GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association of *HTTLPR* and *5-HT$_{2A}$* T102C polymorphisms with smoking characteristics and anthropometric profiles of Thai males

K. Suriyaprom[1], B. Phonrat[2], U. Chuensumran[3], A. Tungtrongchitr[4] and R. Tungtrongchitr[5]

[1]Faculty of Medical Technology, Rangsit University, Pathumthani, Thailand
[2]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
[3]Department of Food Science and Technology, School of Culinary Arts, Suan Dusit Rajabhat University, Bangkok, Thailand
[4]Department of Parasitology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand
[5]Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Corresponding author: R. Tungtrongchitr
E-mail: tmrtg@mahidol.ac.th

Genet. Mol. Res. 11 (4): 4360-4369 (2012)
Received February 15, 2012
Accepted June 25, 2012
Published October 15, 2012
DOI http://dx.doi.org/10.4238/2012.October.15.2

ABSTRACT. Nicotine increases serotonin release in the brain. Gene polymorphisms in the serotonergic system have been suggested to be associated with smoking behavior. We investigated a possible association between two polymorphisms in the serotonergic system - *HTTLPR* of a serotonin transporter gene and *5-HT$_{2A}$* at position T102C - with biochemical and anthropometric parameters, and with cigarette smoking in an investigation of 200 smokers and 111 non-smokers. The two polymorphisms, *HTTLPR* and *5-HT$_{2A}$* at position T102C, were genotyped by PCR-RFLP. They were not significantly associated with smoking status in these Thai males. Among the smokers, thiocyanate concentrations and quantity of cigarettes smoked (cigarette pack-years) were significantly higher for individuals with LL/LS genotypes than SS

genotypes of *5-HTTLPR* (all $P < 0.05$), whereas "age at starting smoking" and "duration of smoking" were not significantly different between these two genotypes. Moreover, anthropometric variables, comprising triceps skinfold thickness, arm circumference, waist circumference, hip circumference, and waist-to-hip ratio, were significantly higher for the CC/TC genotypes of *5-HT*$_{2A}$ than the TT genotype (all $P < 0.05$), except for body mass index. *HTTLPR* and *5-HT*$_{2A}$ T102C polymorphisms were not significantly associated with smoking status among Thai males; however, the *HTTLPR* polymorphism among smokers appears to be an indicator of increased smoking intensity consisting of cigarette pack-years and thiocyanate concentrations. The *5-HT*$_{2A}$ T102C polymorphism plays a role in the anthropometric profiles, triceps skinfold thickness, arm circumference, waist circumference, hip circumference, and waist-to-hip ratio, but not smoking status in Thai subjects.

Key words: *HTTLPR* polymorphism; *5-HT*$_{2A}$ T102C polymorphism; Anthropometric profile; Smoking characteristics

INTRODUCTION

Nicotine is a primary component of cigarettes and the addictive component of tobacco (Zeidler et al., 2007). Although environmental factors may contribute to smoking, scientific evidence also supports a role for genetic influences on smoking behavior and the ability to quit smoking (Batra et al., 2003; Li et al., 2003). Nicotine elevates the secretion of serotonin in the brain (Mihailescu et al., 1998; Tyndale, 2003). Therefore, variations in the serotonergic system, including serotonin-producing cells, serotonin transporter, and serotonin receptors, may influence some aspects of smoking behavior (Tyndale, 2003). Moreover, nicotine withdrawal decreases serotonin levels and a selective serotonin reuptake inhibitor antagonizes the response to nicotine (Ishikawa et al., 1999). The serotonin transporter, 5-HTT, has attracted the attention of researchers, because it controls the duration and concentration of serotonin neurotransmission in the synaptic cleft. A polymorphism in the promoter region of the serotonin transporter gene, *HTTLPR*, is related to altered transcriptional efficacy (Heils et al., 1996). However, previous investigations of the association between this polymorphism and smoking behavior have yielded contradictory results (Lerman et al., 1998; Ishikawa et al., 1999; Chu et al., 2009). Therefore, in this study, we looked for an association between the *HTTLPR* polymorphism and smoking behavior among Thai males.

The serotonin 2A receptor gene (*5-HT*$_{2A}$), located on chromosome 13q14-21, has been shown to be particularly responsive to environmental conditions (Keltikangas-Jarvinen and Salo, 2009). The *5-HT*$_{2A}$ gene has also been linked to emotional disorders and alcohol dependence; these two disorders are related to smoking behavior (Abdolmaleky et al., 2004; Kato et al., 2009; Polina et al., 2009). The T102C polymorphism (rs6313) is located in exon 1, near the gene's promoter (Chen et al., 1992); it does not change the amino acid sequence of the 5-HT$_{2A}$ receptor and, thus, may play a role in gene regulation. Postmortem brain studies have found that the C allele of T102C is associated with lower 5-HT$_{2A}$ mRNA and lower protein expression compared to the T allele (Polesskaya and Sokolov, 2002). Previous studies have reported an association between the T102C polymorphism in the *5-HT*$_{2A}$ gene and smoking status (do Prado-Lima et al., 2004; White et al., 2011), but not all studies have consistently produced the same

findings (Terayama et al., 2004; Huang et al., 2005). Therefore, genetic variations in *5-HT*$_{2A}$ among smokers require clarification. The aims of the present study were to investigate the association between two polymorphisms in the serotonergic system - *HTTLPR* of the serotonin transporter gene and *5-HT*$_{2A}$ at position 102 - and biochemical and anthropometric parameters, and to determine whether these polymorphisms are linked to cigarette smoking.

MATERIAL AND METHODS

Subjects

Following approval by the Ethics Committee of Rangsit University, a convenience sample of 311 Thai volunteers (aged 21 to 62 years) were recruited from urban and suburban residential areas of Bangkok, Thailand. The sample excluded subjects taking regular medication, those with psychiatric or neurological diseases, including substance dependence, or a history of diabetes mellitus or liver, kidney or cardiovascular disease or allergy, confirmed by appropriate physical examination and laboratory tests. No subject was taking psychoactive medications or anti-inflammatory drugs. Of these subjects, 200 were smokers, while the remaining 111 were non-smokers. The volunteers were interviewed about lifestyle pattern and medical history. Smoking characteristics - age at onset of smoking, number of cigarettes smoked, and duration of smoking (in years) - were recorded in detail. Cigarette pack-years were computed as duration of smoking (years) multiplied by the number of smoked cigarettes, divided by 20.

Anthropometric measurements

Anthropometric measurements, comprising weight, height, triceps skinfold thickness, arm circumference, waist circumference (WC), and hip circumference, were determined for each subject. The waist-to-hip ratio (W/H ratio) was calculated using the circumferences of the waist and hip. Body mass index (BMI) was determined and expressed as weight (kg)/height (m^2).

Biochemical measurements

Venous blood (10 mL) was taken from the subjects in the morning, after overnight fasting. Glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured using enzymatic methods by DADE Dimension® AR. Serum thiocyanate concentrations were assessed using the colorimetric method (Degiampietro et al., 1987). Low-density lipoprotein cholesterol (LDL-C) was estimated by the Friedewald formula, as follows:

$$\text{LDL-C} = \text{total cholesterol} - \text{HDL-C} - (\text{triglycerides} / 5)$$

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique

DNA was extracted from EDTA-treated whole blood by the Flexi Gene DNA kit (QIAGEN, Hilden, Germany). DNA fragments of the gene polymorphisms, *HTTLPR*

of the serotonin transporter gene and *5-HT*$_{2A}$ T102C, were amplified by PCR (PE Applied Biosystems).

HTTLPR was typed by PCR, as follows: forward primer - 5'-GGCGTTGCCGCTCTG AATTGC-3' and reverse primer - 5'-GAGGGACTGAGCTGGACAACAAC-3'; PCR was performed in 50 μL according to the protocol previously described (Heils et al., 1996). However, *HTTLPR* is known to be extremely difficult to genotype. Yonan et al. (2006) showed that magnesium concentration affects PCR amplification and the long allele of *HTTLPR* amplifies poorly at higher Mg concentrations. Therefore, in our study, the Mg concentration was reduced to 1.0 mM. The L allele of *HTTLPR* was denoted by the presence of a 528-bp fragment and the S allele by the presence of a 484-bp fragment.

For analysis of the T102C polymorphism of the *5-HT*$_{2A}$ receptor gene, forward primer - 5'-TCTGCTACAAGTTCTGGCTT-3' and reverse primer - 5'-CTGCAGCTTTTTCTCTAG GG-3', were used; PCR was conducted in 50 μL according to the protocol described by Coto et al. (2003). Each reaction product was digested with the restriction enzyme *Msp*I, and electrophoresed on a 3% (w/v) agarose gel. Alleles were visualized as fragments of 342 bp (102T) and 216 and 126 bp (102C).

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 11.5 (SPSS, Chicago, IL, USA). Median and 95% confidence interval (95%CI) were calculated. The difference between the two groups was compared by the Mann-Whitney U-Wilcoxon rank sum W-test. Statistical differences in genotype distributions between the two groups were assessed by the chi-square test. $P < 0.05$ was considered to be statistically significant.

RESULTS

The median and 95%CI of the age and anthropometric-biochemical measurements for the smokers and non-smokers are shown in Table 1. The anthropometric and biochemical variables, and the ages of the smokers were not significantly different from the non-smokers. Table 2 shows the median and 95%CI for age and anthropometric measurements for all subjects, by genotype. With regard to the T102C polymorphism of *5-HT*$_{2A}$, there was no difference in age. Anthropometric variables were significantly higher in the CC/TC genotypes than the TT genotype, except for BMI. Subjects with the CC/TC genotypes tended to have higher BMI than those with the TT genotype. For the *5-HTTLPR* polymorphism, no differences were found for age or anthropometric parameters between LL/LS and SS genotypes. Table 3 shows median and 95%CI of the biochemical measurements for the *5-HT*$_{2A}$ T102C and *5-HTTLPR* genotypes. No significant differences were found in biochemical levels between the two genotypes related to these two polymorphisms. The frequency of the *5-HT*$_{2A}$ polymorphism at position 102 for all subjects is shown in Table 4. The distribution of *5-HT*$_{2A}$ T102C genotypes was in line with the Hardy-Weinberg principle ($P = 0.425$). Polymorphism at this position showed no significant relationship with smoking status ($P = 0.340$). Table 5 shows the frequency of *5-HTTLPR* genotypes among the smokers and non-smokers. The *5-HTTLPR* genotypes were in Hardy-Weinberg equilibrium ($P = 0.642$). The *5-HTTLPR* genotypes were not significantly different between smokers and non-smokers ($P = 0.412$). The results after analyzing the as-

sociation between smoking characteristics and the two polymorphisms among the smokers are summarized in Table 6. Smoking characteristics were not significantly associated with the *5-HT$_{2A}$* T102C polymorphism. For the *5-HTTLPR* polymorphism, thiocyanate concentrations and quantity of cigarettes smoked (cigarette pack-years) were significantly higher for the LL/LS than the SS genotype, whereas "age at starting smoking" and "duration of smoking" were not significantly different between these two genotypes (Table 6).

Table 1. Median and 95% confidence interval (95%CI) for age and anthropometric-biochemical measurements among smokers and non-smokers.

Variables	Total subjects (N = 311)				P[a]
	Smokers (N = 200)		Non-smokers (N = 111)		
	Median	95%CI	Median	95%CI	
Age (years)	37.0	34.0-40.0	36.0	33.5-38.0	0.080
BMI (kg/m^2)	22.9	22.0-23.5	23.1	22.3-24.1	0.224
AC (cm)	28.5	28.0-29.5	29.0	28.0-29.8	0.913
TSF (mm)	10.8	9.2-12.2	12.0	10.9-13.4	0.278
WC (cm)	80.0	78.0-83.0	83.0	80.0-85.0	0.088
HC (cm)	94.9	94.0-96.7	95.0	94.0-97.5	0.539
W/H ratio	0.85	0.84-0.87	0.87	0.85-0.88	0.117
Glucose (mg/dL)	84.0	81.0-87.0	80.0	79.0-84.0	0.229
Total cholesterol (mg/dL)	200.0	190.0-210.0	203.0	195.0-210.0	0.665
Triglycerides (mg/dL)	120.0	111.0-133.0	126.0	115.0-132.0	0.732
HDL-C (mg/dL)	50.0	47.0-52.0	51.0	49.0-53.0	0.323
LDL-C (mg/dL)	119.0	113.0-128.0	129.0	117.0-137.0	0.202
AST (U/L)	33.0	30.0-37.0	32.0	29.0-34.0	0.425
ALT (U/L)	31.0	29.0-34.0	33.0	30.0-36.0	0.131

[a]Compared by the Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed). BMI = body mass index; AC = arm circumference; TSF = triceps skinfold thickness; WC = waist circumference; HC = hip circumference; W/H = waist-to-hip ratio; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Table 2. Median and 95% confidence interval of age and anthropometric measurements, by genotype of *5-HT$_{2A}$* at position T102C and *5-HTTLPR*.

Variables	*5-HT$_{2A}$* T102C		P	*5-HTTLPR*		P
	TT (N = 157)	CC/TC (N = 154)		SS (N = 152)	LL/LS (N = 159)	
Age (years)	36.0	37.0	0.973	36.0	37.5	0.460
	33.6-39.8	34.0-40.0		33.4-37.6	35.0-40.0	
BMI (kg/m^2)	22.1	23.8	0.080	22.8	23.1	0.183
	21.7-23.5	22.7-24.1		21.9-23.7	22.4-23.8	
AC (cm)	28.0	29.0	0.025*	29.0	28.5	0.762
	27.0-28.9	28.5-30.0		28.0-30.0	28.0-29.4	
TSF (mm)	10.2	12.0	0.014*	11.0	12.0	0.122
	9.2-12.0	11.0-13.8		9.5-12.0	10.5-13.0	
WC (cm)	80.0	84.0	0.020*	81.0	83.0	0.078
	78.0-83.0	81.0-86.0		78.0-84.0	80.0-85.0	
HC (cm)	95.0	97.0	0.024*	95.0	96.5	0.260
	93.0-96.0	94.5-98.0		94.0-96.0	94.0-97.0	
W/H ratio	0.851	0.871	0.045*	0.851	0.872	0.140
	0.840-0.870	0.852-0.880		0.840-0.870	0.850-0.880	

*P < 0.05 compared by the Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed). BMI = body mass index; AC = arm circumference; TSF = triceps skinfold thickness; WC = waist circumference; HC = hip circumference; W/H = waist-to-hip ratio.

Table 3. Median and 95% confidence interval of biochemical parameters, by genotype of *5-HT*$_{2A}$ at position T102C and *5-HTTLPR*.

Variables	*5-HT*$_{2A}$ T102C		P	*5-HTTLPR*		P[a]
	TT (N = 157)	CC/TC (N = 154)		SS (N = 152)	LL/LS (N = 159)	
Glucose (mg/dL)	83.0 80.0-86.0	82.0 79.0-86.5	0.451	83.0 79.0-86.0	83.0 80.0-86.0	0.757
Total cholesterol (mg/dL)	198.0 190.0-209.0	203.0 197.0-213.0	0.156	202.0 189.0-211.0	201.0 194.0-210.0	0.700
Triglycerides (mg/dL)	119.0 112.0-129.0	127.0 110.0-135.0	0.649	124.0 113.0-134.0	123.0 110.0-131.0	0.704
HDL-C (mg/dL)	51.0 48.0-53.0	51.0 49.0-52.0	0.288	51.0 50.0-54.0	51.0 48.0-52.0	0.525
LDL-C (mg/dL)	121.0 113.0-130.0	126.0 117.0-134.0	0.233	122.0 114.0-131.0	126.0 116.0-131.0	0.699
AST (U/L)	32.0 29.0-36.0	33.0 29.0-36.0	0.747	33.0 29.0-35.0	33.0 30.0-36.0	0.297
ALT (U/L)	31.0 28.0-34.0	32.0 30.0-34.0	0.750	30.0 27.0-33.0	33.0 30.0-36.0	0.062

[a]Compared by the Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed). HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Table 4. Genotype frequencies of *5-HT*$_{2A}$ T102C in all subjects (N = 311).

5-HT$_{2A}$ T102C	Genotype frequencies		P[a]
	Smoking (N = 200)	Non-smoking (N = 111)	
CC/TC	30.55% N = 95	18.97% N = 59	0.340
TT	33.76% N = 105	16.72% N = 52	

[a]Compared by the chi-square test.

Table 5. Genotype frequencies of *5-HTTLPR* in all subjects (N = 311).

5-HTTLPR	Genotype frequencies		P[a]
	Smoking (N = 200)	Non-smoking (N = 111)	
LL/LS	34.41% N = 107	17.36% N = 54	0.412
SS	29.90% N = 93	18.33% N = 57	

[a]Compared by the chi-square test.

Table 6. Median and 95% confidence interval of variables related to smoking characteristics, by genotype of *5-HT*$_{2A}$ at position T102C and *5-HTTLPR*.

Variables	*5-HT*$_{2A}$ T102C		P	*5-HTTLPR*		P
	TT (N = 105)	CC/TC (N = 95)		SS (N = 93)	LL/LS (N = 107)	
Thiocyanate (μM)	50.0 39.1-60.0	51.5 41.9-61.4	0.734	41.6 31.3-56.9	57.6 48.0-68.1	0.027*
Quantity of cigarettes smoked (cigarette pack-years)	11.0 8.0-14.8	13.0 11.0-15.0	0.188	10.8 7.8-13.4	14.0 11.2-18.6	0.037*
Age at starting smoking (years)	18.0 18.0-19.0	18.0 17.0-19.0	0.310	18.0 17.0-19.0	18.0 18.0-19.0	0.900
Duration of smoking (years)	19.0 15.0-20.0	20.0 15.0-22.0	0.521	19.0 15.0-21.0	20.0 15.0-21.8	0.325

*$P < 0.05$ compared by the Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed).

DISCUSSION

Nicotine raises serotonin release in the brain, and the symptoms of nicotine withdrawal may be modulated by diminished serotonergic neurotransmission (Ishikawa et al., 1999). The *5-HTT* gene has attracted the attention of researchers because it controls the duration and level of serotonin neurotransmission in the synaptic cleft. A polymorphism in the promoter region of the serotonin transporter gene, *5-HTTLPR*, showed functionally important 44-bp insertion/deletion alleles: long (L) and short (S) (Murphy et al., 2004). The S allele (consisting of 14 repeat elements) is related to reduced *5-HTT* transcription, compared to the L allele (16 repeat elements), resulting in diminished transporter levels and decreased serotonin reuptake efficiency (Lesch et al., 1996; Greenberg et al., 1999). However, previous studies on the association between the *5-HTTLPR* polymorphism and smoking behavior have yielded contradictory results. The studies by Ishikawa et al. (1999) and Chu et al. (2009) showed that the L allele was significantly more prevalent among smokers than non-smokers, while smokers who were homozygous for the S allele were more successful in quitting smoking. Other studies reported that smokers who were heterozygous or homozygous for the S allele were more likely to be dependent on nicotine than subjects who were homozygous for the L allele (Lerman et al., 2000; Gerra et al., 2005). Munafo et al. (2006) predicted that the short carriers (S allele) of the *5-HTTLPR* polymorphism would be associated with a decreased likelihood of successful cessation of smoking. The current study's findings among Thais agree with two studies of Caucasians and African-Americans (Lerman et al., 1998; Iordanidou et al., 2010), which found no association between the *5-HTTLPR* polymorphism and smoking. Interestingly, a large genome-wide association study, aiming to identify genes related to smoking behavior, did not detect any association between the *5-HTT* gene and starting to smoke or currently smoking (Vink et al., 2009). However, the current study found a significant association between the *5-HTTLPR* polymorphism and greater smoking intensity, and that thiocyanate levels were significantly higher among smokers with the L allele than the SS genotype. These findings were similar to a study of male Chinese smokers (Chu et al., 2009). Tobacco smoke contains high concentrations of hydrogen cyanide gas, which is primarily metabolized to thiocyanate, and serum thiocyanate was potentially a better biochemical index of smoking status (Degiampietro et al., 1987). The L allele of *5-HTTLPR* likely expresses a higher number of 5-HTT sites on the serotonergic synapses (Heils et al., 1997), and this could result in higher serotonin reuptake activity, which could lead to decreased serotonin availability in the synaptic cleft. Therefore, individuals with the L allele may be vulnerable to a higher intensity of cigarette smoking. Possible reasons for the contradiction between the various studies may include differences in ethnic background, cultural background, and subject recruitment methods.

Nicotine exerts direct and indirect effects on a range of receptor systems, including the serotonergic system. 5-HT_{2A} receptor antagonism could facilitate the cessation of tobacco smoking by relieving nicotine-withdrawal symptoms (Zaniewska et al., 2010). Some studies have indicated that the T allele of the *5-HT_{2A}* T102C polymorphism (and, by analogy, the A allele at -1438 in the regulatory region of *5-HT_{2A}*) is linked to higher 5-HT_{2A} receptor binding, but lower receptor binding related to the C allele, based on postmortem brain samples (Turecki et al., 1999). The T102C polymorphism in *5-HT_{2A}* has been associated with several psychiatric disorders, including schizophrenia, alcoholism, and smoking addiction (Hwu and Chen, 2000;

do Prado-Lima et al., 2004; Ebdrup et al., 2011), while anti-psychotic agents act as antagonists at the 5-HT$_{2A}$ receptor (Ebdrup et al., 2011). Previous data on the relationship between the *5-HT$_{2A}$* T102C polymorphism and smoking behavior are inconsistent. Some studies have suggested that the *5-HT$_{2A}$* polymorphism is linked to cigarette smoking (do Prado-Lima et al., 2004; White et al., 2011), while others have failed to replicate this finding (Terayama et al., 2004; Huang et al., 2005). White et al. (2011) reported that young Caucasian adults with the TT genotype have a greater likelihood of being a current smoker compared to the CC genotype, whereas a Brazilian study found that the CC genotype was associated with current cigarette smoking status (do Prado-Lima et al., 2004). Our findings were consistent with studies of UK adolescents and the Japanese population, in which the T102C genotype was not found to be associated with smoking status (Terayama et al., 2004; Huang et al., 2005). Erritzoe et al. (2009) also supported the contention that cerebral cortex 5-HT$_{2A}$ binding did not significantly correlate with tobacco use. Therefore, our data suggest that the *5-HT$_{2A}$* gene polymorphism at position T102C may not be associated with risk of developing smoking behavior.

The serotonergic system is involved in the regulation of feeding behaviors and satiety control in the central nervous system (CNS). 5-HT$_{2A}$ receptors are found in CNS areas involved in energy balance (Barnes and Sharp, 1999). The function of 5-HT$_{2A}$ in regulating food intake has been shown in animal models, where specific 5-HT$_{2A}$ agonists decrease neuropeptide Y-stimulated food intake (Currie and Coscina, 1998). Polesskaya and Sokolov (2002) showed that the C allele of the 5-HT$_{2A}$ T102C polymorphism was related to lower 5-HT$_{2A}$ mRNA levels. *5-HT$_{2A}$* gene polymorphisms at position 102 influence an individual's body weight (Lane et al., 2006). Rosmond et al. (2002) found that the A(-1438)G polymorphism of the *5-HT$_{2A}$* gene can increase body mass and abdominal distribution of body fat. Interestingly, our results also verified an association between the *5-HT$_{2A}$* T102C polymorphism and anthropometric alterations in a Thai sample. The C allele was associated with higher abdominal obesity measured as WC and W/H ratio. Therefore, our results suggest that the *5-HT$_{2A}$* T102C polymorphism may influence metabolic abnormalities involved in serotonergic system function.

In conclusion, this is the first study to investigate any association between smoking and *HTTLPR* of the serotonin transporter gene and *5-HT$_{2A}$* T102C among Thai subjects. The *HTTLPR* and *5-HT$_{2A}$* T102C polymorphisms were not found to be associated with smoking status among Thai males. However, *HTTLPR* among smokers was significantly related to greater smoking intensity. *5-HT$_{2A}$* T102C was also significantly associated with changes in anthropometric variables. Further studies should fully evaluate the other polymorphic serotonergic genes involved in serotonin transmission, and their relation to smoking behaviors. A genetic tendency to smoke tobacco is probably influenced by many genes, each contributing a small part to the overall risk of smoking.

ACKNOWLEDGMENTS

Research supported by the Thai Health Professional Alliance Against Tobacco (#52-00-0882-10/2553) and the Rangsit University, Thailand, and an NRU grant through the Center for Biopharmaceutical Development and Innovative Therapy, Mahidol University. We sincerely thank the volunteers, staff of the Faculty of Medical Technology, Rangsit University, and the staff of the Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, for their cooperation in this research, and Mr. Paul Adams for reading the manuscript.

REFERENCES

Abdolmaleky HM, Faraone SV, Glatt SJ and Tsuang MT (2004). Meta-analysis of association between the T102C polymorphism of the 5HT2a receptor gene and schizophrenia. *Schizophr. Res.* 67: 53-62.

Barnes NM and Sharp T (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* 38: 1083-1152.

Batra V, Patkar AA, Berrettini WH, Weinstein SP, et al. (2003). The genetic determinants of smoking. *Chest* 123: 1730-1739.

Chen K, Yang W, Grimsby J and Shih JC (1992). The human 5-HT2 receptor is encoded by a multiple intron-exon gene. *Brain Res. Mol. Brain Res.* 14: 20-26.

Chu SL, Xiao D, Wang C and Jing H (2009). Association between 5-hydroxytryptamine transporter gene-linked polymorphic region and smoking behavior in Chinese males. *Chin. Med. J.* 122: 1365-1368.

Coto E, Reguero JR, Alvarez V, Morales B, et al. (2003). 5-Hydroxytryptamine 5-HT2A receptor and 5-hydroxytryptamine transporter polymorphisms in acute myocardial infarction. *Clin. Sci.* 104: 241-245.

Currie PJ and Coscina DV (1998). 5-Hydroxytryptaminergic receptor agonists: effects on neuropeptide Y potentiation of feeding and respiratory quotient. *Brain Res.* 803: 212-217.

Degiampietro P, Peheim E, Drew D, Graf H, et al. (1987). Determination of thiocyanate in plasma and saliva without deproteinisation and its validation as a smoking parameter. *J. Clin. Chem. Clin. Biochem.* 25: 711-717.

do Prado-Lima PA, Chatkin JM, Taufer M, Oliveira G, et al. (2004). Polymorphism of 5HT2A serotonin receptor gene is implicated in smoking addiction. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 128B: 90-93.

Ebdrup BH, Rasmussen H, Arnt J and Glenthoj B (2011). Serotonin 2A receptor antagonists for treatment of schizophrenia. *Expert. Opin. Investig. Drugs* 20: 1211-1223.

Erritzoe D, Frokjaer VG, Haugbol S, Marner L, et al. (2009). Brain serotonin 2A receptor binding: relations to body mass index, tobacco and alcohol use. *Neuroimage* 46: 23-30.

Gerra G, Garofano L, Zaimovic A, Moi G, et al. (2005). Association of the serotonin transporter promoter polymorphism with smoking behavior among adolescents. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 135B: 73-78.

Greenberg BD, Tolliver TJ, Huang SJ, Li Q, et al. (1999). Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. *Am. J. Med. Genet.* 88: 83-87.

Heils A, Teufel A, Petri S, Stober G, et al. (1996). Allelic variation of human serotonin transporter gene expression. *J. Neurochem.* 66: 2621-2624.

Heils A, Mossner R and Lesch KP (1997). The human serotonin transporter gene polymorphism--basic research and clinical implications. *J. Neural Transm.* 104: 1005-1014.

Huang S, Cook DG, Hinks LJ, Chen XH, et al. (2005). CYP2A6, MAOA, DBH, DRD4, and 5HT2A genotypes, smoking behaviour and cotinine levels in 1518 UK adolescents. *Pharmacogenet. Genomics* 15: 839-850.

Hwu HG and Chen CH (2000). Association of 5HT2A receptor gene polymorphism and alcohol abuse with behavior problems. *Am. J. Med. Genet.* 96: 797-800.

Iordanidou M, Tavridou A, Petridis I, Kyroglou S, et al. (2010). Association of polymorphisms of the serotonergic system with smoking initiation in Caucasians. *Drug Alcohol Depend.* 108: 70-76.

Ishikawa H, Ohtsuki T, Ishiguro H, Yamakawa-Kobayashi K, et al. (1999). Association between serotonin transporter gene polymorphism and smoking among Japanese males. *Cancer Epidemiol. Biomarkers Prev.* 8: 831-833.

Kato M, Zanardi R, Rossini D, De Ronchi D, et al. (2009). 5-HT2A gene variants influence specific and different aspects of antidepressant response in Japanese and Italian mood disorder patients. *Psychiatry Res.* 167: 97-105.

Keltikangas-Jarvinen L and Salo J (2009). Dopamine and serotonin systems modify environmental effects on human behavior: a review. *Scand. J. Psychol.* 50: 574-582.

Lane HY, Liu YC, Huang CL, Chang YC, et al. (2006). Risperidone-related weight gain: genetic and nongenetic predictors. *J. Clin. Psychopharmacol.* 26: 128-134.

Lerman C, Shields PG, Audrain J, Main D, et al. (1998). The role of the serotonin transporter gene in cigarette smoking. *Cancer Epidemiol. Biomarkers Prev.* 7: 253-255.

Lerman C, Caporaso NE, Audrain J, Main D, et al. (2000). Interacting effects of the serotonin transporter gene and neuroticism in smoking practices and nicotine dependence. *Mol. Psychiatry* 5: 189-192.

Lesch KP, Bengel D, Heils A, Sabol SZ, et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274: 1527-1531.

Li MD, Cheng R, Ma JZ and Swan GE (2003). A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. *Addiction* 98: 23-31.

Mihailescu S, Palomero-Rivero M, Meade-Huerta P, Maza-Flores A, et al. (1998). Effects of nicotine and mecamylamine

on rat dorsal raphe neurons. *Eur. J. Pharmacol.* 360: 31-36.
Munafo MR, Johnstone EC, Wileyto EP, Shields PG, et al. (2006). Lack of association of 5-HTTLPR genotype with smoking cessation in a nicotine replacement therapy randomized trial. *Cancer Epidemiol. Biomarkers Prev.* 15: 398-400.
Murphy DL, Lerner A, Rudnick G and Lesch KP (2004). Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol. Interv.* 4: 109-123.
Polesskaya OO and Sokolov BP (2002). Differential expression of the "C" and "T" alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. *J. Neurosci. Res.* 67: 812-822.
Polina ER, Contini V, Hutz MH and Bau CH (2009). The serotonin 2A receptor gene in alcohol dependence and tobacco smoking. *Drug Alcohol Depend.* 101: 128-131.
Rosmond R, Bouchard C and Björntorp P (2002). 5-HT2A receptor gene promoter polymorphism in relation to abdominal obesity and cortisol. *Obes. Res.* 10: 585-589.
Terayama H, Itoh M, Fukunishi I and Iwahashi K (2004). The serotonin-2A receptor polymorphism and smoking behavior in Japan. *Psychiatr. Genet.* 14: 195-197.
Turecki G, Briere R, Dewar K, Antonetti T, et al. (1999). Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide. *Am. J. Psychiatry* 156: 1456-1458.
Tyndale RF (2003). Genetics of alcohol and tobacco use in humans. *Ann. Med.* 35: 94-121.
Vink JM, Smit AB, de Geus EJ, Sullivan P, et al. (2009). Genome-wide association study of smoking initiation and current smoking. *Am. J. Hum. Genet.* 84: 367-379.
White MJ, Young RM, Morris CP and Lawford BR (2011). Cigarette smoking in young adults: the influence of the HTR2A T102C polymorphism and punishment sensitivity. *Drug Alcohol Depend.* 114: 140-146.
Yonan AL, Palmer AA and Gilliam TC (2006). Hardy-Weinberg disequilibrium identified genotyping error of the serotonin transporter (SLC6A4) promoter polymorphism. *Psychiatr. Genet.* 16: 31-34.
Zaniewska M, McCreary AC, Wydra K and Filip M (2010). Effects of serotonin (5-HT)2 receptor ligands on depression-like behavior during nicotine withdrawal. *Neuropharmacology* 58: 1140-1146.
Zeidler R, Albermann K and Lang S (2007). Nicotine and apoptosis. *Apoptosis* 12: 1927-1943.