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Smoking Genes: A Case–Control Study of Dopamine Transporter Gene (*SLC6A3*) and Dopamine Receptor Genes (*DRD1*, *DRD2* and *DRD3*) Polymorphisms and Smoking Behaviour in a Malay Male Cohort

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Received: 17 October 2020; Accepted: 30 November 2020; Published: 3 December 2020

Abstract: Dopamine receptor and dopamine transporter genes polymorphisms have been associated with cigarette smoking behaviour in different populations. The aim of this case–control study was to evaluate polymorphisms in the dopamine transporter gene (*SLC6A3* (rs27072)) and the dopamine receptor genes (*DRD1* (rs686), *DRD2* (rs1800497) and *DRD3* (rs7653787)) and their contribution to smoking behaviour in a Malay male population. We identified 476 participants over the age of 18 years comprising 238 smokers and 238 non-smokers. Information such as age, height, weight, body mass index, systolic and diastolic blood pressures, marital status, and smoking status of close family members were taken. For the genetic study, we genotyped four genes (*SLC6A3* (rs27072), *DRD1* (rs686), *DRD2* (rs1800497) and *DRD3* (rs7653787)) using the polymerase chain reaction–restriction fragment length polymorphism method and further confirmed our findings with sequencing. Dopamine receptor genes (*DRD1*, *DRD2* and *DRD3*) were found to be associated with smoking behaviour in a Malay male population. The dopamine transporter gene (*SLC6A3*) did not show this association. Significant differences were observed between smokers' and non-smokers' age, systolic blood pressure, marital status and family members who smoke. Smoking behaviour is significantly influenced by genetic variations of *DRD1*, *DRD2* and *DRD3* in a Malay male population.

Keywords: smoking behaviour; dopamine transporter; dopamine receptor; gene; polymorphism

1. Introduction

Tobacco smoking is highly addictive because of nicotine. The role of genetics in nicotine addiction supports the known associations of smoking behaviour and cessation with genetic variations [1–4]. Nicotine continuously exerts "rewarding effects" by increasing the production of dopamine in the nucleus accumbens of the brain and thus alters the mesolimbic dopamine pathway [3,5,6]. Interestingly, nicotine addiction has been significantly associated with polymorphisms in the dopamine receptor or dopamine transporter genes, which play roles in the dopamine pathway [7,8]. These genes encode for the proteins involved in the synthesis or metabolism of dopamine [8].

For instance, the dopamine transporter gene *SLC6A3* (previously known as *DAT1*; genomic location: 5p15.33, 52,651 bases) encodes a sodium- and chloride-dependent neurotransmitter that pumps the neurotransmitter dopamine out of the synaptic cleft back into the cytosol where vesicular monoamine transporter 2 confiscates it into vesicles for storage and further release [9]. The 3′ untranslated region (UTR) of *SLC6A3* contains 3–11 copies of a 40 bp tandem repeat. Variation in the number of repeats is associated with dependence on alcohol and cocaine and protection against nicotine dependence [10,11]. Polymorphisms in the coding regions of *SLC6A3* (rs27072) have been associated with bipolar disorder and tobacco addiction [12–14]. Some dopamine receptor genes, such as *DRD1* [15], *DRD2* [16,17], *DRD3* [18,19], *DRD4* [18] and *DRD5* [20], have been associated with smoking behaviour in various populations. All five of the dopamine receptor genes are G-protein-coupled receptors.

DRD1 (genomic location: 5q35.2 4147 bases) encodes for the D1 subtype of the five (D1–D5) dopamine receptors, the most abundant dopamine receptor in the central nervous system (CNS) [21]. It functions by stimulating adenylyl cyclase and activating cyclic AMP-dependent protein kinases. The D1 subtype also mediates behavioural disorders, such as pathological gambling, schizophrenia, Tourette's syndrome, and amphetamine addiction [22–25]. Polymorphisms in this gene (rs686) have been associated with depressive symptoms, alcohol dependence, bipolar disorder, and nicotine dependence [26–28].

DRD2, previously known as *Taq1A* (genomic location: 11q23.2, 66,097 bases), encodes for the D2 subtype of the dopamine receptor and functions by inhibiting adenylyl cyclase activity. *DRD2* has been associated with some psychological disorders, such as pathological gambling, schizophrenia, and cocaine dependence, [29] and is the major receptor for most antipsychotic drugs. Specific polymorphisms in this gene (rs1800497) have been associated with mood disorders, migraine, and nicotine dependence [30,31].

DRD3 (genomic location: 3q13.31, 71,611 bases) encodes the D3 subtype of the dopamine receptor that inhibits adenylyl cyclase. Genetic variations have been associated with different neurological abnormalities, such as tremors, schizophrenia, Parkinson's disease, and drug addiction [32–34]. Polymorphisms in this gene (rs7653787) have been associated with nicotine dependence [35].

In Malaysia, the prevalence of cigarette smoking is approximately 22.5% [36]. Among the major three ethnic groups, smoking was found to be highly prevalent among Malays [37]. This study aimed to evaluate the association of gene polymorphisms in the dopamine transporter *SLC6A3* (rs27072) and dopamine receptors *DRD1* (rs686), *DRD2* (rs1800497) and *DRD3* (rs7653787) with smoking behaviour in a Malay male population.

2. Materials and Methods

2.1. Participants

Overall, 476 participants participated in this case–control study, including smokers (n = 238) and non-smokers (n = 238) as defined by their self-reported smoking status. The participants were Malay males between 18 and 50 years of age. The participants were classified as smokers based on whether they currently smoke cigarettes and had smoked >100 cigarettes in their lifetime as per the National Health Interview Survey (NHIS) [38]. Non-smokers identified themselves if they were not active smokers and had smoked <100 cigarettes in the past. Nicotine dependence was assessed using a validated Malay version of the six-item Fagerstrom Test for Nicotine Dependence [39]. Basic information such as age, height, weight, body mass index, systolic and diastolic blood pressures, marital status, and smoking status of close family members were taken. Ethics approval was obtained from the Universiti Sains Malaysia (USM/JEPeM/14110480).

2.2. Genetic Study

We collected venous blood (3 mL) from the participants for the genetic study. Genomic DNA was isolated from whole blood using a DNA extraction kit (G-spin™ Total DNA Extraction, iNtRON

Biotechnology, Seongnam, South Korea). The genotyping of the four genes (*SLC6A3* (rs27072), *DRD1* (rs686), *DRD2* (rs1800497) and *DRD3* (rs7653787)) was performed using the polymerase chain reaction (PCR)–restriction fragment length polymorphism method in a thermal cycler (Eppendorf MasterCycler Nexus Gradient, Eppendorf, Germany). The PCR reaction was conducted in a final volume of 25 µL, followed by the digestion of the PCR products with certain restriction enzymes, including MspI, TaqIA, Cac8I and PsiI. The digested fragments were then analysed on 1% agarose gel using ethidium bromide staining followed by visualisation under ultraviolet light. Some PCR products were randomly selected for sequencing using the Applied Biosystems 3730 XL Genetic Analyser (Applied Biosystems, Foster City, CA, USA) and the BigDye® Terminator v3.1 cycle sequencing kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was used for the sequence confirmation.

2.3. Data Analyses

Data were analysed using SPSS (version 24, IBM, Armonk, NY, USA) and Stata (version 16, StataCorp, College Station, TX, USA) software. The allele and genotype distributions were calculated to equilibrium using the Hardy–Weinberg equation ($p^2 + 2pq + q^2 = 1$). Non-parametric chi-square test was used to calculate the significance of the genotype and allele frequencies. Risk factors were assessed between cases and controls using both simple logistic regression (crude odds ratio (OR)) and multiple logistic regression analyses (adjusted OR). We considered a *p*-value of <0.05 to be statistically significant.

3. Results

3.1. Allele and Genotype Frequencies

We did not find a significant association either at the genotypic level ($\chi^2 = 0.88$, $p = 0.64$) or the allelic level ($\chi^2 = 0.09$, $p = 0.75$) of *SLC6A3* (rs27072) by comparing smokers to non-smokers (Tables 1 and 2; Figures S1, S2 and S9). Among the smokers, a significant difference was found in the *DRD1* (rs686) genotype ($\chi^2 = 54.70$, $p < 0.001$) and allele frequencies ($\chi^2 = 48.498$, $p < 0.001$) compared with the non-smokers (Table 1; Figures S3, S4 and S10). The prevalence of AG genotype in *DRD1* (rs686) was significantly higher in smokers in compared with non-smokers (OR: 7.07, 95% CI: 3.71–13.42; $p < 0.001$; Table 2). We found a significant association of *DRD2* (rs1800497) with smoking at the genotypic level ($p < 0.001$); however, no significant association was observed at the allelic level ($p = 0.36$; Table 2; Figures S5, S6 and S11). At the genotypic level, A1/A2 was significantly associated with smoking behaviour (OR: 4.25, 95% CI: 2.24–8.05; $p < 0.001$; Table 2). In the case of *DRD3* (rs7653787), a significant difference was found at the genotypic level ($\chi^2 = 12.81$, $p = 0.002$) but not at the allelic level ($\chi^2 = 2.62$, $p = 0.10$) among the smokers (Table 1; Figures S7, S8 and S12). Interestingly, among the different *DRD3* genotypes (CC, CT and TT), the homozygous mutant CC was detected as a significantly protective genotype (OR: 0.11, 95% CI: 0.02–0.53, $p = 0.006$) in the smokers (Table 2).

Table 1. Genotype and allele frequencies of *SLC6A3* (rs27072), *DRD1* (rs686), *DRD2* (rs1800497) and *DRD3* (rs7653787) polymorphisms in smokers and non-smokers.

Genes (SNPs)	Smoking Status	Genotype (%)			*p*-Value [a]	Allele		*p*-Value [a]
		CC	CT	TT		C	T	
SLC6A3 (rs27072)	Smoker	5 (2.1)	192 (80.7)	41 (17.2)	0.64	202 (42.4)	274 (57.6)	0.75
	Non-smoker	5 (2.1)	184 (77.3)	45 (20.6)		194 (41.5)	274 (58.5)	
		AA	AG	GG		A	G	
DRD1 (rs686)	Smoker	157 (66.0)	81 (34.0)	0 (0.0)	**<0.001**	395 (83.0)	81 (34.0)	**<0.001**
	Non-smoker	222 (93.3)	16 (6.7)	0 (0.0)		460 (96.6)	16 (6.7)	
		A1/A1	A1/A2	A2/A2		A1	A2	
DRD2 (rs1800497)	Smoker	46 (19.3)	168 (70.6)	24 (10.1)	**<0.001**	260 (54.6)	216 (45.4)	0.36
	Non-smoker	67 (28.2)	112 (47.1)	59 (24.8)		246 (51.7)	230 (48.3)	
		CC	CT	TT		C	T	
DRD3 (rs7653787)	Smoker	22 (9.2)	211 (88.7)	5 (2.1)	**0.002**	255 (53.6)	221 (46.4)	0.10
	Non-smoker	7 (2.9)	216 (90.8)	15 (6.3)		230 (48.3)	246 (51.7)	

[a] chi-square test. Significant *p*-values ($p < 0.05$) are in bold.

Table 2. Factors associated with smoking status.

Independent Variables	Crude OR [a] (95% CI)	Wald Statistics [a] (df)	*p*-Value [a]	Adjusted OR [b] (95% CI)	Wald Statistics [b] (df)	*p*-Value [b]
Age (year)	1.07 (1.05–1.09)	49.87 (1)	**<0.001**	1.09 (1.07–1.12)	56.60 (1)	<0.001
Height	0.42 (0.03–6.42)	0.39 (1)	0.531			
Weight	1.01 (1.00–1.02)	0.91 (1)	0.339			
Body mass index	1.03 (0.99–1.08)	1.85 (1)	0.174			
Systolic	0.98 (0.97–1.00)	7.17 (1)	0.007	0.96 (0.94–0.98)	21.49 (1)	<0.001
Diastolic	1.00 (0.98–1.02)	0.07 (1)	0.788			
Marital status						
Single			1.00 (Reference)			
Married	0.60 (0.41–0.88)	7.02 (1)	**0.008**			
Family members who smoke						
No			1.00 (Reference)			
Yes	0.11 (0.06–0.19)	59.33 (1)	**<0.001**			
SLC6A3 (rs27072)						
CC			1.00 (Reference)			
TT	0.83 (0.23–3.10)	0.07 (1)	0.789			
CT	1.04 (0.30–3.66)	0.00 (1)	0.947			
DRD1 (rs686)						
AA			1.00 (Reference)			
AG	7.16 (4.03–12.71)	45.20 (1)	**<0.001**	7.07 (3.71–13.42)	35.80 (1)	**<0.001**
DRD2 (rs1800497)						
A1/A1			1.00 (Reference)			
A2/A2	1.69 (0.92–3.09)	2.88 (1)	0.090	1.94 (0.94–4.01)	19.60 (1)	**<0.001**
A1/A2	3.69 (2.17–6.27)	23.17 (1)	**<0.001**	4.25 (2.24–8.05)		
DRD3 (rs7653787)						
TT			1.00 (Reference)			
CC	9.43 (2.51–35.37)	11.07 (1)	**0.001**	0.11 (0.02–0.53)	7.71 (1)	**0.006**
CT	2.93(1.05–8.21)	4.19 (1)	**0.041**	0.38 (0.14–1.02)		

OR: odds ratio. Significant *p*-values ($p < 0.05$) are in bold. [a] Simple logistic regression. [b] Multiple logistic regression.

3.2. Factors Associated with Smoking Behaviour

Interestingly, we found smoking behaviour to be associated with age ($p < 0.001$) and systolic blood pressure ($p < 0.001$; Table 2). Married males were significantly less likely to smoke compared with single males ($p < 0.008$); smoking behaviour was also significantly associated with family members who smoke ($p < 0.001$; Table 2).

4. Discussion

To the best of our knowledge, this is the first study to investigate the associations between the genetic polymorphisms within the dopamine transporter *SLC6A3* (rs27072) and dopamine receptors *DRD1* (rs686), *DRD2* (rs1800497) and *DRD3* (rs7653787) and smoking behaviour in the Malaysian population. Due to the social stigma of women smoking in Malaysia, we focused on males in our study population.

We found no significant association between the rs27072 polymorphism of *SLC6A3* and smoking behaviour in male Malay smokers. Several studies have explored the association between 3′ UTR polymorphism of *SLC6A3* and smoking behaviour [12,40–43]. However, only a few studies have investigated the smoking behaviour association with the rs27072 polymorphism. Ling et al. [44] and Breitling et al. [45] found that *SLC6A3* (rs27072) plays a significant role in the onset of smoking but is not significantly associated with nicotine dependence in Chinese and German populations, respectively. Recently, in a Russian population, no significant association was determined between *SLC6A3* (rs27072) and smoking behaviour [46]. However, a study on a Chinese population found that a higher *SLC6A3* score was associated with the possibility of quitting smoking among those with a lower level of nicotine dependence [47]. Therefore, the rs27072 polymorphism of *SLC6A3* may play a role in smoking behaviour. However, more studies with large sample sizes are warranted.

According to our study, the AG genotype of *DRD1* (rs686) was significantly associated with smoking behaviour (OR: 7.07, 95% CI: 3.71–13.42; $p < 0.001$) in the Malay male population. Novak et al. [35] reported that rs686 of *DRD1* was associated with the quantity of tobacco smoked ($p = 0.005$) in a Canadian cohort; thus, supporting the contribution of the rs686 polymorphism of

DRD1 in smoking behaviour. An interesting study in an American population [48] found that rs686 polymorphism in *DRD1* increased the risk of lung cancer in those who were exposed to second-hand smoke during childhood. Huang et al. [27] demonstrated that the rs686 polymorphism of *DRD1* is significantly associated with nicotine dependence in an African American population, which eventually affects the expression of *DRD1*. Therefore, our results are in line with previous reports, and the rs686 polymorphism of *DRD1* is significantly associated with smoking behaviour in our Malay male population.

With *DRD2* (rs1800497), the A1/A2 genotype was significantly associated with smoking behaviour in our cohort. In a few American cohorts, this gene was significantly associated with smoking behaviour [49–51]. Wilcox et al. [52] observed that the $A1^+$ genotype (A1/A1, A1/A2) had more nicotine dependence compared with the $A1^-$ (A2/A2) genotype. In contrast, a study with Japanese male smokers showed that the A2/A2 genotype had a higher risk of smoking behaviour [53]. A study on Czech patients with coronary heart disease found that the *DRD2* polymorphism (rs1800497) was significantly associated with failure to cease smoking [54]. Interestingly, *DRD2* polymorphism (rs1800497) did not have any relationship with smoking behaviour in Taiwanese men [55]. Although in a British cohort (n = 878), *DRD2* rs1800497 genotype was not associated with smoking cessation [56], in a one-year follow-up study on a German cohort (n = 577), *DRD2* rs1800497 genotype was found to be a predictor of smoking cessation [57]. Contradicting these findings, a second study on German subjects found no association between *DRD2* and heavy smoking [58]. These results suggest that in some populations, there is a significant association pattern between *DRD2* (rs1800497) and smoking behaviour.

Our study found that the TT genotype of *DRD3* (rs7653787) was under-represented in Malay smokers. A single prospective study on female participants established no relationship between *DRD3* polymorphism and smoking cessation when the participants were on d,1-fenfluramine [59]. In another study involving a cohort of 341 patients with schizophrenia [35], *DRD3* polymorphism (rs1025398) showed an association with the quantity of tobacco smoked (p = 0.002). Therefore, to the best of our knowledge, ours is the first study to demonstrate a protective role of rs7653787 polymorphism of *DRD3* in Malay smokers.

In our study, marital status was significantly associated with smoking behaviour; married participants were less likely to smoke than single participants, which is similar to the findings of Morton et al. [60]. However, some studies on Egyptian [61,62], Thai [63] and American [64] populations did not find any significant difference. Our study found that age was significantly different between smokers and non-smokers; smokers were younger, which is similar to a study on Thai males [60]. However, no significant difference was found in studies on Greek [65], Thai [60], Egyptian [62] and American [64] populations. Moreover, although our study determined that systolic blood pressure values were significantly lower in smokers, no such difference was observed in a Greek population [65]. We also observed a significant contribution to smoking behaviour when any of the family members smoked, which is in line with previous studies [66–69].

5. Conclusions

In summary, we found that gene polymorphisms in the dopamine receptors *DRD1*, *DRD2* and *DRD3* alter the dopaminergic response to nicotine, which contributes to smoking behaviour. A similar result was not observed with the dopamine transporter *SLC6A3*. We observed significant differences in age, systolic blood pressure, marital status and family members who smoke between Malay male smokers and non-smokers.

Supplementary Materials: The following are available online at http://www.mdpi.com/2218-273X/10/12/1633/s1, Figure S1: PCR product (460 bp) of *SLC6A3* gene (rs27072), Figure S2: RFLP result of *SLC6A3* gene (rs27072), Figure S3: PCR product (460 bp) of *DRD1* gene (rs686), Figure S4: RFLP result of *DRD1* gene (rs686), Figure S5: PCR product (300 bp) of *DRD2* gene (rs1800497), Figure S6: RFLP result of *DRD2* gene (rs1800497), Figure S7: PCR product (488 bp) of *DRD3* (rs7653787), Figure S8: RFLP result of *DRD3* gene (rs7653787), Figure S9: Sequencing analysis of *SLC6A3* gene (rs27072) (A) the chromatograms of *SLC6A3* gene (rs27072) homozygous wild type sequence and (B) *SLC6A3* gene (rs27072) heterozygous mutant sequence, Figure S10: Sequencing analysis of *DRD1*

(rs686) (A) the chromatograms of *DRD1* gene (rs686) homozygous wild type sequence and (B) *DRD1* gene (rs686) heterozygous mutant sequence, Figure S11: Sequencing analysis of *DRD2* (rs1800497) (A) the chromatograms of *DRD2* gene (rs1800497) homozygous wild type sequence and (B) *DRD2* gene (rs1800497) heterozygous mutant sequence, Figure S12: Sequencing analysis of *DRD3* (rs7653787) (A) the chromatograms of *DRD3* gene (rs7653787) homozygous wild type sequence and (B) *DRD3* gene (rs7653787) heterozygous mutant sequence.

Author Contributions: Conceptualization, A.B.R. and I.A.; methodology, A.B.R., M.A.I. and S.K.S.M.; software, A.B.R. and S.K.S.M.; validation, I.A., A.B.R. and M.A.I.; formal analysis, S.K.S.M.; investigation, A.B.R. and S.K.S.M.; resources, A.B.R. and M.A.I.; data curation, A.B.R. and M.A.I.; writing—original draft preparation, A.B.R., M.A.I. and S.K.S.M.; writing—review and editing, A.B.R. and I.A.; visualization, M.A.I.; supervision, I.A.; project administration, I.A.; funding acquisition, A.B.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Universiti Sains Malaysia (USM) short term grant, 304/PPSP/61313050 and USM Research University grant, 1001/PPSP/8012256.

Conflicts of Interest: The authors declare no conflict of interest.

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