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Original Article

# A single-nucleotide polymorphism of GRIN1 in heroin and methamphetamine addicts at a rehabilitation sanatorium in Markazi province, Iran

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**Abstract**

**Introduction:** Using addictive drugs can change the amount of neurotransmitters, especially dopamine and glutamate. Glutamate has been known to trigger the relapse and tendency toward addictive drugs. The glutamate receptor ionotropic NMDA type subunit 1 (GRIN1) contains the single- nucleotide polymorphism C1001G (rs11146020) and encodes N-methyl-D-aspartic acid (NDMA) receptor subunit 1 (NR1). The present study was conducted to investigate the relationship between the rs11146020 polymorphism in GRIN1 and addiction to heroin and methamphetamine.
**Methods:** The present case-control study recruited 90 male heroin and methamphetamine addicts treated with methadone and 100 healthy men. Genomic DNA was extracted from peripheral blood using Iraizol kits. Four pairs of specific primers were designed using AlleleID 7.5, and the T-ARMS PCR was optimized.
**Results:** The genotype distribution of GG, GC and CC was respectively found to be 66%, 31% and 3% in the control group and 58%, 31% and 11% in the patient group. The statistical analysis suggested no significant differences between these two groups.
**Conclusion:** No significant relationships were observed between the C1001G polymorphism in GRIN1 and addiction to heroin and methamphetamine.

## Introduction

Drug addiction, with an increasing prevalence across the world, can damage many parts of the body, including oral cavity, the lung, the liver, the brain and the heart (1). Drug use is a hereditary disease that is caused by combined effects of environmental and genetic factors and the drug (2). The effects induced by drugs such as cocaine and morphine include molecular neurobiological changes that involve gene expression and protein concentration and ultimately cause behavioral changes (3-4). Tsuang et al., who studied heritability in 3372 Vietnamese twins, found genetic changes to play a key role in drug use (2 and 5). Addictive drugs affect many reward-associated molecular properties of the nervous system (6). Research on neuronal addiction is mainly focused on the dopamine mechanism. Furthermore, glutamate has recently been found to play a key role in the fundamental processes associated with developing and maintaining addiction, including sensitization, reinforcement learning and craving. Although most of the glutamate activity is associated with the interaction with the dopaminergic system, some glutamatergic mechanisms independently contribute to addiction (7).

GRIN1 encodes N-methyl-D-aspartic acid (NDMA) receptor subunit 1 (NR1) (8). GRIN1 has been proposed as a candidate for drug addiction. GRIN1 locus was determined during metaphase by using fluorescence in situ hybridization (FISH). A single strong signal was detected on both chromatids of the 9q34,3 chromosome (9).

The relationship between the rs11146020 polymorphism in GRIN1 and susceptibility to schizophrenia (8) has been found to be both significant and insignificant depending on the study population. There is ample evidence associating opioid effects such as painkilling, enduring and dependence to NMDA receptors and suggesting cellular adaptations in glutamate-associated mechanisms that occur in learning and memory (10-11). Evidence also suggests that glutamate plays a key role in anxiety-related behaviors and triggers the relapse and tendency toward addictive drugs (12).

Drugs cause structural and functional changes in the brain. Abnormal behaviors caused by the brain dysfunction are a tangible target for identifying the biological pillars of addiction. Relatively large chromosomal regions contribute to addiction vulnerability, but a single gene may leave hardly noticeable effects (13). The present study was conducted to determine the relationship between the rs11146020 polymorphism in GRIN1 and addiction to heroin and methamphetamine.

## Materials and Methods

The present case-control study was approved by the Ethics Committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1394.162) and recruited 190 men, including 90 heroin and methamphetamine addicts in the patient group who were treated with methadone in the addicts' rehabilitation center of Ebrahimabad, Arak, Iran and 100 healthy men without a history of addiction selected from those presenting to the blood transfusion center in Arak. Five ml of peripheral blood was taken from the subjects and collected in tubes containing anticoagulants after they signed informed consent forms. Genomic DNA was extracted using Iraizol kits (RNA Biotechnology Co., Iran) according to the manufacturer's protocol. In order to evaluate the extracted DNA, 1% agarose gel and biophotometers (Eppendorf) were used (Figure 1).

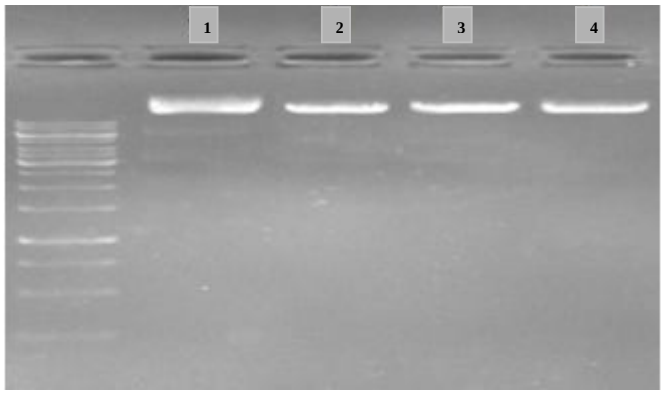


**Figure 1.** 1% agarose gel image of the DNA samples extracted from peripheral blood; from the left: the first well: 1 kbp, lane 1 and 3: patient samples, lane 2 and 4: control samples

Tetra-primer amplification refractory mutation system (T-ARMS) PCR used to investigate genotype distribution is a strong technique for detecting point mutations using mutated and normal primers in two separate tubes. Polymerization in the tube containing mutated primers or normal primers respectively indicates the presence or absence of point mutations in the base. Two pairs of primers were designed using Primer3 and AlleleID 7.5. Table 1 shows the sequence of the primers used to amplify G and C alleles.

**Table 1.** The primer sequence associated with the rs11146020 polymorphism

| Primer | Sequence (3…5) | Tm (˚C) |
|---|---|---|
| Outer Forward | CAGAATCCTCAGTTGCTATTGGAAAT | 58.7 |
| Inner Forward | CTACTCGGGCTAAGAGGAATAG | 58.9 |
| Inner Reverse | CCATGTAACTTGGGACCCGCC | 59.9 |
| Outer Reverse | TCGTCACCCACAGTCAGCGATATTT | 60 |

A thermocycler made by the BioWorld Co. was used in the PCR of the gene. The initial denaturation comprised 4 min at 94 ˚C, followed by 40 cycles (35 sec) at 94 ˚C, 40 sec at 58 ˚C to let the primer bind to DNA, 45 sec at 72 ˚C and 10 min at 72 ˚C for the final amplification of the DNA fragment.

The PCR product was detected on 2% agarose gel using 0.5 X TBE buffers in a gel doc system (Gene Fiash company). The results obtained were analyzed in SPSS-24. The Chi-square test and the logistic regression were used to investigate the significance of the differences. $P<0.05$ was set as the level of statistical significance. Odds ratio was also measured using a confidence interval of 95%.

## Findings

The common fragment derived from the amplification of outer primers OF and OR is a 559-base-pair sequence; that obtained from allele G specific primers IR and OF is a 401-base-pair sequence and from allele C specific primers OR and IF is a 200-base-pair sequence. The GC genotype contains the common fragment and two fragments associated with C and G alleles (Figure 2).

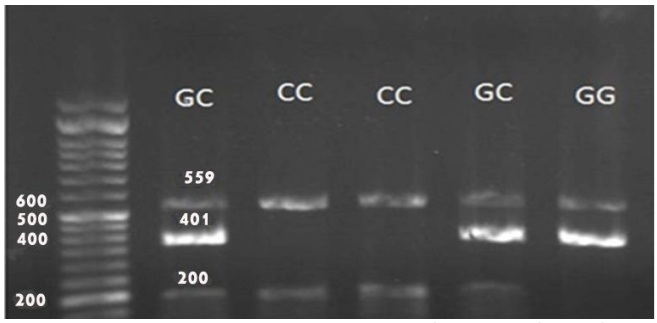


**Figure 2.** Genotype determination using T-ARMS PCR on 2% agarose gel; from the left: the first well contains 50 bp markers; lane 1 and 4 well comprises the GC genotype; lane 2 and 3 well contains the CC genotype and well 5 contains the GG genotype

The genotype distribution of the G/Crs11146020 polymorphism was respectively calculated as 66%, 31% and 3% for genotypes GG, GC and CC in the control group and 58%, 31% and 11% in the heroin and methamphetamine addicts (Table 2). The relationship of genotype distribution with marital status and level of education was found to be significant, but no significant relationships were found between occupation and the study polymorphism (Tables 2-4).

Table 5 and 6 present the results associated with the genotype distribution and allele frequency of the single-nucleotide G/Crs11146020 polymorphism. The GG genotype was observed in 66 healthy subjects and 52 of the heroin and methamphetamine addicts and the GC genotype in 31 healthy subjects and 28 addicts. Moreover, the CC genotype was observed in 3 healthy subjects and 10 addicts. Statistical analysis showed that the relationship of the rs11146020 polymorphism with heroin and methamphetamine addiction was insignificant.

**Table 2.** The relationship between the genotype distribution of the G/Crs11146020 polymorphism and marital status

| Subjects | | Marital status and the rs11146020 polymorphism | | | Total |
|---|---|---|---|---|---|
| | | Single | Married | Divorced | |
| Healthy | GG | 11 | 55 | 0 | 66 |
| | GC | 18 | 13 | 0 | 31 |
| | CC | 1 | 2 | 0 | 3 |
| | Total | 30 | 70 | 0 | 100 |
| Addicted | GG | 30 | 11 | 11 | 52 |
| | GC | 7 | 15 | 6 | 28 |
| | CC | 3 | 4 | 3 | 10 |
| | Total | 40 | 30 | 20 | 90 |

| The Chi-square test | | | |
|---|---|---|---|
| Subjects | X2 | df | Sig |
| Healthy | 17 | 2 | <0.001 |
| Addicted | 11 | 4 | 0.025 |

**Table 3**. The relationship between the genotype distribution of the G/Crs11146020 polymorphism and level of education

| Subjects | | Level of education and the rs11146020 polymorphism | | | Total |
|---|---|---|---|---|---|
| | | Junior high school | High school diploma | University | |
| Healthy | GG | 13 | 35 | 18 | 66 |
| | GC | 11 | 4 | 16 | 31 |
| | CC | 0 | 1 | 2 | 3 |
| | Total | 24 | 40 | 36 | 100 |
| Addicted | GG | 51 | 1 | 0 | 52 |
| | GC | 24 | 0 | 4 | 28 |
| | CC | 8 | 2 | 0 | 10 |
| | Total | 83 | 3 | 4 | 90 |

| The Chi-square test | | | |
|---|---|---|---|
| Subjects | X2 | df | Sig |
| Healthy | 15 | 4 | 0.003 |
| Addicted | 18 | 4 | 0.001 |

**Table 4.** The relationship between the genotype distribution of the G/Crs11146020 polymorphism and occupational status

| Subjects | | Occupational status and the rs11146020 polymorphism | | | | Total |
|---|---|---|---|---|---|---|
| | | Unemployed | Self-employed | Employee | Indefinite | |
| Healthy | GG | 2 | 39 | 16 | 9 | 66 |
| | GC | 1 | 17 | 7 | 6 | 31 |
| | CC | 1 | 2 | 0 | 0 | 3 |
| | Total | 4 | 58 | 23 | 15 | 100 |
| Addicted | GG | 4 | 36 | 0 | 12 | 52 |
| | GC | 2 | 22 | 0 | 4 | 28 |
| | CC | 1 | 7 | 0 | 2 | 10 |
| | Total | 7 | 65 | 0 | 18 | 90 |

| The Chi-square test | | | |
|---|---|---|---|
| Subjects | X2 | df | Sig |
| Healthy | 8 | 6 | 0.209 |
| Addicted | 1 | 4 | 0.908 |

**Table 5**. The genotype distribution of the polymorphism in the addicts and the controls

| Row | Genotype | Number of patients | Number of controls | P | df | $X^2$ |
|---|---|---|---|---|---|---|
| 1 | GG | 52 (58%) | 66 (66%) | | | |
| 2 | GC | 28 (31%) | 31 (31%) | 0.079 | 2 | 5 |
| 3 | CC | 10 (11%) | 3 (3%) | | | |

**Table 6**. The allele frequency of the polymorphism in the addicts and the controls

| Row | Allele type | Allele frequency in the patients | Allele frequency in the controls | P | df | $X^2$ |
|---|---|---|---|---|---|---|
| 1 | G | 66 (73.3%) | 82 (82%) | 0.151 | 1 | 2 |
| 2 | C | 24 (26.7%) | 18 (18%) | | | |

## Discussion

Research suggests that glutamate receptor antagonists have therapeutic potential in many acute and chronic central nervous system diseases including epilepsy, Parkinson's, depression, Alzheimer's, anxiety and drug dependence (14-15). Moreover, NDMA receptors in amygdala play a key role in learning and memory, particularly in reward learning and memory as well as drug dependence and relapse (16). Reduced glutamate neurotransmission also causes cognitive defects and memory loss in Alzheimer's patients (15). The present study found the frequency of the C allele to be respectively 18% and 26.7% and that of the G allele to be 82% and 73.3% in the healthy and addicted groups. The relationship of the single-nucleotide rs11146020 polymorphism with heroin and methamphetamine addiction was found to be insignificant. Statistical calculations revealed that the relationship of marital status and level of education with heroin and methamphetamine addiction was significant; however, no significant relationships were observed between occupational status and addiction to these drugs. Alexander Georgi (2006) found no significant relationships between this polymorphism and bipolar disorder (17).

Galehdari (2008) investigated the association between schizophrenia and the G1001C polymorphism in GRIN1. The G1001C polymorphism has recently been reported to affect the GRIN1 expression and the NMDA activity. Sequence changes in the binding site for the p50 subunit of the nuclear factor kappaB in GRIN1 reduce the expression and activity of NDMA. GRIN1 comprises two functional regions including six upstream AUG codons and four encoded upstream open reading frames (ORFs) in the large 5′ untranslated region (5′ UTR). The 5′ UTR contains multiple rare upstream AUG codons as the only encoders of proto-oncogenes, growth factors, transcription factors and their receptors. The translational level of these regions should be controlled. The G1001C polymorphism was also found to be a key factor in schizophrenia (18).

Shengying Qin et al. (2005) used the microarray platform method to investigate the association of schizophrenia with 11 GRIN1 polymorphisms, 5 GRIN2B polymorphisms and their combination. They found that the genetic interactions of G1001C in GRIN1 with T4197C and T5988C in GRIN2B contribute to schizophrenia (19).

Silvia Gina et al. found the rs11146020 polymorphism to contribute to the sequence changes in the p50 subunit of NF-kB. They also proposed the transcriptional regulation of NF-kB by the tumor necrosis factor (TNF) as a risk factor to schizophrenia in human (20). Previous studies also suggest the association of this polymorphism with schizophrenia (21-22).

Rachanee Chanasong et al. observed no significant relationships between the G1001C polymorphism and psychological dependence on methamphetamine. They also reported that the increased frequency of the A allele in this polymorphism is associated with schizophrenia in different people and that a meta-analysis confirmed the A allele as a marker of increased risk for schizophrenia (8).

Addiction is a multifactorial disease affected by different genetic and environmental factors. The role of other genes is therefore recommended to be investigated in drug use initiation, continued use, propensity and dependence. Genetic factors may also contribute to the relationship between polymorphisms and addiction to methamphetamine and heroin, depending on the study population and its size. Larger sample sizes are therefore required to obtain more definite results.

The association of the rs11146020 polymorphism with heroin addiction or simultaneous addiction to heroin and methamphetamine has not been well addressed yet. The results obtained by Rachanee Chanasong, as one of the rare authors investigating the relationship between this polymorphism and methamphetamine addiction, are consistent with the present study.

## Conclusion

The present study suggests no significant relationships between the rs11146020 polymorphism in GRIN1 and addiction to heroin and methamphetamine; however, statistical investigations found the relationship of this polymorphism with education level and marital status to be significant, but with occupational status to be insignificant. The study limitations comprised small sample size and the lack of similar studies on Iranian populations, which made it difficult to properly compare, draw conclusions and conduct a powerful analysis.

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