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Norepinephrine Transporter (SLC6A2) Gene Polymorphisms in Relation to Drug Addiction among Mixed Amphetamine-Type Stimulant and Opioid Dependent in Malay Male Subjects

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Abstract: The aim of this study was to determine the frequency of SLC6A2 gene (rs3785157) polymorphism among mixed amphetamine-type stimulant and opioid dependent in Malay male subjects. A total of 50 Malay male subject with mixed amphetamine-type-stimulant and opioid dependence and 188 control subject were recruited. The DNA was extracted from leucocytes. Genotyping of NET1 gene polymorphism was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The frequencies for the SLC6A2 allele were 47.87% for CC, 39.89% for CT and 12.23% for TT allele respectively in the normal group while in the drug dependent group, the CC genotype has the highest frequencies compared to the other two which are 48% while the genotype of CT and TT are 42% and 10% respectively. There is no significant difference in SLC6A2 polymorphism ($X^2 = 0.211$, $P = 0.900$) observed between the drug dependent and normal group. A larger sample size are needed in order to confirm the association of SLC6A2 gene polymorphism with the drug addiction behavior among mixed amphetamine-type stimulant and opioid dependent in Malay male subjects.

Key words: SLC6A2 polymorphisms · Opioid · Amphetamine-type-stimulant (ATS)

INTRODUCTION

Drug abuse is the chronic disease which involve the compulsive seeking and drug use which can be influenced by both genetic and environmental factorsIt can change the brain structure and its function [1, 2]. A quarter of a billion people in the world population is estimated to have been using at least one drug in 2014 and among that, over 29 million people are suffering from a drug use disorder but only 1 in 6 people actually seeking treatment for drug use disorder [3, 4]. Opioid and amphetamine-type-stimulant are the main drugs that have been abused among the drug users in Malaysia [5].

Opioid predominantly heroine and morphine are drugs that can produce a morphine-like effect. It can occur in both natural and synthetic forms and producing an effect that can mimic the action of endogenous peptide neurotransmitter when binding to a specific opioid receptor in the central nervous system [6, 7]. The euphoric characteristic that comes with the drug which is used to treat pain has led for it to be abused.

Amphetamine-type-stimulant on the other hand is a synthetic drug that can be manufactured everywhere whether in a small scale in a so called "kitchen laboratories" using a simple recipes or on a large scale inside the cladestine laboratories which have a more sophisticated equipment for the ATS production. It can be categorized as a stimulant[8]. It can produce an amphetamine-like effect and usually is taken together with alcohol and opioid. Over the past decade, amphetamine-type stimulant (ATS) has also emerged as a major drug problem. In the year 2014 only, 173 tons of ATS was seized worldwide [4].

Norepinephrine transporter also known as SLC6A2 and NET1 is one of the cathecolamines generated from amino acid tyrosine which plays an important roles in fight and flight response. Norepinephrine transporter is responsible for the reuptake of norepinephrine. Reuptake of the extracellular norepinephrine is competitive as it competes with variety of naturally occurring amines and drugs to bind with the norepinephrine transporter.

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Failure to bind with the norepinephrine transporter blocks the transport of the norepinephrine, causing the increment of the neurotransmitter concentration in the synaptic cleft therefore enhancing the activation of the postsynaptic receptor [9]. The polymorphism in SLC6A2 gene are associated with mood response to the d-amphetamine. rs47958, rs36017, and rs2270935 genotype were found to be associated with the increment in positive mood and elation. [10].

To date, there is no researchregardingthe allelic frequencies and genotypes of norepinephrine transporter (SLC6A2) effects on drug abuse. Therefore the objective of this study is to determine the possible association between the frequency of SLC6A2 gene (rs3785157) polymorphismswith amphetamine-type stimulants and opioid dependence among Malay male population in Kelantan, Malaysia.

MATERIAL AND METHODS

Recruitment of Subjects: The study protocol was approved by the Ethics Committee, School of Medical Sciences, UniversitiSains Malaysia, Malaysia. All the subjects were informed about the experimental procedures and the study aim before being given the consent form.

For drug abusers (n=50), subjects were enrolled from methadone clinic, Hospital UniversitiSains Malaysia. All the drug abusers that were recruited were confirmed to be abusers by the ATS and morphine drugs testing. The demographic data that were collected includes age, weight, height and blood pressure. The demographic data of the subject are shown in Table 1.

For the normal control (n=188) subjects, the recruitment of the subjects were done at Hospital UniversitiSains Malaysia and several places around the state of Kelantan, Malaysia, based on inclusions and exclusions criteria. They were medically healthy with no history of chronic medical or surgical illness, had no previous history of psychiatric illness and did fulfil the DSM-IV criteria for amphetamine-type stimulants and opioid dependence.

DNA Extractions: 3 ml of blood was drawn into a sterile tube containing EDTA and was then stored at -20°C until the blood was extracted. The blood was extracted to isolate the genomic DNA by using the G-spin Total DNA Extraction Kit (Intron, Korea). The concentration and the purity of the DNA were measured using a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, USA) at 280nm absorbance.

Table 1: Demographic characterization of subjects

	Control (n=188) Mean(SD)	Drug abuser (n=50) Mean (SD)	p-value
Age (years)[a]	29.81 (10.55)	37.16 (9.83)	
Height (m)[b]	1.684 (0.064)	1.678 (0.061)	0.027
Weight (kg)[b]	69.04 (11.89)	70.12 (11.77)	2.640
Body mass index (kg/m^2)[b]	24.32 (3.90)	24.87 (3.74)	0.657

[a]Mann-Whitney test.; bIndependent-samples T-test ; [c]Median (IQR)

Table 2: Alleles and genotypes frequency of SLC6A2 polymorphism in the drug abusers and control subjects

	Control n (%)	Drug n (%)	χ^2 (p)
Genotype			
CC	90 (47.87)	24 (48)	0.21 (0.900)
CT	75 (39.89)	21 (42)	
TT	23 (12.23)	5 (10)	
Allele			
C	255 (67.81)	69 (69)	T
	121 (32.18)	31 (31)	

Genotyping of SLC6A2 gene (rs3785157) using PCR-RFLP: Genotyping of SLC6A2 (rs3785157) polymorphism was performed in a 25 µl of master mix which consist of 2.5µl of 10X PCR buffer with KCI, 0.3 µmol/l of each primer (forward 5'-GGA AGA CTG AGA TGC AAG CTA -3' and reverse 5'-AGC ATG AAC TTA CAG CTC ACC T-3'), 0.16 µmol/l of dNTPs, 0.7 mmol/l of MgCI2 and 0.5 U Taq DNA polymerase (Vivantis, Malaysia) were carried out for PCR reaction. After an initial incubation at 95°C for 15 min, the PCR products were amplified for 35 cycles of 30 sec at 94°C, annealing at 5°C for 30 sec, extension at 75°C for 1 min and final extension at 72°C for 7 min. (Figure 1a) The PCR product was then digested with *BsrD1* enzyme (BioLabs Inc., New Zealand) overnight at 65°C for 1 hour. The digested products were then visualized under the UV light on 1.4% ethidium bromide agarose gel with 100 bp ladder. The heterozygous C/T wild-mutant alleles were digested into three fragments which are 125 bp, 325 bp and 343bp fragments and the homozygous mutant T/T were digested into two fragment (125 bp and 325bp). The homozygous wild-type C/C remained uncut with 343 bp fragment (Figure 1b).

Statistical Analysis: All the data were compiled and differentiated according to the genotype and the allele frequencies. The non-parametric chi square was used to test the significance association between the genotype and allele frequencies distribution with the amphetamine-type stimulants and opioid dependence among the tested subject. $p<0.05$ were considered to be significant. All statistics were performed using the SPSS package 21 (IBM. Armonk, NY).

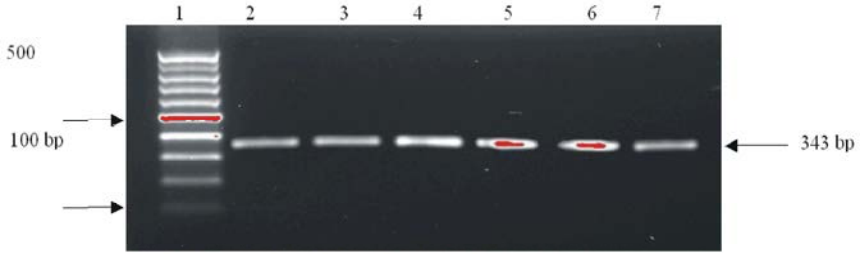

Fig. 1a: PCR product for amplification using SLC6A2forward 5'-GGA AGA CTG AGA TGC AAG CTA -3' and reverse 5'-AGC ATG AAC TTA CAG CTC ACC T-3'primers.

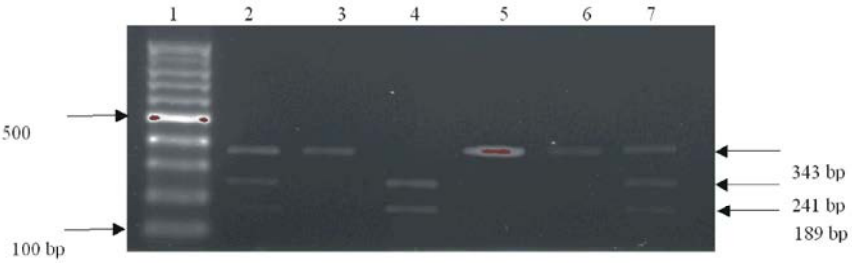

Fig. 1b: PCR-RFLP result after digestion with *BsrD1* restriction enzyme. Lane 2 and 7 show heterozygous C/T wild-mutant alleles with 189 bp, 241 bp and 343bp fragments. Lane 3, 5 and 6 show homozygous wild-type C/C with 343 bp fragment. Lane 4shows homozygous T/T with 189 bp and 241 bp fragments.

RESULT AND DISCUSSION

Genotyping of NET1 gene polymorphism was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The frequencies for the SLC6A2 allele were 47.87% for CC, 39.89% for CT and 12.23% for TT allele respectively in the normal group while in the drug dependent group, the CC genotype has the highest frequencies compared to the other two which are 48% while the genotype of CT and TT are 42% and 10% respectively. There is no significant difference in SLC6A2 polymorphism (X2 = 0.211, P = 0.900) observed between the drug dependent and normal group.

Norepinephrine is a neurotransmitter involved in many aspects that can affect the mood, alertness and also behaviour. It plays a significant role in stress response, maintaining attention and vigilance and also anorexia nervosa [11, 12]. A few polymorphism in a region of SLC6A2 have been found to have affected the behaviour, mood and also drug response in human [13-16].

Several studies had been done to determine the association of SLC6A2 gene with drug addiction but there was no previous study had been conducted to determine whether the specific rs3785157 polymorphism can be associated with drug addiction or not [9, 10, 17].

The result of this study is contradictory with a few studies that have been done. It may be due to the number of drug abuser sample is small. A larger sample can be used in order to confirm whether there is an association between the rs3785157 and drug addiction.

CONCLUSION

A larger sample size is needed in order to confirm the association of SLC6A2 gene polymorphism with the drug addiction behavior.

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