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Association SLC6A3 gene rs2652511 polymorphism with methamphetamine abuse disorder in Iranian population

Mohaddeseh Aminzadeh Khosroshahi, Ali Farrokhi, Hossein Soltanzadeh

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Abstract

Background: In addition to its physical and psychological effects on consumers, addiction is one of the world's most significant personal and social problems, endangering the sociocultural, political, and societal health. Important roles are played by genetic factors in susceptibility to methamphetamine dependence. This study aimed to examine the relationship between the rs2652511 polymorphism in the promoter region of the SLC6A3 gene and methamphetamine use disorder in Iranian males.

Methods: In this case-control study, we recruited 100 men with methamphetamine use disorder as cases and 100 age- and ethnically-matched normal men from East Azerbaijan, Tabriz, Iran as healthy controls. From peripheral blood leukocytes, genomic DNA was extracted. PCR-RFLP was utilized for genotyping.

Results: The genotype distribution of rs2652511 polymorphism in case group was 56% CC, 33% CT, and 11% 44, whereas in controls group was 25% CC, 42% CT, and 33% TT. Statistical analysis showed that the genotype and allele frequencies of rs2652511 polymorphism were significantly different between patients and healthy controls ($p>0.05$).

Conclusion: Our research revealed that the rs2652511 polymorphism in the SLC6A3 gene was associated with methamphetamine misuse disorder in the Iranian population. To determine the precise function of this polymorphism in the pathology of methamphetamine use disorder, however, more research is needed on various racial and geographical groups.

Keywords: Methamphetamine use disorder, SLC6A3 gene, Polymorphism

Introduction

Methamphetamine, a well-known, potent central nervous system (CNS) psychostimulant, is the most commonly used illicit substance in China.[1] Multiple organ, including the heart, intestines, and brain, are susceptible to harm from methamphetamine.[2] Additionally, individuals with a history of chronic methamphetamine use are more likely to develop psychosis related to methamphetamine, such as auditory hallucinations and paranoid thinking.[3,4] Methamphetamine

addiction places a substantial financial burden on individuals and their families. In addition, it can trigger a series of violent incidents, leading to an array of social issues. It has been demonstrated that methamphetamine-induced changes in gene expression are closely associated with severe disruption of normal neurophysiological brain activity, despite the fact that the precise mechanism underlying methamphetamine dependence remains unknown. Recent genome-wide association studies.[5,6] have been utilized to identify associations between genetic variations and complex disorders, such as schizophrenia, coronary heart disease, and height in samples from various populations. Numerous loci that influence these traits have allowed us to gain a greater understanding of the genetic mechanisms underlying complex behaviors like alcohol and other substance use.[7,8] However, despite numerous reports of candidate genes, data on methamphetamine dependence are scant.[9]

Dopamine (DA) is essential for multiple brain functions and is implicated in circadian rhythms, sleep, inflammation,[10] heart failure, and cancer.[11-13] The human dopamine transporter protein (hDAT) located in the plasma membrane is a crucial regulator of synaptic dopamine transmission. Variations in the coding gene SLC6A3 (DAT1) on chromosome 5 (chr5) may impact SLC6A3 function, leading to changes in hDAT's density, dopamine reuptake activity, and the dynamics of dopamine neurotransmission. These alterations may contribute to pathophysiology in both the central (CNS) and peripheral nervous systems.[14,15]

The sequence of the SLC6A3 gene has been associated with various psychiatric disorders that are sensitive to environmental factors, including Parkinson's disease (PD), substance use disorders (SUDs), major depressive disorder (MDD), and attention deficit hyperactivity disorder (ADHD). The presence of comorbidity gives rise to significant clinical concerns.[16,17] Various environmental risk factors, including drugs, stressors, high-fat meals, stimulant medications, and environmental enrichment, have been found to impact the in vivo activity of Slc6a3 (written in lower case to denote animal genes).[18,19] However, these regulations remain unclear from a mechanical standpoint for humans with the associated diseases. Genetic variation of SLC6A3 has also been associated with some drugs abuse although other studies have failed to confirm these findings.[20,21]

In the Iranian population, no systematic study on SLC6A3 gene variants in methamphetamine users has been conducted as of yet. In this case-control study, we investigate the association

between the SLC6A3 gene rs2652511 polymorphism and methamphetamine use in Iranian Azeri men.

Methods

Patients and sample collection

In present case-control study, we enrolled 100 men from educational hospitals of Tabriz, Iran, during 2018 to 2019. All studied women aged 20-40 years. The case group consists of 60 patients with methamphetamine use which were newly diagnosed and had not received any treatment for drug use. The subjects with severe medical diseases, such as cardiovascular disease, brain disease, major psychiatric disorders, and chronic disease were excluded from the study. Moreover, subjects with use other drugs and substances than methamphetamine were excluded. The control group consists of 60 gender- and age-matched healthy subjects who were referred to as routine physical examination and health check-up. Participants in the case and control groups were selected from the Iranian region of East Azerbaijan, matched for age and ethnicity, and genetically unrelated. All participants' demographics, clinical characteristics, and lifestyle were gathered through interviews and questionnaires. The collected information included age, gender, literacy levels, marital status, syphilis infection status, and drug use history. All subjects were informed about the study and signed a consent form according to the Declaration of Helsinki ethical standards.

Genotyping analysis

Each participant's peripheral blood (5 mL) was drawn into EDTA (Ethylenediaminetetraacetic Acid)-containing receptacles. Leukocytes in peripheral blood were used to extract DNA using the salting-out technique. The genotyping was conducted using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. The respective forward and reverse primer sequences were as follows: 5′-GGAGCATCGAGGGTACAC-3′ and: 5′-GACGGCCTGGAAAGCCCTG-3′. The obtained PCR products (252 bp) were digested with the MspI restriction enzymes and then incubated at 37°C. After digestion, five fragments (97bp, 46

bp, 44 bp, 34 bp, and 31 bp) produced in presence of C allele, and four fragments (141 bp, 46 bp, 34 bp, and 31 bp) produced in presence of T allele. The PCR reaction was carried out in a total volume of 25 µL: each primer (25 pmol), template DNA (1 µg), and PCR master mix (12.5 µL) in the following condition: initial denaturation (1 cycle in 94°C for 4 minutes), denaturation (40 cycles in 94°C for 40 seconds), annealing (40 cycles in 50°C for 30 seconds), extension (40 cycles in 72°C for 25 seconds), and final extension (1 cycle in 72°C for 5 minutes). The digested fragments were separated using electrophoresis on 3% agarose gel stained by safe stain. A 50bp size marker (ladder) was used to estimate the size of DNA bands. Finally, a gel documentation instrument was used to visualize the bands of digested PCR products.

Statistical analysis

The statistical analysis of obtained data was carried out using Statistical Package for the Social Sciences (SPSS) software (version 21.0). The logistic regression was used to analyze the association between SLC6A3 gene rs2652511 polymorphism with methamphetamine use disorder. Hardy-Weinberg equilibrium (HWE) in genotypes distribution of patients and healthy controls were analyzed using the chi-square ($\chi 2$) test and Fisher's exact test. Also, the odds ratio (OR) and 95% confidence intervals (CI) were evaluated. The difference between demographic and clinical features between patients and healthy controls were analyzed using independent sample t-test. The statistically significant was considered as $p<0.05$.

Results

Table 1 outlines the demographic and clinical characteristics of the patients studied and the healthy controls. The statistical analysis revealed a significant difference between patients and healthy controls in terms of age, marital status, and syphilis infection status (p0.05); however, there was no significant difference between patients and healthy controls in terms of body mass index (BMI) and educational degree ($p>0.05$).

The statistical analysis revealed that the SLC6A3 gene rs2652511 polymorphism was HWE in both the case and control groups ($p>0.05$). The genotype and allele frequency distributions for the SLC6A3 rs2652511 polymorphism in the case and control groups are shown in Table 2.

In the case group, the frequencies of homozygous CC, heterozygous CT, and homozygous TT were 56%, 33%, and 11%, respectively. In the control group, 25% of individuals were homozygous CC, 42% were heterozygous CT, and 33% were homozygous TT. Statistical analysis revealed that patients and healthy controls had substantially different genotype frequencies for the rs2652511 polymorphism ($p=0.002$; OR=0.83; 95% CI=0.54-1.68).

72.5 percent of patients and 46.0 percent of healthy persons carried the C allele. In addition, the prevalence of the T allele in patients was 27.5%, compared to 54% in healthy controls. By analyzing allele frequencies, it was possible to statistically differentiate between cases and controls ($p=0.003$; OR=0.60; 95% CI=0.49-1.13).

Discussion

Addiction or dependence on drugs can be defined as the nonmedical self-administration of drugs accompanied by intoxication or withdrawal symptoms. Dopamine is known to play a role in reward and motivation,[22-24] and genetic factors are known to play a large role in addiction. Individual tendencies towards methamphetamine use disorder may therefore be affected by alterations in dopaminergic signaling. We assessed the association between the rs2652511 polymorphism in the promoter region of the SLC6A3 gene, a key protein in the dopaminergic signaling pathway, and the risk of methamphetamine use disorder in Iranian men for the first time. According to the findings of this study, conducted on 100 men with methamphetamine use disorder and 100 healthy men, there was a significant association between rs2652511 polymorphism and methamphetamine use disorder in Iranian men.

Previous research indicates that the SLC6A3 gene plays a crucial function in drug dependence.[25,26] Guo et al.[25] reported that the 40 bp 3 -untranslated polymorphism (rs28363170) was associated with risky behavior, such as problematic use of cannabis, alcohol, tobacco, cocaine, heroin, and other illegal substances. This polymorphism was substantially associated with crack cocaine use in another study by Stolf et al.[26] In accordance with the aforementioned

research, our study revealed a significant association between the rs2652511 polymorphism and methamphetamine use disorder.

The rs2652511 polymorphism is located in the promoter region of the transporter protein and has no effect on the protein's amino acid sequence. Nevertheless, it is a functional polymorphism that may impact transporter protein density.[27] Striatal dopamine is essential for positive symptoms of substance use disorders, such as increased drug intake and addiction; it also causes impaired decision-making, which underlies compulsive behavior, reduced sociality, and risk-taking.[25]

Researchers went even further by conducting a Genome Wide Association Study (GWAS) for personality characteristics, as the GWA analysis is the most effective method for demonstrating biological pathways in the context of addiction. The research was conducted on 1089 Korean women. In the genetic analysis, 1042 pathways comprising 8297 genes were analyzed. In this study, no correlations were found between the genes analyzed and personality trait scores.[28,29] Both populations differ in terms of the gender and ethnicity of their subjects, which may influence the results. Since all of our subjects are of Iranian ancestry, we strongly believe that our study requires replication in a female study group and in populations other than Iranians.

Conclusion

In conclusion, this study increased our understanding of methamphetamine use disorder as a multifactorial disorder and suggests that the SLC6A3 gene rs2652511 polymorphism may be associated with the risk of methamphetamine addiction in Iranian men. However, the precise function and effects of the rs2652511 polymorphism in methamphetamine use disorder remain unknown. For a better understanding of the relationship between this polymorphism and methamphetamine addiction, it is recommended that additional research be conducted on other populations and races with larger sample sizes.

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Author Contributions

Conceptualization: HS.

Data curation: HS.

Formal Analysis: HS.

Investigation: MAK and AF.

Methodology: MAK and AF.

Project administration: HS.

Supervision: HS.

Writing – original draft: MAK and AF.

Writing – review & editing: HS.

Conflict of Interests

The authors declare no conflict of interest in this study.

Ethics Approval

The research was conducted in accordance with the Helsinki Declaration and approved by the Committee of Ethics in Research, Tabriz University of Medical Sciences (the ethical code: IR.IAU.TABRIZ.REC.1398.082).

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