Carcinogenesis vol.22 no.1 pp.11–16, 2001

ACCELERATED PAPER

CYP 1A1 polymorphism and risk of lung cancer in relation to tobacco smoking: a case–control study in China

Nan Song, Wen Tan, Deyin Xing and Dongxin Lin[1]

Department of Etiology and Carcinogenesis, Cancer Institute, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

[1]To whom correspondence should be addressed
Email: dlin@public.bta.net.cn

The impact of genetic polymorphisms in *CYP1A1* on susceptibility to lung cancer has received particular interest in recent years since this enzyme plays a central role in activation of major classes of tobacco carcinogens. Several polymorphisms in the *CYP1A1* locus have been identified and their genotypes appear to exhibit population frequencies that depend on ethnicity. We have assessed the role of *CYP1A1* genotype in lung cancer risk in the Chinese population via a case–control study. Three polymorphisms, *m1* (*Msp*I), *m2* (exon 7 Ile→Val) and *m4* (exon 7 Thr→Asn), were determined by PCR–RFLP in 404 controls and 217 lung cancer cases. While no polymorphic alleles were detectable in the m4 site among our study subjects, the allele frequencies for *CYP1A1 m1* and *CYP1A1 m2* were found to be 35.6 and 25.6% among controls, compared with 42.6 and 34.2% among cases. Multivariate analysis showed an elevated risk for lung cancer in subjects having at least one *m1* allele [odds ratio (OR) = 2.0, 95% confidence interval (CI) = 1.4–2.8] or having at least one *m2* allele (OR = 1.9, 95% CI = 1.3–2.7). However, this increased risk was limited to squamous cell carcinoma (SCC), but not adenocarcinoma or other histological types of lung cancer. Stratified analysis indicated a multiplicative interaction between tobacco smoking and variant *CYP1A1 m1* genotypes on the risk of SCC. The ORs of SCC for the variant *CYP1A1 m1* genotype, tobacco smoking and both factors combined were 2.8, 9.1 and 29.9, respectively. When the data was stratified by the pack-year values, this joint effect was consistent and stronger among the heaviest smokers. The interaction between tobacco smoking and the variant *CYP1A1 m2* genotypes followed the same pattern. Our findings support the conclusion that *CYP1A1 m1* and *CYP1A1 m2* polymorphisms are associated with smoking-related lung cancer risk in Chinese.

Introduction

In the last decade the incidence and mortality rates of lung cancer in China have increased significantly and constantly. Tobacco smoke is an established major cause of lung cancer, resulting in an ~3-fold increased risk among individuals who have ever smoked and about 50% lung cancer death in China (1,2). Since China accounts for one third of one billion of the world's tobacco smokers, a major epidemic of lung cancer is predicted (3,4). However, although risk of lung cancer has been conclusively associated with tobacco smoking, fewer than 20% of smokers develop the disease, indicating that there may be important genetic components in the etiology. Thus, identification of genetic susceptibility factors for lung cancer has broad implications in understanding and preventing occurrence of the cancer.

Accumulating evidence has suggested that genetic polymorphisms in genes controlling carcinogen metabolism underlie individual variation in cancer susceptibility (5,6). In recent years the impact of inherited polymorphisms in the *CYP1A1* gene on susceptibility to lung cancer has received particular interest since this enzyme plays a central role in the metabolic activation of major classes of tobacco procarcinogens such as polycyclic aromatic hydrocarbons (PAHs) and aromatic amines (7–9). Several important single nucleotide polymorphisms have been identified in the *CYP1A1* locus. The *CYP1A1 m1* allele has a T→C mutation in the 3′ non-coding region, which has been associated with elevated enzyme activity (10–13). An A→G transition in exon 7 creates the second allelic variant (*m2*), which leads to an amino acid substitution of Val for Ile in the heme-binding region and results in an increase in microsomal enzyme activity (12,14,15). The variant *CYP1A1 m3* has a mutation in intron 7 and appears to be African-American specific (16). Another polymorphism (*m4*), located two bases upstream of the *m2* site, also causes an amino acid substitution of Thr for Asn in the heme-binding region of the enzyme (17), but the effect of this polymorphism on enzyme activity is not yet clear. The *CYP1A1* genotype has been shown to exhibit population frequencies that depend on ethnicity and significantly higher frequencies of *CYP1A1 m1* and *CYP1A1 m2* alleles have been reported among Asians compared with Caucasians and African-Americans (18,19).

The relationship between *CYP1A1* genetic polymorphism and lung cancer risk in various ethnic populations has been investigated in more than 20 studies (reviewed in ref. 20). In most reports from Japan the *CYP1A1 m1* and *m2* polymorphisms were shown to be strongly associated with the risk of lung cancer, especially in relation to tobacco smokers and in lung squamous cell carcinoma (SCC) (21–24). These findings were not confirmed in studies conducted in Caucasian populations, where the prevalence of the *CYP1A1 m1* and *m2* alleles is very low (25–28). However, larger studies in mixed American populations do point to an elevated risk of lung cancer in relation to the *m1* allele (29–31). In two Brazilian populations an increase in lung cancer risk was significantly associated with the presence of the *m2* allele (32,33). An elevated risk for lung adenocarcinoma (AC) among African-Americans carrying the *m3* allele was also observed (34,35), although the polymorphism was not associated with overall lung cancer risk (36,37). However, little is known about the impact of *CYP1A1* polymorphisms on the risk of lung cancer in the Chinese population (38,39).

Abbreviations: AC, adenocarcinoma; BP, benzo[*a*]pyrene; BPDE, benzo[*a*]pyrene-7,8-diol-9,10-epoxide; CI, confidence interval; CYP, cytochrome P450; OR, odds ratio; PAHs, polycyclic aromatic hydrocarbons; RFLP, restriction fragment length polymorphism; SCC, squamous cell carcinoma.

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In view of the prevalence of tobacco smoking and lung cancer in China and the lack of data on the biggest population in the world, a larger case–control study was warranted to evaluate the role of *CYP1A1* polymorphism as a genetic modifier in the etiology of lung cancer, especially in relation to tobacco smoking. Here we report a large contribution of *CYP1A1 m1* and *m2* polymorphisms to the risk of lung cancer, especially lung SCC, in a case–control study conducted in China.

Materials and methods

Study subjects

This case–control study consisted of 217 patients with lung cancer and 404 cancer-free controls. All subjects were unrelated Chinese. The cases with histologically confirmed primary lung cancer were recruited from January 1997 to December 1999 in the Cancer Hospital, Chinese Academy of Medical Sciences, Beijing. All cases were newly diagnosed and previously untreated. Population controls were accrued from a nutritional survey conducted in the same region. Randomly selected controls were frequency matched to the cases by age and sex. At recruitment, each participant was personally interviewed to obtain detailed information on demographic characteristics and lifetime history of tobacco use. The research protocol was approved by the Institutional Internal Review Board.

CYP1A1 genotyping

Genomic DNA was isolated, using standard methods (40), from peripheral blood samples of controls or from surgically resected normal tissues adjacent to the tumor of lung cancer patients. *CYP1A1* genotypes at the *m1*, *m2* and *m4* sites were analyzed by PCR-based restriction fragment length polymorphism (RFLP) methods as previously described (17,41). Genotyping was conducted with blinding to case/control status. The primers for the *m1* site were M1F (5′-CAG TGA AGA GGT GTA GCC GCT-3′) and M1R (5′-TAG GAG TCT TGT CTC ATG CCT-3′), which produce a 340 bp fragment. The primers for the *m2* and *m4* sites were 5′-TTC CAC CCG TTG CAG CAG GAT AGC C-3′ and 5′-CTG TCT CCC TCT GGT TAC AGG AAG-3′, which generate a 204 bp fragment. These fragments were amplified separately but under the same conditions as follows: a 25 µl reaction mixture consisted of ~100 ng template DNA, 10 µM each primer, 0.2 mM each dNTP, 2.4 mM $MgCl_2$, 1.0 U *Taq* DNA polymerase with 1× Reaction buffer (Promega, Madison, WI) and 2% dimethylsulfoxide. PCR was performed in a GeneAmp 2400 thermocycler (Perkin-Elmer, Norwalk, CT). To amplify the fragment containing the *m1* site the PCR profile consisted of an initial melting step of 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 61°C and 1 min at 72°C, and a final elongation step of 10 min at 72°C. PCR conditions for the fragments containing the *m2* and *m4* sites were 35 cycles of 94°C for 30 s, 63°C for 30 s and 72°C for 30 s.

The restriction enzyme *Msp*I (New England BioLabs, Beverley, MA) was used to distinguish the *m1* polymorphism; gain of a *Msp*I restriction site occurs in the polymorphic allele. The wild-type allele has a single band representing the entire 340 bp fragment and the variant allele results in two fragments of 200 and 140 bp. The restriction enzymes *BsrD*I and *Bsa*I (New England BioLabs) were used to distinguish the *m2* and *m4* polymorphisms, respectively, from the same 204 bp fragment. Both cleavage sites were lost in the case of the mutations and give a single band, whereas the wild-type alleles generate 149 and 55 bp (for the *m2* site) or 139 and 65 bp (for the *m4* site) bands, respectively. The restricted product was analyzed by electrophoresis in 3% agarose gel containing ethidium bromide.

Statistical analysis

Pearson's χ^2 test was used to examine differences in distributions of genotypes studied between cases and controls. Odds ratios (ORs) with 95% confidence interval (CI) calculated using unconditional logistic regression and adjusted for age, gender and tobacco smoking were computed to estimate the association between certain genotypes or tobacco smoking and diseases. The gene–smoking interaction, adjusted for age and gender, was also analyzed by logistic regression methods and fit models (42). Smokers were considered current smokers if they smoked up to one year before the date of diagnosis for cancer or up to the date of the interview for controls. Information was collected on the usual number of cigarettes smoked per day, the age at which the subject started smoking and the age at which the subject stopped smoking if the person was an ex-smoker. Pack-years smoked was calculated to indicate cumulative cigarette dose and lighter and heavier smokers were categorized by the approximate 50th percentile pack-years value among controls, i.e. <20 pack-years and ⩾20 pack-years. All of the statistical analyses were performed with Statistical Analysis System software v.6.12 (SAS Institute, Cary, NC).

Table I. Characteristics of the study subjects

Variable	Cases (n = 217)	Controls (n = 404)
Gender, n (%)		
Male	174 (80.2)	338 (83.7)
Female	43 (19.8)	66 (16.3)
Age (years)		
Mean (SD)	57.5 (9.2)	58.3 (6.8)
Range	31–83	41–76
Smoking status, n (%)		
Never	49 (22.6)	166 (41.1)
Current	168 (77.4)	238 (58.9)[a]
<20 pack-years	38 (22.6)	110 (46.2)
⩾20 pack-years	130 (77.4)	128 (53.8)[a]
Mean years smoked (SD)	29.4 (11.1)	34.7 (10.8)[b]
Histological type, n (%)		
SCC	130 (59.9)	
AC	64 (29.5)	
Other[c]	23 (10.6)	

[a]Two-sided χ^2 test, $P < 0.0001$.
[b]Mann–Whitney rank sum test, $P < 0.0001$.
[c]Other includes non-differentiated cancer (n = 11), bronchioalveolar carcinoma (n = 7) and mixed cell carcinoma (n = 5).

Results

The relevant characteristics of the study subjects are shown in Table I. There were no significant differences among cases and controls in terms of mean age and gender distributions. Although an effort was made to obtain a frequency match on smoking status between cases and controls, more smokers were present in the case group as compared with the controls (χ^2 = 21.4, $P < 0.001$). Moreover, the cancer cases had a higher value of pack-years smoked than controls; 77.4% of cases smoked ⩾20 pack-years compared with 53.8% of controls (χ^2 = 23.7, $P < 0.001$). The mean duration of smoking was significantly shorter among cases with lung cancer (29.4 ± 11.1 years) than that among controls (34.7 ± 10.8 years) (t-test, $P < 0.0001$), which might reflect the fact that cases were more susceptible to lung carcinogenesis induced by tobacco smoke since the mean age of the cases and controls were very similar.

Genotyping results (Table II) show that the allele frequencies for *CYP1A1 m1* and *CYP1A1 m2* were 35.6 and 25.6% among the control population compared with 42.6 and 34.2% among lung cancer cases. The *m2* mutation is in close linkage disequilibrium with the *m1* mutation in this study; 73% *m1* mutants had the mutation in the *m2* site. The expected allele frequencies of both *m1* and *m2* were not significantly different from the observed frequencies, indicating that they were in Hardy–Weinberg equilibrium. However, no polymorphic alleles were detectable in the *m4* site of the *CYP1A1* locus among our 621 study subjects. The distributions of *CYP1A1* genotypes at the *m1* and *m2* sites were then compared, respectively, among lung cancer cases and controls. It was found that 72.3% of cancer cases carried the *CYP1A1 w1/m1* or *CYP1A1 m1/m1* genotype; this was significantly higher than that of controls (57.2%) (χ^2 test, $P < 0.001$). Similarly, the *CYP1A1 m2* variant alleles were more prevalent among cancer cases than controls (64.1 versus 48.0%, $P < 0.001$). Those who carried at least one *CYP1A1 m1* or *CYP1A1 m2* variant allele were at a 2-fold elevated risk overall for lung cancer (OR = 2.0, 95% CI = 1.4–2.8 and OR = 1.9, 95% CI = 1.3–2.7, respectively). However, an elevated risk was observed only among SCC patients; no significant association between

Table II. *CYP1A1* genotype frequencies in lung cancer cases and healthy controls

	CYP1A1 m1 genotype[a]				*CYP1A1 m2* genotype[a]			
	w1/w1	*w1/m1*	*m1/m1*	P[b]	*w2/w2*	*w2/m2*	*m2/m2*	P[b]
Controls	173 (42.8)	175 (43.3)	56 (13.9)		210 (52.0)	181 (44.8)	13 (3.2)	
Lung cancer	60 (27.7)	129 (59.4)	28 (12.9)	0.0003	78 (35.9)	130 (59.9)	9 (4.2)	0.0008
SCC	32 (24.6)	83 (63.9)	15 (11.5)	0.0002	45 (34.6)	81 (62.3)	4 (3.1)	0.002
AC	22 (34.4)	33 (51.6)	9 (14.0)	0.41	26 (40.6)	35 (54.7)	3 (4.7)	0.23
Other[c]	6 (26.1)	13 (56.5)	4 (17.4)	0.29	7 (30.4)	14 (60.9)	2 (8.7)	0.08

[a]*w* refers to wild-type genotype and *m* refers to mutant genotype at the studied polymorphic site, respectively.
[b]P for χ^2 test for comparison with controls.

Table III. Risk of lung cancer associated with the *CYP1A1* genotypes

	CYP1A1 m1 genotype[a]			*CYP1A1 m2* genotype[a]		
	w1/w1	*w1/m1* or *m1/m1*	OR (95% CI)[b]	*w2/w2*	*w2/m2* or *m2/m2*	OR (95% CI)[b]
Controls	173	231		210	194	
Lung cancer	60	157	2.0 (1.4–2.8)	78	139	1.9 (1.3–2.7)
SCC	32	98	2.2 (1.4–3.5)	45	85	1.9 (1.3–2.9)
AC	22	42	1.5 (0.8–2.6)	26	38	1.6 (0.9–2.7)
Other[c]	6	17	2.2 (0.8–5.8)	7	16	2.4 (0.9–6.0)

[a]*w* refers to wild-type genotype and *m* refers to mutant genotype at the studied polymorphic site, respectively.
[b]ORs and 95% CIs were calculated by logistic regression, with the *CYP1A1* wild-type genotypes (*w1/w1* or *w2/w2*) as the reference groups. ORs are adjusted for age, gender and tobacco smoking.
[c]Other includes non-differentiated cancer (n = 11), bronchioalveolar carcinoma (n = 7) and mixed cell carcinoma (n = 5).

Table IV. Interaction of *CYP1A1* genotypes and tobacco smoking on the overall risk of lung cancer

Smoking status	*CYP1A1 m1* genotype				*CYP1A1 m2* genotype			
	w1/w1[a]	OR[b] (95% CI)	*w1/m1* or *m1/m1*[a]	OR[b] (95% CI)	*w2/w2*[a]	OR[b] (95% CI)	*w2/m2* or *m2/m2*[a]	OR[b] (95% CI)
Non-smokers	11/76	1.0	38/90	3.2 (1.5–6.7)	19/90	1.0	30/76	1.8 (0.9–3.5)
Smokers	49/97	4.5 (1.8–11.1)	119/141	8.4 (3.7–19.0)	59/120	4.0 (1.9–8.4)	109/118	6.9 (3.4–14.3)
<20 pack-years	11/44	2.6 (0.9–7.4)	27/66	3.6 (1.5–8.6)	15/55	2.0 (0.8–4.8)	23/55	3.1 (1.4–7.1)
⩾20 pack-years	38/53	4.2 (1.7–10.6)	92/75	11.4 (4.8–27.1)	44/65	4.8 (2.2–10.7)	86/63	9.3 (4.3–20.1)

[a]No. of cases/no. of controls.
[b]ORs and 95% CIs were calculated by logistic regression, with the *CYP1A1* wild-type genotypes (*w1/w1* or *w2/w2*) as the reference groups and adjusted for age and gender.

these *CYP1A1* genetic polymorphisms and risk of AC or other histological types of lung cancer was found (Table III).

The risk of lung cancer related to *CYP1A1* genotypes was further examined with stratification of smoking status and cumulative smoking dose. Among smokers, those carrying the *CYP1A1 w1/m1* or *CYP1A1 m1/m1* genotype had an elevated risk that was nearly twice that of those carrying the *CYP1A1 w1/w1* genotype ($P < 0.05$, test for homogeneity). Interestingly, there was also an elevated risk (OR = 3.2, 95% CI = 1.5–6.7) among non-smokers who had the variant *CYP1A1 m1* genotypes as compared with those with the *CYP1A1 w1/w1* genotype (Table IV). When the OR for the *CYP1A1 m1* polymorphism was investigated within strata of pack-years smoked, a joint effect of tobacco smoking and the *CYP1A1 m1* allele was seen only among individuals who consumed ⩾20 pack-years. Among individuals who consumed ⩾20 pack-years and carried the *CYP1A1 w1/w1* genotype the OR for lung cancer was 4.2 (95% CI = 1.7–10.6). However, the OR was increased to 11.4 (95% CI = 4.8–27.1) among individuals who consumed ⩾20 pack-years and carried at least one variant *CYP1A1 m1* allele ($P < 0.05$, test for homogeneity). This pattern of gene–smoking interaction, although somewhat less strong, was also seen among the *CYP1A1 w2/m2* and *CYP1A1 m2/m2* alleles (Table IV).

The interaction of *CYP1A1* polymorphisms and tobacco smoking was also assessed separately for SCC and AC. Compared with individuals with the *CYP1A1 w1/w1* genotype who had never smoked, the ORs of SCC for the variant *CYP1A1 m1* genotypes alone and smoking alone were 2.8 (95% CI = 0.7–10.6) and 9.1 (95% CI = 2.1–40.0), respectively, however, the OR for combined smoking and having the variant *CYP1A1 m1* genotypes was 29.9 (95% CI = 7.0–127.3), indicating that the joint effect was multiplicative. When the data was stratified by cumulative smoking dose, a strong and significant interaction between the susceptible genotypes and pack-years of tobacco consumption existed, with the heaviest smokers being at a remarkably elevated risk (Table V). The joint effects of the variant *CYP1A1 m2* genotype and tobacco smoking followed the same pattern, although the extent of increased risk appears to be less pronounced (Table

Table V. Interaction of *CYP1A1* genotypes and tobacco smoking on the risk of SCC

Smoking status	*CYP1A1 m1* genotype				*CYP1A1 m2* genotype			
	w1/w1[a]	OR[b] (95% CI)	*w1/m1* or *m1/m1*[a]	OR[b] (95% CI)	*w2/w2*[a]	OR[b] (95% CI)	*w2/m2* or *m2/m2*[a]	OR[b] (95% CI)
Non-smokers	3/76	1.0	10/90	2.8 (0.7–10.6)	7/90	1.0	6/76	1.1 (0.3–3.3)
Smokers	29/97	9.1 (2.1–40.0)	88/141	29.9 (7.0–127.3)	38/120	8.5 (2.7–26.3)	79/118	13.1 (4.6–37.6)
>20 pack-years	5/44	5.1 (1.3–19.6)	16/66	7.9 (1.8–35.6)	9/55	3.7 (1.0–13.4)	12/55	4.3 (1.3–14.5)
⩾20 pack-years	24/53	11.8 (2.1–42.1)	72/75	48.7 (9.4–252.7)	29/65	9.4 (2.9–30.0)	67/63	17.8 (6.0–53.0)

[a]No. of cases/no. of controls.
[b]ORs and 95% CIs were calculated by logistic regression, with the *CYP1A1* wild-type genotypes (*w1/w1* or *w2/w2*) as the reference groups and adjusted for age and gender.

Table VI. Interaction of *CYP1A1* genotypes and tobacco smoking on the risk of AC

Smoking status	*CYP1A1 m1* genotype				*CYP1A1 m2* genotype			
	w1/w1[a]	OR[b] (95% CI)	*w1/m1* or *m1/m1*[a]	OR[b] (95% CI)	*w2/w2*[a]	OR[b] (95% CI)	*w2/m2* or *m2/m2*[a]	OR[b] (95% CI)
Non-smokers	8/76	1.0	22/90	2.7 (1.1–6.6)	12/90	1.0	18/76	1.8 (0.8–4.1)
Smokers	14/97	1.6 (0.5–5.0)	20/141	1.6 (0.6–4.5)	14/120	1.5 (0.5–4.3)	20/118	2.1 (0.8–5.7)
<20 pack-years	5/44	1.4 (0.4–5.5)	5/66	0.8 (0.2–3.0)	4/55	0.9 (0.3–3.6)	6/55	1.3 (0.4–4.5)
⩾20 pack-years	9/53	1.7 (0.5–5.5)	15/75	2.4 (0.8–7.6)	10/65	1.9 (0.6–6.0)	14/63	2.8 (0.9–8.4)

[a]No. of cases/no. of controls.
[b]ORs and 95% CIs were calculated by logistic regression, with the *CYP1A1* wild-type genotypes (*w1/w1* or *w2/w2*) as the reference groups and adjusted for age and gender.

V). In contrast to SCC, however, no significant joint effect between tobacco smoking and the *CYP1A1* polymorphisms on risk of AC was observed, although an excess risk (OR = 2.7; 95% CI = 1.1–6.6) related to the variant *CYP1A1 m1* genotypes was seen among non-smokers (Table VI).

Discussion

In the present study we have investigated the prevalence of three single nucleotide polymorphisms in the *CYP1A1* locus and the association between these genetic polymorphisms and lung cancer risk in the Chinese population. We observed a significant difference in the distribution of *CYP1A1 m1* and *CYP1A1 m2* genotype frequencies among healthy controls and lung cancer patients. Our data clearly demonstrate an association between these genetic polymorphisms in the *CYP1A1* locus and elevated risk of lung SCC among Chinese. While a number of studies have been conducted in various ethnic populations to examine the impact of genetic polymorphisms in *CYP1A1* on risk of lung cancer (reviewed in ref. 20), few studies carried out in the Chinese population have been published. Persson *et al.* (38) analyzed the *CYP1A1 m1* and *m2* polymorphisms among 76 Chinese lung cancer cases and 122 healthy controls and found no association of lung cancer with these polymorphisms, which is in contrast to our findings. However, small sample size and perhaps inappropriate sampling of lung cancer cases and controls in their study may have biased the results obtained by these authors. Although the allele frequencies for the *CYP1A1 m1* and *CYP1A1 m2* alleles among control subjects in the two studies are similar, estimation of the allele frequencies for these variant alleles among cases in the study by Persson *et al.* was based on only 76 patients with lung cancer (38). In contrast, our findings were obtained from a relatively large sample consisting of 217 lung cancer cases, which should provide more confident results. More importantly, in the case–control study by Persson *et al.* 50% of cases were AC and 45% were women. This may not reflect the natural distribution of histological types of lung cancer in the Chinese population, where SCC is predominant, although the rates of AC are increasing (43). While AC of the lung is the most common histological type in women, the incidence of lung cancer is far higher in men than in women, both in China and world wide. Because most patients in their small case group were AC and women (38) and because AC among Chinese women was shown not to be strongly related to tobacco smoking (44,45), it was not surprising for Persson *et al.* to note a negative association between lung cancer and the *CYP1A1* polymorphisms. In fact, in our study, despite demonstrating that an elevated risk for SCC was related to polymorphisms in *CYP1A1*, we failed to observe such an association for overall risk of AC. However, when we stratified the data by smoking status, an elevated risk for AC was seen among non-smokers. This finding suggested that other carcinogenic factors involved in the development of AC among non-smokers might also be substrates of CYP1A1 because most AC patients (57%) in our study were women who had never smoked. Recently, Lin *et al.* (39) reported a case–control study among Chinese in Taiwan and their data are essentially consistent with ours, showing that elevated risk of lung SCC but not AC was significantly associated with polymorphisms in the *CYP1A1* and microsomal epoxide hydrolase (*HYL1*) genes. Together, these findings suggest that caution should be taken in interpreting results concerning the complex origin of lung cancer and specific exposures relevant to the investigated genetic polymorphism.

Our results showing that the susceptible effect of the *CYP1A1* polymorphisms on lung cancer among Chinese was mainly limited to SCC are consistent with those of previous studies conducted in other ethnic populations. In Japanese data an association between these *CYP1A1* polymorphisms and lung cancer was clearly stronger for SCC than for AC (21–23).

Although the results obtained from Caucasians are inconsistent, most likely due to the rarity of the *CYP1A1* polymorphisms in this ethnic population (25–28), it is worth noting that Le Marchand *et al*. have recently shown, in a population-based study including Caucasians, Japanese and Hawaiians, that an increased OR for lung SCC was associated with the *CYP1A1 m1* polymorphism, especially when combined with a *GSTM1* deletion, whereas lung AC was closely linked with the *CYP2E1* wild-type genotype (31). Tobacco smoke contains numerous carcinogens, among them PAHs such as benzo[*a*]pyrene (BP), which may play an important role in lung carcinogenesis. It was shown in a study with experimental animals that lung cancer induced by BP and other PAHs were almost exclusively SCC (46). Furthermore, mutational analysis of the tumor suppressor gene *p53* in human lung cancers revealed certain profiles with specific histological types. A G:C→T:A transversion in the *p53* gene is much more common in SCC than in AC and this profile has been linked to the presence of BP in tobacco smoke (47,48). A recent study has also shown that the metabolically activated form of BP, benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE), preferentially binds to guanine residues in codons 157, 248 and 273 of *p53*, which are mutational hot-spots in lung cancer (49). Taken together, these findings provide strong evidence that BP is involved in the etiology of lung SCC. In this context, one may expect that the *CYP1A1* polymorphisms, which result in altered inducible expression or catalytic activity of the enzyme, are an important modifier of genetic susceptibility to lung cancer, especially lung SCC induced by tobacco smoking, since this enzyme is primarily responsible for activation of BP and other PAHs. In fact, *CYP1A1* polymorphisms have been shown to be linked to increased BPDE–DNA adduct levels and prevalence of tobacco-induced *p53* mutations in lung cancer and in oral SCC (50–55), suggesting a link between carcinogen metabolism pathways leading to DNA damage and target gene mutations in individuals.

In early studies among a Japanese population it was shown that susceptibility to lung cancer of individuals with a *CYP1A1* polymorphism was remarkably high at a low level of tobacco smoking and that the difference in susceptibility of high risk genotypes was reduced at high dose levels (21,22). However, in the present study we observed an apparent interaction between cigarette dose and the variant *CYP1A1* genotypes among Chinese. The heaviest smokers who had the variant *CYP1A1 m1* genotypes were at the highest risk for lung SCC. This effect was not seen with lung AC. Our findings were similar to those reported by Wu *et al*., who found a greater than multiplicative interaction on risk of lung cancer between cumulative cigarette dose and the *c1*/*c1* genotype of *CYP2E1*, another polymorphic enzyme responsible for the activation of carcinogens in tobacco smoke (56).

In summary, our results demonstrate an association between genetic polymorphism in *CYP1A1* and elevated risk for lung cancer, especially lung SCC, among Chinese. These data provide additional evidence that *CYP1A1* is an important determinant in susceptibility to tobacco-induced lung carcinogenesis. These data may also support the hypothesis that susceptibility to certain cancers may depend on ethnic-specific gene polymorphisms.

Acknowledgements

This work was supported by Grants 39825122 and 39990570 from National Natural Science Foundation, and Grant G1998051200 from State Key Basic Research Program.

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Received on August 18, 2000; revised on October 16, 2000; accepted on October 17, 2000