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Substantial Reduction in Risk of Lung Adenocarcinoma Associated with Genetic Polymorphism in CYP2A13, the Most Active Cytochrome P450 for the Metabolic Activation of Tobacco-Specific Carcinogen NNK

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ABSTRACT

Cytochrome P450 2A13 (CYP2A13), an enzyme expressed predominantly in the human respiratory tract, exhibits high efficiency in the metabolic activation of tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). A C→T transition in the *CYP2A13* gene causes Arg257Cys amino acid substitution and, thus, results in a significantly reduced activity toward NNK and other substrates. In this case-control study, we genotyped 724 patients with lung cancer and 791 controls for this polymorphism to examine the hypothesis that the variant *CYP2A13* may have impact on risk of lung cancer in relation to tobacco smoking. A gene deletion polymorphism (*CYP2A6*4*) in CYP2A6, another enzyme involved in the metabolic activation of tobacco nitrosamines, was also analyzed as a comparison. We found that, compared with the *CC* genotype, the variant *CYP2A13* genotype (*CT* + *TT*) was associated with substantially reduced risk for lung adenocarcinoma [odds ratio (OR), 0.41; 95% confidence interval (CI), 0.23–0.71], but not squamous cell carcinoma (OR, 0.86; 95% CI, 0.57–1.29) or other types of lung cancer (OR, 0.58; 95% CI, 0.32–1.09). Stratification analysis shows that the reduced risk of lung adenocarcinoma related to the variant *CYP2A13* genotype was limited to smokers, especially light smokers (OR, 0.23; 95% CI, 0.08–0.68) but not nonsmokers or heavy smokers. No association was observed between *CYP2A6* genotype and risk of lung cancer. Our results demonstrate for the first time that the variant *CYP2A13* allele is associated with reduced risk of lung adenocarcinoma, suggesting the role of NNK-CYP2A13 interaction as a causative factor for the cancer.

INTRODUCTION

Lung cancer is one of the most frequent cancers in many countries including China. Tobacco smoking is an established major cause of lung cancer, resulting in >50% deaths of lung cancer in the Chinese population (1). Because China accounts for one third of one billion of the world's smokers, a major epidemic of lung cancer is predicted (2). Tobacco smoke contains a number of carcinogens; among them are *N*-nitrosamines such as *N*-nitrosodiethylamine and tobacco-specific nitrosamine, NNK.[3] NNK is presented in tobacco smoke at a substantial level and has been shown to be a potent lung carcinogen in all of the commonly used experimental animals (3). Furthermore, NNK has been strongly linked to the etiology of human lung adenocarcinoma (4, 5), a fast increasing subtype of lung cancer among tobacco smokers. However, lung cancer in relation to tobacco smoking is generally characterized by various individual susceptibility, showing that only part of smokers develop lung cancer in their normal life span. This suggests that individual genetic traits may also be important in the etiology of this malignancy.

It is well known that most chemical carcinogens including NNK require metabolic activation to form their ultimate electrophiles, which can damage DNA and consequently induce carcinogenesis. Several isoforms of CYP enzymes are involved in the metabolic activation of NNK and *N*-nitrosodiethylamine; among them CYP2A6 is thought to be an important one because of its relatively high activity toward these compounds (6, 7). However, although CYP2A6 may be important in the activation of tobacco smoke-related nitrosamines, its expression in the lung, if any, appears to be relatively low (8–10). Another member of the CYP2A subfamily, CYP2A13, has been found recently to be expressed predominantly in the human respiratory tract including the peripheral lung (10). This enzyme exhibits high activity in the activation of NNK and some other carcinogens, with a catalytic efficiency much greater than that of CYP2A6 (10). Because of the importance of CYP2A13 and CYP2A6 in the metabolic activation of NNK and other nitrosamines in tobacco smoke, it is reasonable to postulate that these enzymes may play a role in lung carcinogenesis, and their activities may mediate susceptibility to lung cancer among smokers.

Great interindividual variation in the activity of CYP2A6 is well documented (7, 11, 12), and this has been proposed to be largely due to genetic polymorphisms in the *CYP2A6* locus (13–15). The association between *CYP2A6* gene polymorphism and lung cancer risk has been investigated in several studies (16–19). However, little is known about functional polymorphism in the *CYP2A13* gene. Recently, a C/T variation in exon 5 of the *CYP2A13* gene has been identified, and this single nucleotide polymorphism leads to an Arg257Cys amino acid change (20). Functional analysis showed that the 257Cys variant has significantly reduced activity toward NNK and other substrates compared with the wild-type 257Arg, suggesting that this polymorphism might provide protection against genetoxicity and carcinogenicity in the target tissues to individuals carrying the *257Cys* allele (20). However, the relevance to any cancer of this functional polymorphism in *CYP2A13* has not been investigated to date.

The aim of this study was to examine the contribution of the Arg257Cys polymorphism in the *CYP2A13* gene to the risk of lung cancer in a large molecular epidemiological study. In this study, we also assessed the role of *CYP2A6* deletion alone and in combination with the *CYP2A13* polymorphism in lung cancer risk.

MATERIALS AND METHODS

Study Subjects. This hospital-based, case-control study consisted of 724 patients with lung cancer and 791 population controls. All of the subjects were unrelated ethnic Han Chinese and participants in a molecular epidemiological study of lung cancer reported previously (21). Briefly, the cases with primary lung cancer were recruited from January 1997 to November 2001, at the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing). Because the histological classification of the cancer is definitely important in this study,

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[3] The abbreviations used are: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; CYP, cytochrome P450; SCC, squamous cell carcinoma; OR, odds ratio; CI, confidence interval.

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surgically resectable patients (91%) were mostly recruited, which allowed us to obtain accurate histological diagnosis by extensive postoperative histopathological examination. The histological types of the cancer in other patients (9%) who were not suitable for curative resection were determined by biopsy via bronchoscopy. All of the histological classifications were determined by senior pathologists of the hospital. Patients were from Beijing City and its surrounding regions, and there were no age, sex, and histology restrictions. Because this was a study of genotypes and the markers were constitutional ones, both incident ($n = 701$) and prevalent ($n = 23$) cases were included. The response rate was 93% due to reluctance of 54 patients to participate the study. The prevalent cases were patients who underwent a follow-up examination within 12 months after surgical treatment. The exclusion criteria included previous cancer, metastasized cancer from other organs, and previous radiotherapy or chemotherapy. Population controls were accrued from a nutritional survey conducted in the same region during the period of case collection, with a response rate of 94% (21). Briefly, these controls were randomly selected from the nutritional survey database consisting of 2500 individuals. They had no history of cancer and were frequency-matched to case on age (±5 years) and sex. At recruitment, informed consent was obtained from each subject, and each participant was then interviewed to collect detailed information of demographic characteristics and lifetime history of tobacco use. This study was approved by the Institutional Review Board of the Chinese Academy of Medical Sciences Cancer Institute.

Polymorphism Analysis. Genomic DNA from controls and most of case subjects was isolated from the peripheral blood lymphocytes. Approximately 30% of the DNA samples from cases were isolated from surgically resected normal tissues adjacent to the tumor of lung cancer patients. Genotypes were analyzed using PCR-based methods as described below, performed without knowledge of case/control status of the subjects. A 15% masked, random sample of cases and controls was tested twice by different persons, and the results were concordant for all of the masked duplicate sets.

CYP2A13. The genotypes of *CYP2A13* at the C3375T (Arg257Cys) site were analyzed by PCR-RFLP assays on the basis of that reported previously (20) with some modifications. The primers used were 5′-TAA CTC CGT TCC TTC CTT GCT/5′-TAA TTT GAA TGG GCC TGT GTC. Because of the highly homologous genomic sequences of the *CYP2A* family, we used a hot start and touchdown PCR procedure to specifically amplify the *CYP2A13* gene. Amplification was accomplished with a 25-μl reaction mixture containing 20 ng DNA, 0.2 μM each primer, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, and 1.0 unit HotStarTaq DNA polymerase with 1× reaction buffer (Qiagen, Chatsworth, CA). The reaction was carried out in the following conditions: an initial melting step of 15 min at 95°C, followed by 13 cycles of 30 s at 94°C, 30 s at 63°C, (step-down 0.5°C every cycle), and 50 s at 72°C. The conditions of the following stage of cycling were the same as above but with an annealing temperature of 57.5°C and a final elongation step of 5 min at 72°C. The 375-bp PCR products were then digested with *Hha*I (New England BioLabs, Inc., Beverly, MA) and separated on a 1.5% agarose gel. The wild-type *C* allele had a *Hha*I restriction site that resulted in two bands (217 and 158 bp), and the *T* allele lacked the *Hha*I restriction site and, thus, produced a single 375-bp band.

CYP2A6. Deletion allele of the *CYP2A6* gene (*CYP2A6*4*) was identified by a two-step PCR method (22). Briefly, the first PCR was carried out using the primer pair 5′-CCAAGA TGC CCT ACA TG and 5′-TTG TGA GAC ATC AGA GAC AA. This reaction produced a 1961-bp fragment from exon 7 to 400 bp downstream of exon 9 of the *CYP2A6* or *CYP2A6/CYP2A7* hybrid from all of the individuals with or without the deleted *CYP2A6* gene. The second PCR, which specifically detected the deleted *CYP2A6* gene, used the product resulting from the first PCR amplification as a template. The primer pairs used were 2A6ex8F (5′-CAC TTC CTG AAT GAG-3′) or 2A7ex8F (5′-CAT TTC CTG GAT GAC-3′) and 2A6R2 (5′-AAA ATG GGC ATG AAC GCC C-3′). Amplified products were analyzed by electrophoresis in 1% agarose gel. The presence of *CYP2A*-specific 1181-bp product amplified with the primer pair 2A6ex8F/2A6R2 indicated the *CYP2A6* wild genotype, whereas the presence of the product with primer pair 2A7ex8F/2A6R2 indicated the deleted *CYP2A6* genotype; the presence of product in both reactions indicated the heterozygote genotype.

Statistical Analysis. χ^2 tests were used to examine the differences in the distributions of genotypes between cases and controls. The association between the *CYP2A13* or *CYP2A6* polymorphisms and risk of lung cancer was estimated by ORs and their 95% CIs, which were calculated by unconditional logistic regression. Smokers were considered current smokers if they smoked up to 1 year before the date of diagnosis for cancer patients or the date of the interview for control subjects. Never-smokers were defined as subjects smoked <10 cigarettes lifetime. Information was collected on the number of cigarettes smoked per day, the age at which the subjects started smoking, and the age at which ex-smokers stopped smoking. Pack-year smoked was calculated to indicate cumulative cigarette dose. Lighter or heavier smokers were categorized by the approximate 50th percentile pack-years value among controls, *i.e.,* <27 pack-years and ≥27 pack-years. Because only 26 patients and 42 controls were ex-smokers, they were combined with current smokers for analysis. The ORs were all adjusted for age, sex, smoking status, or pack-years where it was appropriate. Tests for interaction between *CYP2A13* and *CYP2A6* polymorphisms were performed by using the likelihood ratio test. All of the statistical analyses were performed using Statistical Analysis System software (version 6.12; SAS Institute, Cary, NC).

RESULTS

The analysis included 724 lung cancer patients (319 with adenocarcinoma, 263 with SCC, and 142 with other histological types) and 791 controls. The distributions of age, sex, and smoking status among the subjects are summarized in Table 1. There were no significant differences between case patients and control subjects in terms of mean age and sex distribution, suggesting that the frequency matching was adequate. However, although an effort was made to obtain a frequency match on smoking status between cases and controls, more smokers were presented in the case group compared with controls (58.1% *versus* 42.2%; $\chi^2 = 38.3$; $P = 0.000$). In addition, cases had a higher value of pack-years smoked than controls; 62.9% smokers among cases smoked ≥27 pack-years compared with 43.7% among controls ($P = 0.000$).

Table 2 shows allele frequencies and genotype distributions of *CYP2A13* and *CYP2A6* in cases and controls. Relatively low frequencies were observed for the minor alleles of both *CYP2A13* and *CYP2A6* genes (*i.e., CYP2A13 T* and *CYP2A6*4*) in our study population, with the former being 0.07 and 0.09, and the latter being 0.07 and 0.08 in cases and controls, respectively. For *CYP2A13*, we observed genotype frequencies of 82.4% (*CC*), 16.4% (*CT*), and 1.2% (*TT*) in controls and respective 87.3%, 11.7%, and 1.0% in overall lung cancer cases, both of which were not deviated from those expected from the Hardy-Weinberg equilibrium ($\chi^2 = 0.31$ and 1.11, $P = 0.86$ and 0.58, respectively). Although the rare *TT* homozygotes in overall cases failed to differ from controls (1.0% *versus* 1.2%; $\chi^2 = 0.11$; $P = 0.745$), the frequency of the heterozygotes (*CT*) was significantly lower in cases than in controls (11.7% *versus* 16.4%; $\chi^2 = 6.84$; $P = 0.009$). This difference, however, was mainly due to

Table 1 *Distributions of select characteristics by case-control status*

Variable	Cases ($n = 724$)	Controls ($n = 791$)
Sex, (%)		
Male	479 (66.2)	519 (65.6)
Female	245 (33.8)	272 (34.4)
Mean age in years (SD)	57.0 (10.2)	56.6 (8.8)
Smoking status (%)		
Never	303 (41.9)[a]	457 (57.8)
Ever[b]	421 (58.1)[a]	334 (42.2)
<27 pack-years	156 (37.1)[a]	188 (56.3)
≥27 pack-years	265 (62.9)[a]	146 (43.7)
Mean pack-years smoked (SD)	34.8 (23.0)[a]	27.4 (16.3)
Histological type (%)		
Squamous cell carcinoma	263 (36.3)	
Adenocarcinoma	319 (44.1)	
Other[c]	142 (19.6)	

[a] $P < 0.0000$, compared with controls.
[b] Includes 68 former smokers (26 cases and 42 controls).
[c] Other includes undifferentiated cancer ($n = 60$), bronchioalveolar carcinoma ($n = 27$), and small cell carcinoma ($n = 55$).

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Table 2 *Genotype and allele frequencies of CYP2A13 and CYP2A6 among cases and controls, and their association with lung cancer*

	Controls (*n* = 791)		All cases (*n* = 724)		Cases with SCC[a] (*n* = 263)		Cases with AC[a] (*n* = 319)		Case with other[a] (*n* = 142)	
Genotype	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
CYP2A13										
CC	652	(82.4)	632	(87.3)	219	(83.3)	288	(90.3)	125	(88.0)
CT	130	(16.4)	85	(11.7)	37	(14.1)	31	(9.7)	17	(12.0)
TT	9	(1.2)	7	(1.0)	7	(2.6)	0	(0.0)	0	(0.0)
T allele frequency	0.09		0.07		0.10		0.04		0.06	
Adjusted OR (95% CI)[b]			0.62 (0.45–0.87)		0.86 (0.57–1.29)		0.41 (0.23–0.71)		0.58 (0.32–1.09)	
CYP2A6										
**1/*1*	680	(86.0)	630	(87.0)	235	(89.4)	276	(86.5)	119	(83.8)
**1/*4*	102	(12.9)	84	(11.6)	23	(8.7)	40	(12.6)	21	(14.8)
**4/*4*	9	(1.1)	10	(1.4)	5	(1.9)	3	(0.9)	2	(1.4)
**4* allele frequency	0.08		0.07		0.06		0.07		0.09	
Adjusted OR (95% CI)[b]			0.97 (0.72–1.31)		0.76 (0.48–1.21)		0.95 (0.65–1.40)		1.21 (0.73–1.98)	

[a] SCC, squamous cell carcinoma; AC, adenocarcinoma; other includes undifferentiated cancer (*n* = 60), bronchioalveolar carcinoma (*n* = 27), and small cell carcinoma (*n* = 55).
[b] ORs were calculated with the *CYP2A13 CC* or *CYP2A6 *1/*1* genotype as the reference group and adjusted for age, sex, smoking status, or pack-years (<27 or ≥27). The *CYP2A13 TT* or *CYP2A6 *4/*4* genotype was combined with the *CT* or **1/*4* genotype for the calculation.

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lower frequency of the *CT* genotype in patients with adenocarcinoma (9.7%; $P = 0.03$) but not SCC (14.1%; $P = 0.36$), or other types consisted of undifferentiated cancer, bronchoalveolar carcinoma, and small cell carcinoma (12.0%; $P = 0.18$; Table 2). Furthermore, none of the 319 patients with adenocarcinoma was homozygous *TT* genotype. The frequencies of three *CYP2A6* genotypes in controls were **1/*1*, 86.0%; **1/*4*, 12.9%; and **4/*4*, 1.1%, which were not significantly different from those in overall cases ($\chi^2 = 0.74$; degrees of freedom = 2; $P = 0.69$) and in cases with different subtype of the cancer. The distributions of *CYP2A6* genotypes in both controls and cases also fit in with the Hardy-Weinberg equilibrium law ($\chi^2 = 1.54$ and 3.53, $P = 0.46$ and 0.17, respectively). Because the *CYP2A13 TT* and *CYP2A6 *4/*4* homozygotes were extremely rare in our study, these genotypes were respectively combined with the *CYP2A13 CT* or *CYP2A6 *1/*4* genotype for subsequent estimation of lung cancer risk.

By using logistic regression analysis (Table 2), we found a significant reduction in risk of overall lung cancer among subjects carrying the *CYP2A13 CT* or *TT* genotype (adjusted OR, 0.62; 95% CI, 0.45–0.87) compared with those carrying the *CC* genotype, suggesting that the variant allele is the protective allele. Additional analysis with stratification of major histological subtypes of the cancer revealed that this protection was mainly due to markedly reduced risk of adenocarcinoma (adjusted OR, 0.41; 95% CI, 0.23–0.71) but not SCC (adjusted OR, 0.86; 95% CI, 0.57–1.29) or other types of lung cancer (adjusted OR, 0.58; 95% CI, 0.32–1.09). The difference of the ORs related to the *CC* genotype between adenocarcinoma and SCC was significant ($P = 0.012$, test for homogeneity). However, no significant association between *CYP2A6* genotype and risk of overall lung cancer or the major subtypes of the cancer was found.

The risk of lung cancer related to the *CYP2A13* polymorphism was additionally assessed with stratification by smoking status and pack-years smoked (Table 3). It was found that the *CT* and *TT* genotypes had no effect on risk for overall lung cancer as well as the major subtypes of the cancer among nonsmokers. Among smokers, however, a substantial protective effect of the variant *CT* and *TT* genotypes on risk of adenocarcinoma (adjusted OR, 0.34; 95% CI, 0.17–0.69) was observed. Nevertheless, this protective effect was not evident on SCC and other types of lung cancer among smokers. When smoking was additionally stratified by pack-years smoked, the protective effect of the variant genotypes was seen among subjects who were light smokers (smoked <27 pack-years) but not heavy smokers (smoked ≥27 pack-years) and, again, this effect was restricted to a subgroup of lung adenocarcinoma (OR, 0.23; 95% CI, 0.08–0.68) but not SCC (OR, 0.65; 95% CI, 0.30–1.40). Although a reduced OR related to the variant genotypes for other types of lung cancer was also found in

Table 3 *Risk of lung cancer related to CYP2A13 genotypes by smoking status and pack-years smoked*

	CC genotype	*CT* + *TT* genotype		
Smoking status	Cases/controls	Cases/controls	OR (95% CI)[a]	*P*
Adenocarcinoma				
Nonsmokers	165/381	21/76	0.60 (0.35–1.01)	0.086
Smokers	123/271	10/63	0.34 (0.17–0.69)	0.003
<27 pack-years	56/147	4/41	0.23 (0.08–0.68)	0.008
≥27 pack-years	67/124	6/22	0.47 (0.18–1.25)	0.130
Squamous cell carcinoma				
Nonsmokers	44/381	11/76	1.31 (0.64–2.68)	0.456
Smokers	175/271	33/63	0.83 (0.52–1.33)	0.445
<27 pack-years	56/147	10/41	0.65 (0.30–1.40)	0.272
≥27 pack-years	119/124	23/22	1.13 (0.59–2.16)	0.704
Other[b]				
Nonsmokers	55/381	7/76	0.59 (0.25–1.34)	0.206
Smokers	70/271	10/63	0.62 (0.30–1.28)	0.198
<27 pack-years	29/147	1/41	0.09 (0.01–0.72)	0.023
≥27 pack-years	41/124	9/22	1.25 (0.53–2.97)	0.612
All cases				
Nonsmokers	264/381	39/76	0.71 (0.47–1.09)	0.110
Smokers	368/271	53/63	0.62 (0.42–0.93)	0.020
<27 pack-years	141/147	15/41	0.37 (0.19–0.69)	0.002
≥27 pack-years	227/124	38/22	0.95 (0.53–1.69)	0.857

[a] ORs and 95% CIs were calculated by logistic regression with the *CC* genotype as reference group and adjusted for age, sex, and pack-years within the strata.
[b] Other includes undifferentiated cancer (*n* = 60), bronchioalveolar carcinoma (*n* = 27), and small cell carcinoma (*n* = 55).

Table 4 *Distribution of CYP2A13 and CYP2A6 genotypes by tobacco smoking in controls*

	CYP2A13 genotype (%)			*CYP2A6* genotype (%)		
	CC	*CT + TT*	P^a	**1/*1*	**1/*4 + *4/*4*	P^a
Smoking status			0.416			0.204
Nonsmokers (n = 457)	381 (83.4)	76 (16.6)		399 (87.3)	58 (12.7)	
Smokers (n = 334)[b]	271 (81.1)	63 (18.9)		281 (84.1)	53 (15.9)	
Daily cigarette consumption			0.687			0.436
< 15 cigarettes (n = 122)	98 (80.3)	24 (19.7)		102 (83.6)	20 (16.4)	
≥ 15 cigarettes (n = 212)	173 (81.6)	39 (18.4)		179 (84.4)	33 (15.6)	
Duration of smoking			0.115			0.664
< 30 years (n = 135)	104 (77.0)	31 (23.0)		115 (85.2)	20 (14.8)	
≥ 30 years (n = 199)	167 (83.9)	32 (16.1)		166 (83.4)	33 (16.6)	

[a] P for χ^2 test.
[b] Includes 42 former smokers.

light smokers (OR, 0.09; 95% CI, 0.01–0.72), it is most likely to be due to chance because of the small number involved in the analysis and wide range of confidence interval of the OR.

In contrast to *CYP2A13*, no association between *CYP2A6* genotype alone and risk of lung cancer was observed at any cumulative smoking dose levels. The potential interaction between the *CYP2A6* and *CYP2A13* in terms of genotype or haplotype on risk of lung cancer was additionally examined, and the results do not show any evidence for the interaction (data not shown). The *CYP2A6 *4* is not in linkage disequilibrium with the *CYP2A13* polymorphism.

The overall association between *CYP2A6* and *CYP2A13* polymorphisms and smoking status among 791 control subjects was also examined (Table 4). Among 457 nonsmokers, 87.3% harbored *CYP2A6 *1/*1* genotype, which was similar to that (84.1%) among 334 smokers (P = 0.204). Similarly, the frequencies of *CYP2A13* genotypes among nonsmokers did not significantly differ from those among smokers (P = 0.416). Furthermore, the distributions of *CYP2A6* and *CYP2A13* alleles among smokers who had consumed <15 cigarettes per day and smoked <30 years were not significantly different from those among smokers who had consumed ≥15 cigarettes per day and smoked ≥30 years (Table 4). The potential interaction between *CYP2A6* and *CYP2A13* genotypes on smoking in control subjects was also examined, and the results do not show any evidence for the interaction (data not shown).

DISCUSSION

In the present study, we examined whether genetic polymorphisms in *CYP2A13* and *CYP2A6*, alone and in combination, could have an impact on risk for developing lung cancer. On the basis of analysis of 724 patients with lung cancer and 791 healthy controls in a Chinese population, we observed that polymorphism in *CYP2A13* but not *CYP2A6* influenced risk of developing lung cancer. Subjects having the variant *CYP2A13* allele (*CT* or *TT* genotype) were at a reduced overall risk of lung cancer, especially lung adenocarcinoma in relation to tobacco smoking. To our best knowledge, this is the first study to investigate the effect of the *CYP2A13* polymorphism on susceptibility to cancer.

Our results in the present study are parallel in several lines to the laboratory findings and, therefore, are biologically plausible. Firstly, it has been shown that CYP2A13, which is predominantly expressed in human respiratory tract (8–10), is highly active in the metabolic activation of NNK with a catalytic efficiency much greater than that of other human CYPs examined previously (10). Secondly, the investigated polymorphism in *CYP2A13* has functional significance. It was reported that the 3375C→T transition, which causes an amino acid substitution at the 257 site, significantly reduces the enzyme activity toward several different substrates. Of particular interest, the variant 257Cys protein shows 2–3-fold reduction in the metabolic activation of NNK compared with the 257Arg protein (20). These findings strongly support our observation that subjects carrying the variant allele of *CYP2A13* had a significantly reduced risk for the development of tobacco smoking-related lung cancer. Thirdly, our results showed that the protective effect of the variant *CYP2A13* allele on risk of lung cancer achieves formal significance in adenocarcinoma (and presumably extends to other subtypes) but not SCC. This is also biologically plausible because lung adenocarcinoma is likely to be linked to NNK, whereas lung SCC could be induced by polycyclic aromatic hydrocarbons such as benzo(*a*)pyrine (3–5, 23). Polycyclic aromatic hydrocarbons are well known to be activated primarily by CYP1A1 and, therefore, risk of lung SCC seems to be mostly influenced by genetic polymorphism in the *CYP1A1* locus (24–26) in regard to carcinogen-metabolizing enzymes. Taken together, these data hold up the speculation that NNK-CYP2A13 interaction might be one of the causative factors for the development of lung adenocarcinoma.

In the present study, we found that the protective effect of the *CYP2A13* polymorphism on risk of lung adenocarcinoma depends on smoking dose. A reduced risk of the cancer for subjects with the *CYP2A13* variant genotypes is evident merely at low dose of smoking (<27 pack-years in this study); however, it seems to be absent at high dose. This observation may reflect the biochemical feature of CYP2A13–257Cys, which exhibits only ~3-fold decrease in catalytic efficiency toward NNK compared with the CYP2A13–257Arg counterpart (20). This observation may also emphasize an overwhelming risk of tobacco smoking for lung cancer and limited protective effect of genetic components. On the other hand, we did not observe any significant effect of the *CYP2A13* variant genotypes on risk of lung cancer including adenocarcinoma among nonsmokers. In our study, 61.2% cases among nonsmokers were adenocarcinoma, and most of them (67.0%) were women (data not shown). Because lung adenocarcinoma among Chinese women was shown not to be strongly linked to tobacco smoking (27, 28), it is not surprising to see a null association between risk of this cancer among nonsmokers and the genetic polymorphism in CYP2A13, a metabolic enzyme mainly toward tobacco-specific NNK. These findings might also indicate that carcinogenic factors other than smoking involved in the etiology of the cancer among nonsmokers in this population were unlikely to be substrates of CYP2A13. The factors resulting in lung cancer among nonsmokers are poorly understood, although it has been suggested that indoor air pollution derived from Chinese-style cooking and/or coal burning may play a role (29–33). It would be important to investigate genetic susceptibility factors, especially metabolism polymorphisms for this group of lung cancer, to additionally understand the etiology.

In addition, we also found a reduced risk (OR, 0.09, 95% CI, 0.01–0.72) related to the variant genotypes of *CYP2A13* for other subtypes of lung cancer in light smokers. Despite the uncertainty of this result due to the few cases with the variant genotypes involved in the analysis, it would be interesting to pursue whether this effect of the *CYP2A13* polymorphism may extend to other histological subtypes of lung cancer in other studies with larger sample size.

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The published studies regarding the association between the *CYP2A6* deletion (*CYP2A6*4*) and lung cancer risk are quite conflicting. Tan *et al.* (17) reported an increased risk of lung cancer related to the combined *CYP2A6 *1/*4* and **4/*4* genotypes in a Chinese population. However, the homozygous *CYP2A6*4/*4* genotype was shown to be associated with reduced risk of lung cancer in a Japanese population (19). In addition, this defective *CYP2A6* allele showed no effect in a French population (18). These discrepant results led us to reexamine the association in this more comprehensive study with large sample size. Although genotype frequencies among controls in the present study are similar to those reported in our pilot study (17), no association was observed between this *CYP2A6* polymorphism and risk of overall or different subtypes of lung cancer. This inconsistency between our present and previous studies on the *CYP2A6* polymorphism may simply be due to the small sample size in the previous one, which often results in overestimate of the OR. Lack of association between the *CYP2A6* gene deletion and risk of lung cancer indicates that this enzyme may not be as important as CYP2A13 regarding metabolic activation of tobacco carcinogens in the lung. This suggestion is supported by the fact that CYP2A6 was detected at very low levels, if any, in the target lung tissues (8–10).

Because *CYP2A6* genotype was claimed to be associated with smoking behavior (34) but subsequent studies by other investigators failed to demonstrate this association (reviewed in Ref. 35), we also assessed this issue in the present study. On the basis of analysis of 791 control subjects, we failed to find any significant effect of *CYP2A6* genotypes alone or combined with those of *CYP2A13* on self-reported smoking behavior in this study population. These results are consistent with our previous findings (17), and most of the studies reported in other ethnic populations such as Caucasians (18, 36) and Japanese (37, 38).

In summary, our study provides the first evidence that the functional polymorphism in the *CYP2A13* gene is associated with reduced risk of lung adenocarcinoma. However, this association seems to depend on smoking dose. Because this is the first report and because CYP2A13 is predominantly expressed in human respiratory tract, additional studies on lung cancer and other types of cancer such as nasopharyngeal cancer would be warranted in different ethnic populations.

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