Genetic Association Analysis of NOS1 and Methamphetamine-Induced Psychosis Among Japanese

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Abstract: The neuronal nitric oxide synthase gene (NOS1) is located at 12q24, a susceptibility region for schizophrenia, and produces nitric oxide (NO). NO has been reported to play important roles as a gaseous neurotransmitter in brain. NO is a second messenger for the N-methyl-D-aspartate (NMDA) receptor and is related to the dopaminergic system. Because the symptomatology of methamphetamine (METH) use disorder patients with psychosis is similar to that of patients with schizophrenia, NOS1 is a good candidate gene for METH-induced psychosis. Therefore, we conducted a case-control association study between NOS1 and METH-induced psychosis with Japanese subjects (183 with METH-induced psychosis patients and 519 controls). We selected seven SNPs (rs41279104, rs3782221, rs3782219, rs561712, rs3782206, rs6490121, rs2682826) in NOS1 from previous reports. Written informed consent was obtained from each subject. This study was approved by the Ethics Committee at Fujita Health University School of Medicine and each participating institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA). No significant association was found between NOS1 and METH-induced psychosis in the allele/genotype-wise or haplotype-wise analyses. In conclusion, we suggest that NOS1 might not contribute to the risk of METH-induced psychosis in the Japanese population.

Keywords: Methamphetamine-induced psychosis, neuronal nitric oxide synthase 1 gene (NOS1), case-control association study.

1. INTRODUCTION

Methamphetamine (METH) is a drug that is used widely in the world and causes psychiatric disorder. METH causes abnormalities of the dopamine neural transmission in the mesolimbic system, and is thought to cause psychotic symptoms such as hallucinations and delusions [1, 2].

Nitric oxide (NO) is involved in a variety of mechanisms, regulating the release of neurotransmitters such as dopamine and serotonin, activating N-methyl-D-aspartate (NMDA) receptor, and participating in oxidative stress [3-5]. Therefore, NO functions may be considered to induce psychotic disorders. The nitric oxide synthase 1 gene (NOS1) is a complex gene located at 12q24, consisting of 12 alternative untranslated first exons, termed exon 1a_1l, and 28 exons in a genomic region spanning 149.404Kb. NOS1 is considered to be a likely candidate gene for schizophrenia owing to its chromosomal location, 12q24, which has been reported to be a susceptibility locus from several linkage studies, and to play a role in producing NO in the human brain [6-8].

Several genetic association studies showed that single nucleotide polymorphisms (SNPs) in NOS1 were associated with schizophrenia. Reif et al. identified functional SNP (rs41279104) in the promoter region and found an association with schizophrenia [9]. Two other genetic association studies showed a significant association between rs2682826 in exon 29 and haplotype (rs3782221-rs3782219-rs561712-rs3782206) and schizophrenia. Recently, a whole genome association study reported an association between rs6490121 in intron 2 of NOS1 and schizophrenia [12]. Therefore, NOS1 is recognized to be a candidate gene for schizophrenia [10, 11].

Because the symptoms of METH-induced psychosis are similar to those of paranoid type schizophrenia, it may be that the METH-induced psychosis and schizophrenia have common susceptibility genes. Therefore, it would be of in-
terest to examine the association between \textit{NOS1} and METH-induced psychosis. We conducted a genetic association analysis in the Japanese population.

2. MATERIALS AND METHODS

2.1. Subjects

The subjects in the association analysis were 183 patients (all patients were diagnosed as having METH-induced psychosis; 151 males and 32 females: mean age ± SD 36.7±11.6 years) and 519 healthy controls (268 males and 251 females: mean age ± SD 37.5 ±14.4 years). All subjects were unrelated to each other, ethnically Japanese, and lived in Japan. Among the subjects with METH use disorder, all subjects had a comorbid diagnosis of METH-induced psychosis. One hundred forty-nine subjects with METH use disorder abused or had dependence on drugs other than METH. Cannabinoids were the most frequently abused drugs (31.4%), followed by cocaine (9.09%), LSD (9.09%), opioids (7.69%), and hypnotics (7.69%). Subjects with METH use disorder were excluded if they had a clinical diagnosis of psychotic disorder, mood disorder, anxiety disorder, or eating disorder. The patients were diagnosed according to DSM-IV or ICD-10 criteria with consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. All healthy controls were also psychiatrically screened through unstructured interviews, and those with past individual or family history of drug dependence or an axis I disorder such as psychotic or mood disorder were excluded. After describing the study, written informed consent was obtained from each subject. This study was approved by the Ethics Committee at Fujita Health University and each participating institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNP Selection and Genotyping

We selected seven SNPs (rs2682826, rs3782221, rs3782219, rs61712, rs3782206, rs1279104, rs6490121) in \textit{NOS1} from previous association studies for schizophrenia [9-11]. We used TaqMan assays (Applied Biosystems) for all SNPs.

2.3. Statistical Analysis

Genotype deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated with the chi-square test (SAS/Genetics, release 8.2, SAS Japan INC, Tokyo, Japan). Marker-trait association was also evaluated with the chi-square test in allele- and genotype-wise analyses. Haplotype frequencies were estimated in a two- to four-marker sliding window fashion and log likelihood ratio tests were performed for global P-values with COCAPHASE program version 3.0.6 [13]. In these haplotype-wise analyses, rare haplotypes (less than 0.05) of either cases or controls were excluded from the association analysis. Power calculation was performed using a statistical program prepared with the Genetic Power Calculator (http://pngu.mgh.harvard.edu/∼purecell/gpc/). The level of significance for all statistical tests was 0.05.

3. RESULTS

Genotype frequencies of subjects and controls did not deviate significantly from HWE. No significant association was found between \textit{NOS1} and METH-induced psychosis in the allele/genotype-wise analysis, or in the haplotype analysis (Table 1, 2). Our METH samples were unmatched gender

| SNP ID    | Phenotype               | MAF\(^b\) | N    | Genotype Distribution\(^c\) | P-value | HWE\(^d\) | Genotype | Allele |
|-----------|-------------------------|-----------|------|-----------------------------|---------|----------|----------|--------|
| rs41279104| METH-induced psychosis  | 0.175     | 183  | M/M 123, M/m 56, m/m 4     | 0.413   | 0.684    | 0.983    |
|           | CON                     | 0.175     | 519  | M/M 354, M/m 148, m/m 17   | 0.751   |          |          |
| rs3782221 | METH-induced psychosis  | 0.421     | 183  | M/M 61, M/m 90, m/m 32     | 0.903   | 0.912    | 0.891    |
|           | CON                     | 0.425     | 519  | M/M 175, M/m 247, m/m 97   | 0.55    |          |          |
| rs3782219 | METH-induced psychosis  | 0.423     | 183  | M/M 61, M/m 89, m/m 33     | 0.956   | 0.611    | 0.332    |
|           | CON                     | 0.453     | 519  | M/M 154, M/m 260, m/m 105  | 0.803   |          |          |
| rs561712  | METH-induced psychosis  | 0.161     | 183  | M/M 128, M/m 51, m/m 4     | 0.679   | 0.716    | 0.435    |
|           | CON                     | 0.179     | 519  | M/M 346, M/m 160, m/m 13   | 0.274   |          |          |
| rs3782206 | METH-induced psychosis  | 0.265     | 183  | M/M 100, M/m 69, m/m 14    | 0.663   | 0.745    | 0.44     |
|           | CON                     | 0.245     | 519  | M/M 300, M/m 184, m/m 35   | 0.351   |          |          |
| rs6490121 | METH-induced psychosis  | 0.407     | 183  | M/M 62, M/m 93, m/m 28     | 0.475   | 0.523    | 0.532    |
|           | CON                     | 0.426     | 519  | M/M 175, M/m 246, m/m 98   | 0.484   |          |          |
| rs2682826 | METH-induced psychosis  | 0.380     | 183  | M/M 67, M/m 93, m/m 23     | 0.286   | 0.175    | 0.0754   |
|           | CON                     | 0.329     | 519  | M/M 230, M/m 237, m/m 52   | 0.424   |          |          |

\(\text{a} \text{ METH}: \text{methamphetamine} \quad \text{CON}: \text{control} \quad \text{b} \text{ MAF}: \text{minor allele frequency} \quad \text{c} \text{ M: major allele, m: minor allele} \quad \text{d} \text{ Hardy-Weinberg equilibrium.}
samples for METH-induced psychosis. Therefore, we performed an explorative analysis of gender effects, but no association was detected between any of the SNPs and either sex (Table 3).

In a power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.37-1.6, under a multiplicative model of inheritance.

4. DISCUSSION

We did not find an association between the seven SNPs in NOS1 and METH-induced psychosis in the allele/genotype-wise or haplotype-wise analysis in these subjects. In several genetic studies of METH, gender effects were found in METH use disorder [14, 15]. Because we recognize that our gender samples were unmatched, our negative result may have mainly reflected the effect of male METH-induced psychosis. We therefore conducted an explorative analysis of gender effects, but found none. Since NO has an important role in regulating the release of neurotransmitters such as dopamine and serotonin, NOS1 is recognized to be a good candidate gene for disorder with psychosis. However, a previous study did not report an association between NOS1 and schizophrenia [16]. Other genes involved in the activity of NOS1 may be related to the pathophysiology of psychotic disorders such as schizophrenia and METH-induced psychosis.

Nitric oxide synthase 1 adaptor protein (NOS1AP) encodes an adapter protein that binds to NOS1 and links to a specific target. Recent studies reported evidence of a significant association between NOS1AP and schizophrenia [17, 18]. Considering these positive results, it will be necessary to replicate the studies using other larger population samples and other phenotypes, such METH-induced psychosis.

A few points of caution must be mentioned with regard to our present negative findings. (1) It is important to evaluate associations between METH use disorder with and without psychosis. However, since we had only a small number of subjects without psychosis, we did not evaluate this association to avoid type I error due to small sample size. (2) We could not adopt an LD-based strategy and mutation scan, because NOS1 has a massive gene structure. Therefore, in future studies it will be necessary to evaluate associations between other common variants or rare variants with functional effects and NOS1 in METH use disorder.

In conclusion, our results suggest that NOS1 does not play a major role in METH use disorder with psychosis in the Japanese population. However, the number of METH samples used in this study was small, and even though it is difficult to find samples of METH use disorder, it will be necessary to validate or replicate our association in other, larger population samples.

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| SNP ID     | Gender | Phenotype*                  | MAF* | N      | Genotype Distribution* | P-value |
|-----------|--------|-----------------------------|------|--------|------------------------|---------|
|           |        |                             |      |        | M/M | M/m | m/m | HWEc | Genotype | Allele |
| rs41279104| male   | METH-induced psychosis      | 0.172| 151    | 102 | 46  | 3   | 0.399 | 0.977    | 0.853  |
|           | female | METH-induced psychosis      | 0.187| 32     | 21  | 10  | 1   | 0.884 | 0.597    | 0.452  |
| rs3782221 | male   | METH-induced psychosis      | 0.423| 151    | 50  | 74  | 27  | 0.966 | 0.988    | 0.882  |
|           | female | METH-induced psychosis      | 0.453| 32     | 7   | 21  | 4   | 0.0667| 0.106    | 0.574  |
| rs3782219 | male   | METH-induced psychosis      | 0.423| 151    | 50  | 74  | 27  | 0.966 | 0.541    | 0.326  |
|           | female | METH-induced psychosis      | 0.515| 32     | 8   | 15  | 9   | 0.727 | 0.572    | 0.293  |
| rs561712  | male   | METH-induced psychosis      | 0.188| 151    | 100 | 45  | 6   | 0.741 | 0.253    | 0.119  |
|           | female | METH-induced psychosis      | 0.147| 32     | 23  | 9   | 0   | 0.354 | 0.343    | 0.175  |
| rs3782206 | male   | METH-induced psychosis      | 0.264| 151    | 82  | 58  | 11  | 0.865 | 0.956    | 0.860  |
|           | female | METH-induced psychosis      | 0.296| 32     | 17  | 11  | 4   | 0.317 | 0.243    | 0.174  |
| rs6490121 | male   | METH-induced psychosis      | 0.417| 151    | 53  | 70  | 28  | 0.565 | 0.182    | 0.0736 |
|           | female | METH-induced psychosis      | 0.375| 32     | 13  | 14  | 5   | 0.706 | 0.281    | 0.0937 |
| rs2682826 | male   | METH-induced psychosis      | 0.403| 151    | 50  | 80  | 21  | 0.218 | 0.360    | 0.935  |
|           | female | METH-induced psychosis      | 0.515| 32     | 9   | 13  | 10  | 0.290 | 0.190    | 0.0952 |
|           |        |                             |      |        |    |     |     | 0.352 |          |

* METH: methamphetamine  CON: control  
* MAF: minor allele frequency  
* M: major allele, m: minor allele  
* Hardy-Weinberg equilibrium

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