

Genetic Polymorphism of *CYP2A6* Gene and Tobacco-induced Lung Cancer Risk in Male Smokers¹

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Abstract

Cytochrome P450 2A6 (*CYP2A6*) is the principal enzyme involved in the metabolic activation of tobacco-specific nitrosamines to their ultimate carcinogenic forms and metabolism of nicotine. We investigated the effects of the *CYP2A64, an entire *CYP2A6* gene deletion-type polymorphism, on lung cancer risk and daily cigarette consumption in Japanese male smokers via a hospital-based case control study. The frequency of the *CYP2A6**4 variant was compared in 370 lung cancer patients and 380 control smokers. A markedly reduced adjusted odds ratio for lung cancer risk, 0.23 [95% confidence interval, 0.08–0.67], was seen in the group with homozygous deletion (*4/*4) when the odds ratio for a group with homozygous wild (*1A/*1A) was defined to be 1.00 by logistic regression. The subjects with lung cancer were additionally divided into three groups according to the histological classification of the cancer and examined for an association with the *CYP2A6* polymorphism. The *4/*4 genotype was not found in patients with squamous cell carcinoma (0 of 105) or small cell carcinoma (0 of 44), indicating that subjects with the *4/*4 genotype have low risk for lung cancers, particularly those caused by tobacco smoke. Furthermore, a significant reduction of daily cigarette consumption was observed in smokers with the *4/*4 genotype, suggesting a possibility that**

complete lack of *CYP2A6* appeared to affect the smoking behavior. These data suggest that male smokers possessing the *1A/*1A genotype have higher risk for tobacco-induced lung cancers.

Introduction

*CYP2A6*³ is one of the forms of CYP expressed in the human respiratory tract (1, 2) and is responsible for the metabolic activation of tobacco-specific nitrosamines, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (1, 3, 4), a potent pulmonary-specific carcinogen (5), to yield genotoxic metabolites. This enzyme was also found to be involved in nicotine metabolism (6).

A genetic polymorphism of *CYP2A6* was recognized as one of the causes for the interindividual differences in the metabolism of coumarin (7, 8). The wild type of the *CYP2A6* gene was termed *CYP2A6**1A. The *CYP2A6**2 variant, which has 1-base substitution in exon 3 leading to amino acid change L160H, appears to be one of the causal polymorphisms accounting for poor metabolizers of coumarin in Caucasians (9). Recently, we found that an entire *CYP2A6* gene deletion, a novel genetic polymorphism (*CYP2A6**4), was responsible for the lack of *CYP2A6* activity in Japanese (10–12). Upon consideration of these observations, it appeared possible that a lack of or reduced *CYP2A6* activity caused by genetic polymorphism might lead to a decrease in tobacco-induced lung cancer risk, either by reduced smoking because of lower nicotine catabolism or as a result of a decreased capacity to activate carcinogens such as nitrosamines present in tobacco smoke, or both.

The *CYP2A6**1B (13–15) is recognized as another wild-type allele and is not thought to lead to a decreased enzyme activity thus far because the mutation is located in the 3'-untranslated region of the *CYP2A6* gene. If *CYP2A6* activity affects genetic susceptibility to tobacco-induced lung cancer, individuals possessing the *CYP2A6**4 allele, but not the *CYP2A6**1A or the *CYP2A6**1B allele, would be expected to have low risk for lung cancer, particularly squamous cell carcinoma and small cell carcinoma, which are believed to be caused by smoking (16).

Materials and Methods

Study Population. This study was approved by the ethics committee of the National Cancer Center Hospital and Hokkaido University. All subjects used in this study were unrelated male Japanese smokers. Smokers contained current and ex-smokers and were defined as individuals who have ever smoked cigarettes with a minimum smoking history of 0.5 pack/day for

Received 10/19/01; revised 4/23/02; accepted 5/14/02.

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¹ Supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan, a grant from the Japan Health Science Foundation, a Grant-in-Aid from the Ministry of Health and Welfare for the 2nd-term Comprehensive 10-Year Strategy for Cancer Control, and Organization for Pharmaceutical Safety and Research Grant No. 99-2.

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³ The abbreviations used are: *CYP2A6*, cytochrome P450 2A6; CYP, cytochrome P450; OR, odds ratio; CI, confidence interval.

Table 1 Characteristics of lung cancer patients and control subjects

| | Case | Control |
|-------------------------|------|---------|
| Total (n) | 370 | 380 |
| Gender | Male | Male |
| Age (yr) | | |
| <40 | 5 | 0 |
| 40–49 | 46 | 0 |
| 50–59 | 140 | 259 |
| 60–69 | 179 | 88 |
| ≥70 | 0 | 33 |
| Pack/day × yr | | |
| <500 | 46 | 50 |
| 500–1000 | 153 | 199 |
| 1000–2000 | 138 | 121 |
| 2000–3000 | 30 | 8 |
| 3000–4000 | 2 | 2 |
| ≥4000 | 1 | 0 |
| Histological cell type | | |
| Small cell carcinoma | 44 | |
| Squamous cell carcinoma | 105 | |
| Adenocarcinoma | 193 | |
| Others | 28 | |

at least 1 year. Most cases were recruited from 1997 to 1999 in the National Cancer Center Hospital (Tokyo, Japan). Both incidental and prevalent cases were included without any selections. Among the lung cancer cases, incidental cases were ~90% of the total population, with the remaining 10% consisting of prevalent cases. Control subjects recruited were composed of healthy volunteers who visited one of the hospitals that took part in the research within the same time period described above for a health checkup. All subjects were then randomly selected. This procedure provided a natural balance between cases and controls regarding possible confounding factors such as birthplace because the patients who visited the National Cancer Center Hospital were from all regions of Japan. The age of the lung cancer patients was defined when the lung cancer was first pathologically diagnosed. Pathological classification of lung cancer was determined according to the International Association for the Study of Lung Cancer/WHO criteria (17). Tumors were classified into small cell carcinoma, squamous cell carcinoma, adenocarcinoma, and others (poorly differentiated non-small cell carcinoma). Participants were interviewed using a structured questionnaire, which included queries about city of residence, birthplace, occupational history, smoking status, and previous family (parents and siblings) history of cancer. Each patient in this study was required to sign a consent form. Variables of smoker population used are summarized in Table 1. No difference in distribution of the *CYP2A6* genotypes in Japanese with various ages was confirmed in this study and also by another study conducted in our laboratory (data not shown).

Genotyping. The *CYP2A6* gene was analyzed for the wild-type (*CYP2A6*1A*), another wild-type (*CYP2A6*1B*), and *CYP2A6*4* by a method established in our laboratory (15). The *CYP2A6*4* allele genotyped in this study is the *CYP2A6*4A*, which is equal to the *CYP2A6*4C* (15), a major causal allele in Japanese lacking *CYP2A6* activity (12). The nomenclature system for the human polymorphic *CYP* genes and *CYP2A6* are described in detail on line.^{4,5} Genotyping of the *GSTM1* gene

⁴ Internet address: www.imm.ki.se/CYPalleles/criteria.htm.

⁵ Internet address: www.imm.ki.se/CYPalleles/cyp2a6.htm.

Table 2 Difference in distribution of *CYP2A6* genotypes in lung cancer patients

| Genotype | Case (n = 370) | Control (n = 380) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) ^a |
|----------|----------------|-------------------|-------------------------------|-----------------------------------|
| *1A/*1A | 80 (21.3%) | 54 (14.2%) | 1.00 | 1.00 |
| *1A/*1B | 127 (34.6%) | 123 (32.4%) | 0.70 (0.46–1.07) | 0.68 (0.44–1.06) |
| *1B/*1B | 60 (16.2%) | 67 (17.6%) | 0.60 (0.37–0.99) ^b | 0.56 (0.34–0.93) ^b |
| *1A/*4 | 43 (11.6%) | 51 (13.4%) | 0.57 (0.33–0.97) ^b | 0.59 (0.34–1.02) |
| *1B/*4 | 55 (14.9%) | 66 (17.4%) | 0.56 (0.34–0.92) ^b | 0.59 (0.35–0.97) ^b |
| *4/*4 | 5 (1.4%) | 19 (5.0%) | 0.18 (0.06–0.50) ^b | 0.23 (0.08–0.67) ^b |

^a Age and smoking amount were adjusted in this analysis. *1A, *CYP2A6*1A*; *1B, *CYP2A6*1B*; *4, *CYP2A6*4*.

^b Significant decrease in OR is indicated by 95% CI.

(null-type) and the *CYP1A1* gene (*2A and *2C variants) was carried out according to the method reported by Bell *et al.* (18) and Cascorbi *et al.* (19), respectively.

Statistical Analysis. To determine whether an association existed between *CYP2A6* genotypes and lung cancer risk, the significance of the difference in the distribution of genotypes between cases and controls was calculated by χ^2 test and shown by *P*. All *P*s were two-sided. *P* < 0.05 was considered to be statistically significant. Difference in allele frequency between cases and controls was calculated by Fisher's exact test. OR and 95% CI were calculated by logistic regression analysis with the statistical package, Stat View version 5.0 (Abacus Concepts, Inc., Berkeley, CA). A relationship between the number of cigarettes smoked and each *CYP2A6* genotype was evaluated by Student-Newman-Keuls test.

Results

The distribution of *CYP2A6* genotypes in lung cancer patients was significantly different (*P* < 0.01) from that in controls, indicating existence of a relationship between *CYP2A6* genotypes and lung cancer risk (Table 2). The frequency of the individuals with the *4/*4 genotype was lower in lung cancer patients than in controls, whereas the frequency of patients homozygous for the *CYP2A6*1A* allele (*1A/*1A) was higher than that of control subjects. Consequently, a markedly decreased OR, 0.18 (95% CI, 0.06–0.50) for lung cancer risk, was seen in the *4/*4 group. There was also a statistically significant difference in prevalence of the *CYP2A6*4* allele (*P* = 0.0018) between cases and the controls (data not shown). After adjustment for age and smoking status by logistic regression analysis, a significant difference in the distribution of *CYP2A6* genotypes was also seen between controls and cases. The adjusted OR was significantly lower (0.23; 95% CI, 0.08–0.67) in the group with the *4/*4 genotype and was almost similar to the unadjusted OR. A slight but significant decrease in OR in the group with the *1B/*1B and the *1B/*4 genotypes was also observed (Table 2). Because the case and control groups were not strictly matched for age, we reexamined the effect of the *4/*4 genotype, adopting subjects only in the age group of 50–69 years (case, *n* = 319; control, *n* = 347). The adjusted OR was 0.24 (95% CI, 0.07–0.78) and was statistically significant, suggesting that the presence of young cases are not distorting the results.

To determine the possible association between *CYP2A6* genotypes and the risk of histological types of lung cancer, cases were divided into three groups according to a pathological classification. Table 3 summarizes the association between various *CYP2A6* genotypes and the risk of two representative histological cell types, small cell carcinoma and squamous cell

Table 3 Association between CYP2A6 polymorphism and risk for lung cancer cell types

| | Smcc ^a (n = 44) | OR (95% CI) ^b | Sqcc (n = 105) | OR (95% CI) | Smcc + Sqcc (n = 149) | OR (95% CI) | Adenocarcinoma (n = 193) | OR (95% CI) | Control (n = 380) |
|-----------|-------------------------------|-----------------------------|-------------------|-------------------------------|--------------------------|-------------------------------|-----------------------------|-------------------------------|----------------------|
| Genotype | | | | | | | | | |
| *1A/*1A | 10 (22.7%) | 1.00 | 22 (21.0%) | 1.00 | 32 (21.5%) | 1.00 | 42 (21.8%) | 1.00 | 54 (14.2%) |
| *1A/*1B | 16 (36.4%) | 0.70 (0.28–1.75) | 42 (40.0%) | 0.87 (0.46–1.66) | 58 (38.9%) | 0.79 (0.45–1.41) | 61 (31.6%) | 0.63 (0.38–1.05) | 123 (32.4%) |
| *1B/*1B | 8 (18.2%) | 0.56 (0.20–1.59) | 17 (16.2%) | 0.60 (0.29–1.27) | 25 (16.8%) | 0.59 (0.31–1.15) | 30 (15.5%) | 0.49 (0.26–0.90) ^c | 67 (17.6%) |
| *1A/*4 | 5 (11.4%) | 0.59 (0.18–1.91) | 10 (9.5%) | 0.44 (0.18–1.09) | 15 (10.0%) | 0.49 (0.23–1.05) | 23 (11.9%) | 0.58 (0.30–1.10) | 51 (13.4%) |
| *1B/*4 | 5 (11.4%) | 0.42 (0.13–1.35) | 14 (13.3%) | 0.48 (0.21–1.07) | 19 (12.8%) | 0.47 (0.23–0.95) ^c | 32 (16.6%) | 0.63 (0.35–1.13) | 66 (17.4%) |
| *4/*4 | 0 (0.0%) | NC | 0 (0.0%) | NC | 0 (0.0%) | NC | 5 (2.6%) | 0.37 (0.12–1.11) | 19 (5.0%) |
| Allele | | | | | | | | | |
| CYP2A6*1A | 41 (46.6%) | 1.00 | 96 (45.7%) | 1.00 | 137 (46.0%) | 1.00 | 168 (43.5%) | 1.00 | 282 (37.1%) |
| CYP2A6*1B | 37 (42.0%) | 0.79 (0.49–1.26) | 90 (42.9%) | 0.82 (0.59–1.14) | 127 (42.6%) | 0.81 (0.61–1.08) | 153 (39.6%) | 0.80 (0.61–1.04) | 323 (42.5%) |
| CYP2A6*4 | 10 (11.4%) | 0.51 (0.25–1.04) | 24 (11.4%) | 0.52 (0.32–0.85) ^c | 34 (11.4%) | 0.51 (0.34–0.79) ^c | 60 (15.5%) | 0.74 (0.52–1.06) | 136 (17.9%) |

^a Smcc, small cell carcinoma; NC, not calculated; Sqcc, Squamous cell carcinoma; *1A, CYP2A6*1A; *1B, CYP2A6*1B; *4, CYP2A6*4.

^b OR for genotype was calculated by logistic regression analysis considering the variation of age and smoking amount.

^c Significant decrease in OR is indicated by 95% CI. Non-small and non-squamous cell carcinomas (n = 221) were divided into adenocarcinoma (n = 193) and others.

carcinoma. Because of the limited number of patients compared with controls, a significant difference ($P = 0.0117$) in the distribution of CYP2A6 genotypes between controls and cases could be seen when the patients who suffered from either small cell carcinoma or squamous cell carcinoma were combined. No individuals with the *4/*4 genotype were found in any of 105 patients with squamous cell carcinoma or in any of 44 patients with small cell carcinoma (Table 3). Because the OR of the group with the *4/*4 genotype could not be calculated, an OR of the CYP2A6*4 allele was determined. Significantly reduced OR was observed for the CYP2A6*4 allele in a patient group with squamous cell carcinoma (Table 3).

The risk of adenocarcinoma in relation to CYP2A6 genotypes was also investigated (Table 3). No significant association between CYP2A6 polymorphism and the risk of adenocarcinoma was found in either allele basis or genotype basis, except for a group with the *1B/*1B genotype in which its OR was apparently reduced.

Finally, an association between CYP2A6 genotypes and daily cigarette consumption in 380 control subjects was examined to elucidate possible effects of the CYP2A6 gene deletion on smoking behavior. The number of daily cigarette consumption in a group with the *4/*4 genotype was significantly smaller when compared with the other genotype groups (Fig. 1).

Discussion

In this study, we found a possible association between the genetic polymorphism of CYP2A6 and lung cancer risk in Japanese male smokers (Table 2). This association was essentially unchanged when we analyzed a larger population, 672 cases and 706 controls, including various ages of males and females (data not shown). On the contrary, our data with a smaller number of nonsmoker subjects have shown no significant difference in prevalence of the CYP2A6*4 allele between cases (n = 33) and controls (n = 40; $P = 0.703$). Additional studies are needed to confirm the latter point with a larger number of nonsmoker subjects.

Previous studies from other laboratories demonstrated that there was an association between lung cancer risk and the genotypes of CYP1A1 (20, 21) or GSTM1 (22, 23). Thus, it seemed reasonable to expect that the polymorphism of the CYP2A6 gene in combination with those of the CYP1A1 and GSTM1 genes was associated more clearly with the lung cancer risk. Analyzing the data using the same subjects used in this

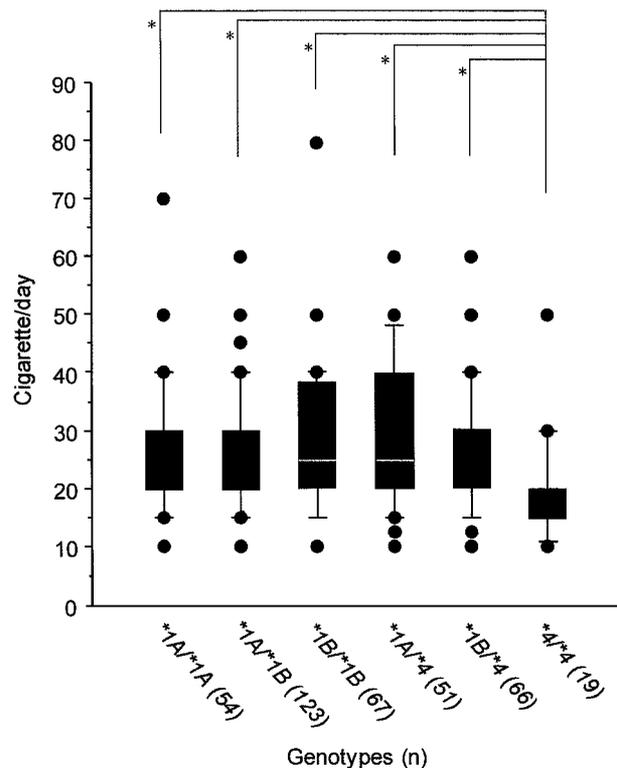


Fig. 1. Relationship between CYP2A6 genotypes and the amount of daily cigarette consumption. The same control subjects as shown in Table 1 were used in this analysis. 1A, CYP2A6*1A; 1B, CYP2A6*1B; 4, CYP2A6*4; *, $P < 0.05$. The amount of daily cigarette consumption was also significantly smaller only in the group with the *4/*4 genotype by Wilcoxon's rank-sum test.

study, we obtained no clear evidence to support this supposition (data not shown).

London *et al.* (24) reported that no significant association was seen between the CYP2A6 inactive allele, particularly focusing on the CYP2A6*2, and lung cancer risk. The discrepancy between their study and the present one may be explained by several possibilities. First, the frequency of the causal allele for the lack of CYP2A6 activity in their study was too small to detect a potential relationship with sufficient statistical power. The CYP2A6*2 allele examined in their study, which encodes

an inactive enzyme (9, 25), was reported to be one of the major variant alleles in Caucasians; the allele frequency of this variant was reported to be only 1–3% (9, 14, 26). On the other hand, the frequency of the *CYP2A6**4 allele examined in Japanese is 18–20% (13, 15). Similar discussion on the importance of the allele frequency in the analysis of the data has been made in other reports concerning a possible relationship between the genetic polymorphism of *CYP1A1* and lung cancer risk (27). Because the frequency of individuals possessing the *CYP2A6**4 allele in Caucasians was much lower than that in Japanese (15), a recent study performed using a French population did not detect the positive relationship between *CYP2A6* polymorphism and susceptibility to lung cancer, probably because of insufficient statistical power (28). If the *CYP2A6**4 is one of the important factors decreasing lung cancer risk, Caucasians may have a higher susceptibility to developing tobacco-induced lung cancer than Asians. Second, cancer patients used in their study were a mixture of patients who suffered from different histological types of lung cancer. Third, the genotyping method used in their study may not be accurate (9, 26) because it was recognized that primers used in their study amplify not only the *CYP2A6* gene but also other *CYP2A6*-related genes (9). More recently, Tan *et al.* (29) demonstrated no association between *CYP2A6* genotypes and lung cancer risk in a Chinese population. However, as mentioned by the authors, the frequency of the *CYP2A6**4 allele in control subjects (7–9%) was much lower than that in Asians (15–21%) previously reported by at least three different laboratories (13–15, 30). Although the reasons for this low frequency of the *CYP2A6**4 allele in their study population was uncertain, this appeared to give an opposite conclusion in their study. The frequency of the *CYP2A6**4 allele in the control Chinese population should be reexamined.

The squamous cell carcinoma has been the most frequent type of lung cancer in smokers, whereas adenocarcinoma is recognized as a major type of lung cancer in smokers in recent years (16, 27). This shift is reportedly the result of a decrease in the amount of polycyclic aromatic hydrocarbons and an increase in the relative content of nitrosamines in smoke inhaled from filtered cigarettes. Thus, the increase in adenocarcinoma is thought to be nitrosamine related. The epithelium at the branches of the central bronchi is a region where squamous cell carcinoma predominantly occurs, whereas the peripheral lung is the original site of most adenocarcinomas. Because measurable amounts of *CYP2A6* mRNA are expressed in human bronchial epithelial cells (2, 31), nitrosamines in tobacco smoke may be activated by *CYP2A6* present in bronchi. This mechanism may account, at least in part, for the significant association of *CYP2A6* polymorphism with the risk of squamous cell carcinoma. On the other hand, the existence of functional *CYP2A6* in the peripheral lung is still a matter of controversy (1, 2, 32). This may be the reason for the apparently less clear effects of the *4/*4 genotype in adenocarcinoma (Table 3) as compared with small or squamous cell carcinoma. Because the difference was modest and suggestive, it must be confirmed in larger cohort studies. Alternatively, the risk of adenocarcinoma may be more closely associated with the activity of *CYP2A13*, which was reported to have higher metabolic capacity to activate 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and is expressed higher in the peripheral lung tissues compared with *CYP2A6* (33). Moreover, although we have not obtained enough number of samples, we examined the possibility of whether similar results could be seen in female smokers in a preliminary study because adenocarcinoma was more preferentially seen in females. To avoid marked age imbalance, subjects of 40–69 years (case, $n = 58$; control, $n =$

50) were used. Because of a higher frequency of patients with the *1/*1 genotype (15.5%) compared with that of controls (4.0%), an adjusted OR was 0.13 (95% CI, 0.01–2.46) in the *4/*4 genotype. However, in contrast to males, a substantial number of lung cancer patients with *4/*4 genotype was found. It is noted that all of the cancer patients with the *4/*4 genotype suffered from adenocarcinoma. Therefore, the possibility that *CYP2A6* may not play important roles for developing adenocarcinoma cannot be excluded. Studies using female smokers should be performed with a larger number of populations. These possibilities are currently under investigation.

CYP2A6 catalyzes nicotine metabolism (6, 9). Thus, individuals who are deficient in this enzyme activity are expected to have a reduced consumption of cigarettes (34), probably because of higher plasma concentration caused by the poor capacity of nicotine metabolism. We demonstrated in this study that the *CYP2A6* gene deletion lowered daily cigarette consumption in controls (Fig. 1). Therefore, the lesser consumption of cigarettes may explain, in part, the reduced risk for lung cancer in smokers with the *4/*4 genotype because of a lower dose of tobacco-derived carcinogens such as nitrosamines and nicotine. This idea is consistent with a recent study, which demonstrates that the slower metabolism and the reduced intake of nicotine from cigarette smoking may explain lower lung cancer rates in Asians compared with Caucasians (35). In contrast to the previous concept, Tan *et al.* (29) found no significant association between *CYP2A6* genotypes and smoking status in which they analyzed their data combining the *1/*4 and the *4/*4 genotypes. However, the *1/*4 and the *4/*4 genotypes should not be combined because only the *4/*4 genotype but not the *1/*4 genotype (*1A/*4 and *1B/*4 in our study) appeared to affect daily cigarette consumption (Fig. 1). It was unexpected that the *1A/*1A group did not show the highest number of daily cigarette consumption. At least two possibilities may be present. First, because of an insufficient number of subjects, the results did not show the precise relationship between *CYP2A6* genotype and smoking dose. Second, effects of unidentified polymorphism of *CYP2A6* on nicotine metabolism may exist. These possibilities are worth investigating in the future. The effects of the heterozygote on the reduction of lung cancer, as compared with the *1A/*1A genotype, remains unclear and needs to be elucidated in future studies.

In conclusion, we demonstrated in this study that individuals with the *1A/*1A genotype of the *CYP2A6* gene have the highest risk for tobacco-related lung cancer in Japanese male smokers. Because the population of individuals with the functionally active *CYP2A6* gene is much higher in Caucasians than in Asians, the genetic polymorphism of *CYP2A6* may be one of the factors accounting for an interethnic difference in susceptibility to lung cancer in smokers.

Acknowledgments

We thank Dr. Robert A. Neal and Dr. Frank J Gonzalez for their advice and helpful comments to prepare this manuscript.

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Cancer Epidemiol Biomarkers Prev 2002;11:890-894.

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