Association of single nucleotide polymorphism and methylation of dopamine system-related genes with psychotic symptoms in patients of methamphetamine use disorders

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Abstract

Background: Methamphetamine use disorders (MAUD) can substantially jeopardize public security due to their high-risk social psychology and behavior. Given that the dopamine reward system is intimately associated with MAUD, we investigated the association of single nucleotide polymorphisms (SNPs), as well as methylation status of DRD4, COMT genes and paranoid, motor impulsive symptoms in MAUD patients. Methods: A total of 189 MAUD patients participated in our study. Samples of peripheral blood were used to detecte for 3 SNPs and levels of 35 CpG units of methylation in the DRD4 gene's promoter region, 5 SNPs and 39 CpG units in the COMT gene. Results: MAUD patients with rs1800955 C allele have a lower percentage of paranoid symptom than those with rs1800955 TT. Individuals with paranoid symptom exhibited reduced methylation degree at particular DRD4 CpG2.3 unit. The interaction of the DRD4 rs1800955 C allele and the reduced DRD4 CpG2.3 methylation degree resulted in the lower occurrence of the paranoid symptom. Meanwhile, those with COMT rs4818 CC allele have lower motor impulsivity scores in MAUD patients, but greater COMT methylation levels in the promotor region and methylation degree at COMT CpG 51.52 unit. Therefore, based only on COMT rs4818 CC polymorphism, there was a negative correlation between COMT methylation and motor impulsivity scores in the MAUD patients. Conclusions: Our results found that the combination of SNP genotyping and methylation status of the DRD4 and COMT genes may serve as biological indicators to evaluate the prevalence of relatively high-risk psychotic symptoms in MAUD patients.

Introduction:

Methamphetamine (MA) abuse is a significant social and public health issue on a global scale [1]. According to the World Drug Report 2022 (UNDOC) [2], 34 million persons between the ages of 15 and 64, or 0.7% of the world's population, are expected to have used amphetamines (mainly amphetamine and MA) in the last year. Recent estimates indicate that up to approximately 40% of MA users suffer from psychotic symptoms, such as delusions, impulsivity, and violence, etc. These symptoms can even persist long after MA use is discontinued and may prove refractory to antipsychotic medications[2-4]. More importantly, regarded as social high-risk factors, these symptoms frequently lead to prominent violence, widespread infectious disease transmission, and other undesirable social negative outcomes [5, 6]. Therefore, it's very necessary to find biological indicators which can warn of these psychotic symptoms in MAUD patients.

The development of MAUD results from the interaction of genetic and environmental variables, with the

genetic factors accounting for 33%˜79% of the disease [7, 8]. Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms brought on by variations in a single nucleotide at the genome level. They constitute the third generation marker of the human genetic map and were first identified in 1996. Comprising more than 90% of all known polymorphisms, SNPs are the most common type of heritable variation. Protein expression or function will be significantly impacted by SNPs mutations that occur in the gene coding region or gene regulatory region. Some SNPs can lead to abnormalities in the development and function of related key brain nerves. Studies have shown that the SNPs rs3916965, rs2391191, rs947267, and rs1421292 in the G72 gene[9], which encodes the D-amino acid oxidase activator, are associated with MA-induced psychosis, whereas the SNP rs130058, rs1228814, and rs1228814 in the 5HT1bR gene [10], and the SNP rs2070744, rs1799983 in the NO3 gene have no significant association with MA-induced psychosis [11].

The function of epigenetics in disease development, which is considered to be an outcome of environmental influences, has also received considerable attention recently. Compared with genetics, epigenetics, including DNA and histone modifications, refers to the regulation of function without changing the nucleotide sequence of gene[12]. One of the best known DNA modification processes is DNA methylation, a key epigenetic mechanism that regulates gene expression by altering chromatin structure, DNA conformation, stability, and DNA protein interaction mode [13, 14]. DNA methylation is linked to pathophysiological changes in a variety of psychiatric disorders, such as eating disorders [15], depressive disorders [16], schizophrenia[17], cocaine use disorder [18], and alcohol dependence [19]. DNA methylation has also been shown to be an important gene regulatory mechanism for MA-induced alterations in learning and memory in mouse models [20]. Epigenetics plays an important role in regulating MA addiction[21]. However, few studies have reported about its association with MAUD-related psychotic symptoms.

Dopaminergic neurons and their projections are involved in reward circuits, which are crucial for the development of MAUD. MA and dopamine (DA) share a similar structure. Soon after entering the brain, MA not only promotes the release of DA but also inhibits DA reuptake, thus causing the depletion of DA in nerve terminals, disrupting the balance of DA in the brain, and leading to continuous neuronal excitation and euphoric effect [22]. Therefore, the dopamine system-related genes of dopamine receptor type 4 (DRD4) and catechol-O-methyltransferase (COMT), a subtype of dopamine receptors and an enzyme catalyzing dopamine degradation, were chosen for our present study. We sought to determine the relationship between gene polymorphisms, as well as promoter methylation status in COMT and DRD4 genes and psychotic symptoms in MAUD patients.

Materials and methods:

Study recruitment

MAUD patients aged 18 to 60 years old with at least 14 days of MA abstinence at the time of research participation were recruited for this investigation. A total of 189 MAUD participants were ultimately enrolled in this study. All participants were Han Chinese in ethnicity, and all participants were male. Written informed consent was obtained from each participant.

MAUD diagnosis and symptom assessment

All subjects were interviewed by trained interviewers using the Chinese version of the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA). The SSADDA was used to assess a variety of psychiatric disorders, including MAUD [23], according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-4); the Chinese version of SSADDA has good reliability and validity in diagnosing MA dependence (according to DSM-4 criteria) and MAUD (according to DSM-5 criteria)[24, 25]. We used the DSM-5 criteria in this study. In the MAUD section, the participants were asked whether they had ever had ever experienced paranoia, tolerance, or withdrawal. The data were scored using a computer algorithm to determine the presence of a lifetime diagnosis of MAUD according to DSM-5 criteria..

The Aggression Questionnaire developed by Buss and Perry was used to assess the level of aggression in participants with MAUD[26]; this scale assesses individual aggression characteristics in terms of cognitive,

emotional, and behavioral traits and is widely used and is recognized as a standard tool worldwide. The 11-item Barratt Impulsiveness Scale (BIS)-11 [27] is used to assess the level of impulsivity in people with MAUD and is recognized as the standard tool for assessing impulsivity. For nearly 50 years, the BIS-11 has been the most widely cited impulsivity measure. In China, different scholars have translated and revised the BIS-11. In this study, we used the revised Chinese version of the BIS-11. The revised version kept the original items and the number of dimensions, and only revising only the language settings of the items[28].

Genotyping

DNA was extracted from ethylenediaminetetraacetic acid (EDTA)-treated peripheral blood (3ml) collected from participants through venipuncture and stored at - 80 °C. There have been many studies of polymorphisms in dopaminergic reward pathway related gene and disease associations, and some SNPs may affect the susceptibility to or severity of various substance use disorders [29]. Information on the DRD4 and COMT genes was downloaded from the HapMap database and the SNPs with a minimum allele frequency (MAF) [?] 1%. Biologically SNPs, which affect gene function, are generally located in gene promoters, exons, and 5' untranslated regions (5' UTRs). Based on the above screening criteria, a total of 8 SNP units in the DRD4 and COMT genes were selected in this study. DRD4 rs1800955, rs747302, rs3758653 and COMT rs4680, rs4818, rs6267, rs4633, rs6269 were genotyped by the TaqMan method using the ABI PRISM 3730XL Sequence Detection System (ABI, Foster City, CA, USA) carried by BGI Genomics Co., Ltd (Beijing, China). Two-tailed Pearson's chi-square (χ^2) tests were used to compare the distribution of genotypes with those predicted under Hardy–Weinberg equilibrium.

Methylation detection by MassARRAY

Two assays covering DRD4 and COMT promoters were performed, as these promoters have been the most widely implicated in the context of epigenetics. Methylation assays were designed using Epidesigner software (http://www.epidesigner.com/). A total of 35 CpG units in the promoter region of DRD4were evaluated (Supplementary Figure 1). For the promoter region of COMT, 39 CpG units were investigated (Supplementary Figure 2). DNA samples were amplified by polymerase chain reaction technique using the designed primers (Supplementary Figures 1 and 2), and DNA methylation was detected by BGI Genomics Co., Ltd. The methylation levels were quantified using MassARRAY and methylation ratios were calculated using EpiTyper software. The level of methylation is the average of the degrees of methylation of all CpG units in one gene. The degrees of methylation were expressed by the methylation percentage [mC/(mC+C)] for each CpG unit (mC is methylated cytosine and C is unmethylated cytosine).

Statistical analysis

All quantitative variables were tested for the normality using the skewness/kurtosis test, all of which were nonnormally distributed and expressed as the median (interquartile range) [M(IQR)]. χ^2 tests were used to compare genotypic differences in SNPs between MAUD patients with/without MA-induced psychotic symptoms. The nonparametric Mann-Whitney U test was used to compare the differences in total impulsivity scores, total aggression scores, and their scores on each dimension among participants in the MAUD group with different genotypes at the 8 SNPs. In the analysis of the degree of methylation in the promoter regions of the two genes, the following statistical methods were used. The nonparametric Mann-Whitney U test was used to compare the differences in the methylation of each CpG unit of the promoter regions of the two genes between patients in the MAUD group with and without MA-induced paranoid symptoms; Spearman correlation analysis was used to explore the correlation between the methylation levels and psychotic symptoms.

Results

Hardy-Weinberg balance test

A total of 189 MAUD patients were recruited in this study, aged from 18 to 60 years old (mean age=34 years [SD=7]). In case-control studies, the Hardy-Weinberg equilibrium serves as a preliminary quality check and constitutes the cornerstone of population genetic data research[30, 31]. χ^2 tests were performed to compare the

distribution of observed genotypes with that was predicted under Hardy–Weinberg equilibrium. All 8 SNPs in our genotype study were under Hardy-Weinberg equilibrium (p>0.01), indicating that the 8 SNPs were stable in the two population groups, which allowed us to perform further investigations on the relationship between the phenotype variants and the particular disease (Table 1).

The associations of DRD4 rs1800955 and DRD4 CpG2.3 unit methylation with the prevalence of the paranoid symptoms.

Three SNPs of DRD4 and 5 SNPs of COMT were analyzed in our study, but only the genotype distribution of DRD4 rs1800955 was significantly different between the groups with and without MA-induced paranoid symptoms (χ^2=4.268, p=0.039) (Table 2). MAUD patients carrying the major allele (TT) of DRD4 rs1800955 had a significantly higher proportion of MA-induced paranoid symptoms (51.47%) than those carrying the minor C allele (CC and CT individuals: C carriers) (35.58%) (Figure 1A). However, there was no difference in the methylation level of DRD4 between these two groups (TT & C carriers; z=-1.668, p=0.0955) (Figure 1B). Because the methylation level of DRD4 showed no significant difference between patients with and without paranoid symptoms (z=-0.400, p=0.689, Figure 1C), we further compared the methylation degrees of all 35 DRD4 CpG units. Figure 1D and 1E show that the methylation degrees of CpG_2.3 in the promoter region of the DRD4 gene were lower in patients with MA-induced paranoid symptoms compared to those without paranoid symptoms (z=-2.273, p= 0.023), and this difference was mainly driven by MAUD patients carrying C alleles (z=-1.994, p=0.046). The interaction of DRD4 rs1800955 C allele and decreased DRD4 CpG2.3 methylation degree contributed to the lower prevalence of the paranoid symptom (Figure 1F).

The association of the COMT rs4818 G allele and COMT CpG51.52 methylation with the motor impulsivity symptom in MAUD patients.

The relationships of the 8 SNPs in the DRD4 and COMT genes with the motor impulsive symptoms in MAUD patients were also investigated. The Mann-Whitney U nonparametric test revealed that there were statistically significant differences in motor impulsivity scores between COMT rs4818 genotypes (Table 3). Patients carrying the minor alleles had significantly higher motor impulsivity scores (GG and CG, 42.61) than patients with the major allele (CC, 36.89) (z=-2.271, p=0.023, Figure 2A), indicating that the G allele carriers were more impulsive in this study. As shown in Figure 2B, there was a significant difference in the methylation level in the promoter region of COMT gene between MAUD patients carrying the minor allele (CG and GG) and the major allele (CC), and this difference was identified to the methylation degree of the CpG51.52 unit (Figure 2B, C, p=0.035 & p=0.023, respectively). Neither the methylation level of COMT nor the methylation degrees at the CpG 51.52 unit of the COMT gene had a correlation with motor impulsivity score among MAUD patients (Figure 2D, E). Depending on the COMT rs4818 CC polymorphism, there were negative correlations between COMT methylation status and motor impulsivity scores in MAUD patients (r= -0.315, p=0.011) (Figure 2F). However, as shown in Figure 2G showed that, depending on COMT polymorphism, there was a weak correlation between the degree of methylation of the CpG51.52 unit at the promoter region of the COMT gene and the motor impulsivity score (r= -0.268, p=0.042). The results indicated that the interaction of the COMT rs4818 G allele and COMT methylation status was associated with motor impulsivity symptom prevalence in MAUD patients.

Discussion:

Substance use disorders are chronic brain diseases that have serious effects on people's health and socioeconomic status worldwide. Genetic, epigenetic, and environmental variables all play a role in the neuropsychiatric state of drug dependence [7, 32, 33]. The DA system is the most well-known neurobiological component of substance use disorders. DA primarily exerts its influence by interacting with and activating dopamine receptors, which belong to the G-protein-coupled receptor superfamily. To date, few studies have examined the synergistic impact of genetic-epigenetic (G×E) regulatory variables on the symptoms in patients with MAUD. Here, we discovered that DRD4 rs1800955-CpG2.3 and COMT rs4818-CpG51.52 interactions were associated with paranoid and motor impulsive symptoms of MAUD, respectively.

DRD4, a member of Gi protein-coupled subtype that is expressed in areas of the limbic system associated

with cognitive and emotional function, inhibits adenosine cyclase, thereby reducing the concentration of the second intracellular messenger cAMP. According to our findings (Table 2, Figure 1A, B), individuals with the DRD4 rs1800955 C allele, but not DRD4 rs747302 or rs3758653, have a decreased prevalence of paranoid symptoms. The polymorphism rs1800955 (-521 T>C) is located at the 5'-promoter region of DRD4; in this gene, the T allele decreases the transcriptional efficiency by 40% compared with the C allele[34], which may be related to differences in psychological and behavioral symptoms. The DRD4 rs1800955 TT polymorphism was associated with higher rates of avoidant and obsessive personality disorder symptomatology [35]. When exposed to neutral or negative (but not positive) social stimuli, DRD4 rs1800955 CC carriers show higher mean positive affect ratings than T carriers[36]. Although the DRD4 gene's SNP rs1800955 C allele showed a nominal association with heroin dependence[37], it was strongly related to cigarette smoking in comparisons of heavy smokers to non-smokers and light smokers to non-smokers [38]. The DRD4 rs1800955 TT and CT genotypes were associated with low reward dependence in juniors ($p<0.001$) and seniors ($p=0.010$), respectively[39]. In contrast, there was no significant connection between the DRD4 rs1800955 SNPs and the progression of attention-deficit /hyperactivity disorder [40]. However, the prefrontal cortex-controlled executive function of humans was significantly impacted by the combination of two SNPs COMT rs4680 and the DRD4 rs1800955 [41].

As a major epigenetic regulatory pathway that controls gene expression, DNA methylation is involved in adaptive changes in neuroplasticity after prolonged drug use [42, 43]. Despite the lack of significant correlations between overall methylation levels in the DRD4 promoter regions and paranoid symptoms in MAUD patients (Figure 1C, D), the methylation degree at the DRD4 CpG2.3 unit was significantly lower in this group.

Similar to our study, several studies have discovered a connection between drug addiction and DRD4 methylation. According to Duan's research, MA users have greater levels of DRD4 CpG1 and CpG4 methylation than controls [44]. Elevated DRD4 promoter methylation raises the risk of Alzheimer's disease in men [45]. A study revealed that there were several correlations of DRD4 rs3758653 and rs11246226 with the methylation levels of some CpG loci in the same gene [46]. Consistent with the SNP sequencing results, the DRD4 rs1800955 C allele had high efficiency in gene transcription, while also exhibiting a lower methylation degree at the DRD4 CpG2.3 unit, DNA methylation is generally thought to inhibit gene transcription. This result suggests that the combination of the methylation and SNPs contributes to the paranoid symptoms (Figure 1F).

The COMT enzyme methylates and thereby inactivates catecholamine neurotransmitters, such as norepinephrine, epinephrine, and DA. COMT rs4818 is associated with cognitive processing, psychological function, pain modulation, brain metabolic activity, etc. According to our data (shown in Table 3 and Figure 2), MAUD patients who were COMT rs4818 G carriers had higher motor impulsivity scores. It has been reported that COMT rs4818 GG carriers have higher levels of COMT in the prefrontal cortex than CC carriers [47]. In addition, COMT gene expression in the prefrontal cortex differs significantly between GG and CC carriers [48]. Moreover, rs4818 GG carriers had an increased level of CHRM1 expression in the human dorsolateral prefrontal cortex [49]. These changes may influence the modulation of human cognitive ability. COMT SNP rs4818 is associated with variation in performance on cognitive tasks, including the Stockings of Cambridge and the Iowa Gambling Task [50]. The Pivac team reported that the presence of the G allele or GG genotype of COMT rs4818 was associated with an increase in several dimensions of negative symptoms and anhedonia [51]. Patients receiving treatment for persistent low back pain displayed a substantial association between COMT rs4818 and their baseline level of impairment ($p = 0.02$)[52].

We also examined the methylation status of the COMT promoter region (Supplementary Figure 2). The results demonstrated that the COMT rs4818 CC genotype was correlated with a greater overall methylation level and greater methylation degree at COMT CpG51.52 in MAUD patients (Figure 2B, 2C). In contrast, the COMT rs4818 GG genotype was strongly associated with a higher level of methylation in children with ADHD[53]. Although the methylation status of the COMT promoter region did not correlate with the motor impulsivity symptom in MAUD patients (Figure 2C, D), when combined with the COMT rs4818 CC genotype, the decreased methylation level of the COMT promoter region and decreased methylation degree

at COMT CpG51.52 unit elevated the motor impulsivity scores (Figure 3E, F). COMT rs4818 G allele and lower level of methylation at the promoter region are both associated with higher levels of COMT, which may result in decreased DA regulation activity in the brain synaptic networks, and thus lay the molecular foundation for the heightened motor impulsive symptoms.

In summary, based on data from 189 MAUD patients, our investigation discovered that DRD4 rs1800955-CpG2.3 and COMT rs4818-CpG51.52 are related to the paranoid and motor impulsivity symptom of MAUD, respectively. Our findings demonstrated that certain genetic-epigenetic regulatory factor combinations may function as biological markers for early illness detection and a target for therapy.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of the privacy of all the participants. Requests to access the datasets should be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Xiangya Hospital of Central South University. The patients/participants provided written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to this study. T.F. and QM.L. prepared the clinical samples and analyzed the data. NM.L. X.T. and F.L. helped in preparing the analyzed data. X.Z and W.H. performed data interpretation, T.F. prepared the figures, H.L. conceptualized the research and wrote the manuscript, J.L. and N.W. critically revised revision of the manuscript. All authors critically reviewed the content and approved the final version for publication.

ETHICAL STATEMENT

This study was performed under the guidelines established by the Ethics Committee of the Second Xiangya Hospital of Central South University (No. 2017-064).

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Richards JR, Laurin EG. Methamphetamine toxicity. *Statpearls* . Treasure Island (FL); 2022.

2. Freckelton I. Methamphetamine-induced psychosis and mental impairment: A challenge from new zealand. *J Law Med* . 2019;27:284-293

3. Hsieh JH, Stein DJ, Howells FM. The neurobiology of methamphetamine induced psychosis. *Front Hum Neurosci* . 2014;8:537

4. McKetin R, Lubman DI, Baker AL, Dawe S, Ali RL. Dose-related psychotic symptoms in chronic methamphetamine users: Evidence from a prospective longitudinal study. *JAMA Psychiatry* . 2013;70:319-324

5. Homer BD, Solomon TM, Moeller RW, Mascia A, DeRaleau L, Halkitis PN. Methamphetamine abuse and impairment of social functioning: A review of the underlying neurophysiological causes and behavioral implications.*Psychol Bull* . 2008;134:301-310

6. Springer AE, Peters RJ, Shegog R, White DL, Kelder SH. Methamphetamine use and sexual risk behaviors in u.S. High school students: Findings from a national risk behavior survey. *Prev Sci* . 2007;8:103-113

7. Lopez-Leon S, Gonzalez-Giraldo Y, Wegman-Ostrosky T, Forero DA. Molecular genetics of substance use disorders: An umbrella review.*Neurosci Biobehav Rev* . 2021;124:358-369

8. Bousman CA, Glatt SJ, Everall IP, Tsuang MT. Genetic association studies of methamphetamine use disorders: A systematic review and synthesis. *Am J Med Genet B Neuropsychiatr Genet* . 2009;150B:1025-1049

9. Kotaka T, Ujike H, Okahisa Y, Takaki M, Nakata K, Kodama M, et al. G72 gene is associated with susceptibility to methamphetamine psychosis.*Prog Neuropsychopharmacol Biol Psychiatry* . 2009;33:1046-1049

10. Ujike H, Kishimoto M, Okahisa Y, Kodama M, Takaki M, Inada T, et al. Association between 5ht1b receptor gene and methamphetamine dependence.*Curr Neuropharmacol* . 2011;9:163-168

11. Okochi T, Kishi T, Ikeda M, Kitajima T, Kinoshita Y, Kawashima K, et al. Genetic association analysis of nos3 and methamphetamine-induced psychosis among japanese. *Curr Neuropharmacol* . 2011;9:151-154

12. Zhang L, Lu Q, Chang C. Epigenetics in health and disease. *Adv Exp Med Biol* . 2020;1253:3-55

13. Moore LD, Le T, Fan G. DNA methylation and its basic function.*Neuropsychopharmacology* . 2013;38:23-38

14. Nestler EJ, Luscher C. The molecular basis of drug addiction: Linking epigenetic to synaptic and circuit mechanisms. *Neuron* . 2019;102:48-59

15. Hubel C, Marzi SJ, Breen G, Bulik CM. Epigenetics in eating disorders: A systematic review. *Mol Psychiatry* . 2019;24:901-915

16. Chen D, Meng L, Pei F, Zheng Y, Leng J. A review of DNA methylation in depression. *J Clin Neurosci* . 2017;43:39-46

17. Ovenden ES, McGregor NW, Emsley RA, Warnich L. DNA methylation and antipsychotic treatment mechanisms in schizophrenia: Progress and future directions. *Prog Neuropsychopharmacol Biol Psychiatry* . 2018;81:38-49

18. Fonteneau M, Filliol D, Anglard P, Befort K, Romieu P, Zwiller J. Inhibition of DNA methyltransferases regulates cocaine self-administration by rats: A genome-wide DNA methylation study.*Genes Brain Behav* . 2017;16:313-327

19. Liu C, Marioni RE, Hedman AK, Pfeiffer L, Tsai PC, Reynolds LM, et al. A DNA methylation biomarker of alcohol consumption. *Mol Psychiatry* . 2018;23:422-433

20. Fan XY, Yang JY, Dong YX, Hou Y, Liu S, Wu CF. Oxytocin inhibits methamphetamine-associated learning and memory alterations by regulating DNA methylation at the synaptophysin promoter. *Addict Biol* . 2020;25:e12697

21. Wang H, Dong X, Awan MUN, Bai J. Epigenetic mechanisms involved in methamphetamine addiction. *Front Pharmacol* . 2022;13:984997

22. Stout KA, Dunn AR, Lohr KM, Alter SP, Cliburn RA, Guillot TS, et al. Selective enhancement of dopamine release in the ventral pallidum of methamphetamine-sensitized mice. *ACS Chem Neurosci* . 2016;7:1364-1373

23. Pierucci-Lagha A, Gelernter J, Chan G, Arias A, Cubells JF, Farrer L, et al. Reliability of dsm-iv diagnostic criteria using the semi-structured assessment for drug dependence and alcoholism (ssadda).*Drug Alcohol Depend* . 2007;91:85-90

24. American Psychiatric Association. *Diagnostic and statistical manual of mental disorders: Dsm-5* . Arlington, VA: American Psychiatric Publishing; 2013.

25. Fan B, Ma J, Zhang H, Liao Y, Wang W, Zhang S, et al. Association of fkbp5 gene variants with depression susceptibility: A comprehensive meta-analysis. *Asia Pac Psychiatry* . 2021;13:e12464

26. Buss AH, Perry M. The aggression questionnaire. *Journal of personality and social psychology* . 1992;63:452

27. Patton JH, Stanford MS, Barratt ES. Factor structure of the barratt impulsiveness scale. *J Clin Psychol* . 1995;51:768-774

28. Zhang J, Su H, Tao J, Xie Y, Sun Y, Li L, et al. Relationship of impulsivity and depression during early methamphetamine withdrawal in han chinese population. *Addict Behav* . 2015;43:7-10

29. Chen D, Liu F, Shang Q, Song X, Miao X, Wang Z. Association between polymorphisms of drd2 and drd4 and opioid dependence: Evidence from the current studies. *Am J Med Genet B Neuropsychiatr Genet* . 2011;156B:661-670

30. Royo JL. Hardy weinberg equilibrium disturbances in case-control studies lead to non-conclusive results. *Cell J* . 2021;22:572-574

31. Stark AE, Seneta E. A reality check on hardy-weinberg. *Twin Res Hum Genet* . 2013;16:782-789

32. Deak JD, Johnson EC. Genetics of substance use disorders: A review.*Psychol Med* . 2021;51:2189-2200

33. Volkow ND, Michaelides M, Baler R. The neuroscience of drug reward and addiction. *Physiol Rev* . 2019;99:2115-2140

34. Okuyama Y, Ishiguro H, Toru M, Arinami T. A genetic polymorphism in the promoter region of drd4 associated with expression and schizophrenia. *Biochem Biophys Res Commun* . 1999;258:292-295

35. Joyce PR, Rogers GR, Miller AL, Mulder RT, Luty SE, Kennedy MA. Polymorphisms of drd4 and drd3 and risk of avoidant and obsessive personality traits and disorders. *Psychiatry Res* . 2003;119:1-10

36. Gilman TL, Ford MT, Jasnow AM, Coifman KG. Drd4 polymorphism associated with greater positive affect in response to negative and neutral social stimuli. *Ann Hum Genet* . 2022;86:218-223

37. Vereczkei A, Demetrovics Z, Szekely A, Sarkozy P, Antal P, Szilagyi A, et al. Multivariate analysis of dopaminergic gene variants as risk factors of heroin dependence. *PLoS One* . 2013;8:e66592

38. Perez-Rubio G, Ramirez-Venegas A, Noe Diaz V, Garcia Gomez L, Elvira Fabian K, Garcia Carmona S, et al. Polymorphisms in htr2a and drd4 predispose to smoking and smoking quantity. *PLoS One* . 2017;12:e0170019

39. Abrahams S, McFie S, Lacerda M, Patricios J, Suter J, September AV, et al. Unravelling the interaction between the drd2 and drd4 genes, personality traits and concussion risk. *BMJ Open Sport Exerc Med* . 2019;5:e000465

40. Li Y, Baker-Ericzen M, Ji N, Chang W, Guan L, Qian Q, et al. Do snps of drd4 gene predict adult persistence of adhd in a chinese sample?*Psychiatry Res* . 2013;205:143-150

41. Mitaki S, Isomura M, Maniwa K, Yamasaki M, Nagai A, Nabika T, et al. Impact of five snps in dopamine-related genes on executive function.*Acta Neurol Scand* . 2013;127:70-76

42. Cadet JL, Jayanthi S. Epigenetics of addiction. *Neurochem Int* . 2021;147:105069

43. Lax E, Szyf M. The role of DNA methylation in drug addiction: Implications for diagnostic and therapeutics. *Prog Mol Biol Transl Sci* . 2018;157:93-104

44. Ji H, Xu X, Liu G, Liu H, Wang Q, Shen W, et al. Dopamine receptor d4 promoter hypermethylation increases the risk of drug addiction.*Exp Ther Med* . 2018;15:2128-2133

45. Ji H, Wang Y, Jiang D, Liu G, Xu X, Dai D, et al. Elevated drd4 promoter methylation increases the risk of alzheimer's disease in males.*Mol Med Rep* . 2016;14:2732-2738

46. Zhang R, Dang W, Zhang J, He R, Li G, Zhang L, et al. Methylation quantitative locus rs3758653 in the drd4 gene is associated with duration from first heroin exposure to addiction. *Brain Res* . 2022;1775:147746

47. Parkin GM, Udawela M, Gibbons A, Scarr E, Dean B. Catechol-o-methyltransferase (comt) genotypes are associated with varying soluble, but not membrane-bound comt protein in the human prefrontal cortex. *J Hum Genet* . 2018;63:1251-1258

48. Dean B, Parkin GM, Gibbons AS. Associations between catechol-o-methyltransferase (comt) genotypes at rs4818 and rs4680 and gene expression in human dorsolateral prefrontal cortex. *Exp Brain Res* . 2020;238:477-486

49. Dean B, Scarr E. Comt genotype is associated with differential expression of muscarinic m1 receptors in human cortex. *Am J Med Genet B Neuropsychiatr Genet* . 2016;171:784-789

50. Roussos P, Giakoumaki SG, Pavlakis S, Bitsios P. Planning, decision-making and the comt rs4818 polymorphism in healthy males.*Neuropsychologia* . 2008;46:757-763

51. Madzarac Z, Tudor L, Sagud M, Nedic Erjavec G, Mihaljevic Peles A, Pivac N. The associations between comt and mao-b genetic variants with negative symptoms in patients with schizophrenia. *Curr Issues Mol Biol* . 2021;43:618-636

52. Omair A, Mannion AF, Holden M, Fairbank J, Lie BA, Hagg O, et al. Catechol-o-methyltransferase (comt) gene polymorphisms are associated with baseline disability but not long-term treatment outcome in patients with chronic low back pain. *Eur Spine J* . 2015;24:2425-2431

53. Fageera W, Chaumette B, Fortier ME, Grizenko N, Labbe A, Sengupta SM, et al. Association between comt methylation and response to treatment in children with adhd. *J Psychiatr Res* . 2021;135:86-93

Figure Legends:

Figure 1. **Association between DRD4 and paranoid symptoms in MAUD patients.**

A: Proportion of MAUD individuals with (red) or without (blue) paranoid symptom in the DRD4 rs1800955 TT and C+ groups.

B: Methylation level at DRD4 promoter region of the DRD4 rs1800955 TT and C+ MAUD patients.

C: Methylation level at DRD4 promoter region of the MAUD individuals with or without paranoid symptom.

D: Respective methylation degree of the CpG units in the DRD4 promoter region among the MAUD individuals with (red) and without (blue) paranoid symptom.

E: the methylation degree at the DRD4 CpG2.3 unit among the MAUD individuals with and without paranoid symptom.

F: the interaction effects of DRD4 rs1800955 polymorphism and DRD4 CpG2.3 unit methylation on the paranoid symptoms in the MAUD patients.

Figure 2. **Association between COMT and impulsivity symptom in MAUD.**

A: Motor impulsivity scores in MAUD individuals withthe COMT rs4818 CC and G+ genotypes.

B: Methylation level of COMT promoter region in MAUD individuals with the COMT rs4818 CC and G+ genotypes.

C: Methylation degree of the COMT CpG51.52 unit in the COMT promoter region among MAUD individuals with the COMT rs4818 CC and G+ genotypes.

D: Correlation of total methylation level of the COMT promoter region and the motor impulsivity symptoms in MAUD patients.

E: Correlation of methylation degree of the COMT CpG51.52 unit in the COMT promoter region and the motor impulsivity symptoms in MAUD patients.

F: Synergistic effects of COMT rs4818 polymorphisms and COMT methylation level on the motor impulsivity symptoms in MAUD patients.

G: Synergistic effects of COMT rs4818 polymorphisms and COMT CpG51.52 methylation on the motor impulsivity symptom in MAUD patients.

Table 1. Hardy-Weinberg equilibrium test for SNP genotyping

SNP	**Genotype**	**Observed N(%)**	**Expected N(%)**	χ^2	**p**
DRD4					
rs1800955				2.625	0.269
	TT	68(39.5%)	32(30.5%)		
	CT	86(50.0%)	58(55.2%)		
	CC	18(10.5%)	15(14.3%)		
rs747302				8.551	0.014
	GG	69(40.4%)	31(29.5%)		
	CG	72(42.1%)	63(60.0%)		
	CC	30(17.5%)	11(10.5%)		
rs3758653				0.016	0.992
	TT	85(49.7%)	52(49.5%)		
	CT	74(43.3%)	46(43.8%)		
	CC	12(7.0%)	7(6.7%)		
COMT					
rs4680				3.084	0.214
	GG	99(53.2%)	55(52.4%)		
	AG	80(43.0%)	41(39.0%)		
	AA	7(3.8%)	9(8.6%)		
rs4818				4.268	0.118
	CC	69(37.1%)	52(49.5%)		
	CG	93(50.0%)	42(40.0%)		
	GG	24(12.9%)	11(10.5%)		
rs6267				1.915	0.166
	GG	162(87.1%)	97(92.4%)		
	GT	24(12.9%)	8(7.6%)		
	TT	0(0%)	0(0%)		
rs4633				2.132	0.344
	CC	97(52.2%)	58(55.2%)		
	CT	81(43.5%)	39(37.1%)		
	TT	8(4.3%)	8(7.6%)		
rs6269				2.338	0.311
	AA	72(40.2%)	52(49.5%)		
	AG	84(46.9%)	42(40.0%)		
	GG	23(12.8%)	11(10.5%)		

Table 2. Association between SNPs and paranoid symptoms symptoms in MAUD patients

SNP	Genotype	Without MA-induced paranoid symptoms N(%)	With MA-induced paranoid symptoms N(%)	χ^2	p
DRD4					
rs1800955				4.268	0.039
	TT	33(48.53%)	35(51.47%)		
	C+	67(64.42%)	37(35.58%)		
rs747302				0.042	0.837
	GG	41(59.42%)	28(40.58%)		
	C+	59(57.84%)	43(42.16%)		
rs3758653				1.775	0.183
	TT	54(63.53%)	31(36.47%)		
	C+	46(53.49%)	40(46.51%)		
COMT					
rs4680				3.318	0.069
	GG	53(53.54%)	46(46.46%)		
	A+	58(66.67%)	29(33.33%)		
rs4818				0.454	0.500
	CC	39(56.52%)	30(43.48%)		
	G+	72(61.54%)	45(38.46%)		
rs6267				1.072	0.300
	GG	99(61.11%)	63(38.89%)		
	T+	12(50.00%)	12(50.00%)		
rs4633				1.535	0.245
	CC	54(55.67%)	43(44.33%)		
	T+	57(64.04%)	32(35.96%)		
rs6269				0.258	0.612
	AA	41(56.94%)	31(43.06%)		
	G+	65(60.75%)	42(39.25%)		

Table 3. Association between SNPs and Motor impulsivity symptom in MAUD patients

SNP	Genotype	Count	Motor impulsivity score, median (IQR)	z	p
DRD4					
rs1800955				-0.564	0.572
	TT	68	42.25(19.48)		
	C+	104	40.22(15.67)		
rs747302				-0.005	0.996
	GG	69	40.40(17.58)		
	C+	102	41.30(16.98)		
rs3758653				-0.238	0.812
	TT	85	41.00(17.09)		
	C+	86	40.87(17.35)		

SNP	Genotype	Count	Motor impulsivity score, median (IQR)	z	p
COMT					
rs4680				-1.268	0.205
	GG	99	41.75(16.83)		
	A+	87	39.05(17.47)		
rs4818				-2.271	0.023
	CC	69	36.89(16.34)		
	G+	117	42.61(17.31)		
rs6267				-1.940	0.052
	GG	162	39.56(17.28)		
	T+	24	46.76(14.99)		
rs4633				-1.358	0.174
	CC	97	41.78(17.04)		
	T+	89	39.07(17.23)		
rs6269				-1.898	0.058
	AA	72	37.47(16.12)		
	G+	107	42.57(17.68)