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Research Article

Study the genetic variations of GRIAs genes to investigate association to methamphetamine addiction

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Abstract

Objectives: Drug addictions are chronic complicated disorders characterized by the repeated and irresistible intake of specific drugs that produce transient bliss. Methamphetamine, an amphetamine derivative, is an addictive psychostimulant substance used all over the world, especially in South East Asia. Chronic Methamphetamine consumption not only causes dependence but also frequently promotes psychotic symptoms, auditory hallucinations, and paranoid delusions, which are similar to the positive symptoms of schizophrenia. This study looked at the genetic variation of the *GRIA1* and *GRIA3* genes to see if they were associated with methamphetamine addiction in Iraqi males.

Methods: A total of 150 male participants were enrolled in this case-control study (100 methamphetamine-dependent and 50 control), ages between 20 to 45 years. *GRIA1* (rs2195450) and *GRIA3* (rs3761555) polymorphism detection was achieved by using high-resolution melting (HRM) real-time PCR analysis.

Results: The findings of the current study showed a highly significant difference in genotype distribution and allele frequency of rs2195450 SNP between the methamphetamine-dependent and the control groups, according to GA genotype and A allele, and in rs3761555 based on CC genotype and C allele, displaying a positive association with addiction.

Conclusion: According to the result of present study GA and CC genotypes have a positive correlation with disease these could be considered as a risk factor that makes people more susceptible to methamphetamine addiction.

Keywords: Drug addiction, genetic variation, *GRIA1*, *GRIA3*, methamphetamine

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Methamphetamine is the second most often abused substance worldwide. Methamphetamine addiction can lead to weight loss, memory loss, tremors, convulsions, psychosis, paranoia, hallucinations, Parkinson's-like symptoms, and cardiovascular collapse. The intensity of effects of withdrawal differs from person to person [1, 2]. The α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptor (AMPA or GluR) is an ionotropic glutamate receptor subtype with four subunits: GluR1–4. The functional unit of the AMPA receptor is made up of GluR1R2, GluR2R3, or GluR2-lacking combinations. These subunits are expressed in various brain areas, including the hippocampus, nucleus accumbens, dorsal striatum, and prefrontal cortex [3, 4]. The AMPA receptor has an essential role in activities of brain plasticity of synapses, including learning, memory, and drug addiction [5, 6]. Thus, genes encoding AMPA glutamate receptors (GRIAs) are intriguing candidate genes for vulnerability to methamphetamine use and related psychosis [7]. Glutamate Ionotropic Receptor AMPA Type Subunit 1 (*GRIA1*) is situated on chromosome 5q33; earlier research suggested that *GRIA1* may be implicated in the therapeutic effect of major depressive disorder (MDD). Furthermore, it has been reported to be responsible for psychotic characteristics in schizophrenia and bipolar disorder [3, 8]. *GRIA3* is located on Xq25 and is frequently linked to the risk of schizophrenia, bipolar disorder, and alcoholism [9]. Thus, genetic variations in GRIA subunit genes have been linked to both mental disease and

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drug dependence. However, there is no indication that they are associated with either METH dependency or METH-dependent psychosis [9, 10]. Therefore, the goal of this study was to evaluate the relationship of common and previously investigated polymorphisms in *GRIA1* and *GRIA3* with METH dependency in the Iraqi male population. Addiction is connected to both hereditary and environmental causes. Examining the significance of genetic differences in the etiology of addiction might enhance the effectiveness of medications and assist in avoiding diseases. Both genome-wide techniques and candidate gene investigations seek for the fundamental significance of the genetic factor that causes drug addiction by identifying genes that are critical to neural adaption [11, 12].

Materials and Methods

This study was approved by the Ethics Committee of the Ministry of justice in Iraq/ Iraqi correction service (No: 24303/3/1/13, Date: 15/09/2021). This study involving human subjects is in accordance with the Helsinki Declaration of 1975 as revised in 2000.

Study groups and sample collection

This case-control study included 150 participants (100 methamphetamine dependent, 50 control) males ethically aged 20–45. Methamphetamine dependent subjects came from the Iraqi Correction Service. The primary inclusion criterion was diagnosis by a psychiatrist as meeting the criteria for substance dependence according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) [13]. The control subjects were free from any reported personal or family history of drug misuse or psychiatric disorders. All subjects were given a complete illustration of the study. The signed written consent was taken from all participants in the study and the personal information for each participant was presented in questionnaire form under the supervision of the consultant and after obtaining approval for obtaining samples from all participants. The venous blood samples were collected in an EDTA tube from both study groups and genomic DNA was extracted by using ReliaPrep™ Blood gDNA Miniprep System and kept at -20°C for genotyping [14]. The purity and concentration of DNA solu¬tion extracted from blood samples were determined using Nanodrop at 260 nm, and the DNA purity was calculated as a ratio of A260/A280.

Genotyping analysis

The primers of *GRIA1* and *GRIA3* genes were designed for the current study. These primers were supplied by Alpha DNA company in a lyophilized form. Lyophilized primer was dissolved in free nuclease water to give a final concentration of 10 Pmol/ µl as a stock solution. *GRIA1* (rs2195450) and *GRIA3* (rs3761555) polymorphisms were chosen to investigate, these SNPs detection were achieved by using high-resolution melting (HRM) real-time PCR analysis. The reaction of HRM analysis for genotype using quantitative real-time PCR was performed in 20 µL total volume which comprised: 2xTransStart® Tip Green qPCR Super Mix, DNA template, 10 Pmol of Primers for rs2195450 genotyping (forward: 5`GAGGCAGTGCTTTCTCTGCT 3`) and reverse: (5`TTCAGTGGCTGCAATATACCC3`). While (forward: 5` CCTTCACTCCTGTCCATGAAA 3`) and reverse:(5` TCATTCTGATAATGCATAATTTCCTC 3`) for rs3761555 genotyping, then complete volume with nuclease free water. The HRM-PCR program of rs2195450 and rs3761555 polymorphisms displayed in Table 1.

Data analysis

The Statistical Package of Social Science (SPSS) version 26 was used to evaluate the effect of Diverse factors on study parameters. Chi-square was employed to test count variance. Alleles and genotype of gene SNPs. were presented as number and percentage frequencies. Hardy-Weinberg equilibrium analysis of genotype frequency was performed using Fisher test. A mean and standard deviation of continuous variables were calculated (SD). The student's t-test was applied to compare group differences. Statistical differences were defining significant as * $p \leq 0.05$ or ** $p \leq 0.01$ [15, 16].

Results

Demographic results

In this study, the results of some demographic features of two investigated groups are described in Table 2. They were measured by analyzing a questionnaire that was completed during a direct interview with all participants. According to the age mean±SD of the study groups was 28.62 ±8.25 for the methamphetamine dependent group and 27.01±5.14 for the control group, the result found that there was no significant association between both groups studied p=0.08. As mentioned in the Marital status, results showed no significant differences between the control and methamphetamine dependent groups (p=0.1). Then, depending on the occupation the findings of the present study showed significant differences between two studied groups (p=0.001). According to anxiety and depression, self-confidence, improve memory, self-hart and smoking the methamphetamine dependent was comprised of (53%, 56%, 43%, 60%, 86%, 8%) respectively.

Table 1. The HRM-PCR program of rs2195450 and rs3761555 polymorphisms

Step	Temperature (°C)	Time (sec.)	Cycles
Enzyme activation	94	30	1
Denaturation	94	5	
Annealing			
(rs2195450)	60	15	40
(rs3761555)	58		
Extension	72	20	
HRM	55–95	0.2sec for 1 degree	

HRM: High resolution melting; PCR: Polymerase chain reaction.

Table 2. The study groups' anthropometric characteristics

Variables	Methamphetamine dependent n=100		Control n=50		p
	n	%	n	%	
Age	28.62±8.25		27.01±5.14	0.08	NS
Marital status					
Single	54	54.00	28	56.00	0.1 NS
Married	46	46.00	22	44.00	
Occupation					
No	77	77.00	18	36.00	0.001*
Employ	23	23.00	32	64.00	
Anxiety and depression	53	53	–	–	
Self confidence	56	56	–	–	
Improve memory	43	43	–	–	
Self-hart	60	60	–	–	
Smoking	86	86	–	–	
Drink	8	8	–	–	

*: Significant. NS: Non-significant.

GRIA1 and *GRIA3* genotyping using HRM real-time PCR

DNA samples from all research groups were genotyped for rs2195450 and rs3761555 SNPs using HRM real-time PCR reaction. The picture depicts the thermocycler output from the HRM analysis process for the amplification of DNA, as shown in Figure 1.

The rs2195450 was the SNP of the *GRIA1* gene, located on chromosome 5 (5:153491449 (GRCH38)) with allele variation G>A. The genotype result for SNP rs2195450 displayed in Figure 2, where A refer to the HRM result, while B represents the wild, heterozygous and mutant genotypes.

In contrast, the rs3761555 was the SNP of the *GRIA3* gene, located on chromosome X at locus123182584, located in the promotor region with allele variation T>A,C. The genotype result for SNP rs3761555 clarified in Figure 3, where A refer to the HRM result, while B represents the wild, heterozygous and mutant genotypes.

The results displayed in Table 3, there were a highly significant differences in genotype distribution and allele frequency of rs2195450 SNP between the methamphetamine dependent and the control groups, according to GA genotype and A allele, These have a positive correlation with disease (odds ratio

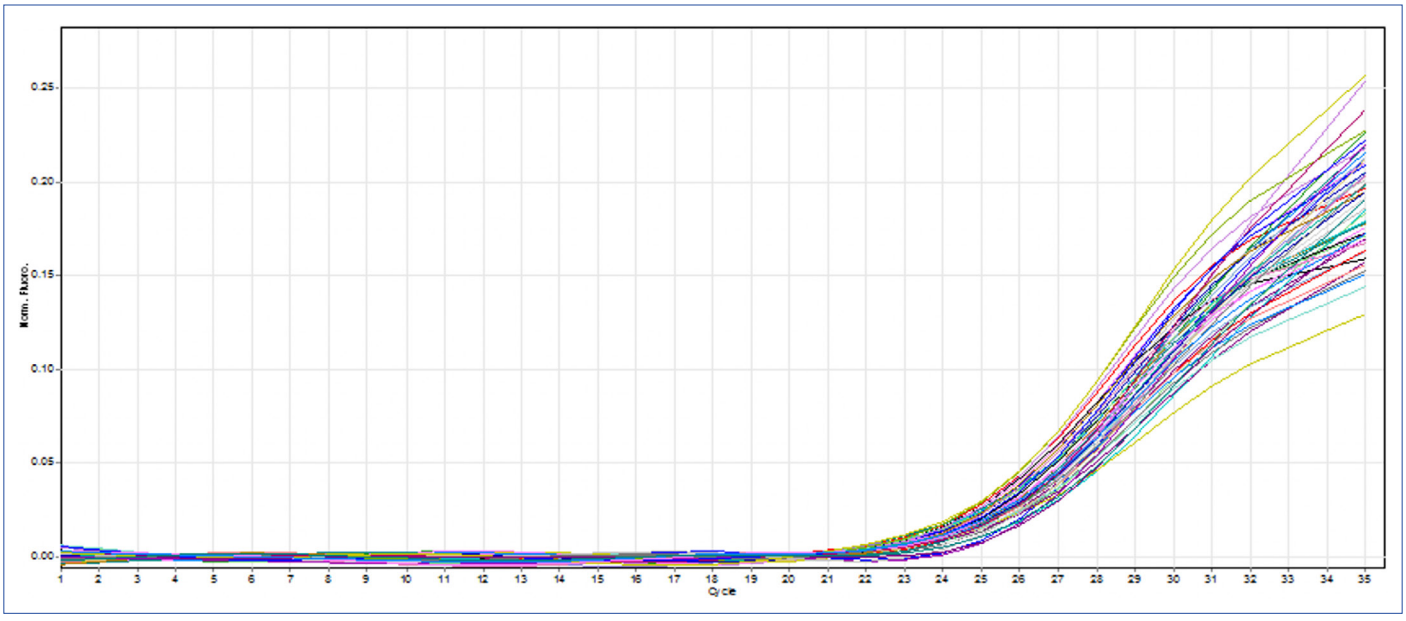

Figure 1. Amplification of DNA, the picture was taken directly from HRM analysis software.

HRM: High-resolution melting.

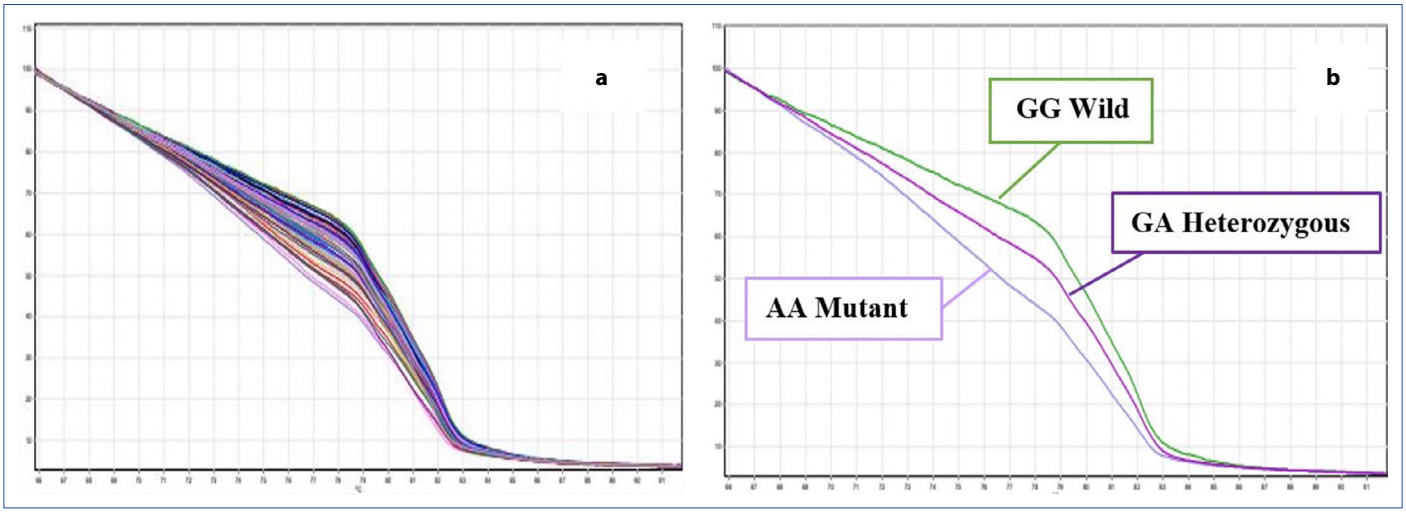

Figure 2. The genotype result for SNP rs2195450, (a) represents the HRM result, (b) represents the wild, heterozygous and mutant genotypes.
GG: Wild genotypes; GA: Heterozygous genotypes; AA: Mutant genotypes; HRM: High-resolution melting.

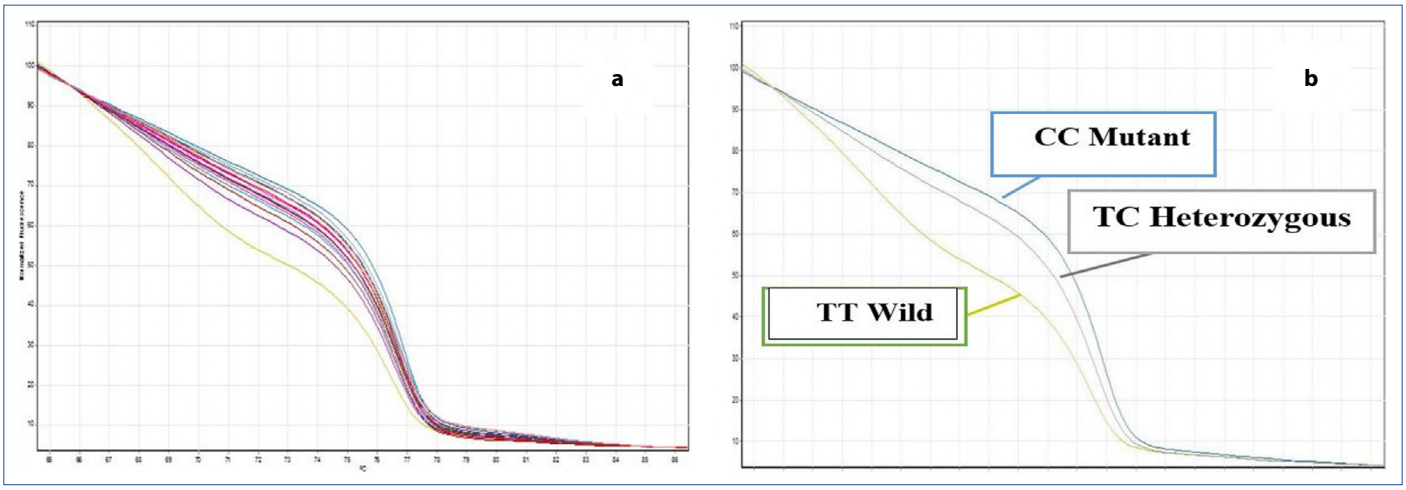

Figure 3. The genotype result for SNP rs3761555, (a) represents the HRM result, (b) represents the wild, heterozygous and mutant genotypes.
TT: Wild genotypes; TC: Heterozygous genotypes; CC: Mutant genotypes.

6 and 4.36 respectively) these could be considered as a risk factor that makes people more susceptible to drug addiction. On the other hand, there were a highly significant differences in rs3761555 genotype distribution and allele frequency between two studied groups, based on CC genotype and C allele, The odd ratio of these (6.09 and 6.75 respectively), displaying a positive association with addiction, which might be seen as a risk factor that makes people more vulnerable to addiction.

The observed genotype frequencies of rs2195450 SNP exhibited no significant differences from those predicted, it was 1.65 in Methamphetamine dependent group and 1.71 in controls, respectively, according to the Hardy-Weinberg Equilibrium. Because the total observed recorded for this locus was highly ratio at the genotype AA, it was 93 may be considered the common genotype in the Iraqi population, the other genotype, GA and, GG were 51, 6 correspondingly. In contrast, the genotype frequencies of rs3761555 SNP observed in methamphetamine dependent group were much greater than those expected by the Hardy-Weinberg Equilibrium hypothesis it was 37.69 while it was less than expected by H.W.E in the control group it was 26.52. TT genotype was shown to be the most common genotype in the Iraqi population with a total observed 99 followed by TC genotype with a total observed 20 CC genotype was present with a total observed 31, as shown in Table 4.

Discussion

This study analyzed the genetic variation of *GRIA1* and *GRIA3* genes and investigated the genetic susceptibility to methamphetamine addiction among sample of Iraqi males. No significant effects were found for AA and GG genotype of *GRIA1* SNP also, TT and TC genotype of *GRIA3* SNP that leading to indicate no impact of these SNPs on methamphetamine addiction. On the other hand, GA and CC have a favorable link with addiction, these could be regarded risk factors for methamphetamine addiction. The results are consistent with the extensive evidence for a disruption of glutamatergic neu-

Table 3. Genotype distribution of *GRIA1* and *GRIA3* polymorphisms in methamphetamine dependent and control groups

Subjects	Genotype frequency						Allele frequency			
***GRIA1* rs2195450**	**AA**		**GA**		**GG**		**A**		**G**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Methamphetamine dependent (n=100)	5	5	45	45	50	50	55	27.5	145	72.5
Control (n=50)	1	2	6	12	43	86	8	8	92	92
p	0.5 NS		0.0001**		–		0.0001**		–	
Odd ratio (95% CI)	2.58 (0.34–62.54)		6.00 (2.4–16.58)		1.00		4.36 (2.05–10.17)		1.00	
GRIA3 rs3761555	TT		TC		CC		T		C	
Methamphetamine dependent (n=100)	54	54	18	18	28	28	126	63	74	37
Control (n=50)	45	90	2	4	3	6	92	92	8	8
p	–		0.01**		0.001**		–		0.0001**	
Odd ratio (95% CI)	1.00		5.27 (1.32–34.61)		6.09 (1.90–26.25)		1.00		6.75 (3.20–15.60)	

**: Significant. GRIA: Glutamate Ionotropic receptor AMPA gene; GG: Wild genotypes; GA: Heterozygous genotypes; AA: Mutant genotypes; NS: non-significant; CI: Confidence interval.

Table 4. Expected frequencies of *GRIA1* and *GRIA3* polymorphisms in methamphetamine dependent and control groups using Hardy-Weinberg Equilibrium

***GRIA1* rs2195450**	**AA**	**GA**	**GG**	**χ^2**	**p**
Methamphetamine dependent genotype					
Observed no.	50	45	5	1.65	0.4 NS
Expected no.	52.56	39.87	7.56		
Control genotype					
Observed no.	43	6	1	1.71	0.4 NS
Expected no.	42.32	7.36	0.32		
Total observed	93	51	6		
***GRIA3* rs3761555**	**TT**	**TC**	**CC**	**χ^2**	**p**
Methamphetamine dependent genotype					
Observed no.	54	18	28	37.69	0.0001**
Expected no.	39.69	46.62	13.69		
Control genotype					
Observed no.	45	2	3	26.52	0.0001**
Expected no.	42.32	7.36	0.32		
Total observed	99	20	31		

**: Significant. NS: non-significant; GRIA: Glutamate Ionotropic receptor AMPA gene; GG, TT: Wild genotypes; GA, TC: Heterozygous genotypes; AA, CC: Mutant genotypes.

rotransmission in the brain in METH-induced psychosis [17]. As previously stated, this glutamatergic dysregulation is similar in mechanism and pathophysiology to that postulated for schizophrenia. The positive symptoms are similar to those of METH dependency [18, 19]. Furthermore, *GRIA3* DNA hypermethylation, as well as a significant decrease in *GRIA3* relative gene expression evaluated in blood samples, has been associated to the increased likelihood of schizophrenia [20]. The BDNF promotes the production of dopamine D3 receptors, which is very crucial in addiction [21, 22]. Taken together, these results reveal potential interaction effects of BDNF and AMPA receptor in boosting dopaminergic neurotransmission, critically altering the mesolimbic dopaminergic pathway in response to exposure to drugs of abuse. On the other hand, the results of this study did not support previous evidence implicating a role for *GRIA1* in METH addiction susceptibility [7], who described was an absence of substantial relationships between *GRIA1* gene polymorphisms and METH dependency in male Thai subjects. Another study by [23] have indicated the role of a *GRIA1* polymorphism in psychotic symptoms, while [8] displayed that GRIA1 has been related with schizophrenia in a large Korean sample. The previous studies by [24, 25], who

discovered a statistically significant result is that the *GRIA1* rs2195450 variant and the rs3761555 SNP of *GRIA3* gene is a potential genetic risk factor for female migraine in the Chinese Han population from the southern Fujian province of China.

Conclusion

According to the result of the present study GA genotype of rs2195450 SNP in *GRIA1* gene and the CC genotype of rs3761555 SNP in *GRIA3* gene have a positive correlation with disease these could be considered as a risk factor that makes people more susceptible to methamphetamine addiction.

Ethics Committee Approval: The study was approved by the Ministry of justice in Iraq/Iraqi correction service Ethics Committee (no: 24303/3/1/13, date: 15/09/2021).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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