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Brief communication (Original)

Association of cytochrome P450 2A6 polymorphism, anxiety, and environmental factors with cigarette smoking by Thai adults

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Background: The effects and associations of genetic variation, psychological, and environment factors associated with cigarette smoking and nicotine dependence remain largely unknown.

Objective: To determine the influence and association of functional genetic polymorphisms of cytochrome P450 2A6 (CYP2A6), anxiety, and environmental factors on cigarette smoking and nicotine dependence.

Method: A cross-sectional study was conducted at King Chulalongkorn Memorial Hospital, Thailand between October 2014 and June 2015. We recruited 127 Thai adult smokers when they visited for an annual physical check-up. Participants completed questionnaires regarding demographic characteristics, The Fagerstr m Test for Nicotine Dependence, and The Thai Hospital Anxiety and Depression Scale. Blood was collected for CYP2A6 genotyping to determine the enzyme metabolism level/group.

Results: Factors associated with significantly greater cigarette consumption were age and being ultrarapid/extensive metabolizers (UM/EM). Anxiety and smoking by household family members were significantly associated with the degree of nicotine dependence. We observed associations between severe nicotine dependence and genotype (UM/EM) and age (b = 0.037; P = 0.005), intermediate metabolizers (IM) and age (b = 0.031; P = 0.43), UM/EM and anxiety (b = 0.258; P < 0.001), IM and anxiety (b = 0.285; P < 0.001), UM/EM and household smoking in the family members (b = 1.427; P = 0.003), and IM and smoking by household family members (b = 1.293; P = 0.024).

Conclusions: Information regarding the association between the gene encoding enzyme metabolism, anxiety, and their interactions may be beneficial for selecting treatment choices for smoking cessation for individual genotypic metabolizers.

Keywords: Anxiety, cigarette smoking, cytochrome P450 2A6 polymorphism

Cigarette smoking is a leading cause of morbidity and mortality worldwide. Several studies have shown that many factors including genetic variation, psychological, and environmental factors are associated with cigarette smoking and nicotine dependence [1].

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Genetic variation directly involved in the metabolism of drugs such as rs1051730 genetic variants in the nicotine acetylcholine receptor gene cluster (*CHARNA5–CHARNA3–CHARNB4*) and cytochrome P450s (CYPs). CYP2A6 is highly polymorphic with reduced function alleles leading to metabolic inactivation of nicotine and cotinine clearance [2-5]. Genetic polymorphisms of CYP2D6 affect cigarette consumption and nicotine dependence. Individuals with wild-type CYP2A6, classified as normal metabolizers, usually require more daily

cigarettes than poor metabolizers, and were more likely to progress to nicotine dependence [6-9].

Psychological factors, especially anxiety and depression, are related to a greater level of smoking, cigarette consumption, and degree of nicotine dependence [10, 11]. The relationships between anxiety, depression, cigarette consumption, and nicotine dependence have been explained either by nicotine intake causing an increase in the chances of developing anxiety and depression, or that anxiety and depression may induce smoking behavior [10, 12, 13]. The prevalence of cigarette consumption among persons with anxiety or mood disorders may be higher than individuals without psychiatric problems [14-16]. The finding from the Thai National Survey on mental health in 2008 reported that the prevalence of anxiety and major depressive disorder in the Thai population were 1.7% and 2.2% respectively. However, the prevalence of anxiety and major depressive disorders among Thais with illicit drug use and alcohol abuse were 1.8%–16.5% and 1.3%–17.7%, respectively [17]. Thus, the prevalence of anxiety and depression is increased in Thai populations with substance use. Other psychosocial factors may be related to cigarette use. The role of a smoking household member, or a peer or colleague smoking, have been demonstrated to be environmental factors that highly influence smoking behavior and nicotine dependence [18, 19]. However, to our knowledge, studies that determine the effects and associations of genetic variation, anxiety, and other environment factors on cigarette smoking are still largely lacking. Therefore, the objectives of this study were to determine the associations between genetic polymorphisms of CYP2A6, anxiety, and environment factors with cigarette consumption and nicotine dependence, and to examine the associations between genetic polymorphisms of CYP2A6 and anxiety, genetic polymorphisms of CYP2A6 and environmental and other related factors among Thai adult smokers.

Materials and methods

After approval by the Ethics Committee and the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (approval No. 367/2014), we conducted a cross-sectional study at King Chulalongkorn Memorial Hospital, Thailand between October 2014 and June 2015. We recruited 127 participants from Thai adult smokers who visited for a check-up at the Preventive and Social Medicine Clinic. The inclusion criteria were smokers who were willing to disclose their smoking behavior, aged from 18 to 60 years, having daily cigarette use and had smoked more than 100 cigarettes in the past 6 months. Exclusion criteria were subjects who had used any medication that may induce or inhibit CYP2A6 activity within 14 days of the study. The present study did not include pregnant women, or women with oral or injected contraception, or individuals with kidney or liver disease. All participants provided their written informed consent to participate.

All participants completed questionnaires to determine demographic data, smoking behavior in the past month (to control recall bias), environmental factors regarding smoking household members and colleagues, The Fagerstr m Test for Nicotine Dependence, and The Thai Hospital Anxiety and Depression Scale for evaluation of anxiety and depression. The Fagerstr m Test for Nicotine Dependence [20, 21] is a widely used 6 item self-report instrument to measure the degree of nicotine dependence. The total scores range from 0–10, with higher scores indicating a higher degree of dependence. In this study, the scores 0–3, 4–5, and 6–10 indicated low, moderate, and high dependence status, respectively. The Thai Hospital Anxiety and Depression Scale (The Thai-HADS), a reliable and valid instrument for screening anxiety and depression in both Thai patients and general populations were applied [22-24]. The test is composed of 14 items (each item scored 0–3). Seven items relate to depressive symptoms and the rest of 7 items relate to anxiety symptoms. A cut-off point of $\geq$11 was interpreted as a clinical case of both depression and anxiety. The sensitivity of anxiety and depression subscales of the Thai HADS are 100% and 85.71%, respectively, while the specificity is 86.0% for anxiety, and 91.3% for depression. Both subscales also showed good internal consistencies with a Cronbach's alpha coefficient of 0.85 for the anxiety subscale and 0.83 for the depression subscale.

CYP2A6 genotyping

Blood samples were collected into ethylenediaminetetraacetic acid containing tubes and transported on dry ice to a –80 °C freezer within 3 h of collection. Genomic DNA was extracted using a Purelink Genomic DNA mini kit (Invitrogen, USA). The genotyping of CYP2A6*9 alleles was assessed using real-time PCR with Stepone software, version 2.2, and an Applied Biosystems 7500 Real Time PCR System.

The genotyping of CYP2A6*1A, CYP2A6*1B, and CYP2A6*4 alleles were determined using a restriction fragment length polymorphism method (PCR-RFLP) [25].

CYP2A6 genotypes were categorized into 4 groups: ultrarapid metabolizers (UM) including persons with more than 2 CYP2A6*1x2A or CYP2A61x2B alleles; extensive metabolizers (EM) included those having 2 CYP2A6*1A/x1A or CYP2A6*1A/x1B or CYP2A6* 1A/x1B alleles; intermediate metabolizers (IM) included those having either one CYP2A6*1A/x4C, CYP2A6*1B/x4C, CYP2A6*1A/x*9, or CYP2A6*1B/x*9; and poor metabolizers (PM) including participants having 2 copies of the inactive variants (CYP2A6*4C) or having one or 2 CYP2A6*4C/x*9, CYP2A6*9A/x10 variants [9].

Statistical analysis

The frequency of categorical variables was analyzed using descriptive variables. Mean and standard deviation (SD) were used to describe continuous variables. Univariate analysis for associations of independent variables with nicotine dependence and cigarette consumption were analyzed using an independent sample *t* test, one-way analysis of variance (one-way ANOVA), Kruskal–Wallis test, and Spearman correlation. Multiple linear regressions were used to examine factors predicting nicotine dependence and cigarette consumption. The associations were also estimated in multiple linear regression to examine the effect between genotype and age, genotype and anxiety, and CYP2A6 metabolizers genotype and environment factors on the degree of nicotine dependence (score) and amount of cigarette consumption. All data analyses were conducted using STATA, version 11.0 (STATA Corp, College Station, Texas).

Results

We recruited 127 Thai adult smokers (96% men) with a mean age (SD) = 37.5 (9.99) years, range 18–59 years. Baseline data regarding socioeconomic status are presented in **Table 1**. The prevalence of individuals with low, moderate, and high nicotine dependence were 46%, 22%, and 32%, respectively. On average, participants were moderately nicotine dependent with a Fagerstr m Test for Nicotine Dependence score of 3.69 (2.56). The mean age (SD) at which participants starting smoking was 17.0 (3.82) years (range 9–35 years); the mean duration (SD) of their smoking was 20.5 (9.99) years (range 1–44 years); the mean (SD) quantity of cigarette consumption was 11.86 (6.86) per day (range 1–40 cigarettes per day). **Table 1** presents a comparison of the demographics of participants in relation to daily cigarette consumption and nicotine dependence. Being female was significantly associated with higher nicotine dependence. By contrast, education, marital status, occupation, alcohol, and caffeine consumption were not associated with amount of cigarette smoking or severity of nicotine dependence ($P > 0.05$).

CYP2A6 genotype, anxiety, depression, environmental and related factors according to daily cigarette consumption and nicotine dependence

Prevalence of anxiety was 13% and depression was 9% among adult smokers. Most participants exposed to a household member smoking (85%) or colleague smoking (94%) in their environment.

Based on CYP2A6 genotype, there were UM (28%); (mean (SD) cigarette consumption = 13.14 (7.97)), EM (32.3%); (mean (SD) cigarette consumption = 13.02 (7.65)), IM (27.6%); (mean cigarette consumption 10.77 (4.71)) and PM (12.6%); (mean cigarette consumption = 8.50 (4.66)). Because the average cigarette consumption per day between UM and EM groups were not different, UM and EM were combined for inferential statistical analysis.

Table 2 presents comparisons of the means of CYP2A6 genotype, anxiety, depression, environmental factors, and smoking information according to daily cigarette consumption and nicotine dependence. We found differences in the average number of cigarette consumed between each type of CYP2A6 genotype. A post hoc comparison test indicated that participants with UM/EM smoked a greater number of cigarettes per day. This difference from participants who are poor metabolizers was significant. However, we found that there were no significant differences in the degree of nicotine dependence between each CYP2A6 genotype. Anxiety was not associated with cigarette consumption, but was associated with the degree of nicotine dependence. Likewise, a household member smoking was not significantly associated with cigarette consumption, but was statistically associated with degree of nicotine dependence.

Table 3 demonstrates the correlations between continuous variables and cigarette consumption and nicotine dependence. Age and duration of smoking

were positively correlated with cigarette consumption. Positive correlations between age, duration of smoking, and anxiety score were found with nicotine dependence. The multicollinearity between age and duration of smoking habit were seen. Age was then selected into the multiple regression analysis.

Table 1. Comparisons of demographic characteristics according to daily cigarette consumption and nicotine dependence (Fagerstr m Test for Nicotine Dependence score)

	Cigarette consumption						Nicotine dependence				
	n	Mean	SD	Statistic (df)		†*P*	Mean	SD	Statistic (df)		†*P*
Sex											
Male	122	11.8	6.87	*t* (125)	–0.64	0.522	3.6	2.58	*t* (7.18)	–3.83	0.006
Female	5	13.8	6.87				5.4	0.89			
Education											
Primary school	31	14.4	9.39	$\chi 2$	2.11	0.347	4.2	2.87	*F* (2,124)	1.77	0.18
Secondary/vocational	77	11.0	5.84				3.4	2.54			
Bachelor'sdegree/higher	19	11.4	4.85				4.2	1.93			
Marital status											
Single/divorced/widowed	65	11.6	5.84	*t* (112.69)	–0.42	0.677	3.7	2.53	*t* (125)	0.07	0.95
Married	62	12.1	7.82				3.7	2.61			
Employment											
Employee/ laborer	108	12.3	7.01	*F* (2,124)	1.67	0.193	3.6	2.53	*F* (2,124)	0.30	0.74
Government and state enterprise	10	10.0	5.03				4.3	2.87			
Own business	9	8.6	5.96				3.7	2.79			
Salary (baht)											
≤10,000	50	13.1	7.22	*F* (2,124)	1.65	0.196	4.1	2.64	*F* (2,124)	2.95	0.06
10,001–15,000	36	10.4	6.05				2.8	2.47			
>15,000	41	11.7	6.95				4.0	2.43			
Alcohol consumption											
No	37	13.6	6.92	*t* (125)	1.84	0.068	4.1	2.35	*t* (125)	1.25	0.21
Yes	90	11.2	6.74				3.5	2.64			
Caffeine consumption											
No	33	11.2	5.75	*t* (125)	–0.64	0.526	3.6	2.81	*t* (125)	–0.15	0.88
Yes	94	12.1	7.22				3.7	2.48			

df = Degrees of freedom. †*P* was based on an independent sample *t* test, one-way ANOVA, or Kruskal–Wallis test as appropriate.

Table 2. CYP2A6 genotype, anxiety, depression, environmental factors according to daily amount of cigarette consumption and severity of nicotine dependence

	Cigarette consumption						Nicotine dependence				
	n	Mean	SD	Statistic (df)		†*P*	Mean	SD	Statistic (df)		†*P*
Smoking household member											
No	19	9.8	5.29	*t* (125)	–1.40	0.164	2.2	2.25	*t* (125)	–2.81	0.006
Yes	108	12.2	7.06				4.0	2.53			
Smoking colleague											
No	10	15.2	10.35	*t* (9.61)	1.09	0.303	4.1	2.64	*t* (125)	0.52	0.60
Yes	117	11.6	6.46				3.7	2.56			
Anxiety											
No	111	11.6	6.46	*t* (17.22)	–1.00	0.331	3.4	2.46	*t* (125)	–3.96	<0.001
Yes	16	13.9	9.15				5.9	2.11			
Depression											
No	116	12.2	6.88	*t* (125)	1.98	0.05	3.7	2.56	*t* (125)	–0.17	0.87
Yes	11	8.0	5.42				3.8	2.75			
CYP2A6 genotype											
UM/EM	76	13.1	7.75	c2	6.09	0.048	3.9	2.53	*F* (2,124)	2.11	0.13
IM	35	10.8	4.72				3.7	2.66			
PM	16	8.5	4.66				2.5	2.28			

df = Degrees of freedom. †*P* were based on independent sample *t* test, one-way ANOVA or Kruskal–Wallis test as appropriate. UM/EM, ultrarapid/extensive metabolizers.

Table 3. Means, standard deviation, and bivariate correlation of the study variables

	Mean	SD	1 Age	2 Duration of smoking habit	3 Anxiety	4 Depression	5 Cigarette consumption	6 Nicotine dependence
1. Age	37.5	9.99	–					
2. Duration of smoke (years)	20.5	9.98	$r = 0.93$ $P < 0.001$	–				
3. Anxiety	6.5	3.43	$r = 0.15$ $P = 0.09$	$r = 0.08$ $P = 0.36$	–			
4. Depression	5.1	3.45	$r = 0.11$ $P = 0.23$	$r = 0.04$ $P = 0.65$	$r = 0.42$ $P < 0.001$	–		
5. Cigarette consumption	11.9	2.56	$r = 0.22$ $P = 0.01$	$r = 0.22$ $P = 0.013$	$r = 0.13$ $P = 0.14$	$r = –0.17$ $P = 0.06$	–	
6. Nicotine dependence	3.7	2.56	$r = 0.241$ $P = 0.006$	$r = 0.23$ $P = 0.009$	$r = 0.47$ $P < 0.001$	$r = 0.08$ $P = 0.36$	$r = 0.63$ $P < 0.001$	–

*Bivariate correlation analysis was performed using a Spearman correlation

Predictors of cigarette consumption and nicotine dependence

Table 4 shows predictors for cigarette consumption and degree of nicotine dependence. We analyzed the associations between gene and age, gene and anxiety, and gene and environmental factors on the degree of nicotine dependence and on cigarette consumption upon different adjustments of covariate, and using genetically poor metabolizers as a reference group.

In model 1, the predictors for cigarette consumption were age and UM/EM. Older age was significantly associated with greater cigarette consumption. UM/EM was also associated with greater cigarette consumption. Model 2 presented the associations of gene and anxiety on cigarette consumption after adjusting for sex, age, and a smoking household member. UM/EM and anxiety were associated with greater cigarette consumption. In Model 3, after adjusting for sex, age and anxiety, UM/EM, and a smoking household member, were associated with greater cigarette consumption. In Model 4, after adjusting for sex, a smoking household member, and anxiety, UM/EM, and age were associated with greater cigarette consumption.

In Model 1, the predictors for a greater degree of nicotine dependence score were age, anxiety, and a smoking household member. By contrast, metabolizer groups were not significantly associated with the degree of nicotine dependence. However, in Model 2 where the associations of genes and anxiety on the degree of nicotine dependence were examined, after adjusting for sex, age, and smoking household member, UM/EM and anxiety, and IM and anxiety were associated with a greater nicotine dependence score. In Model 3, after adjusting for sex, age, and anxiety, UM/EM and smoking household member, and IM and smoking household member were associated with an increased in nicotine dependence score. In Model 4, after adjusting for sex, smoking household member, and anxiety, UM/EM and age were associated with a greater degree of nicotine dependence indicated by a higher nicotine dependence score.

Discussion

We found that the proportion of UM/EM in participants was 60%. This is higher than that of the previous study in Thailand, where the prevalence of UM/EM group in Thai smokers was 37.5% [26]. This higher proportion of UM/EM in Thai Adult smokers in our study probably reflects that all of our participants were current smokers, while data of the previous study was collected on general population. Associations between UM/EM and cigarette consumption were found. This finding is consistent with a previous study that found that the nicotine and cotinine clearance in UM/EM smokers is faster than that by IM or PM and might lead to higher cigarette consumption [6-9, 27]. However, CYP2A6 polymorphisms were not a predictor of the degree of nicotine dependence.

Previous studies described that female sex is associated with a higher rate of nicotine biotransformation compared with male sex, leading to higher rate of withdrawal symptoms [28, 29]. Consistent with this, our study found that female smokers had an average degree of nicotine dependence score that was higher than that for male smokers.

Table 4. Predictors of cigarette consumption and nicotine dependence

	Cigarette consumption				Nicotine dependence			
	b	**SE(*b*)**	**95%CI (*b*)**	***P***	***b***	**SE(*b*)**	**95%CI (*b*)**	***P***
Model 1								
Female sex	–0.626	3.057	–6.68, 5.43	0.838	0.756	1.022	–1.27, 2.78	0.461
Age [a]	0.155	0.061	0.03, 0.276	0.012	0.036	0.020	–0.005, 0.08	0.082
Smoking household member[b]	2.047	1.669	–1.26, 5.35	0.222	1.208	0.558	0.10, 2.31	0.032
Anxiety	0.168	0.177	–0.18, 0.52	0.344	0.309	0.059	0.19, 0.43	<0.001
UM/EM[c]	3.929	1.821	0.32, 7.53	0.033	0.977	0.609	–0.23, 2.18	0.111
IM[c]	2.127	1.988	–1.81, 6.06	0.287	0.970	0.665	–0.35, 2.29	0.147
Model 2[d]								
Anxiety and UM/EM[c]	0.363	0.167	0.03, 0.69	0.031	0.258	0.057	0.15, 0.37	<0.001
Anxiety and IM[c]	0.049	0.20	–0.35, 0.44	0.808	0.285	0.068	0.15, 0.42	<0.001
Model 3[e]								
Smoking household member								
Smoking and UM/EM[c]	4.127	1.408	1.34, 6.14	0.004	1.427	0.471	0.49, 2.36	0.003
Smoking household member								
Smoking and IM[c]	1.574	1.686	–1.76, 4.91	0.352	1.293	0.563	0.18, 2.41	0.024
Model 4[f]								
Age and UM/EM[c]	0.149	0.039	0.07, 0.23	<0.001	0.037	0.013	0.01, 0.06	0.005
Age and IM[c]	0.082	0.046	–0.01, 0.17	0.071	0.031	0.015	0.001, 0.06	0.043

[a]Age and anxiety variables as a continuous data into the model.
[b]Dummy variable (reference with "no")
[c]Dummy variable (reference with "Poor metabolizers")
[d]Model 2 was adjusted for gender, age, and smoking household member.
[e]Model 3 was adjusted for gender, age, and anxiety.
[f]Model 4 was adjusted for gender, smoking household member, and anxiety.
UM/EM, ultrarapid/extensive metabolizers; IM, intermediate metabolizers.

To our knowledge, the associations between UM/EM and age, anxiety, and environmental factors were associated with cigarette consumption and nicotine dependence. The effect of the associations between UM/EM and a smoking household member was the strongest predictor for greater cigarette consumption and the degree of nicotine dependence. This might be the consequence of genetic variations and the individual habits of smokers in their family environment. Environmental factors highly influence the initiation of smoking by adolescents and lead to persistent smoking and resulting nicotine dependence [19, 30]. Determining associations between genetic variation and environmental factors is important knowledge for health care professionals in all areas. Prevention of cigarette smoking in a home environment can reduce the number of new smokers and might also be a strategies for smokers who are attempting to quit smoking. Apart from environmental factors, the associations between UM/EM and anxiety were also associated with cigarette use, as similarly found in a previous study that described associations between anxiety and nicotine dependence. The relationship between anxiety, depression, cigarette consumption, and nicotine dependence were studied as co-occurring mental health disorders. Anxiety and depression may be associated with an increase in cigarette consumption as a self-medicating behavior in order to cope with their stress [10, 12, 13]. Moreover, one study found that cigarette consumption may play a role in the onset of mental illness [31].

Information on the association of genes, anxiety, and their interactions may be of benefit for selecting available treatments in smoking cessation programs in regard to individual CYP2A6 genotypes. A urine testing method to determine CYP2A6 genotypes may be practical for physicians in the clinic in considering the benefit of various treatments for smokers. Nonetheless, health care providers should still consider the important role of psychological counseling, relaxation techniques, or cognitive behavior therapy to help their smoking patients to cope with anxiety associated with nicotine dependence.

Our participants were current smokers who visited for their annual medical check-up. This means they might have a physical or health status that differs from

smokers in other settings. The relatively small sample size may also have led to difficulty in identifying some predictors of cigarette use. In addition, we used a candidate gene approach in this study and only one gene was selected. However, we might be able to generalize the results of the association between their CYP2A6 genotype and cigarette smoking to the Thai population. A further study with more intensive genotyping including genome-wide associations study may provide better insight into the influence of genetic variation on nicotine use and dependence.

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Conflict of interest statement

The authors declare that there is no conflict of interest in this research.

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