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Associations of *CYP2A6* genotype with smoking behaviors in southern China

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Abstract

Aims—To investigate the association of *CYP2A6* genetic polymorphisms with smoking-related phenotypes in Chinese smokers.

Design—Case-only genetic association study.

Setting—Southern China

Participants—A total of 1,328 Han Chinese smokers who participated in a community-based chronic disease screening project in Guangzhou and Zhuhai from 2006 to 2007.

Measurements—All participants were answered a structured questionnaire about socio-demographic status and smoking behaviors and informative alleles for the cytochrome P450 2A6 (*CYP2A6*) gene (*CYP2A6* *4, *5, *7, *9 and *10) were genotyped.

Findings—The frequencies of *CYP2A6* *4, *5, *7, *9 and *10 alleles were 8.5%, 1.2%, 6.3%, 13.5% and 2.4%, which corresponded to 48.9%, 15.4%, 24.2% and 11.5% of participants being classified as normal, intermediate, slow and poor metabolizers, respectively. Multivariate analyses demonstrated that compared with normal metabolizers, poor metabolizers reported smoking fewer

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Conflict of interest declaration

Dr. RF Tyndale holds shares in Nicogen, a company focused on the development of novel smoking cessation treatments. No support was provided by Nicogen for this study and the manuscript was not reviewed by other members of the company. Dr RF Tyndale has also consulted for Novartis on tobacco cessation. All other authors have no conflicts of interests to declare in relation to this report.

cigarettes per day (adjusted *OR* = 0.49; 95% *CI*: 0.32–0.76), started smoking regularly later in life (adjusted *OR* = 1.55; 95% *CI*: 1.06–2.26) and, amongst former smokers, reported smoking for a shorter duration prior to quitting (adjusted *OR* = 0.33; 95% *CI*: 0.12–0.94). However, poor metabolizers were less likely to quit smoking and remain abstinent than normal metabolizers (*OR* = 0.54; 95% *CI*: 0.34–0.86).

Conclusions—Reduced metabolism function of CYP2A6 in smokers appears to be associated with fewer cigarettes smoked, later initiation of smoking regularly, shorter smoking duration and lower likelihood of smoking cessation.

Keywords

CYP2A6; genetic polymorphisms; smoking behavior; Chinese smokers

INTRODUCTION

China is the largest tobacco producer and consumer in the world with more than 300 million Chinese smokers consuming roughly one-third of the world's cigarettes (1–3). It is estimated that one million smokers die from tobacco-related diseases each year in China and this number is expected to rise to 2.2 million death by 2020 (4,5). Approximately 66% of Chinese males and 4% of Chinese females are current smokers (4); fewer than 30% of smokers in China report that they intend to quit smoking and less than 10% report quitting successfully and remaining abstinent (6). Therefore, there is an urgent need to advance our understanding of risk factors in smoking and cessation, to complement current population-based tobacco control efforts in China.

Nicotine dependence is influenced by multiple environmental and genetic factors (7–11). Approximately 80% of inhaled nicotine is metabolically inactivated to cotinine in the liver by the cytochrome 2A6 (*CYP2A6*) enzyme (12). Variations in the rate of metabolic inactivation of nicotine, as mediated by *CYP2A6*, are associated with alterations in nicotine's bioavailability, which in turn can alter smoking behaviors (13,14).

Twin and family studies indicate that approximately 80% of the variation in the amount smoked is heritable and variation in some genes has been associated with heaviness of smoking (7–11). Variation in the *CYP2A6* gene (chromosome 19q13.2) moderate *CYP2A6* enzymatic activity (15,16). More than 30 *CYP2A6* alleles have been reported (<http://www.imm.ki.se/CYPalleles/cyp2a6.htm>). Some alleles are associated with absent (e.g., *4) (17,18), reduced (e.g., *9) (19), normal (e.g., *8) (20), or increased (e.g., *1B) (21) *CYP2A6* activity with markedly different frequencies among ethnic groups (22–31).

Previous studies have reported that reduced activity *CYP2A6* genotypes were associated with altered age of onset (22,32,33), shorter duration of smoking (22,34,35), fewer cigarettes per day (22–25,34,36,37), fewer pack-years (22,24,35), and likelihood of quitting smoking (35). However two early studies, that used older assays and evaluated fewer alleles, found no association with cigarettes per day (38,39). Among smokers of European-ancestry two clinical trials (40–44) and two case-control studies (33,45) have shown that reduced activity *CYP2A6* genotypes were associated with higher abstinence rates or history of smoking cessation, respectively. However, in the one published report of *CYP2A6* genotype and smoking cessation in Asians, Minematsu and colleagues reported a lower likelihood of smoking cessation in smokers with reduced activity *CYP2A6* genotypes (35). Thus the influence of *CYP2A6* genotype on smoking behaviours is not completely clear, particularly among less well-studied populations such as the Chinese.

In the present study, we studied five *CYP2A6* alleles (*CYP2A6**4, *5, *7, *9 and *10 alleles) known to reduce the rate of nicotine metabolism and that are prevalent in Chinese (20,22,30,46,47). Our aim was to explore associations between variation in the *CYP2A6* gene and age of onset of smoking regularly, smoking duration, cigarette consumption and smoking cessation in Han Chinese in southern China (1,3,48). We hypothesized that reduced activity *CYP2A6* genotypes would be associated with later onset of smoking regularly, fewer cigarettes per day, a shorter smoking duration and increased likelihood of smoking cessation.

METHODS

Design

The present study was a case-only genetic association study of smokers in Han Chinese of southern China.

Participants

A total of 7,293 local residents aged 20 years or over participated in a community-based chronic disease-screening project conducted in Guangzhou and Zhuhai, China from July 2006 to June 2007. They were randomly selected by a stratified multistage sampling method described in our previous publication (49) resulting in $N = 1,328$ Han Chinese smokers being selected for genotyping [$n = 970$ (73%) current smokers; $n = 358$ (27%) former smokers]. The Ethics Committees of Sun Yat-sen University approved the study and written informed consent was obtained from all participants.

Data collection

Data collection methods incorporated face-to-face interviewing conducted by trained medical students or clinical doctors using a structured questionnaire for acquiring socio-demographic characteristics (e.g. age, gender, income, education level, marital status, occupation) and smoking behaviors. The survey was completed, and blood samples obtained, at local health care centers.

Measurement and definition of smoking behaviors

A 'current regular smoker' was defined by having smoked greater than 100 cigarettes in ones lifetime and having smoked at least one cigarette daily at the time of the interview. A 'former smoker' was someone who had smoked greater than 100 cigarettes in their lifetime but reported that they had quit smoking (50). 'Daily cigarette consumption' was the average number of cigarettes per day reported by current regular smokers. 'Age of starting smoking regularly' referred to the age when an individual started smoking at least one cigarette per day and was analyzed in current and former smokers. 'Smoking cessation' was defined by participant responses of having quit smoking and having reported being abstinent at the time of the interview (former smokers). 'Duration of smoking' was calculated by subtracting the age of onset of daily smoking from the age of smoking cessation among former smokers only.

CYP2A6 genotyping

Blood was collected and transported on ice to a -80° freezer within three hours of collection. Genomic DNA was extracted using the Takara kit according to manufacturer's instructions [Blood Genome DNA Extraction Kit, TaKaRa Biotechnology (Dalian) CO., Ltd. China]. The genotyping of *CYP2A6* *4, *9, *7 and *10 alleles was conducted using methods previously described (20,22,40) with the modification of the first and second amplification primer steps for *7 and *10, respectively (51,52).

A two-step allele-specific PCR assay was developed to genotype for *CYP2A6**5. The 3,128 bp PCR product from the first step of genotyping *7 and *10 was used as a template in the second step PCR for the *5 allele. The 25 µl PCR mixture consisted of 1 µl the first PCR product, 80 nM each primer (26,52), 0.2 mM dNTPs, 2.5 µl 10×buffer [containing Tris-HCl (PH 8.3) 100 mM, KCl 500 mM and MgCl₂ 1.5 mM] and 0.5 U of *Taq* DNA polymerase. The reaction conditions were as the follows: an initial denaturation at 94° for 1 minutes, followed by 30 cycles of denaturation at 94° for 30 seconds, annealing at 57° for 30 seconds, and extension at 72° for 2 minutes, before a final extension at 72° for 10 minutes. The product of the second step PCR was 1,215 bp. *Taq* polymerase was obtained from TaKaRa [TaKaRa Biotechnology (Dalian) CO., Ltd. China]. Primers were synthesized by Sangon (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. China).

The *CYP2A6**9 allele was considered a “decrease of function” allele (D) and *4, *5, *7 and *10 were considered ‘loss of function’ (L) alleles (14,17,18,20,26,47,53–58) resulting in the following genotype grouping based on these predicted pharmacokinetic impacts. (1) ‘Normal metabolizers’ were defined as having neither a D nor an L allele (i.e., */*1); (2) ‘Intermediate metabolizers’ had only one D allele (e.g., */*9) which was associated with approximately 75% of the activity of normal metabolizers; (3) ‘Slow metabolizers’ had either one L allele or two D alleles (e.g. */*4 or */*9) which was associated with 50% of the activity of normal metabolizers; and (4) ‘Poor metabolizers’ had either one L and one D allele or two L alleles (e.g. */*4, or */*4) which was associated with less than 25% of the activity of normal metabolizers (16,22).

Statistical analysis

Means and standard deviations (SD) were used to describe continuous variables, while percentages were used to describe categorical variables. Binary logistic regression analyses were conducted to assess the associations between *CYP2A6* genotype and smoking phenotypes (age of starting to smoke regularly, duration of smoking, daily cigarette consumption and smoking cessation); in these analyses, each phenotype was classified into binary variables as dependent variables according to their median (0 ≤ median, 1 > median) and for smoking cessation, (0 = current regular smokers, 1 = former smokers). The means of smoking behavior phenotypes were compared among the four *CYP2A6* genotype groups by Kruskal-wallis test, followed by Least-Significance-Difference test for multiple comparisons. Confounding was defined as a change in the odds ratio (OR) of more than 10%. Only variables meeting the criteria for confounding were included in a final model [age (categorical), nationality, occupation, education level, and family income] and the only covariate meeting this criterion for confounding was age. Thus, age was adjusted for in all logistic regression models.

The frequencies of *CYP2A6* genotypes for each allele were each consistent with the Hardy-Weinberg distribution. All *p* values were two-sided, and statistical significance was *p* = 0.05. We used SPSS 13.0 software package (SPSS, Inc., Chicago, IL USA) for all analyses and calculation.

RESULTS

Socio-demographic characteristics

The mean age of participants was 54.6 (SD = 11.9) years, ranging from 20 to 85. The majority of smokers (92.4%) were males; other demographic characteristics are shown in Table 1.

Frequency *CYP2A6* genotypes and alleles

The frequency of the *CYP2A6**4 allele was 8.5%, the *5 allele was 1.2%, the *7 allele was 6.3%, the *9 allele was 13.5% and the *10 allele was 2.4%. The most common genotypes were *CYP2A6**1/*1 (48.9%), *1/*9 (15.4%), and *1/*4 (10.6%). Allele and genotype-predicted metabolic group frequencies are presented in Table 2. Based on the predicted pharmacokinetic impact of the variant alleles, 48.9% of the subjects were regarded as normal metabolizers, 15.4% as intermediate metabolizers, 24.2% as slow metabolizers, and 11.5% as poor metabolizers.

Smoking history

The mean age of becoming a regular smoker was 21.9 (SD = 7.7) years with a mean cigarette consumption for current smokers of 14.6 (SD = 9.0) cigarettes per day. The mean smoking duration for former smokers was 29.8 (SD = 12.8) years; former smokers were older than current smokers [59.7 (SD = 10.2) years vs. 52.7 (SD = 11.9) years; $p < 0.001$]. There were 928 (70.0%) individuals who reported that they had never quit smoking and 358 (26.9%) reported they quit smoking in the past and were currently not smoking (former smokers); and there were 42 (3.1%) current smokers who reported quitting in the past. The mean age of quitting smoking amongst former smokers was 49.3 (SD = 12.6) years.

Association of *CYP2A6* genotypes with smoking behavior

Table 3 presents the results of logistic regression analyses of the association of *CYP2A6* with smoking behaviors stratified by *CYP2A6* genotype-predicted metabolic category (i.e., normal, intermediate, slow, poor metabolizer) and sex. The logistic regression analyses are also reported by genotype (e.g., *1/*4, etc.) in the Supplementary Table.

Crude and age-adjusted *ORs* are presented for each phenotype of interest. *CYP2A6* genotype groups were not associated with any phenotype examined in females and logistic regression analyses combining both sexes, and of males alone, did not demonstrate any appreciable differences in observed associations. Results are presented stratified by sex. However, it should be noted that the number of female smokers was quite low ($n = 101$) rendering analytic results relatively less reliable. The following, statistically significant, results represent age-adjusted analyses of males only: Compared with normal metabolizers, poor metabolizers reported smoking fewer cigarettes per day (current smokers) and initiating smoking regularly at an older age (current and former smokers). Slow and poor metabolizers smoked for a shorter duration (former smokers) compared to normal metabolizers. Both slow and poor metabolizers were less likely to have reported quitting smoking than normal metabolizers (former smokers).

Figure 1 illustrates differences in the mean number of cigarettes per day, age of starting smoking regularly, and smoking duration between *CYP2A6* genotype-predicted metabolic groups.

DISCUSSION

To our knowledge, this study represents the first report of *CYP2A6* genotypes and their association with smoking behaviors in southern China. *CYP2A6* genotypes indicative of reduced metabolic function were associated with later smoking initiation, fewer cigarettes smoked per day, shorter smoking duration and reduced likelihood of smoking cessation.

Smoking Initiation

Gu and colleagues observed that smokers possessing a *CYP2A6**2 allele (a loss of activity allele) started smoking regularly three years later than smokers not possessing a *CYP2A6**2

allele (33). Moreover, in a prospective adolescent cohort study by Audrain-McGovern and colleagues (23), the reduced activity metabolizer genotype group was associated with a slower rate of escalation in nicotine dependence. In contrast to the findings here, Schoedel and colleagues reported that slow metabolizers had an earlier age of first smoking (22) and O'Loughlin and colleagues who studied early adolescents (age 13) found that poor metabolizers, once smoking, converted to nicotine dependence at a faster rate, but smoked fewer cigarettes once dependent (32). Therefore, while none of these studies assessed initiation prospectively, it would appear that those with reduced activity converted to dependence more rapidly, but escalated in level of dependence more slowly, consistent with their lower levels of smoking consumption. Differences in definitions of 'age of starting smoking' contribute to the apparent differences seen in the impact of *CYP2A6* on this variable (59) and should be examined in the prospective adolescent cohorts.

Daily cigarette consumption

CYP2A6 poor metabolizers reported lower daily cigarette consumption than *CYP2A6* normal metabolizers, which is consistent with several previous studies in subjects of a variety of ancestral backgrounds (22–25,32,34,36). Cigarette consumption is the most robust phenotype associated with *CYP2A6* genotype; recently it was also detected in a genome-wide meta-analysis. Thorgeirsson and colleagues examined more than 31,000 European-ancestry smokers and reported a highly significant association between a single nucleotide polymorphism (rs4105144), which is in linkage disequilibrium with *CYP2A6* *2 (rs180127212), and lower smoking quantity (37).

Smoking duration and smoking cessation

Amongst former smokers, *CYP2A6* poor and slow metabolizer genotypes were both associated with reduced smoking duration compared to normal metabolizer genotypes. Slow metabolizer *CYP2A6* genotypes have also been reported to be associated with shorter smoking duration in European-ancestry studies (22,33). However, the only other *CYP2A6* genetic association study of Asians reporting smoking duration found no association (24).

In contrast to results from European-ancestry smokers in clinical trials (40–44) and case-control studies (33,45), the present study demonstrated that *CYP2A6* slow and poor metabolizers were less likely to report having successfully quit smoking than *CYP2A6* normal metabolizers. Minematsu and colleagues found that individuals possessing a null or *2 allele were less likely to have successfully quit smoking even though, as we found, these reduced activity genotypes were lighter smokers (fewer cigarettes per day) (35).

Given the limitations of the data, we are unable to confirm why there appears to be a discrepancy in associations between *CYP2A6* genotype and smoking cessation in Asian and European-ancestry smokers, but there are several potential explanations for this observation. One potential explanation is that in China and Japan, for example, smoking cessation is rarely attempted until one is confronted with a smoking-related health complication (60–63), perhaps because environmental factors including negative societal norms towards smoking (20,22) and clinical treatment of nicotine dependence may be less prevalent (64–66). Lighter smokers would be expected to experience major morbidity prompting a smoking cessation attempt less often than heavier smokers and *CYP2A6* slow and poor metabolizers tend to be lighter smokers and less prone to develop smoking-related illness than *CYP2A6* normal metabolizers (67). Alternatively Minematsu and colleagues suggested that in Japanese smokers slower nicotine metabolism resulted in maintenance of reinforcing levels of nicotine for a longer period of time, thereby making it more difficult to extinguish smoking-related behavior (35). Another possible explanation is that slow metabolizers who continue to smoke may have a higher proportion of other variables associated with reduced smoking

cessation such as lower education and/or may possess additional genetic variants that increase risk of nicotine dependence and may therefore be more resistant to quitting smoking (25). However, even though the present study observed that slow and poor metabolizers were less likely to report quitting smoking, our study did not examine smokers enrolled in a smoking cessation clinical trial with biochemical verification of abstinence and/or response to nicotine replacement or other pharmacotherapies. Clinical trials conducted by our group in North America have demonstrated that European-ancestry smokers with reduced activity *CYP2A6* genotypes, and/or those with slower *CYP2A6* activity, quit well on nicotine patch and with behavioral counseling treatment for smoking cessation (40,42,43), which cannot be explained entirely by higher nicotine plasma levels. Thus, the role of *CYP2A6* and smoking cessation, and the variety of ways this can be evaluated, needs further evaluation among different ethnic groups.

Frequencies of *CYP2A6* genotypes

The frequencies of the *CYP2A6* *4, *5, *7, *9 and *10 alleles were similar to the ranges reported in previous studies of Chinese people outside of Han Chinese of southern China (4.9–15.1%, 0.5–1.2%, 5.7–9.8%, 15.6–15.7%, and 1.7–4.3%, respectively) (22,26,30,47). However, as seen in Table 4, the frequencies of *CYP2A6* alleles vary widely between ancestral groups. In the present study population, reduced activity *CYP2A6* alleles were more prevalent than previously observed in European (20,22,23,25,29), African (27), African-American (22) and Indian ancestry populations (30), and generally comparable for other Asian populations (i.e., Indian, Korean, Malaysian, Thai) (27–30), except for Japanese, for whom the frequency of major reduced-activity alleles combined is about twice as high as those reported among Chinese populations (16,22,24,27,29).

Limitations

There are several limitations to the present study. The data is retrospective and prone to recall bias. The age of first smoking attempt was also not queried and nor was it possible to determine transition time to nicotine dependence. Non-smokers were not included in the study. However, as *CYP2A6* metabolizes nicotine and carcinogens but is not implicated in reward signaling, it is unlikely to play a role in whether or not individuals experiment with smoking and there is no clear justification for including non-smokers as they would mainly be a study population of untested genetic risk. Given our study sample, we would have needed to identify non-smokers who had tried smoking and not become regular smokers so they had a chance for their genetic variability in *CYP2A6* to act (on nicotine) and then follow participants prospectively. Given the way the data was collected, this was not possible since there was no distinction between individuals who had never experimented with smoking and those who had smoked less than 100 cigarettes.

While not all possible *CYP2A6* alleles were genotyped, the selection of alleles were those previously reported to be present in more than 1% of Asians and so we do not anticipate a high ‘false negative’ rate for reduced activity alleles. If anything, the lack of variant allele testing would weaken the power of the present analyses, biasing results toward the null. Another limitation of the data is the lack of biochemical verification of abstinence in former smokers or biomarkers of nicotine exposure and *CYP2A6* metabolic activity. Finally, dietary and medication histories were not available, and so were not able to control the potential effects of diet (68) or drug (69) on induction of *CYP2A6* activity or effects of oral contraceptives on nicotine clearance. (12).

Conclusions

In this study of mostly male smokers of Han ethnicity from southern China, *CYP2A6* poor metabolizer genotypes were associated with lighter smoking, a later age of initiation and a

shorter duration of smoking. In addition, both slow and poor metabolizer genotypes were associated with decreased smoking duration and decreased likelihood of smoking cessation. If replicated these results have important public health implications in order to reduce the harm associated with smoking and to develop more targeted efforts to increase smoking cessation and harm reduction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Comparison of smoking behaviors in smokers grouped according to *CYP2A6* genotype-predicted metabolic status.

□: Normal metabolizers;
 ▨: Intermediate metabolizers;
 ▩: Slow metabolizers;
 ■: Poor metabolizers.

Note. Y scale was the mean + standard deviation of age for the age of starting smoking regularly (all smokers), years for smoking duration (former smokers) & daily cigarette consumption (current smokers).

Table 1Demographic characteristics in smokers from southern China ($N = 1,328$)

Demographic characteristics	<i>N</i>	%
Age (years)		
20–29	38	2.9
30–39	120	9.0
40–49	247	18.6
50–59	434	32.7
60–69	357	26.9
70–85	132	9.9
Gender		
Male	1,227	92.4
Female	101	7.6
Occupation		
Person in charge	156	11.8
Technician	183	13.8
Service personnel	167	12.6
Operator	306	23.0
Retired personnel	368	27.7
Jobless	148	11.1
Education		
Illiteracy or elementary school	278	20.9
Junior middle school	446	33.6
Senior middle school or vocational secondary school	418	31.5
College or above	186	14.0
Marital status		
Single	51	3.8
Married	1,224	92.2
Divorce or widowed	53	4.0
Family monthly total income (yuan)		
<1,000	172	13.0
1,000~	452	34.0
3,000~	363	27.3
5,000~	212	16.0
Don't know/refuse to answer	129	9.7
CYP2A6 allele		
*4 allele	227	8.5
*5 allele	33	1.2
*7 allele	168	6.3
*9 allele	358	13.5
*10 allele	64	2.4

Table 2Frequencies of *CYP2A6* genotypes (above) and genotype-predicted metabolizer status (below)

<i>CYP2A6</i> genotype	<i>N</i>	Proportion (%)
*1/*1	649	48.9
*1/*4	141	10.6
*1/*5	12	0.9
*1/*7	103	7.8
*1/*9	205	15.4
*1/*10	37	2.8
*5/*7	5	0.4
*5/*5	1	0.1
*5/*9	7	0.5
*5/*10	1	0.1
*7/*9	35	2.6
*7/*10	5	0.4
*7/*7	9	0.7
*9/*10	12	0.9
*9/*9	28	2.1
*10/*10	1	0.1
*4/*5	5	0.4
*4/*7	3	0.2
*4/*9	49	3.7
*4/*10	8	0.6
*4/*4	11	0.8
*1/*5 and *7/*7	1	0.1
<i>CYP2A6</i> genotype-predicted metabolizer status		
Normal metabolizers	649	48.9
Intermediate metabolizers	205	15.4
Slow metabolizers	321	24.2
Poor metabolizers	153	11.5

Table 3

Binary logistic regression analyses of smoking phenotypes stratified by *CYP2A6* genotype-predicted metabolizer status and sex

Males (N = 1,227)				
Characteristics related with smoking behavior [#]	N (%)	Crude OR (95% CI)	Adjusted OR [†] (95% CI)	Trend test [‡]
Age of starting smoking regularly				
Normal metabolizer	594 (48.4)	1.00 (reference)	1.00 (reference)	p = 0.015
Intermediate metabolizer	190 (15.5)	1.08 (0.77–1.51)	1.07 (0.76–1.51)	
Slow metabolizer	299 (24.4)	1.19 (0.89–1.58)	1.26 (0.94–1.69)	
Poor metabolizer	144 (11.7)	1.40 (0.97–2.02)	1.55 (1.06–2.26)*	
Smoking duration (years) [§]				
Normal metabolizer	180 (55.7)	1.00 (reference)	1.00 (reference)	p = 0.002
Intermediate metabolizer	57 (17.6)	0.81 (0.45–1.48)	0.75 (0.38–1.48)	
Slow metabolizer	59 (18.3)	0.35 (0.18–0.68)*	0.36 (0.17–0.75)*	
Poor metabolizer	27 (8.4)	0.32 (0.12–0.83)*	0.33 (0.12–0.94)*	
Daily cigarette consumption ^{§§}				
Normal metabolizer	414 (45.8)	1.00 (reference)	1.00 (reference)	p = 0.002
Intermediate metabolizer	133 (14.7)	0.99 (0.67–1.47)	0.99 (0.67–1.47)	
Slow metabolizer	240 (26.5)	0.77 (0.56–1.07)	0.77 (0.56–1.07)	
Poor metabolizer	117 (12.9)	0.49 (0.32–0.76)*	0.49 (0.32–0.76)*	
Smoking cessation				
Normal metabolizer	573 (48.1)	1.00 (reference)	1.00 (reference)	p < 0.001
Intermediate metabolizer	186 (15.6)	0.97(0.67–1.38)	0.95 (0.65–1.38)	
Slow metabolizer	289 (24.2)	0.56(0.40–0.78)*	0.59 (0.41–0.83)*	
Poor metabolizer	144 (12.1)	0.50(0.32–0.79)*	0.54 (0.34–0.86)*	
Females (N = 101)				
Characteristics related with smoking behavior [#]	N (%)	Crude OR (95% CI)	Adjusted OR [†] (95% CI)	Trend test [‡]
Age of starting smoking regularly				
Normal metabolizer	55 (54.5)	1.00 (reference)	1.00 (reference)	p = 0.851
Intermediate metabolizer	15 (14.9)	0.69 (0.22–2.21)	0.67 (0.21–2.16)	
Slow metabolizer	22 (21.8)	1.04 (0.39–2.79)	1.02 (0.38–2.76)	
Poor metabolizer	9 (8.9)	0.83 (0.20–3.42)	0.82 (0.20–3.40)	
Smoking duration (years) [§]				
Normal metabolizer	14 (40.0)	1.00 (reference)	1.00 (reference)	p = 0.866
Intermediate metabolizer	7 (20.0)	1.33 (0.22–8.29)	2.99 (0.34–26.37)	
Slow metabolizer	10 (28.6)	0.67 (0.13–3.45)	0.87 (0.15–5.04)	
Poor metabolizer	4 (11.4)	1.00 (0.11–9.23)	1.92 (0.18–20.93)	
Daily cigarette consumption ^{§§}				
Normal metabolizer	41 (62.1)	1.00 (reference)	1.00 (reference)	p = 0.164

Males (N = 1,227)				
Characteristics related with smoking behavior [#]	N (%)	Crude OR (95% CI)	Adjusted OR [†] (95% CI)	Trend test [‡]
Intermediate metabolizer	8 (12.1)	0.31 (0.03–2.78)	0.30 (0.03–2.72)	p = 0.468
Slow metabolizer	12 (18.2)	0.72 (0.17–3.10)	0.71 (0.16–3.07)	
Poor metabolizer	5 (7.6)	-	-	
Smoking cessation				
Normal metabolizer	50 (53.2)	1.00 (reference)	1.00 (reference)	
Intermediate metabolizer	15 (16.0)	0.88(0.28–2.78)	0.91 (0.28–2.96)	
Slow metabolizer	21 (22.3)	0.75(0.27–2.09)	0.77 (0.27–2.15)	
Poor metabolizer	8 (8.5)	0.60 (0.13–2.79)	0.61 (0.13–2.86)	

Note. The smoking cessation was defined by 0 = no, 1 = yes, and the other dependent variables were defined by 0 = less than or equal to the median, 1 = larger than the median;

[†] Binary logistic regression adjusted for age; -: Insufficient small sample size for LR to generate *ORs*.

[§] Smoking duration results for former smokers only;

^{§§} Smoking quantity results for current smokers only; Results for others measures included current regular smokers and former smokers; *p*-value for statistical trend of age-adjusted analyses;

* *p* < 0.05.

Table 4
CYP2A6 allele frequencies from present study and other published reports across multiple ethnic groups

CYP2A6 allele	Caucasian	Black-African	African-American	Chinese	Southern China [†]	Japanese	Korean	Thai	Indian	Malays	Ref.
*4	0-4.2	0.9-1.9	1.9	4.9-15.1	8.5	17.0-24.2	10.8	14.0	1.4	7.0	(22-31,47)
*5	0-0.3	0	0	0.5-1.2	1.2	0	0.5		0.9	0.9	(22,26,27,29-31)
*7	0	0	0	5.7-9.8	6.5	9.8-12.5	9.4-9.8	5.0	0	4.3	(20,24,27-29,47)
*9	5.2-8.0	5.7	7.1-8.5	15.6-15.7	13.8	19.0-20.3	19.6	20.0			(22-25,27,28,46)
*10	0	0	0	1.7-4.3	2.5	2.2-3.2	1.0-4.1	2.0	0	4.3	(27-30,47)

Note. Numbers in columns represent allele frequency ranges, as percent of total alleles, in published studies and in the present study;

[†] Allele frequencies from the present study.