Addiction & Health

https://ahj.kmu.ac.ir
10.34172/ahj.1484
AHJ. 2025;17: 1484

Original Article

Association of the SLC6A3 Gene rs2652511 Polymorphism with Methamphetamine Abuse Disorder in the Iranian Population

Mohaddeseh Aminzadeh Khosroshahi[1], Ali Farrokhi[1], Hossein Soltanzadeh[1,2*]

[1]Department of Cellular and Molecular Biology, Bonab Branch, Islamic Azad University, Bonab, Iran
[2]Medicinal Plants Research Center, Maragheh University of Medical Sciences, Maragheh, Iran

***Corresponding Author:** Hossein Soltanzadeh, Email: hossien4040@gmail.com

Abstract

Background: Besides its physical and psychological effects on individuals, addiction is a major personal and societal issue that threatens cultural, political, and community well-being. Genetic factors play essential roles in susceptibility to methamphetamine dependence. The purpose of this study was to investigate the correlation between methamphetamine use disorder in Iranian males and the rs2652511 polymorphism in the promoter of SLC6A3.

Methods: 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.

Findings: The genotype distribution of rs2652511 polymorphism in the case group was 56% CC, 33% CT, and 11% 44, whereas in the control group it was 25% CC, 42% CT, and 33% TT. According to statistical analysis, there was a substantial variation in genotype and allele frequencies of the rs2652511 polymorphism between the case group and the healthy control group ($P>0.05$).

Conclusion: Our research revealed that the rs2652511 polymorphism in the SLC6A3 gene was associated with methamphetamine misuse disorder in the Iranian population. To clarify the exact role of this polymorphism in the pathology of methamphetamine use disorder, further research is required across different racial and geographic groups.

Keywords: Methamphetamine use disorder, SLC6A3 gene, Polymorphism

Citation: Aminzadeh Khosroshahi M, Farrokhi A, Soltanzadeh H. Association of the SLC6A3 gene rs2652511 polymorphism with methamphetamine abuse disorder in the Iranian population. *Addict Health*. 2025;17:1484. doi:10.34172/ahj.1484

Received: July 7, 2023, **Revised:** October 2, 2023, **Accepted:** May 1, 2024, **ePublished:** June 22, 2025

Introduction

In China, methamphetamine, a potent central nervous system (CNS) psychostimulant, is the most commonly utilized illicit substance.[1] Multiple organs, including the heart, intestines, and brain, are susceptible to methamphetamine damage.[2] Additionally, methamphetamine-related psychoses, such as auditory hallucinations and paranoid thinking, are becoming more prevalent in those with a history of chronic methamphetamine consumption.[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 regular neurophysiological brain activity, even though the precise mechanism underlying methamphetamine dependence remains unknown. Recent investigations of genome-wide associations have been employed in different populations to identify correlations between genetic variations and complicated illnesses, including height, coronary heart disease, and schizophrenia.[5,6] We have recently gained a better understanding of the genetic pathways underlying complex behaviors such as alcohol and other substance use, which can be attributed to numerous loci that influence these traits.[7,8] However, despite numerous reports of candidate genes, data on methamphetamine dependence are sparse.[9,10]

Dopamine is involved in various brain functions, including inflammation, sleep, cancer, heart failure, and circadian rhythms, as well as numerous additional functions.[11-13] The plasma membrane's human dopamine transporter protein (hDAT) is a vital regulator of synaptic dopamine transmission. Dopamine reuptake activity,

the density of hDAT, and the dynamics of dopamine neurotransmission can all be altered by variations in the coding gene SLC6A3 (DAT1) on chromosome 5 (chr5). These alterations may influence the pathophysiology of the peripheral and CNSs.[14,15]

The SLC6A3 gene sequence has been associated with a broad spectrum of environmentally sensitive mental health conditions, such as Parkinson's disease, substance use disorders, attention deficit hyperactivity disorder, and major depressive disorder. Serious health concerns arise when comorbidity is present.[16,17] Various environmental risk factors, including drugs, stressors, high-fat meals, stimulant medications, and environmental enrichment, can 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. Although research has not yet been capable of validating these findings, genetic variation of SLC6A3 has also been associated with specific drug misuse.[20,21]

There is currently no thorough investigation of SLC6A3 gene variants among methamphetamine users in the Iranian population. We investigated the causal relationship between the rs2652511 polymorphism in the SLC6A3 gene and methamphetamine use among Iranian Azeri men in this case-control study.

Methods

Sample collection

In this case-control study conducted in 2018 and 2019, we recruited 100 males from the educational hospitals in Tabriz, Iran. All of the women in the investigation were between the ages of 20 and 40. The 60 patients in the case group had just been identified as methamphetamine users who had not undergone any rehabilitation. Participants with serious illnesses were not allowed to participate in the study; this included individuals with chronic conditions, neurological problems, cardiovascular disease, and severe mental illnesses. Additionally, individuals who consumed drugs or substances other than methamphetamine were excluded. The 60 individuals who participated in the control group were matched for age and gender and were referred to as routine physical examinations and health check-ups.

The case and control group participants were selected from East Azerbaijan, Iran, and matched for ethnic background and age, and were genetically unrelated. Interviews and questionnaires were used to collect information regarding each participant's demographic characteristics, clinical history, and lifestyle. The data gathered included gender, age, syphilis infection status, marriage status, literacy levels, and drug use history. Following the ethical guidelines of the Declaration of Helsinki, each participant signed an authorization document after being briefed about the study.

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 PCR-RFLP technique and specific primers (forward: 5′-GGAGCATCGAGGGTACAC-3′ and reverse: 5′-GACGGCCTGGAAAGCCCTG-3′). The amplified fragment (252 bp) was digested by MspI enzymes and incubated at 37 °C. After digestion, five fragments (97 bp, 46 bp, 44 bp, 34 bp, and 31 bp) were obtained for the C allele, and four fragments (141 bp, 46 bp, 34 bp, and 31 bp) for the T allele (Figure 1). The template DNA (1 µg), primers (25 pmol), and PCR master mix (12.5 µL) were employed in a PCR reaction in a 25 µL total volume. Electrophoresis on a 3% agarose gel, designated with a nontoxic stain, was utilized to separate the digested fragments. The size of DNA bands was estimated using a 50 bp size ladder.

Statistical analysis

SPSS software (version 21.0) was used to analyze the collected data statistically. The correlation between the rs2652511 polymorphism in the SLC6A3 gene and methamphetamine use disorder was studied with logistic regression. The Fisher's exact test and chi-square ($\chi 2$) test were employed to evaluate the Hardy-Weinberg equilibrium (HWE) in the genotype distribution of patients and healthy controls. Additionally, 95% confidence intervals (CIs) and the odds ratio (OR) were calculated. The independent sample t-test was applied to investigate the distinctions in clinical and demographic characteristics between the case and healthy control groups. A P value of less than 0.05 was considered statistically significant.

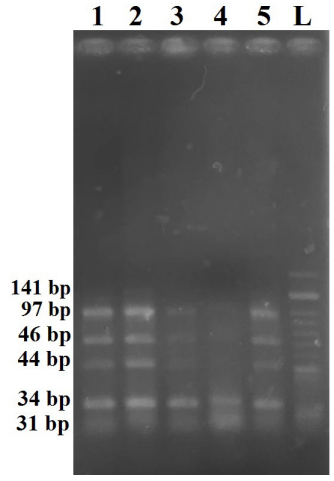

Figure 1. The electrophoresis-digested PCR products of SLC6A3 rs2652511 C/T polymorphism on agarose gel. After digestion, five fragments (97bp, 46 bp, 44 bp, 34 bp, and 31 bp) were produced in the presence of the C allele, and four fragments (141 bp, 46 bp, 34 bp, and 31 bp) were produced in the presence of the T allele

Results

The demographic data of the subjects are presented in Table 1. Age, marital status, and syphilis infection status ($P<0.05$) were significantly different between the patients and the healthy controls. There were no significant differences in body mass index (BMI) or education levels between the two groups ($P>0.05$).

The statistical analysis indicated that the rs2652511 polymorphism in the SLC6A3 gene conformed to HWE for both the case and healthy control groups ($P>0.05$). Allele and genotype frequency distributions for SLC6A3 rs2652511 polymorphism in case and healthy 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 significantly 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. Additionally, the prevalence of the T allele in patients was 27.5%, compared to 54.0% in the healthy controls. By analyzing allele frequencies, it was possible to statistically differentiate between the case and healthy control groups ($P=0.003$; OR = 0.60; 95% CI = 0.49-1.13).

Discussion

Drug addiction is the nonmedical use of illegal substances characterized by symptoms of intoxication or withdrawal. Genetic factors have a significant role in addiction, and dopamine is influential in reward and motivation.[22-24] Individual tendencies towards methamphetamine use disorder may, therefore, be affected by alterations in dopaminergic signaling. In this study, the rs2652511 polymorphism in the promoter of SLC6A3, a crucial protein in the dopaminergic signaling pathway, was evaluated for the first time in its connection with the risk of methamphetamine use disorder in Iranian men. The findings of the study, which was conducted on 100 men with methamphetamine use disorder and 100 healthy men, showed a significant correlation between the rs2652511 polymorphism and methamphetamine use in Iranian men.

Evidence has demonstrated that the SLC6A3 gene is influential in drug dependency.[25,26] A 40 bp 3-untranslated polymorphism (rs28363170) was linked with risky behavior, such as use of cannabis, heroin, cocaine, tobacco, alcohol, and other illicit drugs in the Guo et al.[25] study. In another investigation, Stolf et al[26] discovered a significant correlation between crack cocaine usage and this polymorphism. Our investigation identified a substantial correlation between the rs2652511 polymorphism and methamphetamine use disorder, which is consistent with the previously cited investigations. The functional rs2652511 polymorphism influences the density of transporter protein, and does not influence the amino

Table 1. The clinical features and demographic characteristics of the subjects with methamphetamine abuse and the healthy controls

Variable	Case (n = 100)	Controls (n = 100)	*P* value*
Age, years	28.41 ± 2.51	32.73 ± 9.22	< 0.001
BMI, kg/m^2	22.19 ± 2.18	22.34 ± 2.55	0.529
Marital status			
Married, No (%)	48 (48%)	77 (57%)	
Single, No (%)	23 (30%)	15 (15%)	0.004
Divorced, No (%)	19 (19%)	8 (8%)	
Educational degree			
Under diploma and diploma, No (%)	69 (69%)	56 (56%)	0.089
Higher diploma, No (%)	31 (31%)	44 (44%)	
Drug use history			
Onset age of drug use (years)	24.78 ± 2.28	-	-
Drug use time (years)	4.56 ± 3.24	-	-
Daily frequency of drug use	1.87 ± 2.11	-	-
Drug manner			
Injection, No (%)	20 (20%)	-	-
Oral inhalation, No (%)	80 (80%)	-	-
Syphilis infection status			
Positive, No (%)	15 (15%)	0 (0.0%)	< 0.001
Negative, No (%)	85 (85%)	100 (100.0%)	

Note: BMI: body mass index. *Statistically significant $P<0.05$.

Table 2. Genotype and allele distribution of SLC6A3 gene rs2652511 polymorphism in cases and controls

Inheritance model	Genotype and allele	Patients (n=100)	Controls (n=100)	*P* value*	OR (95% CI)
Codominant	CC	56 (56%)	25 (25%)	Ref	Ref=1
	CT	33 (33%)	42 (42%)	0.801	0.83 (0.54-1.68)
	TT	11 (11%)	33 (33%)	0.002	2.68 (1.10-6.81)
Dominant	CC	56 (56%)	25 (25%)	Ref	Ref=1
	CT+TT	44 (44%)	75 (75%)	0.009	0.73 (0.12-1.03)
Recessive	TT	11 (11%)	33 (33%)	Ref	Ref=1
	CC+CT	89 (89%)	67 (67%)	0.64	1.01 (0.67-1.71)
Overdominant	CT	33 (33%)	42 (42%)	Ref	Ref=1
	CC+TT	67 (67%)	58 (58%)	0.77	1.02 (0.59-1.71)
Allele	C	145 (72.5%)	92 (46.0%)	Ref	Ref=1
	T	55 (27.5%)	108 (54.0%)	0.003	0.60 (0.49-1.13)

Note: OR, odds ratio; CI, confidence interval. *Statistically Significant $P<0.05$.

acid sequence of the protein.[27]

Genome-wide association study (GWAS) is the most effective technique for discovering biological pathways in the context of addiction. Therefore, researchers proceeded and performed a GWAS for personality characteristics. 1089 Korean women participated in the research. This genetic study investigated 1042 pathways consisting of 8297 genes. However, no associations were found between the personality trait scores and the genes examined in this research.[28,29] The gender and ethnicity of the individuals in both populations varied, which could influence the findings. It would be advisable that our research be replicated in a female study group and in populations other than Iranians.

Conclusion

In conclusion, this research elucidated methamphetamine use disorder as a complex condition. The findings indicated that the rs2652511 polymorphism in SLC6A3 may be associated with Iranian men's susceptibility to methamphetamine addiction. The function and consequences of this polymorphism in methamphetamine use disorder are yet to be illuminated. Further study on other groups and races with larger sample numbers is suggested to gain a better understanding of the relationship between this polymorphism and methamphetamine addiction.

Acknowledgments

We would like to extend our gratitude to all participants involved in the study.

Author' Contribution

Conceptualization: Hossein Soltanzadeh.
Data curation: Hossein Soltanzadeh.
Formal analysis: Hossein Soltanzadeh.
Investigation: Mohaddeseh Aminzadeh Khosroshahi, Ali Farrokhi.
Methodology: Mohaddeseh Aminzadeh Khosroshahi, Ali Farrokhi.
Project administration: Hossein Soltanzadeh.
Supervision: Hossein Soltanzadeh.
Writing–original draft: Mohaddeseh Aminzadeh Khosroshahi, Ali Farrokhi.
Writing–review & editing: Hossein Soltanzadeh.

Competing Interests

There was no conflict of interest present.

Ethical Approval

The research was approved by the Committee of Ethics in Research at Tabriz University of Medical Sciences and carried out following the Helsinki Declaration (ethical code: IR.IAU.TABRIZ.REC.1398.082).

Funding

This research was financially supported by the Bonab Branch of Islamic Azad University in Bonab, Iran.

References

1. Liu Y, Hao B, Shi Y, Xue L, Wang X, Chen Y, et al. Violent offences of methamphetamine users and dilemmas of forensic psychiatric assessment. Forensic Sci Res. 2017;2(1):11-7. doi: 10.1080/20961790.2017.1287155.
2. Prakash MD, Tangalakis K, Antonipillai J, Stojanovska L, Nurgali K, Apostolopoulos V. Methamphetamine: effects on the brain, gut and immune system. Pharmacol Res. 2017;120:60-7. doi: 10.1016/j.phrs.2017.03.009.
3. Glasner-Edwards S, Mooney LJ. Methamphetamine psychosis: epidemiology and management. CNS Drugs. 2014;28(12):1115-26. doi: 10.1007/s40263-014-0209-8.
4. Oruji F, Baghbani Arani F, Mahdavi Ortakand M. Evaluation of the gene expression of IL-1β and CASP-1 related to inflammation process in glomerulonephritis patients. J Anim Environ. 2018;10(3):477-82.
5. Asadi Z, Hallajzadeh J, Fathi S, Khanbabaie G, Soltanzadeh H. Expression of microRNA-195 increased significantly in patients with methamphetamine abuse disorder. Gene Cell Tissue. 2021;9(2):e118755. doi: 10.5812/gct.118755.
6. Fathi S, Soltanzadeh H, Tanomand A, Asadi Z, Rezai Moradali S. Investigation of miR-222 as a potential biomarker in diagnosis of patients with methamphetamine abuse disorder. Egypt J Med Hum Genet. 2022;23(1):67. doi: 10.1186/s43042-022-00281-1.
7. Soltanzadeh H, Hojjati Bonab Z. Investigation of MIRs in amphetamine misusers. J Shahid Sadoughi Univ Med Sci. 2024;32(4):7739-53. doi: 10.18502/ssu.v32i4.15837.
8. Khanbabaie G, Soltanzadeh H, Montazem H. High expressions

of microRNA-143 in patients with methamphetamine abuse disorder: case-control study. Res Mol Med. 2022;10(4):255-62. doi: 10.32598/rmm.10.4.1263.1.
9. Rezai Moradali S, Soltanzadeh H, Montazam H, Asadi Z, Fathi S. MicroRNA-127 and microRNA-132 expression in patients with methamphetamine abuse in East Azerbaijan, Iran: a case-control study. Addict Health. 2022;14(3):214-7. doi: 10.34172/ahj.2022.1298.
10. Hashemi A, Bigdeli R, Shahnazari M, Oruji F, Fattahi S, Panahnejad E, et al. Evaluation of inflammasome activation in peripheral blood mononuclear cells of hemodialysis treated patients with glomerulonephritis. Iran J Pharm Res. 2021;20(3):609-17. doi: 10.22037/ijpr.2020.114260.14757.
11. Wu Y, Hu Y, Wang B, Li S, Ma C, Liu X, et al. Dopamine uses the DRD5-ARRB2-PP2A signaling axis to block the TRAF6-mediated NF-κB pathway and suppress systemic inflammation. Mol Cell. 2020;78(1):42-56.e6. doi: 10.1016/j.molcel.2020.01.022.
12. Gaweda G, Iyer RP, Shaver PR, Grilo GA, Dinkins ML, Stoffel HJ, et al. Dopamine receptor D3 agonist (pramipexole) reduces morphine-induced cardiac fibrosis. Biochem Biophys Res Commun. 2020;529(4):1080-5. doi: 10.1016/j.bbrc.2020.06.137.
13. Mehralizadeh H, Nazari A, Oruji F, Roostaie M, Hosseininozari G, Yazdani O, et al. Cytokine sustained delivery for cancer therapy; special focus on stem cell- and biomaterial- based delivery methods. Pathol Res Pract. 2023;247:154528. doi: 10.1016/j.prp.2023.154528.
14. Miller DR, Guenther DT, Maurer AP, Hansen CA, Zalesky A, Khoshbouei H. Dopamine transporter is a master regulator of dopaminergic neural network connectivity. J Neurosci. 2021;41(25):5453-70. doi: 10.1523/jneurosci.0223-21.2021.
15. Reith ME, Kortagere S, Wiers CE, Sun H, Kurian MA, Galli A, et al. The dopamine transporter gene SLC6A3: multidisease risks. Mol Psychiatry. 2022;27(2):1031-46. doi: 10.1038/s41380-021-01341-5.
16. Salatino-Oliveira A, Rohde LA, Hutz MH. The dopamine transporter role in psychiatric phenotypes. Am J Med Genet B Neuropsychiatr Genet. 2018;177(2):211-31. doi: 10.1002/ajmg.b.32578.
17. Jabbarzadeh Kaboli P, Shabani S, Sharma S, Partovi Nasr M, Yamaguchi H, Hung MC. Shedding light on triple-negative breast cancer with Trop2-targeted antibody-drug conjugates. Am J Cancer Res. 2022;12(4):1671-85.
18. Redina O, Babenko V, Smagin D, Kovalenko I, Galyamina A, Efimov V, et al. Gene expression changes in the ventral tegmental area of male mice with alternative social behavior experience in chronic agonistic interactions. Int J Mol Sci. 2020;21(18):6599. doi: 10.3390/ijms21186599.
19. Amato D, Canneva F, Cumming P, Maschauer S, Groos D, Dahlmanns JK, et al. A dopaminergic mechanism of antipsychotic drug efficacy, failure, and failure reversal: the role of the dopamine transporter. Mol Psychiatry. 2020;25(9):2101-18. doi: 10.1038/s41380-018-0114-5.
20. Lohoff FW, Bloch PJ, Hodge R, Nall AH, Ferraro TN, Kampman KM, et al. Association analysis between polymorphisms in the dopamine D2 receptor (DRD2) and dopamine transporter (DAT1) genes with cocaine dependence. Neurosci Lett. 2010;473(2):87-91. doi: 10.1016/j.neulet.2010.02.021.
21. Guindalini C, Howard M, Haddley K, Laranjeira R, Collier D, Ammar N, et al. A dopamine transporter gene functional variant associated with cocaine abuse in a Brazilian sample. Proc Natl Acad Sci U S A. 2006;103(12):4552-7. doi: 10.1073/pnas.0504789103.
22. Safarzadeh Kozani P, Shabani S. Adverse events and side effects of chimeric antigen receptor (CAR) T cell therapy in patients with hematologic malignancies. Trends Med Sci. 2021;1(1):e116301. doi: 10.5812/tms.116301.
23. Hall FS, Chen Y, Resendiz-Gutierrez F. The streetlight effect: reappraising the study of addiction in light of the findings of genome-wide association studies. Brain Behav Evol. 2020;95(5):230-46. doi: 10.1159/000516169.
24. Poisson CL, Engel L, Saunders BT. Dopamine circuit mechanisms of addiction-like behaviors. Front Neural Circuits. 2021;15:752420. doi: 10.3389/fncir.2021.752420.
25. Guo G, Cai T, Guo R, Wang H, Harris KM. The dopamine transporter gene, a spectrum of most common risky behaviors, and the legal status of the behaviors. PLoS One. 2010;5(2):e9352. doi: 10.1371/journal.pone.0009352.
26. Stolf AR, Szobot CM, Halpern R, Akutagava-Martins GC, Müller D, Guimaraes LS, et al. Crack cocaine users show differences in genotype frequencies of the 3′ UTR variable number of tandem repeats of the dopamine transporter gene (DAT1/SLC6A3). Neuropsychobiology. 2014;70(1):44-51. doi: 10.1159/000365992.
27. Shabani S, Forouzandeh Moghadam M, Mousavi Gargari SL. Isolation and characterization of a novel GRP78-specific single-chain variable fragment (scFv) using ribosome display method. Med Oncol. 2021;38(9):115. doi: 10.1007/s12032-021-01561-3.
28. Kim HN, Kim BH, Cho J, Ryu S, Shin H, Sung J, et al. Pathway analysis of genome-wide association datasets of personality traits. Genes Brain Behav. 2015;14(4):345-56. doi: 10.1111/gbb.12212.
29. Agabio R, Campesi I, Pisanu C, Gessa GL, Franconi F. Sex differences in substance use disorders: focus on side effects. Addict Biol. 2016;21(5):1030-42. doi: 10.1111/adb.12395.