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Influence of dopaminergic system gene polymorphisms on mixed amphetamine-type stimulants and opioid dependence in Malaysian Malays

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Abstract

Background: The dopaminergic pathways have previously been reported to be involved in drug dependence. The candidate gene involved in the dopaminergic function has been associated with substance abuse.

Objective: The objective of the study is to investigate the possible association between dopaminergic system gene polymorphisms with mixed amphetamine-type stimulants and opioid dependence in Malaysian Malays. The study has never been done anywhere else, due to its unique population of study subjects.

Subjects and methods

In this study, genetic polymorphisms of dopamine D2 receptor (DRD2) dopamine transporter (SLC6A3), dopamine beta-hydroxylase (DβH), and norepinephrine transporter (SLC6A2) in Malay males (n = 70) having mixed amphetamine-type stimulant (ATS) and opioid dependence were compared with those in control subjects (n = 87). DNA was extracted from leucocytes followed by single nucleotide polymorphism (SNP) determination using PCR-RFLP. The association of the gene with drug dependency was analyzed using chi-squared tests.

Results: There was a significant difference between the genotype (χ^2 = 10.048, p < 0.01) and allele frequency (χ^2 = 14.039, p = 0.000) of the DRD2 rs1800497 gene in the drug dependence group as compared to the control. There was also a significant difference in DβH rs1611115 at the allelic (χ^2 = 4.483, p = 0.034) but not at genotypic levels (χ^2 = 7.572, p = 0.23) in both control and drug dependence groups. There was an association for SLC6A3 rs27072 with drug dependence at the genotypic level (χ^2 = 7.006, p = 0.030) although no significant difference exist at the allelic level (χ^2 = 2.091, p = 0.148). No significant difference was observed in SLC6A2 rs3785157 genes polymorphism at both genotype and allelic level in control and drug dependence group respectively (χ^2 = 0.94, p = 0.954) (χ^2 = 0.29, p = 0.865) indicating that these polymorphisms do not affect drug dependence.

Conclusion: Our study suggests that DRD2 rs1800497, DβH rs1611115, and SLC6A3 rs27072 but not SLC6A2 rs3785157 are associated with drug-dependent behavior among Malaysian Malays.

Keywords: Dopamine D2 receptor (DRD2), Dopamine transporter (SLC6A3), Dopamine beta-hydroxylase (DβH), Norepinephrine transporter (SLC6A2), Amphetamine-type stimulant and opioid dependence

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Background

Drug dependence is influenced by both genetic and environmental factors. Opioids, predominantly heroin and morphine as well as amphetamine-type stimulant (ATS) including methamphetamine, amphetamine, and ecstasy are the primary drugs contributing to drug dependence in Malaysia [1]. The National Anti-Drug Agency of Malaysia identified 7,864 addicts in 2013, of whom the majority (75.72%) were dependent on opioid and the remaining were dependent on ATS [2]. Nevertheless, in the past decade, ATS is becoming a major challenge. Globally, it is now the second most commonly used illicit drug type and is more widely used than heroin. Therefore, ATS dependence is especially problematic in the Asian region. In fact, ATS production, use, confiscation, and demand for treatment continued to rise [3].

Opioids which are available in both natural and synthetic forms produce a morphine-like effect [4] to relieve pain, which mimics the action of endogenous peptide neurotransmitter when it binds to a specific opioid receptor in the central nervous system [4]. The euphoric characteristic that comes with the drug contributes to its dependence. Opioid dependence is diagnosed by symptoms associated with a compulsive behavior and its prolonged use without legitimate medical reasons. Individuals with opioid dependence will normally continue to use the drug despite the adverse effects suffered [5].

ATS is a synthetic drug that is categorized as a stimulant. It acts as a stimulant in the central nervous system which increases the synaptic concentration of monoamine neurotransmitters including dopamine [6]. The substance, which is chemically related to its parent compound amphetamine, produces a similar amphetamine-like effect. Its effects is further enhanced when ATS is taken together with opioid [7, 8]. The prevalence of ATS use in Asia is relatively high, contributing to the increasing number of people requiring treatment for ATS [9]. For example, methamphetamine abuse can cause acute renal failure, altered behavior, neurological damage to the brain, and toxic effect to the cardiovascular and central nervous systems [10].

Dopamine receptor is divided into five subtypes based on their structure and pharmacological and biochemical properties which fall into two big groups: dopamine receptor D1-like (D1 and D5) and D2-like (D2, D3, and D4) groups [11]. Dopamine D2 receptor (DRD2) appears to play a significant role in the rewarding effects of drugs of abuse. There have been reports on the association between the DRD2 locus and drug dependence [12]. It has been postulated that substance dependence is related to a structural deficit in the dopaminergic reward system. The pharmacological perspective suggests that the diminished responsiveness in the dopaminergic system associated with the DRD2 A1 allele poses as a larger genetic risk factor in drug abuse, especially for amphetamines [13]. In fact, the DRD2 gene was previously associated with a range of substance use disorder including nicotine [14, 15], cocaine [16], and alcohol dependences [17]. Dopamine beta-hydroxylase (DβH) is found within the synaptic vesicles of noradrenergic and adrenergic neurons as well as the neuro-secretory cells. It is present in both soluble as well as membrane-bound fractions [18] and is the only enzyme responsible to convert dopamine into norepinephrine.

The dopamine transporter which is encoded by the SLC6A3 gene regulates the reuptake of dopamine back into the presynaptic neuron. Ujike and colleagues indicated that the 9 or fewer repeat variable number of tandem repeats (VNTR) alleles were associated with methamphetamine psychosis lasting 1 month or more following discontinuation of methamphetamine [19]. In a study on Chinese men, there was no association of the dopamine transporter VNTR alleles with methamphetamine dependence [20]. Two less common allelic variants which alter the coding VNTR, consisting of a repeat unit of 40 nucleotides, are found in the 3-untranslated region in exon 15 of the *SLC6A3* gene. In another study, a weak association was established between the SNP rs27072 polymorphism with amphetamine dependence patient in a Han Chinese population [21].

The norepinephrine transporter is responsible for the reuptake of norepinephrine. The reuptake of the extracellular norepinephrine is a challenge since there is competition with a variety of naturally occurring amines and drug for the binding with the norepinephrine transporter. Failure of binding with the norepinephrine transporter leads to the blockage of norepinephrine transport, causing an increase in the neurotransmitter concentration in the synaptic cleft, thereby enhancing the activation of the postsynaptic receptor [22]. A study by Kreek et al. (2005) showed that the norepinephrine transporter gene polymorphism may contribute to opiate addiction [23]. Polymorphism in SLC6A2 gene was also associated with mood response to the D-amphetamine. Also, rs47958, rs36017, rs2270935, and rs47958 genotype polymorphisms were found to be associated with the increment in positive mood and elation [24].

To date, the allelic and genotypic frequencies of D2 receptor gene (DRD2) *TaqlA* rs1800497, dopamine beta-hydroxylase (DβH) rs1611115, dopamine transporter SLC6A3 rs27972, and norepinephrine transporter (SLC6A2) rs3785157 have been reported among drug abusers in Chinese [25], Caucasian [26], Columbian [27], and Han Chinese [28] populations. However, to our knowledge, there is no similar data reported for the Malaysian population, so far which is very ethnically diversified since it consists of the three main ethnic groups (Malays, Indians, and Chinese). Identification of the

genes involved may help guide the implementation of personalized treatment strategies for drug dependence in the future. Therefore, the objective of this study was to determine the possible association between genetic variants of the four genes of the catecholaminergic system, namely DRD2, DβH SLC6A3, and SLC6A2 genes, with ATS and opioid dependence among the Malay male population in Kelantan, Malaysia.

Methods

Subject recruitment

The study protocol was approved by the Research and Ethics Committee of School of Medical Sciences, Universiti Sains Malaysia [USM/JEPeM/15020063] which complies with the Declaration of Helsinki. All subjects were informed about the experimental procedures and the study aim before signing the written informed consents.

For the drug dependence group (n = 70), all subjects were from a pool of patients coming for treatment at the Hospital Universiti Sains Malaysia, Kelantan, Malaysia. All drug dependence subjects recruited were diagnosed using the Diagnostic and statistical manual-IV (DSM-IV) criteria and fulfilled the criteria for ATS and opioid dependence [29]. Semi-structured criteria based on the Addiction Severity Index (ASI) criteria were used to collect the demographic and clinical data from subjects. The collected data included age, body weight, height, and blood pressure. Additionally, the status as well as confirmation of the types of drug being abused, age when the individual first started using drugs, information on treatment strategies, and the drug dependence history were recorded.

Subjects in the healthy control group (n = 87) were recruited from the Hospital Universiti Sains Malaysia based on the inclusion and exclusion criteria. The controls were medically healthy individuals with no history of chronic medical or surgical illnesses and had no previous history of psychiatric illness or drug use.

DNA extractions

Venous blood (3 ml) was drawn into sterile tubes containing ethylenediaminetetraacetic acid (EDTA) and was stored at − 20 °C until DNA extraction. Genomic DNA was isolated by using the G-spin total DNA Extraction Kit (Intron, Korea). DNA concentrations and purities were measured using the NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, USA) at 280 nm.

Genotyping of DRD2 rs1800497 gene using polymerase chain reaction-restriction fragment length polymorphism

Genotyping of DRD2 rs1800497 was conducted in a total volume of 25 μl solution consisting of 2.5 μl of 10× PCR buffer containing potassium chloride (KCI), 0.3 μmol/l of each forward and reverse primers, 0.16 μmol/l of dNTPs, 0.7 mmol/l of magnesium chloride ($MgCI_2$) and 0.5 U *Taq* DNA polymerase (Vivantis, Malaysia). Following an initial incubation step at 95 °C for 15 min, the PCR products were amplified for 35 cycles of 30 s each at 94 °C, an annealing step at 55 °C for 30 s, an extension step at 75 °C for 1 min, and a final extension step at 72 °C for 7 min.

A 300-bp fragment was yielded by running on a 1% agarose gel (Fig. 1a) containing a 100-bp ladder in the presence of ethidium bromide. The PCR product was then digested with *Taq1A* enzyme (BioLabs Inc., New Zealand) for 2 h at 65 °C. The digested products were visualized under the ultraviolet (UV) light on an agarose gel (1.4%) stained with ethidium bromide. The homozygous mutant type alleles (A1/A1) remained uncut even following redigestion. The homozygous wild-type variant (A2/A2) alleles were digested into two fragments (125 bp and 175 bp). The heterozygous A1/A2 alleles were digested into three fragments (125 bp, 175 bp and 300 bp) (Fig. 1b).

Genotyping of DβH rs1611115 gene using PCR-RFLP

Genotyping of DβH gene rs1611115 polymorphism was performed in 25 μl of master mix which consisted of 2.5 μl of 10× PCR buffer containing KCI, 0.3 μmol/l of each forward and reverse primers, 0.16 μmol/l of dNTPs, 0.7 mmol/l of MgCI2, and 0.5 U of *Taq* DNA polymerase (Vivantis, Malaysia). After an initial incubation at 95 °C for 15 min, the PCR products were amplified for 35 cycles for 30 s at 94 °C, annealing at 57 °C for 30 s, extension at 75 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR product (Fig. 2a) was digested with the *Hha1* enzyme (BioLabs Inc., New Zealand) at 37 °C for 1 h. The digested products were visualized under the UV light on an agarose gel (1.4%) stained with ethidium bromide. The homozygous wild type C/C remained uncut with a 131 bp fragment while the homozygous mutant T/T were digested into two fragments (22 bp and 109 bp). On the other hand, the heterozygous C/T wild-mutant alleles were digested into three fragments (22 bp, 109 bp, and 131 bp) (Fig. 2b).

Genotyping of SLC6A3 rs27072 gene using PCR-RFLP

Genotyping of SLC6A3 rs27072 was performed with a total volume of 25 μl of master mix which consisted of 2.5 μl of 10× PCR buffer containing KCI, 0.3 μmol/l of each forward and reverse primers, 0.16 μmol/l of dNTPs, 0.7 mmol/l of $MgCI_2$, and 0.5 U of *Taq* DNA polymerase (Vivantis, Malaysia). After an initial incubation step at 95 °C for 15 min, the PCR products were amplified for 35 cycles of 30 s at 94 °C. This was followed by annealing at 54 °C for 30 s, an extension at 75 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR product (Fig. 3a) was then digested overnight

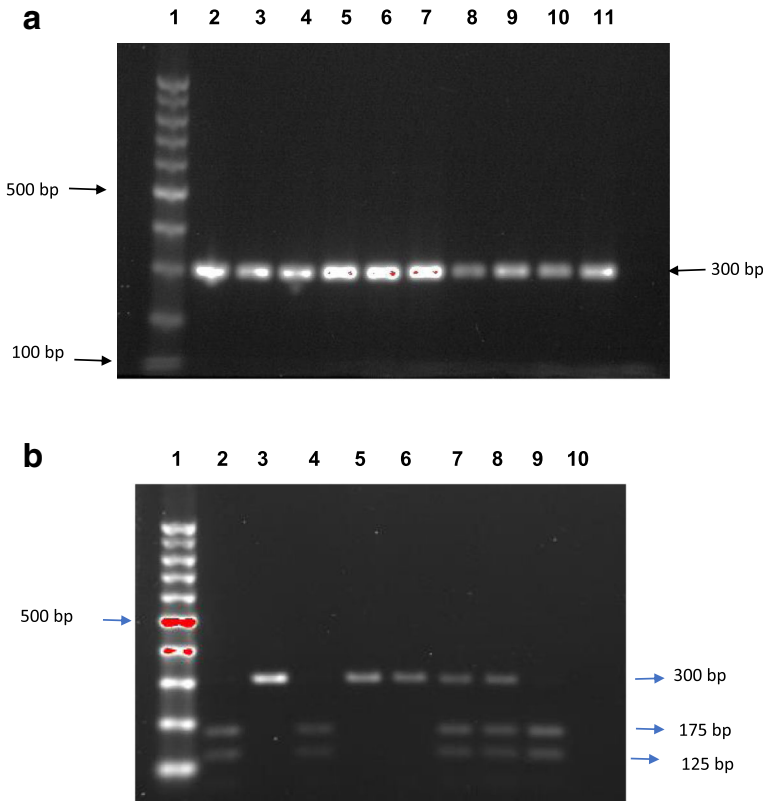

Fig. 1 **a** PCR product for amplification using DRD2 primers. **b** PCR-RFLP result after digestion with *Taq1A* restriction enzyme. Lane 1 shows a 100-bp DNA ladder. Lanes 7 and 8 show heterozygous A1/A2 wild-mutant alleles with 125-, 175-, and 300-bp fragments. Lanes 3, 5, and 6 show homozygous mutant A1/A1 with a 300-bp fragment. Lanes 2, 4, and 9 show homozygous wild type A2/A2 with 125- and 175-bp fragments

with *Msp1* enzyme (BioLabs Inc., New Zealand) at 37 °C. The digested products were visualized under the UV light on an agarose gel (1.4%) stained with ethidium bromide. The homozygous wild-type alleles with the CC genotype remained uncut with a 450-bp fragment. The homozygous mutant which were the TT alleles was digested into two fragments (125 bp and 325 bp) while the heterozygous CT alleles were digested into three fragments (125 bp, 325 bp, and 450 bp) (Fig. 3b).

Genotyping of SLC6A2 rs3785157 gene using PCR-RFLP

Genotyping of SLC6A2 rs3785157 polymorphism was performed in a master mix (25 μl) which consisted of 2.5 μl of 10× PCR buffer containing KCI, 0.3 μmol/l of each forward and reverse primers, 0.16 μmol/l of dNTPs, 0.7 mmol/l of MgCI2, and 0.5 U of *Taq* DNA polymerase (Vivantis, Malaysia). After an initial incubation at 95 °C for 15 min, the PCR products were amplified for 35 cycles at 94 °C of 30 s, annealing at 5 °C for 30 s, extension at 75 °C for 1 min, and a final extension at 72 °C for 7 min. The PCR product (Fig. 4a) was then digested overnight with *BsrD1* enzyme (BioLabs Inc., New Zealand) at 65 °C for 1 h. The digested products were then visualized under the UV light on an agarose gel (1.4%) stained with ethidium bromide. The heterozygous C/T wild-mutant alleles were digested into three fragments (189 bp, 241 bp, and 343 bp) while the homozygous mutant T/T were digested into two fragments (189 bp and 241 bp) (Fig. 4b). The homozygous wild type C/C remained uncut with a 343 bp fragment (Fig. 4b).

Sequencing analysis

All PCR products were sent to the First BASE Laboratories for DNA sequencing analysis. The DNA sequencing was performed using the Applied Biosystem 3730 XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). DNA sequences were confirmed by sequencing both of the DNA strands. The sequencing results for DRD2 rs1800497, DβH rs1611115, SLC6A3 rs27072, and SLC6A2 rs3785157 were as shown in Figs. 5, 6, 7, and 8 respectively.

Statistical analysis

The demographic data were analyzed using Mann Whitney and an independent-sample *t* test followed by calculation of genotype and the allele frequencies using the Hardy-Weinberg equation ($p^2+2pq+q^2$). Subsequently, the non-parametric chi-square test was used to establish

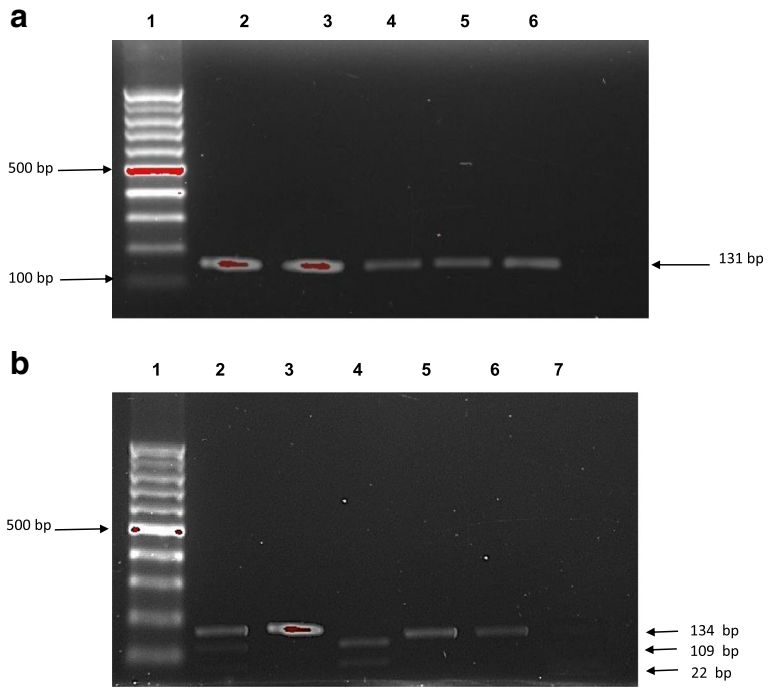

Fig. 2 a PCR product of DβH rs1611115 (131 bp). **b** PCR-RFLP result following digestion with *Hha1* restriction enzyme. Lane 1 shows a 100-bp DNA ladder. Lane 2 shows a heterozygous C/T wild-mutant alleles with 22-, 109-, and 131-bp fragments. Lanes 3, 5, and 6 show homozygous wild type C/C with a 131-bp fragment. Lane 4 shows a homozygous mutant T/T with 22- and 109-bp fragments

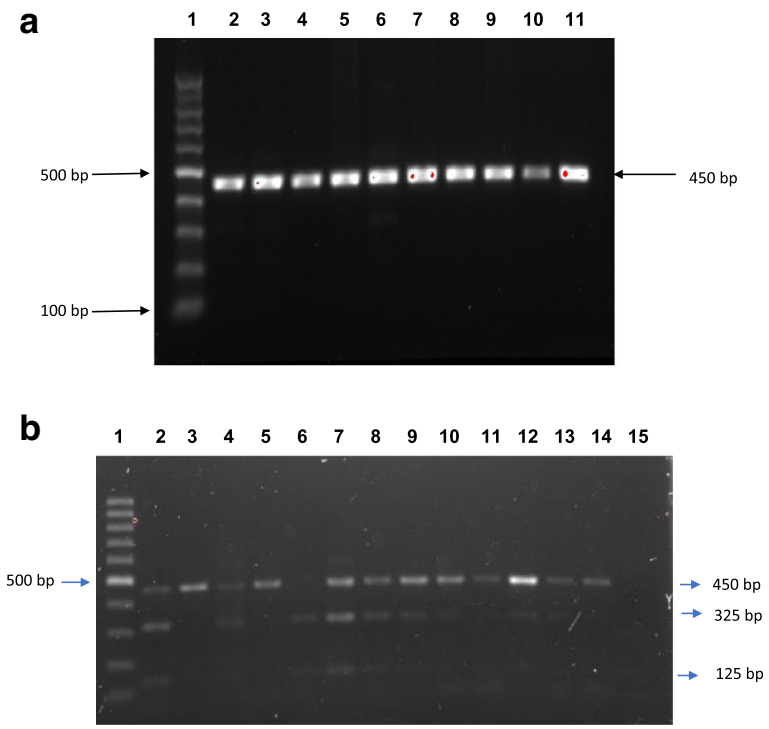

Fig. 3 a PCR product following amplification using SLC6A3 primers. **b** PCR-RFLP result after digestion with *Msp1* restriction enzyme. Lane 1 shows a 100-bp DNA ladder. Lanes 2, 4, 7, 8, 9, 10, and 12 show heterozygous C/T wild-mutant alleles containing 125-, 325-, and 450-bp fragments. Lane 3, 5, 11, 13, and 14 show homozygous wild type C/C with 450 bp fragment. Lane 6 show homozygous mutant T/T with 125 bp and 325 bp fragments

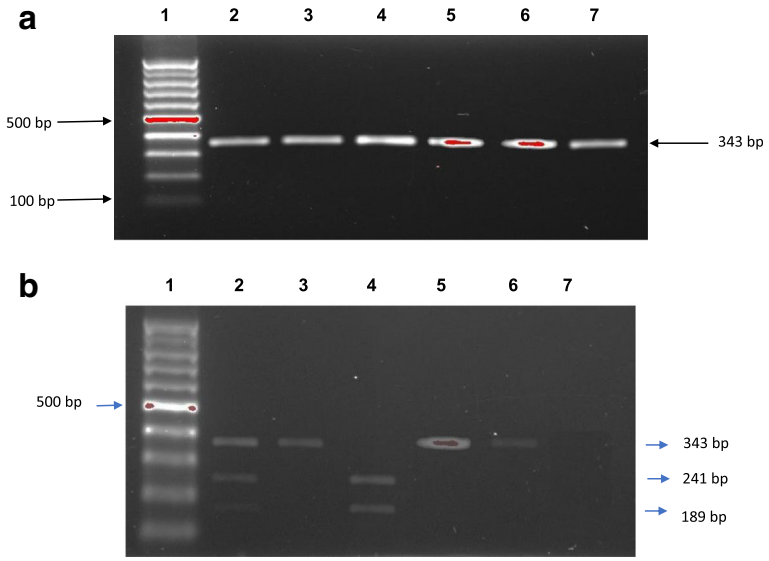

Fig. 4 a PCR product for amplification using SLC6A2 primers. **b** PCR-RFLP result following digestion with *BsrD1* restriction enzyme. Lane 1 shows a 100-bp DNA ladder. Lane 2 shows heterozygous C/T wild-mutant alleles with 189-, 241-, and 343-bp fragments. Lanes 3, 5, and 6 show homozygous wild type C/C with a 343-bp fragment. Lane 4 shows homozygous mutant T/T with 189- and 241-bp fragments

any significant association between the genotype and allele frequencies distribution with ATS and opioid dependence among the subjects. A p value of < 0.05 was considered statistically significant. All statistics were performed using the SPSS (version 23, IBM, Armonk, NY)

Results

The demographic data consisted of age, weight, height, blood pressure, and body mass index (BMI) for both groups (Table 1). The genotypic and allelic frequencies for DRD2 rs1800497, DβH rs1611115, SLC6A3 rs27072,

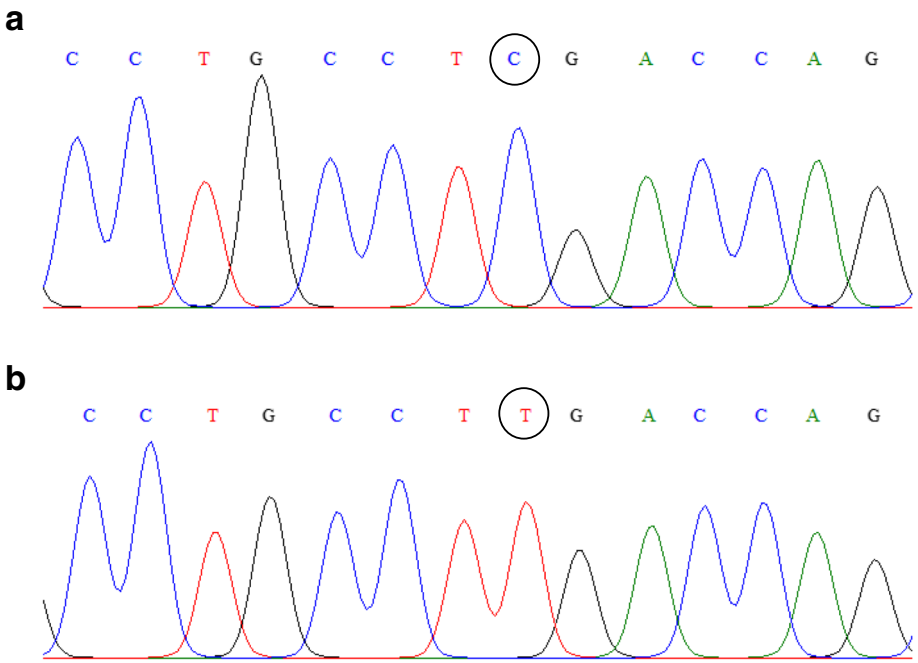

Fig. 5 a Sequencing analysis of the DRD2 rs1800497 (homozygous wild type). **b** Sequencing analysis of the DRD2 rs1800497 (heterozygous mutant)

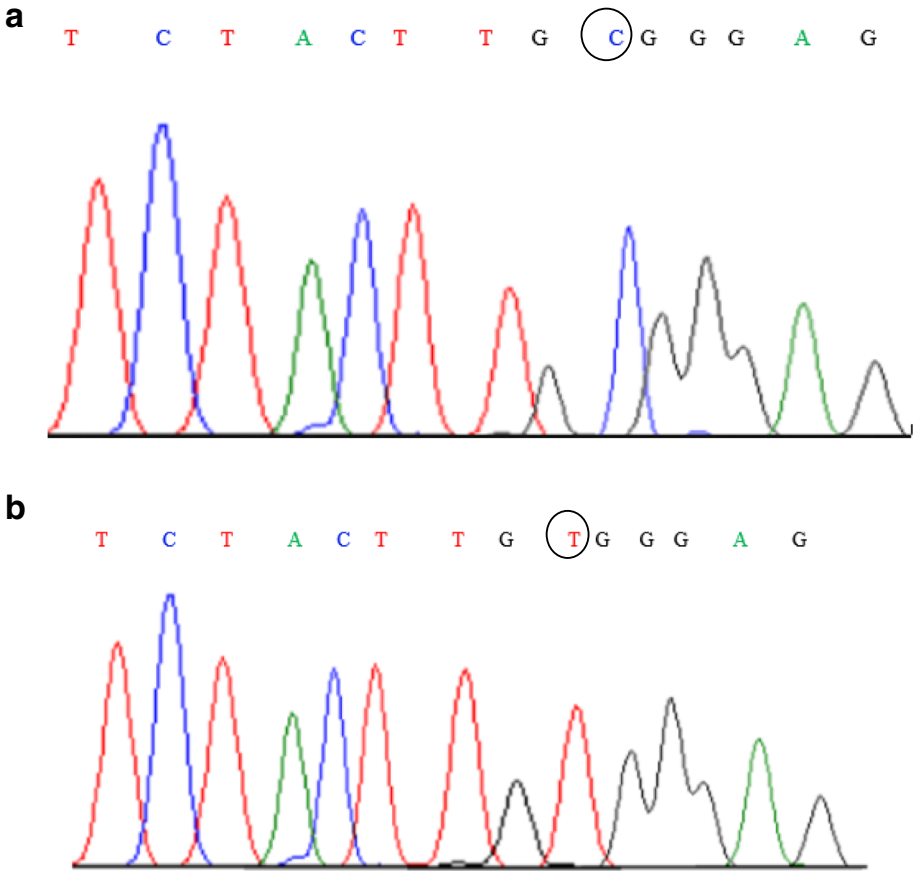

Fig. 6 **a** The chromatograms of DβH rs1611115 homozygous wild type. **b** The chromatograms of DβH rs1611115 heterozygous mutant

and SLC6A2 rs3785157 of the control and drug dependence groups were summarized in Tables 2, 3, 4, and 5.

Discussion

There was a significant difference in DRD2 rs1800497 at both genotypic level (χ^2 = 10.048, p < 0.01) and allelic level (χ^2 = 14.039, p = 0.0000). There was no homozygous wild type (TT genotype) for the DβH rs1611115 gene present in the control group, although the CC genotype (5%) was present in the drug dependence group. There was no significant difference in the genotypic frequencies (χ^2 = 7.572, p = 0.23) when the drug dependence was compared with the control group. However, there was a significant association at the allelic level (χ^2 = 4.483, p = 0.034) when the drug dependence was compared with the control groups. DβH is associated with drug addiction in different ethnic groups including the Caucasian [30], European Americans [31], and Japanese [18]. Randesi et al. [32] in their study showed no association between the SNPs of rs1611115 and opioid dependence in a European population indicating that the variation is ethnic-specific. However, their study showed a strong association between other SNPs in the DβH gene (rs2073837 and rs1611131) when the three groups (healthy control, non-opioid-dependent, and opioid-dependent groups) [32] were compared indicating that the type of gene plays a significant role in establishing any associations. Another study by Isaza et al. [27] involving the Columbian population showed no significant association between the DβH polymorphism and drug addiction which also supported our findings of lack of association between DβH rs1611115 and drug addiction.

As for the SLC6A3 rs27072 gene, there was a significant difference at the genotypic level (χ^2 = 7.006, p = 0.030) when the drug dependence was compared with the control group. However, there was also no significant association detected at the allelic level (χ^2 = 2.091, p = 0.148) when the drug dependence was compared with the control group. Our findings support the results of the previous study which relate the SLC6A3 gene with drug dependence. SLC6A3 polymorphism is more common in the drug dependence group as compared to the control group [27] which may be influenced by ethnicity. A higher frequency of T alleles (28%) was recorded in the Han Chinese population, which was slightly higher than that reported for the Caucasian (18–19%) confirming that the

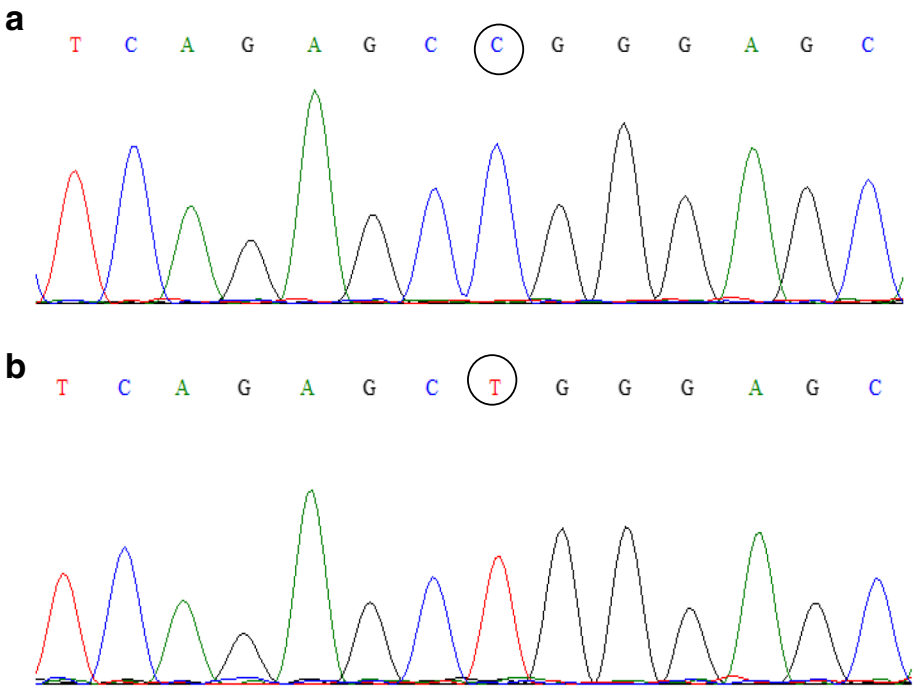

Fig. 7 a Sequencing analysis of the SLC6A3 rs27072 (homozygous wild type). **b** Sequencing analysis of the SLC6A3 rs27072 (heterozygous mutant sequence).

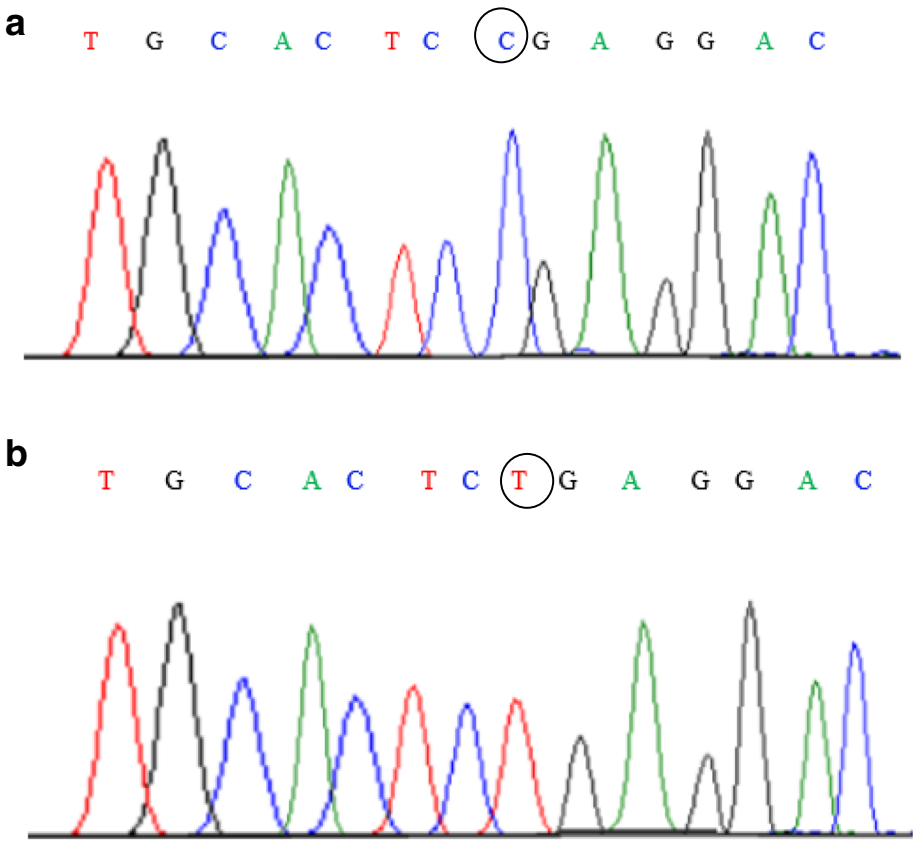

Fig. 8 a The chromatograms of SLC6A3 rs3785157 homozygous wild type. **b** The chromatograms of SLC6A3 rs3785157 heterozygous mutant

Table 1 Demographic data of the subjects

	Control group (n = 87) Mean (SD)	Drug-dependent group (n = 70) Mean (SD)	p value
Age (years)[a]	24.0 (10.55)[c]	28.0 (7.68)[c]	0.000*
Height (m)[b]	1.668 (0.63)	1.683 (0.63)	0.186
Weight (kg)[b]	70.55 (11.30)	70.04 (11.30)	0.784
Body mass index (kg/m^2)[b]	24.66 (3.59)	25.30 (3.72)	0.281
Brachial systolic blood pressure (mm/Hg)	135.44 (17.64)	126.23 (17.63)	0.000*
Brachial diastolic blood pressure (mm/Hg)	76.45 (8.94)	75.40 (8.89)	0.465

*Significant difference between control and drug dependent groups ($p < 0.05$)
[a]Mann-Whitney test
[b]Independent-sample t test
[c]Median (IQR)

polymorphism is lower in Caucasians as compared to the Asian community [28]. Hong et al. [20] reported that there was no significant association between dopamine and serotonin transporter polymorphisms with methamphetamine abuse in the three SNPs of the SLC6A3 genes (DAT 3′-VNTR, 5-HTTLPR, and 5-HTTVNTR) confirming that genetic polymorphism plays an important role in influencing drug dependence. Additionally, a study conducted by Lohoff et al. [33] showed that there was no association between VNTR polymorphism and cocaine dependence among African cocaine-dependent subjects.

As for the SLC6A2 rs3785157 gene, no significant difference was found in the genotypic (χ^2 = 0.94, p = 0.954) and allelic frequencies (χ^2 = 0.29, p = 0.865) for the drug dependence and control groups. Although several studies have attempted to determine the association of the SLC6A2 gene with drug addiction, no previous study determines whether the specific rs3785157 polymorphism is associated with drug addiction or not [22]. In a study conducted by Dlugos et al. [24], the SNPs rs47958 and rs36017 are associated with an increase in the positive mood and mood elation (both related with the pleasurable effect of using ATS and opioid [8]) following consumption of D-amphetamine (20 mg). Toni Kim et al. [34] reported in their study that the SLC6A2 SNPs rs36020 and rs36029 genes were associated with alcoholism based on a study conducted on 21 SNPs. However, the findings were contradictory to those of the study conducted by Samochowiec et al. (2002) [35] in Berlin, Germany, which showed that SLC6A2 (G1287A Sau96I-RFLP) is unlikely to be involved in alcohol susceptibility and severe alcohol withdrawal. Another study by Levran et al. (2015) [36] in a European and Middle Eastern subgroup showed that there is a possible association between the SLC6A2 genotype with opioid and cocaine dependence with two SNPs from SLC6A2 gene (rs10521329 and rs3785155). Nevertheless, no association between SNPs rs3785157 with ATS and opioid dependence was established in our study indicating that the association is ethnic-specific.

In this study, only Malay males were selected because multiple ethnicities require extensive sampling which is beyond the limited resources of this study.

DRD2 gene was chosen for our study because previous studies have reported some positive association between the A2/A2 and A1/A2 genotypes with drug dependence in various populations [25, 26, 37]. Our findings indicate a positive association between the A1/A1 genotype with drug dependence. Similarly, Hou and Li [38] reported that subjects with the DRD2 A1/A1 and A1/A2 genotypes were significantly associated with heroin dependence among the Han Chinese population in China. In addition, a significant difference in the distribution of genotypes exists in heroin-addicted with a genotype

Table 2 Allelic and genotypic frequencies of DRD2 rs1800497 polymorphism in the drug dependence and control subjects

DRD2 rs1800497	Group, n (%)		Statistics[a]	
	Drug dependence (n = 70)	Control (n = 87)	χ^2 (df)	p value
Genotype				
A1/A1	7 (10.10)	22 (25.29)	10.048 (2)	<0.001
A1/A2	14 (20.0)	25 (28.74)		
A2/A2	49 (70.0)	40 (45.98)		
Allele				
A1	28 (20.00)	69 (39.66)	14.039 (1)	0.0000
A2	112 (80.00)	105 (60.34)		

[a]Chi-squared test

Table 3 Allelic and genotypic frequencies of DβH rs1611115 polymorphism in the drug dependence and control subjects

DβH rs1611115	Group, *n* (%)		Statistics[a]	
	Drug dependence	Control	χ^2 (df)	*p* value
Genotype				
CC	37 (52.86)	57 (65.52)	7.572 (2.00)	0.23
CT	28 (40.00)	30 (34.48)		
TT	5 (7.14)	0 (0.00)		
Allele				
C	102 (72.86)	144 (82.76)	4.483 (1.00)	0.034
T	38 (27.14)	30 (17.24)		

[a]Chi-squared test

Table 5 Allelic and genotypic frequencies of SLC6A2 rs3785157 polymorphism in the drug dependence and control subjects

SLC6A2 rs3785157	Group, *n* (%)		Statistics[a]	
	Drug dependence	Control	χ^2 (df)	*p* value
Genotype				
CC	34 (48.57)	42 (48.28)	0.94 (2.00)	0.954
CT	29 (41.43)	35 (40.23)		
TT	7(10.00)	10 (11.49)		
Allele				
C	97 (69.29)	119 (68.39)	0.29 (1.00)	0.865
T	43 (30.71)	55 (31.61)		

[a]Chi-squared test

frequency (χ^2 = 6.957, p = 0.031) as compared to the control group.

Matsusue et al. [39] in their study reported that *Taq1A* rs1800497 SNP showed a significantly (p = 0.030) high association between the control and methamphetamine intoxication cases. The genotypic frequency for the dominant A1/A1 and A1/A2 genotypes were also significantly high compared to the A2/A2 genotype in the methamphetamine intoxication cases. The frequency of the *Taq1A* A1 allele (A1/A1+A1/A2) was higher in the methamphetamine abuse group compared to the control group (χ2 = 4.70, p = 0.03) [40]. A meta-analysis conducted by Chen et al. [41] showed that *Taq1A* A1 allele was associated with a significant increase of opioid dependence risk in cases (n = 2679) as compared to the control (n = 2186). A significant association was observed between A1 allele and addiction in the opium-dependent group (p < 0.0001) indicating that DRD2 is involved in the pathophysiology of opium addiction in an Iranian population [42].

Our study is limited by the small sample size, although some positive associations have been successfully established. We also do not discount the fact that the presence of polymorphisms not investigated in this study may influence the overall findings. The study of the association between genetics and drug dependence behavior is complex and is dependent on many factors, making the association unique to each population. Nevertheless, identification of the DRD2, DβH, SLC6A3, and SLC6A2 polymorphisms among the Malaysian Malays which may affect drug dependence is the first step to guide implementation of personalized treatment strategies in the future.

Table 4 Allelic and genotypic frequencies of SLC6A3 rs27072 polymorphism in the drug dependence and control subjects

SLC6A3 rs27072	Group, *n* (%)		Statistics[a]	
	Drug dependence	Control	χ^2 (df)	*p* value
Genotype				
CC	7 (10.00)	23 (26.44)	7.006 (2.00)	0.030
CT	55 (78.57)	54 (62.07)		
TT	8 (11.43)	10 (11.49)		
Allele				
C	69 (49.29)	100 (57.47)	2.091 (1.00)	0.148
T	71 (50.71)	74 (42.53)		

[a]Chi-squared test

Conclusion

There was a significant association between DRD2 rs1800497, DβH rs1611115, and SLC6A3 rs27072 gene polymorphisms with the mixed amphetamine-type stimulant and opioid dependence at the genotype and allelic levels. However, no association is established between the SLC6A2 rs3785157 gene polymorphism with mixed amphetamine-type stimulant and opioid dependence. To our knowledge, the present study is the first to provide evidence for the association of allelic variants of those genes with mixed amphetamine-type stimulant and opioid dependence in the Malay male population.

Abbreviations

DRD2: Dopamine D2 receptor; DβH: Dopamine beta-hydroxylase; SLC6A2: Norepinephrine transporter; SLC6A3: Dopamine transporter

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Authors' contributions

RAB conceptualized and designed the study and wrote the manuscript. DSMS performed the research and statistical analysis. IA and VBK acted as field supervisors, dealing with people using drugs. SS formulated the hypotheses and helped with the result analysis. GSH provided assistance in manuscript writing. All authors read and approved the final manuscript.

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Availability of data and materials

Data will not be shared as we are still analyzing the data for another publication.

Ethics approval and consent to participate
The study protocol was approved by the Research and Ethics Committee of School of Medical Sciences, Universiti Sains Malaysia [USM/JEPeM/15020063] which complies with the Declaration of Helsinki. All the subjects signed the written informed consents before being included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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