly 10% of the samples were selected and re-genotyped to confirm the authenticity of the result obtained earlier and the results were found to be 100% concordant.

2.2. Statistical analysis

We determined whether genotype or allele frequencies of *CYP1B1* among cases and controls were in Hardy–Weinberg equilibrium (HWE) using standard X2 statistics. The haplotype analyses (haplotype frequency estimation and pair wise linkage disequilibrium between the SNPs) were carried out using Haploview (http://www.broad.mit.edu/mpg/haploview/). The association between individual variable demographic characteristics and environmental factors, or genetic polymorphisms or haplotypes, and risk of lung cancer was estimated by conditional logistic regression. Using multiple logistic regression models, we determined the relationship of *CYP1B1* polymorphisms with lung cancer risk after adjusting for other covariates. These covariates include age, cigarette smoking, tobacco chewing and alcohol drinking in the study of lung cancer. Interaction between genotypes or between genotypes and environmental factors were also estimated by conditional logistic regression. A p-value of <0.05 was considered statistically significant. All statistical analysis was performed with the SPSS software package (Version 11.0 for windows; SPSS Chicago, IL).

3. Results

The main characteristics of the study populations are summarized in Table 1. The mean ages for controls and cases were 43 ± 12 and 56 ± 9 respectively. One of the reasons for the age difference was to include more of the non-smoking controls in the study. However, it is well established that the chances of developing lung cancer increase with age. Majority of the lung cancer patients had the history of cigarette smoking, tobacco chewing and alcohol drinking. However, based on the criteria mentioned earlier, the patients were categorized as smokers or tobacco chewers or alcohol users. As evident from Table 1, cigarette smoking was more prevalent in the lung cancer patients (60%) than in the controls (31%) which resulted in an increased risk to lung cancer (OR: 3.33, 95%CI: 2.21–5.04) in these cases. Individuals with tobacco chewing habit were also more in the cases (31%) as compared to controls (26%), though this did not produce any significant increase in risk for lung cancer in the cases (OR 1.28, 95%CI: 0.82–1.97, $p = 0.268$). Daily alcohol use was also found to be slightly higher in the cases (23%) than in the controls (15%) and this showed an increased risk for lung cancer in cases when compared to the controls (OR 1.76, 95%CI: 1.05–2.94).

Table 2 summarizes the genotype frequencies for four polymorphic variants of *CYP1B1* gene. The genotype frequencies among

Table 1
Frequency distribution of demographic variables and putative risk factors of lung cancer

Variables	Categories	Controls 200 (%)	Patients 200 (%)	OR, 95%CI	*p*-Value
Age	–	43 ± 12	56 ± 9		
Smoking status	Non-smoker	138 (69)	80 (40)	1.0 (Ref.)	
	Smoker	62 (31)	120 (60)	3.33 (2.21–5.04)	**0.00**
Tobacco chewing	Non-tobacco chewer	148 (74)	138 (69)	1.0 (Ref.)	
	Tobacco chewer	52 (26)	62 (31)	1.28 (0.82–1.97)	**0.268**
Alcohol intake	Non-alcoholics	171 (85)	154 (77)	1.0 (Ref.)	
	Alcoholics	29 (15)	46 (23)	1.76 (1.05–2.94)	**0.03**

Ref.: reference category; OR: odds ratio; 95%CI: 95% confidence interval.