

Smoking cessation program and CYP2A6 polymorphism

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1. ABSTRACT

The relationship between CYP2A6 genotype and smoking status remains unclear although several studies have been reported. In this study, we have investigated the significance of CYP2A6 genotype on smoking habit and treatment of nicotine patch. Sixty-one smokers (1.7%) working in a Japanese company (n=3585) participated in this smoking cessation program. Forty-four of 61 (72.1%) smokers were treated by nicotine patch. A genotyping analysis was carried out for 41 (40 men and 1 women) of 61 participants (67.2%). The smoking cessation rate at 90 days was 54.1% (33/61). Age and smoking years in re-smoking group are significantly lower than those in smoking cessation group. The smoking cessation rate of participants treated with nicotine patch (63.6%; 28/44) was significantly higher than that of the group non-treated with nicotine patch (29.4%; 5/17) at 90 days ($p < 0.05$). The incidence of homozygotes of CYP2A6 gene deletion (CYP2A6*4/*4) in 41 cases (9.8%; 4/41) could be higher than that in 894 healthy controls (3.7%; 33/894) ($p = 0.12$), while no other variant alleles (CYP2A6*2, CYP2A6*3 and CYP2A6*6) were found. Age and smoking years of participants with CYP2A6*4/*4 are significantly higher than those with CYP2A6*1 positive. The scores of Fagerstrom test, an analysis for nicotine-dependence, were slightly different between participants with CYP2A6*4/*4 and CYP2A6*1 positive. Although treatment of nicotine patch is efficacious to smoking cessation, cases with CYP2A6*4/*4 might be more sensitive to nicotine adverse effects and more difficult to quit smoking once they have smoking habit.

2. INTRODUCTION

Nicotine is the compound in tobacco primarily responsible for the development and maintenance of tobacco dependence (1). Nicotine increases dopamine levels by virtue of its capacity to stimulate dopamine release, while cocaine acts as a dopamine uptake inhibitor and heroin stimulates opioid receptors although both result in elevated dopamine levels (2-5). The CYP2A6 gene encoding cytochrome P450 2A6 (CYP2A6) is located on chromosome 19. CYP2A6 primarily expressed in the liver is involved in metabolism of a number of endogenous and exogenous compounds, including nicotine. In humans, approximately 70-80% of nicotine is converted to cotinine by C-oxidation that is mostly catalyzed by CYP2A6 in the liver (6-8). Therefore, CYP2A6 may have an influence upon smoking behavior through its role in nicotine metabolism.

At the present time, 16 variant alleles of CYP2A6 have been identified in addition to the wild type, designated CYP2A6*1 (see <http://www.imm.ki.se/CYPalleles/cyp2a6.htm> for CYP2A6 allele nomenclature). The frequency of variant alleles has been reported to differ among ethnic groups (9). Of the variant alleles, CYP2A6*2 encoding an enzyme with a L160H amino acid substitution (10, 11) and CYP2A6*3 generated by CYP2A6-to-CYP2A7 gene conversion (12) produce inactive enzymes. Both variants are more common in people of European descent (1%-3%) (13) although they are exceedingly rare in Asian populations. CYP2A6*4 encoding an inactive enzyme with allele deletion (14, 15) is common in Asian populations

(15%-20%) but comparatively rare in European populations (8). *CYP2A6*6* have a single nucleotide mutation in exon 3, resulting in a R128Q amino acid substitution that decreases coumarin 7-hydroxylation to one eighth of wild type in insect cells probably due to instability of the mutant enzyme (16). People with these variant alleles may be poor metabolizers of nicotine and more sensitive to aversive effects of nicotine (17). Indeed, people with a *CYP2A6* homozygous gene deletion had significantly impaired nicotine metabolism (18, 19).

Many cancers are caused by chemical carcinogens present in our environment (20). *CYP2A6* metabolically activates a number of carcinogens including tobacco-specific N-nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (21, 22). Thus, the capacity of enzymes to activate chemical carcinogens can be a risk-determining factor for cancer. Genetic polymorphism of the genes for such enzymes is a typical factor that alters activities and amounts of the enzymes. Thus, the genetic polymorphism may alter the risk of chemical carcinogenesis (23). Recently, several reports have demonstrated the role of *CYP2A6* genetic polymorphisms in lung cancer risk with some conflicting results in several populations from different ethnicities (24-29). A large-scale epidemiological study showed the relationship between variety of *CYP2A6* polymorphisms and tobacco-related lung cancer risk in male Japanese smokers (30).

On the other hand, the causal link between smoking and increased morbidity/mortality is firmly established that 3 million people globally are estimated to die each year as a result of smoking (31). In a recent study, Vineis, *et al.* demonstrated in their large prospective study that environmental tobacco smoke is a risk factor for lung cancer and other respiratory diseases, particularly in ex-smokers, in which the smoking status was shown to correlate with cotinine measurements (32). Nicotine is a psychoactive substance responsible for tobacco dependence; smokers adjust their cigarette consumption to maintain brain nicotine levels. Several studies on the efficacy of smoking cessation programs have reported that nicotine replacement therapy (NRT) is effective aids to smoking cessation (33). However, relationship between *CYP2A6* genotype and smoking habit has been poorly investigated. In this study, we determined *CYP2A6* genotypes in 61 smokers of our smoking cessation program and studied the significance of *CYP2A6* genotype on smoking habit and treatment of nicotine patch.

3. MATERIALS AND METHODS

3.1. Subjects

Smokers working in Japanese company were raised by the interview and internet. Sixty-one of 3585 (1.7%) participated in this smoking cessation program. There were 52 men and 9 women ranging in age from 25 to 60 years (mean, 45.0 years).

A genotyping analysis was carried out using for 41 individuals (40 men and 1 women). Blood samples (7mL) in 41 of 61 (67.2%) were obtained by venipuncture, and genomic DNA was extracted from the peripheral lymphocytes (34). This study approved by the ethics

committee of medical care and research of the University of Occupational and Environmental Health (UOEH) under the guidelines of the Ministry of Education, Culture, Sports, Science and Technology in Japan. The chi-square test and the t-test were used in this statistical analysis.

3.2. Smoking cessation program

All of 61 smokers were investigated variable smoking factors, such as age, smoking years, cigarettes per day, Fagerstrom test for nicotine dependence (FTND) (35) and carbon monoxide (CO) concentration in the expiration, by interview when they started this smoking cessation program. These smokers decided when they stopped smoking and were provided a self-help booklet by physicians. The treatment of nicotine patch (TNP) was also explained by physician (36) and 44 of 61 (72.1%) smokers chose to use nicotine patch. TNP was performed as follows; a 15 mg patch used for the first 4 weeks, a 10 mg patch for the next 2 weeks and a 5 mg patch for the final 2 weeks. They were followed up by phone 7, 30, 60 and 90 days later when this smoking cessation program was started.

3.3. *CYP2A6* polymorphism

3.3.1. Detection of *CYP2A6* wild, variant alleles and homozygous gene deletion

To identify the *CYP2A6* genotypes, PCR-RFLP analysis was performed as described previously (16, 19). The single PCR and RFLP methods were used to identify the wild *CYP2A6* allele (*CYP2A6*1*), variant alleles (*CYP2A6*2*, *CYP2A6*3* and *CYP2A6*6*) and *CYP2A6* homozygous gene deletion. *CYP2A6* specific PCR was accomplished with the primer pair Kd1F (5'-CCT GAT CGA CTA GGC GTG GTA) and E3R (5'-TCG TCC TGG GTG TTT TCC TTC) yielding a single 215-base pair (bp) product. Forward primer Kd1F was set overlapping intron 2 and exon 3. For the reverse primer, E3R was designed in intron 3 by Fernandez-Salguero *et al.* (12). The reaction mixture contained approximately 100ng genomic DNA, 2mM of each primer, 0.2mM of dNTP, 1.5mM MgCl₂, 1U of rTaq polymerase (TOYOCO, Japan) in a total volume of 50mL. Amplification was performed by denaturing at 94°C for 30 sec, annealing at 56°C for 30 sec and extending at 72°C for 30s for 35 cycles using a program temp control system PC-707 (ASTEC, Japan). Then, 8mL of PCR products was digested by the restriction enzymes, *Msp* I, *Xcm* I, and *Dde* I in a total volume of 20mL. The PCR products with and without the enzymatic digestion were analyzed on a 4% agarose gel visualized by ethidium bromide. Table 1 shows *CYP2A6* genotypes determined by Kd1F/E3R PCR-RFLP analysis. PCR amplification of β -actin as a control was accomplished with the primer pair OKY24 (5'-GGC ATC GTG ATG GAC TCC G) and OKY25 (5'-GCT GGA AGG TGG ACA GCG A) yielding a single 820-base pair (bp) product as described previously (37).

3.3.2. Detection of *CYP2A6* homozygous gene deletion (*CYP2A6*4/*4*)

β -globin PCR was accomplished with the primer pair GH20 (5'-GAA GAG CCA AGG ACA GGT AC) and PCO4 (5'-CAA CTT CAT CCA CGT TCA CC) yielding a

Table 1. CYP2A6 genotypes determined by Kd1F/E3R PCR-RFLP analysis and GH20/PCO4 and Kd1F/E3R PCR-RFLP analysis

	Characteristic RFLP	Site of nucleotide change	Codon exchange ¹	Amino acid substitution
<i>Kd1F/E3R</i> PCR-RFLP analysis				
<i>CYP2A6</i> *1 (wild type)	-	-	-	-
<i>CYP2A6</i> *2	<i>Xcm</i> I	479	CTC to CAC	H160L
<i>CYP2A6</i> *3	<i>Msp</i> II/ <i>Dde</i> I	Many ²	Many ^b	Many ^b
<i>CYP2A6</i> *6	<i>Msp</i> I	383	CGG to CAG	R128Q
<i>CYP2A6</i> variant allele with Silent mutation	<i>Dde</i> I	406	CGG to AGG	Silent mutation
<i>GH20/PCO4</i> and <i>Kd1F/E3R</i> PCR-RFLP analysis				
<i>CYP2A6</i> *4 (deletion type)			Deletion	

¹The exchanged codons are shown in boldface, ²There are many sites of nucleotide and amino acid exchange.

Table 2 Relationship between the status of the smoking habit and five smoking related factors in this smoking cessation program. Sixty-one smokers are divided into two groups

Smoking related factors	Smoking cessation group mean \pm SD	Re-smoking group mean \pm SD	p
	n=33	n=28	
Age	47.3 \pm 7.49	42.4 \pm 10.4	0.04
Smoking years	27.3 \pm 7.43	21.9 \pm 10.0	0.02
Cigarettes per day	27.6 \pm 10.7	25.1 \pm 15.2	0.46
Fagerstrom test ¹ for nicotine dependence	6.0 \pm 2.4	4.9 \pm 2.6	0.10
CO concentration in the expiration. (mg/m ³)	24.4 \pm 12.5	21.9 \pm 11.8	0.43

Smoking cessation group; cases who successfully quit smoking for 90 days, Re-smoking group; cases who fail to quit smoking for 90 days, ¹: see Table 6

single 285-base pair (bp) product (38). The Kd1F/E3R primers were used with GH20/PCO4 primers for β -globin in the same reaction tube. The reaction mixture contained approximately 100ng genomic DNA, 2mM of Kd1F/E3R primer, 0.2mM of GH20/PCO4 primer, 0.2mM of each dNTP, 1.8 mM MgCl₂, 1U of rTaq polymerase (TOYOBO, Japan) in a total volume of 50mL. Amplification reaction was performed as described above. PCR products were displayed on a 4% agarose (Bio-Red) gel, and visualized by ethidium bromide staining.

4. RESULTS

As seen in Table 2, thirty-three smokers of this smoking cessation program successfully quitted smoking for 90 days (SCG; smoking cessation group) and 28 smokers fail to quit smoking for 90 days (RSG; re-smoking group). Age and smoking years in RSG were significantly lower than those in SCG, while no significant difference between SCG and RSG was observed in cigarettes consumption (cigarettes per day), the results of FTND, and CO concentration in the expiration. The time course of smoking cessation is shown in Figure 1. The majority of participants in RSG smoked again in the first week of this program. Forty-four smokers of this smoking cessation program were treated by nicotine patch (TNPG; treatment of nicotine patch group) and 17 smokers did not desire to be treated by nicotine patch (NTNPG; no treatment of nicotine patch group). The smoking cessation rate at 90 days was 54.1% (33/61). The smoking cessation rate of TNPG (63.6%; 28/44) was significantly higher than that of NTNPG (29.4%; 5/17) at 90 days ($p < 0.05$).

CYP2A6 genotyping by PCR-RFLP was carried out for 41 of 61 smokers (67.2%). The *CYP2A6* products in exon 3 were detected at the molecular length of 215 bp (Figure 2A; lane 3 - 6) as well as control products (820 bp) of β -actin (Figure 2B; lane 9 - 12). There are four polymorphic sites in these *CYP2A6* products. Three variant

alleles (*CYP2A6**2, *CYP2A6**3 and *CYP2A6**6) and silent mutation are detected by digesting the 215 bp fragment with three kinds of restriction enzymes (*Msp* I, *Xcm* I, and *Dde* I) as described in Table 1 and our previous reports (16, 19). No polymorphic site of the above four alleles was found in the 41 *CYP2A6* products (Table 3). On the PCR analysis, the *CYP2A6**4/*4 genotype (*CYP2A6* homo-deletion) showed the control 820 bp products of β -actin but no detectable 215 bp signal of *CYP2A6*. These specimens were further analyzed with another PCR method, which used two sets of primers (*CYP2A6* and β -globin primers), to confirm the deletion. Only β -globin products were detected in the representative specimens with *CYP2A6**4/*4 (lane 5 and 6 in Figure 2B) although the control specimens showed both *CYP2A6* and β -globin products (lane 3 and 4 in Figure 2B). The cases of lane 5 and 6 in Figure 2B were diagnosed as *CYP2A6**4/*4. *CYP2A6* genotypes of 41 cases in this smoking cessation program are shown in Table 3. *CYP2A6* genotypes in 894 healthy controls, living in the northern Kyusyu region, are also revealed in Table 3. The incidence of *CYP2A6**4/*4 in 41 cases (9.8%; 4/41) seemed to be higher than that in 894 healthy controls (3.7%; 33/894) ($p = 0.12$).

Table 4 shows the incidences of SCG and TNPG with respect to *CYP2A6* genotypes. There was no significant difference between the incidences of *CYP2A6**1 positive (*CYP2A6**1/*1 or *CYP2A6**1/*4) cases and the incidences of *CYP2A6**4/*4 cases in SCG and TNPG. Table 5 shows relationship between *CYP2A6* genotypes and five smoking related factors in this smoking cessation program. Age and smoking years in cases with *CYP2A6**4/*4 are significantly higher than those in cases with *CYP2A6**1 positive although no significant difference was found between cases with *CYP2A6**4/*4 and cases with *CYP2A6**1 positive in cigarettes consumption, results of FTND, or CO concentration in the expiration. There was no significant difference between *CYP2A6* genotypes and FTND scoring although the score of cases with

Table 3. CYP2A6 genotypes of 41 cases in this smoking cessation program

	Controls ¹	Cases
CYP2A6*1/*1 and CYP2A6*1/*4	855 (95.6%)	37 (90.2%)
CYP2A6*1/*2, CYP2A6*2/*2 and CYP2A6*2/*4	0 (0%)	0 (0%)
CYP2A6*1/*3, CYP2A6*3/*3 and CYP2A6*3/*4	0 (0%)	0 (0%)
CYP2A6*4/*4	33 (3.7%)	4 (9.8%)
CYP2A6*1/*6	4 (0.4%)	0 (0%)
CYP2A6*1/variant allele with Silent mutation	2 (0.2%)	0 (0%)
Total	894	41

¹Health controls living in the northern Kyushyu region as described in previous paper (16).

Table 4. Incidences of smoking cessation group (SCG) and treatment of nicotine patch group (TNPG) with respect to CYP2A6 genotypes

Cases (%)	CYP2A6*1 positive	CYP2A6*4/*4	Total
TNPG	31/37 (83.8)	3/4 (75.0)	34/41 (82.9%)
Smoking cessation group in TNPG	21/31 (67.7%)	1/3 (33.3%)	22/34 (64.7%)
Smoking cessation group in NTNPG	2/6 (33.3%)	1/1 (100%)	3/7 (42.9%)
Smoking cessation group	23/37 (62.2)	2/4 (50.0)	25/41 (61.0%)

CYP2A6*1 positive; CYP2A6*1/*1 or CYP2A6*1/*4, Smoking cessation cases; cases who successfully quit smoking for 90 days
TNPG; Treatment of nicotine patch group, NTNPG; No treatment of nicotine patch group

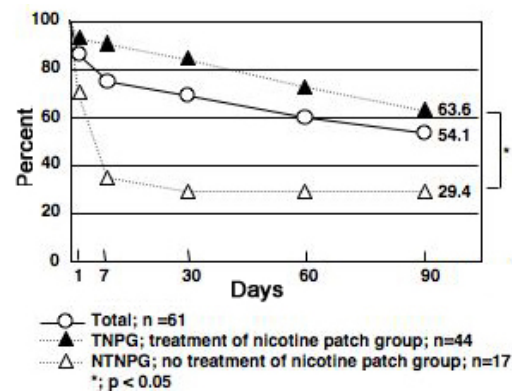


Figure 1. The smoking cessation rate for 90 days on this smoking cessation program. Forty-four smokers of this smoking cessation program were treated by nicotine patch (TNPG; treatment of nicotine patch group) and 17 smokers were not treated by nicotine patch (NTNPG; no treatment of nicotine patch group).

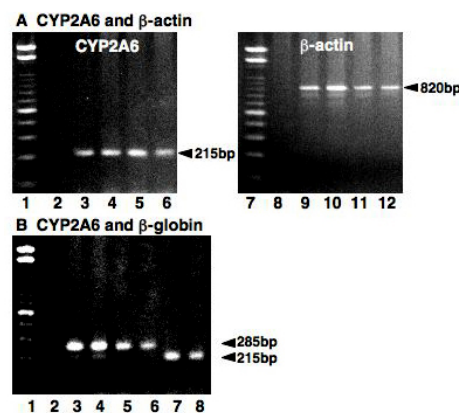


Figure 2. Verification of genotyping for CYP2A6*4/*4. A. Lower arrow indicates Kd1F/E3R PCR products (215bp: CYP2A6 products). Lane 3, 4, 5 and 6 shows CYP2A6 detected DNA subjects. Upper arrow indicates Oky24/Ok25 PCR products (820bp: β-actin products). Lane 9, 10, 11 and 12 shows β-actin detected DNA subjects. Lane 1 and 7 shows 100bp DNA ladder size marker. Lane 2 and 8 shows negative controls. B. CYP2A6 PCR with β-globin as internal PCR control. Upper arrow indicates PCO4/GH20 PCR products (285bp: β-globin products). Lower arrow indicates Kd1F/E3R PCR products (215bp: CYP2A6 products). Lane 3 and 4 shows both CYP2A6 and β-globin detected DNA subjects. Lane 5 and 6 shows PCR results from CYP2A6 deleted DNA subjects. Lane 7 and 8 indicates Kd1F/E3R PCR products. Lane 1 shows 100bp DNA ladder size marker. Lane 2 shows negative controls.

Table 5. Relationship between CYP2A6 genotypes and five smoking related factors in this smoking cessation program

Smoking related factors	CYP2A6*1 positive mean± SD	CYP2A6*4/*4 mean± SD	P
	n=37	n=4	
Age	45.8±6.71	55.3±4.86	0.01
Smoking years	25.9±6.29	33.0±4.08	0.04
Cigarettes per day	28.5±12.7	25.0±10.0	0.59
Fagerstrom test ¹ for nicotine dependence	5.9±2.3	4.3±1.5	0.17
CO concentration in the expiration. (mg/m ³)	24.6±11.3	19.8±5.25	0.40

CYP2A6*1 positive; CYP2A6*1/*1 or CYP2A6*1/*4, ¹: see Table 6

Table 6. Fagerstrom Test for Nicotine Dependence (FTND) and relationship between CYP2A6 genotypes and FTND scoring in this smoking cessation program

Questions	Answers	Points	CYP2A6*1 positive mean± SD n=37	CYP2A6*4/*4 mean± SD n=4	P
1. How soon after you wake up do you smoke your first cigarette?	Within 5 minutes	3	2.22±0.84	2±0	0.64
	6-30 minutes	2			
	31-60 minutes	1			
	After 60 minutes	0			
2. Do you find it difficult to refrain from smoking in places where it is forbidden e.g. in church, at the library, in cinema, etc.?	Yes	1	0.35±0.23	0±0	0.16
	No	0			
3. Which cigarette would you hate most to give up?	The first one in the morning	1	0.35±0.23	0.25±0.25	0.69
	All others	0			
4. How many cigarettes / day do you smoke?	10 or less	0	1.86±0.95	1.5±1.0	0.48
	11-20	1			
	21-30	2			
	31 or more	3			
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?	Yes	1	0.49±0.26	0.25±0.25	0.38
	No	0			
6. Do you smoke if you are so ill that you are in bed most of the day?	Yes	1	0.62±0.24	0.25±0.25	0.16
	No	0			

CYP2A6*1 positive; CYP2A6*1/*1 or CYP2A6*1/*4

CYP2A6*4/*4 might be lower than that with CYP2A6*1 positive in Question 2 and 6 (Table 6).

5. DISCUSSION

Hyland, *et al.* attempted to identify predictors of smoking cessation in a cohort of cigarette smokers followed over 13 years (39). They revealed that measures of nicotine dependence were much more strongly associated with cessation than measures of motivation and other predictors, such as male gender, older age, higher income, and less frequent alcohol consumption. Grotvedt, *et al.* showed that age, gender, education, and the physical component became a predictor to quit smoking in multivariate logistic regression analysis (40). In this study, age and smoking years in re-smoking group are significantly lower than those in smoking cessation group (Table 2). Age and smoking years may be predictors of smoking cessation as described in the previous reports.

A large number of nicotine replacement therapy trials have been reported since 1994 (33, 41). Based on analysis of the results from 39 reports, Stead, *et al.* showed that the odds ratio for smoking cessation with treatment of nicotine patch was 2.07 (95% CI 1.64 – 2.62) in 1994 and 1.73 (95% CI 1.56 – 1.93) in 2000 (41). Based on the results from 123 reports, Silagy, *et al.* also showed that the odds ratio for smoking cessation with nicotine replacement therapy compared to control was 1.77 (95% confidence intervals (CI): 1.66 to 1.88) and with treatment of nicotine

patch was 1.81 (95% CI: 1.63 to 2.02) (42). The smoking cessation rate of TNPG and NTNPG were 63.6% and 29.4%, respectively (Figure 1). The odds ratio for smoking cessation with TNPG compared to NTNPG was 2.16 ($p < 0.05$), which clearly demonstrates that the treatment of nicotine patch is efficacious for smoking cessation.

The half-life of nicotine in human serum is for several hours and that of cotinine is about one day (43). Many of the symptoms of nicotine withdrawal are similar to those of other drug withdrawal syndromes, such as anxiety, awakening during sleep, depression, difficulty concentrating, impatience, irritability/anger and restlessness (44). In most of smokers the symptoms of nicotine withdrawal come out within one day when they start the smoking cessation. Most of smokers usually fail to quit smoking within the two weeks of the beginning in smoking cessation (45). The smoking cessation rates at 30 days and at 90 days were almost the same in NTNPG (Figure 1), indicating that the first week in smoking cessation is important for success to quit smoking.

Any of genotypes having CYP2A6*2, CYP2A6*3, CYP2A6*6 alleles and variant allele with silent mutation were not detected in 41 CYP2A6 products (Table 3), which is not surprising because these polymorphisms are rare in Asian and Japanese populations (13, 16). Although we could not detect those alleles in our PCR-RFLP analysis (Table 1 and 3), the incidence of CYP2A6*4/*4 in 41 cases (9.8%; 4/41) seemed to be higher than that in 894 healthy controls (3.7%; 33/894) ($p =$

0.12) (Table 3). Cases with *CYP2A6**4/*4 is difficult to metabolize the nicotine, resulting in a high level of plasma nicotine when they intake nicotine by smoking (46). Cases with *CYP2A6**4/*4 may feel nicotine aversive effects strongly and continuously (17). Therefore, the apparent high frequency of *CYP2A6**4/*4 in the smoking cessation program compared with control suggests a possibility that cases with *CYP2A6**4/*4 might tend to become the feeling of wish to quit smoking.

As seen in Table 4, no significant difference was observed between the incidences of *CYP2A6**1 positive and *CYP2A6**4/*4 cases in SCG and TNP. However, the smoking cessation rate of cases with *CYP2A6**4/*4 seemed to be lower than that with *CYP2A6**1 positive. Cases with *CYP2A6**4/*4 may be more sensitive to nicotine adverse effects although three cases with *CYP2A6**4/*4 were treated by nicotine patch for 56 days.

In our study, age and smoking years in cases with *CYP2A6**4/*4 are significantly higher than those in cases with *CYP2A6**1 positive (Table 5). The smoking cessation rate of cases with *CYP2A6**4/*4 is likely to be lower than that with *CYP2A6**1 positive (Table 4). Cases with *CYP2A6**4/*4 might be hard to quit smoking when they have smoking habit. At first, Pianezza, *et al.* showed that the *CYP2A6* polymorphism related to smoking in 1998 (47). Their study revealed that cases with variant alleles smoked fewer cigarettes per weeks than those with wild alleles (*CYP2A6**1), and suggested that variant alleles provided a protective effect against nicotine dependence (47). In contrast, some studies reported that smoking behavior may not be related with variant *CYP2A6* alleles (24, 26, 28, 48-52). Carter, *et al.* examined 11 studies providing information on *CYP2A6* genotyping in smokers or non-smoking control subjects to help resolve these conflicting results. This meta-analysis failed to find any empirical evidence of a relationship between variant *CYP2A6* alleles and smoking status (n=4091) or cigarette consumption (n=1537) (9). Our study demonstrates the first example of the relationship between *CYP2A6* polymorphism and smoking cessation.

Fagerstrom test is well known as an indicator of nicotine dependence and also has the correlations with other proposed measures of nicotine dependence, such as carbonyl monoxide in the expiration and nicotine and cotinine levels in serum (53). Scores of Fagerstrom test are also related with NRT outcome although scores of Fagerstrom test are only weakly connected with symptoms of nicotine withdrawal (54). In this study, scores of Fagerstrom test and CO concentration in the expiration in SCG seem to be higher than those in RSG because 44 of 61 (72.1%) smokers were treated with nicotine patch. No significant difference was observed between Fagerstrom Test for Nicotine Dependence (FTND) scoring of participants with *CYP2A6**1 positive and *CYP2A6**4/*4 (Table 5). In questions 2 and 6 of FTND, the scores of participants with *CYP2A6**4/*4 might be lower than that with *CYP2A6**1 positive (Table 6). Since the nicotine clearance is slowest among *CYP2A6* polymorphisms, *CYP2A6**4/*4 may keep a high level of serum nicotine after smoking from several hours to all day long.

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Abbreviations: CYP; cytochrome P450, FTND; Fagerstrom test for nicotine dependence, CO; carbon monoxide, NRT; Nicotine replacement therapy, SCG; smoking cessation group, RSG; re-smoking group, TNPG;

treatment of nicotine patch group, TNTPG; no treatment of nicotine patch group

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