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Genetic variation of *GRIN1* confers vulnerability to methamphetamine-dependent psychosis in a Thai population

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HIGHLIGHTS

- A cohort of male METH dependence with psychosis was genotyped for two SNPs in *GRIN1*.
- G2108A SNP showed a very strong association with METH-induced psychosis.
- These results identify a potential risk factor for drug-induced psychosis.
- Genetic variation in glutamate system plays a role in the emergence of psychosis.

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ABSTRACT

GRIN1 is a gene that encodes the N-methyl-D aspartate (NMDA) receptor subunit1 (NR1). Variations of *GRIN1* have been identified as a risk factor for schizophrenia and drug dependence, supporting hypotheses of glutamatergic dysfunction in these disorders. Methamphetamine (METH) is a psychostimulant drug which can induce psychotic symptoms reminiscent of those found in schizophrenia; thus *GRIN1* is a candidate gene for vulnerability to METH dependence or METH-dependent psychosis. The present study examined two polymorphisms of *GRIN1*, rs11146020 (G1001C) and rs1126442 (G2108A), in 100 male Thai METH-dependent patients and 103 healthy controls using PCR-RFLP techniques. Neither polymorphism was significantly associated with METH dependence, although rs1126442 was highly significantly associated with METH-dependent psychosis, in which the A allele showed reduced frequency ($P < 0.00001$). The present findings indicate that the rs1126442 of *GRIN1* contributes to the genetic vulnerability to psychosis in METH-dependent subjects in the Thai population.

1. Introduction

The fundamental pathophysiology of drug dependence is thought to involve dysfunction of the mesolimbic dopamine system underlying reward mechanisms [19,20,37,39]. However, increasing evidence indicates a role for the glutamatergic system, and particularly the NMDA receptor, in drug addiction [10,11,33,36].

Methamphetamine (METH) is a derivative of amphetamine which is classified as one of the psychostimulant group of addictive drugs. METH can induce psychotic symptoms which closely resemble schizophrenia [2,35]. In addition, METH administration can induce behavioural sensitization which has been useful as an animal model of schizophrenia [5,17]. Certainly, there is substantial evidence implicating a dysfunction of glutamatergic neurotransmission in schizophrenia [21,22,24,32]. Therefore, METH dependence, or particularly the psychosis associated with METH dependence, may share some common underlying glutamatergic neuropathology with schizophrenia.

N-methyl-D aspartate (NMDA) receptors are ionotropic glutamate receptors that play important roles in neurodevelopment and learning and memory [9,26]. In humans, the NR1 receptor subunit is translated from the *GRIN1* gene which is located on chromosome 9q34.3 and is composed of 21 exons. It is required as a key receptor subunit for physiological function combined with one or more of the four NR2 subunits NMDAR2A–D to make the heterodimeric receptor complex [8,15,18,30]. Numerous studies have indicated a dysfunction of NR1 in both schizophrenia and drug

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abuse, particularly METH dependence. In METH-sensitized rats, it was found that NR1, NR2A, and NR2B proteins in the striatum were decreased [40]. Similarly, amphetamine exposure for 5 days and after 14 days of withdrawal exhibited decreased NR1 mRNA and protein in the nucleus accumbens but with increases in prefrontal cortex [16]. Following acute METH treatment, decreased expression of NR1 was found in the frontal cortex while NR2A was increased [31]. Additionally, in other studies where METH was given as either acute or subacute administration, an elevation of NR1 immunoreactivity (IR) was found in the striatum, whereas increased NR1-IR in the frontal cortex was only found in the subacute group [11].

A further approach to understanding how glutamate mechanisms contribute to drug dependence is its association with genetic variation; i.e. how single nucleotide polymorphisms (SNPs) in genes involved in glutamatergic function may contribute to individual differences in vulnerability to drug dependence. In alcohol studies, alcohol-associated anxiolysis and motor impairment were reduced in NR1 mutant mice by a reduction of glycine binding sites [12]. Interestingly, association studies in German alcoholics found the A allele of the G2108A SNP (rs1126442) in exon 7 of the NR1 gene (*GRIN1*) was more common in patients than in controls [27,38]. Furthermore, there are several studies in which SNPs of NMDA receptor genes were reported to be associated with elevated risk in schizophrenia. An increased frequency of the C allele of rs11146020 is reported in schizophrenia in various ethnicities [3,6,41]. This observation was found to be particularly in a subgroup with a lifetime history of depressive symptoms [7]. Additionally, this SNP has revealed an interaction with SNPs in the NR2B gene (*GRIN2B*), T4197C and T5988C, to confer vulnerability to the disease [25], and a meta-analysis also concluded that the C allele of rs11146020 may be a marker for a high risk for developing schizophrenia [28].

Taken together, this evidence suggests that genetic variation of *GRIN1* provides a good candidate for association with METH dependence and its resultant psychosis. We have chosen to test these hypotheses in a Thai population in which METH abuse and dependence is a major social and medical concern. The present study therefore hypothesized that the genetic variation in two SNPs of *GRIN1*, rs11146020 (G1001C) and rs1126442 (G2108A), may confer susceptibility to METH dependence and METH psychosis in the Thai population.

2. Materials and methods

2.1. Subjects

Subjects comprised 100 Thai METH dependent patients recruited from the Central Correctional Institution for Drug Addicts, Bangkok, Thailand, meeting criteria for METH dependence according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria [1]. Subjects were divided into those with or without a history of psychotic symptoms considered to be due to METH use. Psychiatric symptoms were treated according to local practice with antipsychotic, antidepressant or anxiolytic drugs as appropriate. 103 Thai control subjects were recruited, excluding those with a history of drug misuse or psychiatric disorder. Subjects were all male and age-matched (mean age ± SD 29.45 ± 4.55, range 21–45 years in patients, and 28.75 ± 6.39, range 20–46 years in controls). The average age of onset of METH use in patients was 19.52 ± 6.53, range 12–41 years, and the duration of METH use was 9.35 ± 5.26, range 1–23 years. All participants were informed with a complete and extensive explanation of the study, and signed consent forms approved by the Ethical Committees of Naresuan University, Thailand.

2.2. SNP genotyping

Blood samples from both patients and controls were obtained by fingertip venipuncture and were collected on FTA® cards (Whatman, WB120305, USA). The cards were washed with FTA purification reagent (Whatman, WB1202045, USA) until clear to obtain genomic DNA. PCR primers specific to rs11146020 (G1001C) in the 5′ UTR region and rs1126442 (G2108A) in exon 7 of *GRIN1* were designed based on the sequence and information from dbSNP database http://www.ncbi.nlm.nih.gov/SNP/. PCR reaction of each SNPs was performed in a total volume of 25 μl using GoTaqMix® Green Master Mix (Promega, USA) which contained GoTaq® DNA polymerase in 1× Green GoTag® reaction buffer (pH 8.5), 200 μM dNTPs, 2.5 mM $MgCl_2$ (rs11146020) and 2 mM (rs1126442), and 10 pmol of each primer (rs11146020 forward: 5′-GTCCAGTTTCCAGGCTCTC-3′, reverse: 5′-CTCCCCACAAGGTTCAGAAA-3′ and rs1126442 forward: 5′-ACGGGCTCTGAGTCGCAT-3′, reverse: 5′-GAAGTAACAGTGTCCAGAGGATG-3′). The cycling conditions consisted of denaturation at 95 °C for 1 min (rs11146020) and 94 °C for 3 min (rs1126442), 35 cycles at 94–95 °C for 30 s, 57 °C (rs11146020) and 58 °C (rs1126442) for 30 s, 72 °C for 25 s and a final extension at 72 °C for 5 min. The genotype of each SNP was then detected by Restriction Fragment Length Polymorphism (RFLP) analysis. 10–15 μl of each amplified product was incubated with 0.5–1 U of specific restriction enzyme (BseRI for rs11146020 and BtgI for rs1126442). The amplicon of rs11146020 was digested into 96 bp and 55 bp fragments when the G allele was present, while the C allele presented as a 151 bp fragment. For rs1126442, the amplicon was cut in 352 bp, 86 bp and 62 bp fragments for G allele and 352 bp and 148 bp fragments for A allele. The DNA fragments were identified by 2.5% agarose gel electrophoresis and subsequently visualized with ethidium bromide staining. All genotyping was repeated on a separate occasion to confirm the result; any discrepancies were resolved after a further repetition.

2.3. Statistics

Statistical differences in genotype and allele frequencies between patients and controls in both SNPs were evaluated using Fisher's exact test in SPSS version 11.5 Software (Statistical Package for Social Science, SPSS, Inc., Chicago, IL) for single-SNP analysis. Analysis of the Hardy-Weinberg equilibrium (HWE) and, pair-wise linkage disequilibrium (LD) was carried out using SHEsis software (http://analysis2.bio-x.cn/myAnalysis.php). All statistical significances were considered at P-value <0.05.

3. Results

Neither rs11146020 nor rs1126442 SNPs of *GRIN1* significantly deviated from Hardy-Weinberg equilibrium in patient or control groups. Pair-wise linkage disequilibrium of rs11146020 and rs1126442 gave values of $D' = 0.078$, $r = -0.024$ and global haplotype association P-value = 0.32, providing no evidence for linkage disequilibrium between the two SNPs. Fisher's exact test showed that the genotype and allele frequencies between patients and controls were not statistically significant for either polymorphism (Table 1). When METH dependent patients were divided into those with and without psychosis, the genotype and allele frequencies of rs1126442, but not of rs11146020, showed strong and highly significant differences in distribution, in which the minor A allele was strongly associated with the absence of psychosis.