UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI PENYELIDIKAN
LAPORAN AKHIR

CANDIDATE GENES POLYMORPHISMS IN SMOKING BEHAVIOR AMONG MALAY POPULATION

PENYELIDIK

DR. RUZILAWATI ABU BAKAR

PENYELIDIK BERSAMA

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FINAL REPORT
FUNDAMENTAL RESEARCH GRANT SCHEME (FRGS)
Laporan Akhir Skim Geran Penyelidikan Asas (FRGS) IPT
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RESEARCH TITLE : Candidate gene polymorphisms in smoking behavior among Malay population
Tajuk Penyelidikan

PROJECT LEADER : Dr Ruzilawati Abu Bakar
Ketua Projek

PROJECT MEMBERS (including GRA) :
1. Dr Harmy Mohamed Yusoff
2. Dr Imran Ahmad
3. Ms Nur Iwani A. Rozak

Ahli Projek

JECT ACHIEVEMENT *(Prestasi Projek)*

ACHIEVEMENT PERCENTAGE

Project progress according to milestones achieved up to this period	**0 - 50%**	**51 - 75%**	**76 - 100%**
Percentage			99%

RESEARCH OUTPUT

Number of articles/ manuscripts/ books *(Please attach the First Page of Publication)*	**Indexed Journal**	***Non-Indexed Journal***
	1	-
Conference Proceeding *(Please attach the First Page of Publication)*	**International**	***National***
	1	-
Intellectual Property *(Please specify)*	n/a	

HUMAN CAPITAL DEVELOPMENT

Human Capital	**Number**				**Others** (please specify)
	On-going		**Graduated**		
Citizen	Malaysian	Non Malaysian	Malaysian	Non Malaysian	
PhD Student	-				
Master Student	1				
Undergraduate Student	1				
Total	2				

EXPENDITURE *(Perbelanjaan)*

C

ADDITIONAL RESEARCH ACTIVITIES THAT CONTRIBUTE TOWARDS DEVELOPING SOFT AND HARD SKILLS
(Aktiviti Penyelidikan Sampingan yang menyumbang kepada pembangunan kemahiran insaniah)

D

International

Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	1) International Environment and Health Conference, Vistana Hotel, Pulau Pinang, 7-6 June 2012.	Pusat Pengajian Sains Kesihatan, USM.
	2) The 3rd Biennial Conference of the ASEAN Region Primary Care Physicians Organization (ARPAC) Manila, Philippine 6 – 9 March 2013	The Philippine Academy of Family Physicians ,
	3) International Conference on Medical and Health Sciences, Kota Bharu, Kelantan, 22 – 24 May 2013	Pusat Pengajian Sains Perubatan, USM
	4) The XIII International Congress of Toxicology, Coex, Seoul, Korea, 30 June – 4 July, 2013	International Union of Toxicology & The Korean Society of Toxicology
	5) 6th European Conference on Tobacco or Health, Istanbul, Turkey, 26 – 28 March, 2014	Association for European Cancer Leagues

National

Activity	Date (Month, Year)	Organizer
(e.g : Course/ Seminar/ Symposium/ Conference/ Workshop/ Site Visit)	1) Intensive Course On Intermediate Statistic, Scientific Writting And Producing Quality Thesis, 16 -20 Dec 2012	Pusat Pengajian Sains Perubatan, USM
	2) Intensive Course On Basic Statistic,17 – 18 September 2013	Pusat Pengajian Sains Perubatan, USM
	3) Seminar on Droplet PCR, 26 August 2013	INFORMM, USM

OBLEMS / CONSTRAINTS IF ANY *(Masalah/ Kekangan sekiranya ada)*

n/a

COMMENDATION (*Cadangan Penambahbaikan*)

n/a

SEARCH ABSTRACT – Not More Than 200 Words (*Abstrak Penyelidikan – Tidak Melebihi 200 patah perkataan*)

Smoking behavior is influenced by both genetic and environmental factors. Genetic variables appear to play a key role in every aspect of nicotine addiction. Nicotinic acetylcholine receptors (nAChRs), dopamine receptors (DR) as well as serotonin receptor, 5-HT2A and serotonin transporter, 5-HTT are thought to play an important role in nicotine addiction of smokers. The objectives of this study was to examine the frequency of single nucleotide polymorphisms in the CHRNA4 genes (rs2273502 and rs2236196), dopamine receptors (DR), the 5-HT2A serotonin receptor and serotonin transporter, 5-HTTLPR genes among Malay smokers. The study was conducted in 248 Malay smokers and 248 Malay non-smokers. DNA was extracted from leucocytes and the allele was determined by a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The PCR product was digested with restriction enzymes. Our result suggested that the CHRNA4 (rs2273502 and rs2236196), DR, 5-HT2A and 5HTTLPR gene polymorphisms are not major determinants of smoking behavior in Malay populations

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KUMPULAN WANG PENYELIDIKAN FUNDAMENTAL
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Jumlah Geran	RM83,270.00	Ketua Projek	DR RUZILAWATI ABU BAKAR
Peruntukan (Tahun 1) 2011	RM52,020.00	Tajuk Projek	CANDIDATE GENES POLYMORPHISMS IN SMOKING BEHAVIOR AMONG MALAY POPULATION
Peruntukan (Tahun 2) 2012	RM31,250.00	Tempoh	30 BULAN (01/04/2011-30/09/2013)
		No. Akaun	203/PPSP/6171135

Kwgan	Akaun	PTJ	Projek	Peruntukan Projek	Perbelanjaan Terkumpul sehingga Tahun lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
203	11000	PPSP	6171135	17,072.00	11,937.11	5,134.89	-		-	5,134.89
203	14000	PPSP	6171135			-	-		-	-
203	15000	PPSP	6171135			-	-		-	-
203	21000	PPSP	6171135	10,000.00		10,000.00			-	10,000.00
203	22000	PPSP	6171135			-	-		-	-
203	23000	PPSP	6171135	600.00	76.21	523.79	-	83.87	83.87	439.92
203	24000	PPSP	6171135		96.00	(96.00)			-	(96.00)
203	26000	PPSP	6171135			-	-		-	-
203	27000	PPSP	6171135	31,198.00	34,823.70	(3,625.70)		13,401.41	13,401.41	(17,027.11)
203	28000	PPSP	6171135	2,000.00		2,000.00	-		-	2,000.00
203	29000	PPSP	6171135	16,400.00	20,604.50	(4,204.50)		2,229.73	2,229.73	(6,434.23)
203	35000	PPSP	6171135	6,000.00	-	6,000.00	-		-	6,000.00
203	A11102	PPSP	6171135	-	-	-			-	-
203	A11559	PPSP	6171135	-	-	-			-	-
				83,270.00	67,537.52	15,732.48	-	15,715.01	15,715.01	17.47

FRGS PROJECT FINAL REPORT
(GRANT A/C No. 203/PPSP/6171135)

PROJECT TITLE:

Candidate gene polymorphisms in smoking behavior among Malay population

PRINCIPAL INVESTIGATOR:

Dr Ruzilawati Abu Bakar

CO-INVESTIGATORS:

Dr Harmy Mohamed Yusoff
Dr Imran Ahmad

School of Medical Sciences
Universiti Sains Malaysia

KERTAS PENERBITAN

1) Nur Iwani Abd Rozak, Imran Ahmad, Harmy Mohd Yusuff and **Ruzilawati Abu Bakar** (2012) alpha-4 subunit (CHRNA4) rs2236196 polymorphism of nicotinic acetylcholine receptor (nAChR) gene among Malay smokers. Proceeding of International Environment & Health Conference 2012, 159-164.


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8

α-4 Subunit, rs2236196, of Nicotinic Acethylcholine Receptor (CHRNA4) Gene Polymorphism among Malay Smokers

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ABSTRACT: Nicotine is the major addictive substance in cigarettes and genes involved in sensing nicotine are logical candidates for nicotine addiction. One of the genes, α-4 subunit of nicotinic acethylcholine receptor (CHRNA4) rs2236196 gene was reported to be associated with nicotine dependence or smoking behavior in many populations. However, there has been no prior study reported for this gene in Malay smokers, so far. Therefore, the aim of the present study was to investigate the frequencies of CHRNA4 rs2236196 gene polymorphism among Malay smokers. The study was conducted in 105 Malay smokers. DNA was extracted from leucocytes and the allele was determined by a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The PCR product was digested with restriction enzymes *Eco47III*. In this study, 6 subjects (5.7%) were found to be mutants for CHRNA4 rs2236196 gene. Larger sample size is needed to confirm the association of this gene with smoking behavior among Malay smokers.

Keywords: CHRNA4 subunit rs2236196; Malay smokers; nicotinic acetylcholine receptors (nAChR); PCR-RFLP

Introduction

Smoking is a risk factor for the most preventable deaths in the world. Nicotine is the main factor for the addictive behavior to smoking. Previous studies have shown that genetic factors strongly influence on smoking behavior and nicotine addiction (Batra *et al.*, 2003). Nicotine addiction, like many other drug dependencies, is believed to be a complex and multifactorial behavior, with both genetic and environmental determinants (Feng *et al.*, 2004). Over the past few decades, many large-sample twin studies have yielded results consistent with an overall conclusion that genetics contribute to the risk of becoming a regular smoker (Sullivan *et al.*, 1999). A familial aggregation study among siblings of nuclear families also suggested that genetics plays an important role in determining the vulnerability to nicotine addiction (Niu *et al.*, 2000).

The behavioral and physiological effects of nicotine are mediated largely by neuronal nicotinic acetylcholine receptors (nAChRs). Molecular analyses have identified expression of nine α-subunits (α 2– α 10) of nAChRs in the central nervous system (Galzi *et al.*, 1995). α-4 subunit is the most important for nicotine-induced reward, tolerance, and sensitization (Tapper *et al.*, 2004) therefore, the gene encoding the subunit (nicotinic acetylcholine receptor subunit α-4 gene (CHRNA4)) represents logical candidate for smoking behaviors. One of the polymorphisms of

this gene, rs2236196 characterized by an A→G transition located in the 3'-untranslated region of CHRNA4 (Feng *et al.*, 2004).

Considering the evidence for ethnic differences in nicotine metabolism (Perez *et al.*, 1998; Benowitz *et al.*, 1999; Tyndale *et al.*, 2003) , in the response to smoking (Perkins *et al.*, 1999) and on the genetic influences on nicotine dependence (Li *et al.*, 2003), we were interested to investigate the association of α-4 subunit rs2236196 gene with smoking behavior in Malay smokers. Therefore, the aim of this study was to investigate the frequency of this allele in Malay smokers by using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Materials and methods

Subjects

A total of 105 male participants were recruited from Malay population. The subjects were Male smokers with age between 18 to 50 years old, all of Malay origin. All subjects were given informed consent form to participate in the study. The study was approved by Ethics Committee of School of Medical Sciences, Universiti Sains Malaysia.

DNA Extraction

Three ml of venous blood was drawn into a tube containing EDTA and stored at -20°C until the isolation of genomic DNA. Genomic DNA was isolated from the leucocytes using QIAamp DNA Blood Mini Kit (Qiagen, USA) and the allele was determined by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). DNA purity and concentration was measured by spectrophotometer, Biophotometer Uvette (Eppendorf, Germany) at 280 nm absorbance

Genotyping

For the analysis of rs2236196 polymorphism, genomic DNA was PCR-amplified in a final volume of 25µl containing 2.5 µl of 10X PCR buffer with KCl (Vivantis, Malaysia), 0.2µM/l of each primer (forward, 5'-AGCTCCACAAAACTCTGTTC-3'; reverse, 5'-AGTCTCTCAGCAGATCTG-3'), 0.2µmol/l of dNTP (Vivantis, Malaysia), 1.5mmol/l $MgCl_2$ and 2.5U *Taq* DNA polymerase (Vivantis, Malaysia). The following PCR conditions were used: an initial denaturation step for 3 min at 95°C, followed by 25 cycles of 1 min at 95°C, 30 s at 55 °C and 1 min at 72°C with a 10 min final extension at 72°C. A 326bp fragment was yielded by running on a 1% agarose gel (**FIGURE 1**) in a presence of ethidium bromide. The PCR product was digested with 1U restriction enzyme *AfeI* (Vivantis, Malaysia) for 2 hours at 37°C. Digestion of the PCR product with *AfeI* resulted in 326bp fragments for wild type sequence and 2 fragments of 200 bp and 125 bp for the rs2236196 mutant allele (**FIGURE 2**). **FIGURE 3** shows schematic representations of RFLP analysis of CHRNA4 rs2236196. The percentage of the gene polymorphisms was calculated.

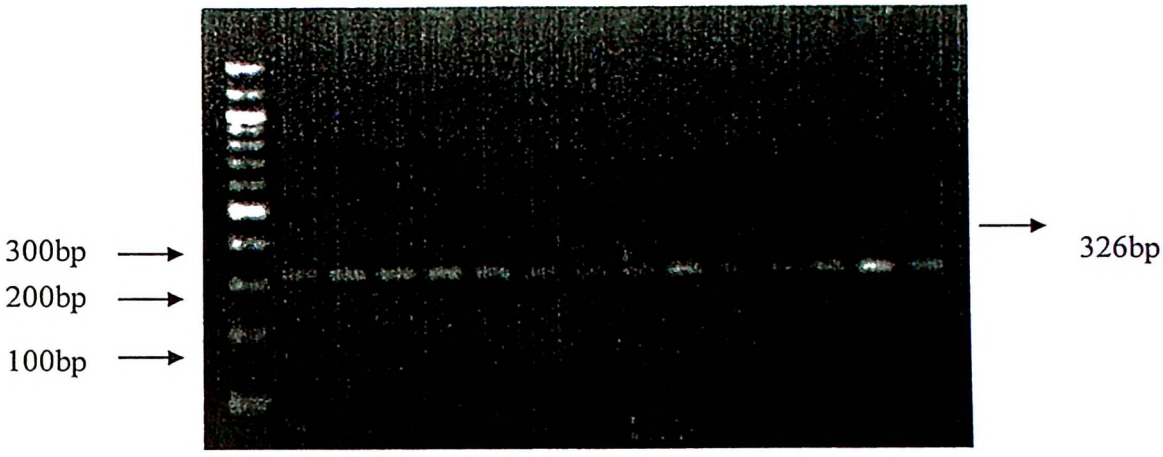

FIGURE 1: PCR products for implications using rs2236196 primers

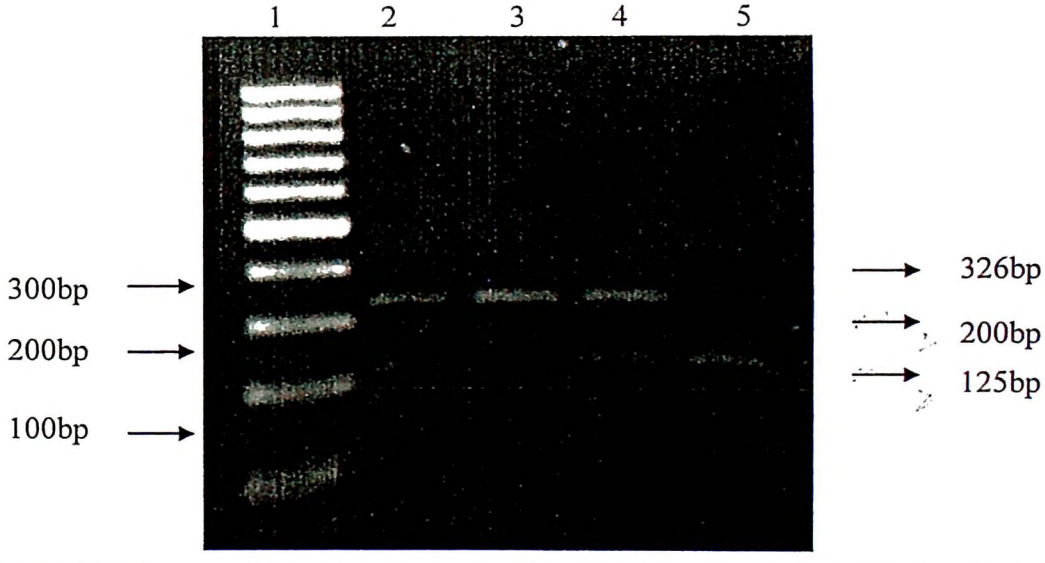

FIGURE 2: RFLP results for wild type (lanes 3) and mutant (lanes 2, 4, 5) after digestion with *AfeI*

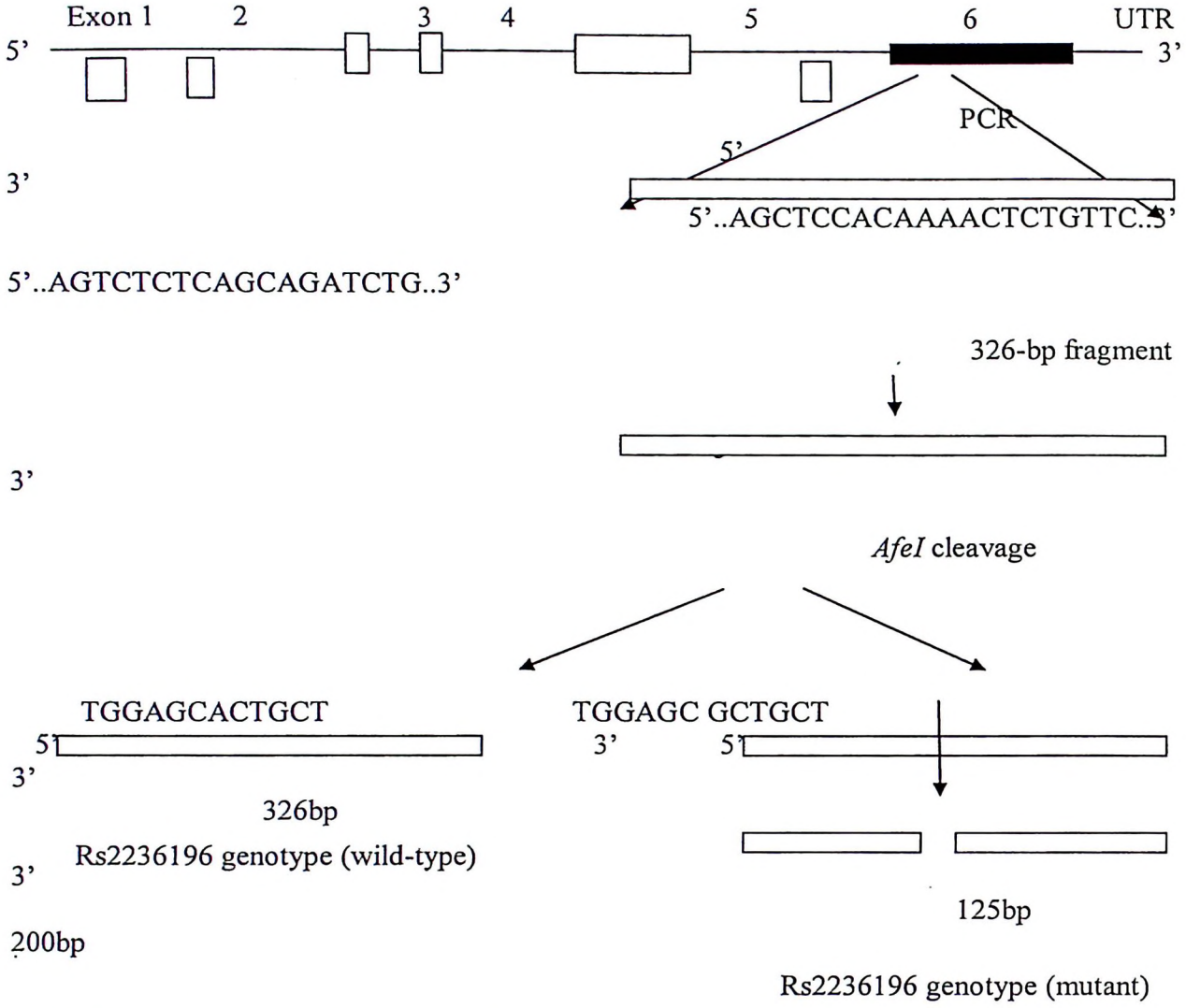

FIGURE 3: RFLP analysis of CHRNA4 rs2236196 gene. In the mutant type, *AfeI* cleaves a 326 bp fragment into 125 bp and 200bp whereas the wild type is not cleaved.

Results and Discussion

In this study, CHRNA4 rs2236196 mutations were detected in 6 (5.7%) subjects out of 105 subjects. In all reactions, correct lengths of expected PCR products were obtained. The restriction enzyme used in the present study was found to have work successfully as depicted in **FIGURE 2.**

CHRNA4 SNPs such as rs2236196 have been associated with smoking in several ethnic groups. Breitling and colleagues (2009) have observed significant association between rs2236196 and smoking-related phenotypes in 5500 Germans. Li and colleagues (2005) also identified a SNP (rs2236196) in African vs. European Americans was associated with nicotine dependence.

Robert and Frank (2011) have shown that SNP at rs2236196 in CHRNA4 can increase receptor binding and increase sensitivity to the acute effects of nicotine.

Furthermore, a SNP (rs2236196) which is located in the 3'untranslated region (UTR) was also associated with nicotine dependence and smoking behavior in African American women (Hutchison et al., 2007). Hutchison and colleagues (2007) have also identified mutation in this gene was associated with subjective responses to smoking. In their study, participants were asked to smoke a cigarette after an 8 hour abstinence period and they were rated on the effects of smoking phenotypes, including the physical, cognitive, and rewarding properties of smoking. They found that, participants with a heterozygous genotype at SNP rs2236196 exhibited an increased sensitivity to all 4 subjective measures of smoking.

In contrast, Etter *et al.* (2009) have found no association of SNP at rs2236196 in CHRNA4 with smoking status or other smoking related variables among smokers in Switzerland.

Conclusion

In conclusion, the present study is the first to provide information of α-4 subunit (CHRNA4) rs2236196 of nicotinic acethylcholine receptor (nAChR) gene polymorphism among Malay smokers. However, larger sample size is needed to confirm the association of this gene with smoking behavior among this population.

Acknowledgement

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2) Nur Iwani Abd Rozak, Imran Ahmad, Harmy Mohd Yusuff, Gan Siew Hua and **Ruzilawati Abu Bakar** (2013).

Lack of Association between Serotonin Transporter (5-HTTLPR) and Serotonin Receptor (5HT2A) Gene Polymorphisms With Smoking Behavior Among Malaysian Malays.

Have been submitted to Journal of Clinical Pharmacy and Therapeutics (ISI-Indexed: Impact Factor - 1.755).

Journal of
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Lack of Association between Serotonin Transporter (5-HTTLPR) and Serotonin Receptor (5HT2A) Gene Polymorphisms with Smoking Behavior among Malaysian Malays

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Keywords:	gene polymorphism, smoking behaviour, serotonin receptor, serotonin transporter

SCHOLARONE
Manuscripts

Lack of Association between Serotonin Transporter (*5-HTTLPR*) and Serotonin Receptor ($5HT_{2A}$) Gene Polymorphisms with Smoking Behavior among Malaysian Malays

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SUMMARY

An insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (*5-HTTLPR*) and a polymorphism in the serotonin 2A receptor gene ($5HT_{2A}$) have previously been linked to smoking habits. The objective of this study was to determine the possible association of the *5-HTTLPR* and $5HT_{2A}$ gene polymorphisms with smoking habits within a population of Malaysian male smokers (n= 248) and non-smokers (n=248). The *5-HTTLPR* genotypes were determined using polymerase chain reaction (PCR) and were classified as short (S) alleles or long (L) alleles. The $5HT_{2A}$ genotypes were determined using PCR–restriction fragment length polymorphisms (PCR-RFLP). No significant differences in the distribution frequencies of the

alleles were found between the smokers and the non-smokers for the *5-HTTLPR* polymorphism (χ^2 = 0.72, P>0.05) or the *5HT$_{2A}$* polymorphism (χ2 = 0.73, P>0.05). The *5-HTTLPR* and *5HT$_{2A}$* polymorphisms were not found to be associated with smoking behavior in Malaysian Malays.

***Keywords*: 5-HTTLPR; 5HT$_{2A}$; Genetic polymorphism; PCR-RFLP; Malay male**

BACKGROUND

Nicotine is the main addictive substance in cigarettes and is responsible for the development, as well as the maintenance, of the smoking addiction.[1] Cigarette smoking is a major preventable cause of disease. Genetic variables appear to play key roles in every aspect of nicotine addiction.[2] Therefore, genes involved in the pharmacodynamics and pharmacokinetics of nicotine are logical targets of nicotine addiction.

Serotonin (5-hydroxtryptamine, 5-HT) is a neurotransmitter derived from the amino acid tryptophan, and it has a wide range of central nervous system activities.[3] Polymorphisms affecting the serotonergic system, such as those in the serotonin transporter (*5-HTT*) and *5HT* genes, have been linked to smoking-related behaviors because nicotine increases serotonin release.[4] Therefore, variations in the serotonergic system may influence some aspects of smoking, such as mood variations during nicotine withdrawal.[5] One study showed that serotonin release was increased in the cortical region of the brain in rats treated with nicotine and that nicotine withdrawal seemed to be related to the subsequent decrease in serotonin.[4]

5-HTT controls the duration and concentration of serotonin neurotransmission in the synaptic cleft.[6] *5-HTT* is encoded by a single gene (*SLC6A4*) that is located on the long arm of chromosome 17 (17q12).[7] The *5-HTT* gene has been linked to psychological traits, such as anxiety-related personality traits[8] and depression,[9] and these traits are also related to nicotine dependence.[10,11] A 44 bp insertion or deletion polymorphism, *5-HTTLPR*, was identified within

this gene, resulting in two allelic variants, the long (L) and short (S) alleles, which alter the transcriptional efficiency of the *5-HTT* gene.[12] The S allele has been associated with reduced serotonin uptake, leading to the hypothesis that individuals with the S allele are not prone to smoking.[8] This hypothesis is supported by two studies in Japanese and Chinese populations that found that individuals with the homozygous S genotype were less likely to initiate smoking behavior and could more easily stop smoking than others.[13,14] However, the hypothesized role of the S allele in smoking behavior has not yet been confirmed; other studies that attempted to replicate these findings have obtained contradictory results,[15-18] indicating that the influence of these genes may vary from one ethnicity to another.

In contrast, *5HT* is classified into seven groups (5HT1-7); one of them, Group 2, can be further divided into three subtypes (A, B, and C). The $5HT_{2A}$ gene is located on chromosome 13q14–q21.[19] The $5HT_{2A}$ gene has been associated with emotional disorders and alcohol dependence, both of which are related to smoking behavior.[20] One of the polymorphisms that has been described in this receptor is *T102C*, which involves the substitution of a cytosine residue with a thymine residue.[19] This polymorphism has been associated with the continuation of smoking behavior but is less likely to be involved in the initiation of smoking behavior.[21] A study conducted in a Brazilian population found that individuals with the CC genotype are more likely to be current smokers than individuals with other genotypes. Interestingly, the opposite finding was obtained in Caucasian Australians; in this study, the occurrence of the TT genotype was associated with current smokers.[22] However, this finding has not been replicated in several other studies in different populations,[23-25] indicating that the influence of these genes may vary from one ethnicity to another.

The present study investigated the possible associations between the *5-HTTLPR* and $5HT_{2A}$ polymorphisms and the smoking behavior of Malaysian Malays, which has not yet been

investigated. To the best of our knowledge, this is the first study conducted on a Malaysian Malay population regarding the association of *5-HTTLPR* and $5HT_{2A}$ polymorphisms and smoking behavior. If such an association is established, it may explain why different individuals have different risks for nicotine dependence and why some individuals are able to quit smoking more easily, suggesting that specific interventional approaches should be used for certain individuals based on their genetic background.

METHODS

Subjects

A total of 496 Malaysian Malay male subjects, both smokers (n=248) and non-smokers (n=248), between 18 and 50 years old were selected. Smoking was defined as having smoked more than 100 cigarettes in a lifetime and being a current smoker at the time of the study. The subjects were confirmed to be of the Malay ethnic group for at least three generations. Only males were recruited to avoid inter-individual variations that may arise due to sex differences and hormonal changes commonly shown by females. The demographic data of the subjects are shown in Table 1. All participants gave written informed consent before they were enrolled, and the study was approved by the Ethics Committee (ethical no: USMKK/PPP/JEPeM[233.3.(05)]/Amend.01) of the School of Medical Sciences, Universiti Sains Malaysia, which complies with the Declaration of Helsinki.

DNA Extraction

Three milliliters of venous blood was drawn into a sterile tube containing EDTA and was stored at -20°C until the isolation of genomic DNA. Genomic DNA was isolated from the blood using the QIAamp DNA Blood Mini Kit (Qiagen, USA). DNA purity and concentration were determined

by measuring the absorbance at 280 nm using a Biophotometer Uvette spectrophotometer (Eppendorf, Germany).

Molecular analysis

Genotyping of the *5-HTTLPR* gene polymorphism was performed using polymerase chain reaction (PCR) as previously described by Heils et al.,[12] with some slight modifications. The PCR reaction was performed in a final volume of 25 µl containing 60 ng of genomic DNA, 200 µM dNTP mix, 0.24 µM of each primer (forward 5'-GGCGTTGCCGCTCTGAATGC-3' and reverse 5'-GAGGGACTGAGCTGGACAACCAC-3'), 0.75 mM magnesium chloride ($MgCl_2$), 1x ammonium sulfate buffer [$(NH_4)_2SO_4$] (Fermentas, Vilnius, Lithuania), and 1.25 U Taq polymerase (Fermentas, Vilnius, Lithuania). After an initial incubation at 95°C for 2 min, the PCR products were amplified for 35 cycles of denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 1 min. The final extension step lasted 7 min at 72°C. The PCR products were then resolved using a 2% agarose gel and were visualized under UV light. Each gel contained one lane of a 100 bp ladder to identify the 528 bp fragment, designated as the L allele, and the 484 bp fragment, designated as the S allele (Fig. 1).

Genotyping of the $5HT_{2A}$ polymorphism was performed as previously described[26], with several modifications. PCR was performed in a 25 µl reaction containing 78 ng of genomic DNA, 100 µM of each dNTP, 1.5 mM $MgCl_2$, 0.25 µM of each primer (forward 5'-TGTGCTACAAGTTCTGGCTT-3' and reverse 5'-GTGCAGTTTTTCTCTAGGG-3'), and 0.75 U DNA Taq polymerase (Fermentas, Vilnius, Lithuania). After an initial denaturation at 95°C for 3 min, the PCR products were amplified for 35 cycles of 1 min at 94°C, 45 s at 53°C, and 1 min at 72°C, followed by a final extension step of 10 min at 72°C. The 342 bp PCR product was digested with *Hpa* II (New England Biolabs, MA, USA) for 3 h at 37°C. The digestion products were then resolved on a 2% agarose gel containing ethidium bromide and were visualized

under UV light. The 102T mutant remained uncut (342 bp), whereas the wild type 102C allele was digested into two bands of 216 and 126 bp; three fragments of 342, 216, and 126 bp were observed for samples containing both the wild type and mutant alleles (Fig. 2).

Statistical analysis

The allele and genotype frequencies were calculated to equilibrium using the Hardy–Weinberg equation ($p^2 + 2pq + q^2 = 1$). The significance of the allele frequency or the genotype distribution among the volunteers with different smoking habits was examined using a non-parametric chi-square test. A $P<0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS package version 20.0 (IBM, Armonk, NY).

RESULTS AND DISCUSSION

The genotype and allele frequencies of the *5-HTTLPR* and *5HT$_{2A}$* polymorphisms in smokers and non-smokers are given in Table 2. The frequencies of the *5-HTTLPR* alleles S/S, L/L, and heterozygous S/L in the non-smoker population were 39.1%, 11.3%, and 49.6%, respectively, whereas in the smokers, the frequencies of the S/S, L/L, and heterozygous S/L alleles were 41.1%, 12.9%, and 46.0%, respectively. The genotype frequencies for the *5HT$_{2A}$* polymorphism in Malay smokers were 10.1% for CC, 46.8% for TT, and 43.1% for CT, whereas in the Malay non-smokers, the frequencies were 8.1% for CC, 46.4% for TT, and 45.6% for CT. No significant differences in the genotype frequencies of the *5-HTTLPR* polymorphism ($\chi2 = 0.73$, $P>0.05$) and the *5HT$_{2A}$* polymorphism ($\chi2 = 0.72$, $P>0.05$) were observed between the smoking group and the non-smoking group. The samples demonstrated Hardy–Weinberg equilibrium. Population stratification was not likely to be a confounder in this study because the samples were carefully limited to individuals with at least three generations of Malay descent.