Lack of Association Between Prokineticin 2 Gene and Japanese Methamphetamine Dependence

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Abstract: Disruption of circadian rhythms may be involved in the pathophysiology of psychiatric disorders, including drug addiction. Recently, we detected the significant association between prokineticin 2 receptor gene (PROKR2) and Japanese methamphetamine dependence patients. Also, prokineticin 2 (PK2) gene deficient mice showed reduced physiological and behavioral parameters, including circadian locomotor activity, circulating glucocorticoid, glucose levels and the expression of peripheral clock genes compared with WT mice. These evidences indicate that PK2 gene (PROK2) is a good candidate gene for the pathogenesis of methamphetamine dependence. To evaluate the association between PROK2 and methamphetamine dependence, we conducted a case-control study of Japanese samples (215 methamphetamine dependence and 232 controls) with four tagging SNPs selected by HapMap database. The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients. Written informed consent was obtained from each subject. This study was approved by the ethics committees at Fujita Health University, Nagoya University Graduate School of Medicine and each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA). We did not detect an association between PROK2 and Japanese methamphetamine dependence patients in allele/genotype-wise analysis, or the haplotype analysis. Our findings suggest that PROK2 does not play a major role in the pathophysiology of methamphetamine dependence in the Japanese population.

Keywords: Prokineticin 2 gene (PROK2), methamphetamine dependence, tagging SNPs, linkage disequilibrium.

1. INTRODUCTION

Disruption of circadian rhythms may be involved in the pathophysiology of psychiatric disorders, including drug addiction [1-7]. Several animal studies have shown that methamphetamine increased expression of circadian clock molecule genes such as Per1, Per2, Bmal1, and Npas2 in the brain [8-10]. Recently, we detected the significant association between prokineticin 2 receptor gene (PROKR2) and Japanese methamphetamine dependence patients [11].

The prokineticin 2 (PK2) gene deficient mice showed reduced physiological and behavioral parameters, including circadian locomotor activity, circulating glucocorticoid, glucose levels and the expression of peripheral clock genes compared with WT mice [12-15]. The expression of PK2 gene in SCN was activated by the Clock/Bmal1 complex, and suppressed by Per/Cry in vitro studies [12]. This evidence indicate that PK2 gene (PROK2) is a good candidate gene for the pathogenesis of methamphetamine dependence. To evaluate the association between PROK2 and methamphetamine dependence, we conducted a case-control study of Japanese samples (215 methamphetamine dependence and 232 controls) with four tagging SNPs selected by HapMap database.

2. MATERIALS AND METHODS

2.1. Subjects

The subjects in the association analysis were 215 METH dependence patients (160 males and 39 females; mean age ± standard deviation (SD) 36.3 ± 11.4 years) and 232 healthy controls (187 males and 45 females; 36.4 ± 11.3 years). The age and sex of the control subjects did not differ from those of the methamphetamine dependence patients. All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan. The patients were diagnosed according to DSM-IV criteria with consensus of at least two
experienced psychiatrists on the basis of unstructured interviews and a review of medical records. One hundred ninety-seven of the subjects with METH dependence had a diagnosis of co-morbid METH induced psychosis. METH-induced psychosis patients were divided into two categories of psychosis prognosis, the transient type and the prolonged type, which showed remission of psychotic symptoms within 1 month and after more than 1 month, respectively, after the discontinuance of methamphetamine consumption and beginning of treatment with neuroleptics; 112 patients (56.9%) were the transient type, and 85 patients (42.1%) were the prolonged type. One hundred eighty-two subjects with METH-induced psychosis also had dependence on drugs other than METH. Cannabinoids were the most frequently abused drugs (21.4%), followed by cocaine (9.09%), LSD (9.09%), opioids (7.69%), and hypnotics (7.69%). Subjects with METH-induced psychosis were excluded if they had a clinical diagnosis of psychotic disorder, mood disorder, anxiety disorder or eating disorder. More detailed characterizations of these subjects have been published elsewhere [4, 16, 17]. All healthy controls were also psychiatrically screened based on unstructured interviews. None had severe medical complications such as liver cirrhosis, renal failure, heart failure or other Axis-I disorders according to DSM-IV.

The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committee at Fujita Health University, Nagoya University School of Medicine and each participating member of the Institute of the Japanese Genetics Initiative for Drug Abuse (JGIDA).

2.2. SNPs Selection and Linkage Disequilibrium (LD) Evaluation

We first consulted the HapMap database (release#23a/phase II, March 2008, www.hapmap.org, population: Japanese Tokyo, minor allele frequencies (MAFs) of more than 0.05) and included 9 SNPs covering PROK2 (5'-flanking regions including about 3100 bp from the initial exon and about 9800 bp downstream (3') from the last exon: HapMap database contig number chr3: 71894788.. 71919925). Four ‘tagging SNPs’ in PROK2 was then selected with the criteria of r² threshold greater than 0.8 in ‘pair-wise tagging only’ mode using the ‘Tagger’ program (Paul de Bakker, http://www/broad.mit.edu/mpg/tagger), in Haploview, for the following association analysis [18].

2.3. SNPs Genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA,) for all SNPs. One allelic probe was labeled with FAM dye and the other with the fluorescent VIC dye. The plates were heated for 2 min at 50°C and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 58°C for 1 min. Please refer to ABI for the primer sequence. Detailed information is available on request.

2.4. Statistical Analysis

Genotype deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan).

Marker-trait association analysis was used to evaluate allele- and genotype-wise association with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan), and haplotype-wise association analysis was conducted with a likelihood ratio test using the COCAPHASE2.402 program [19]. We used the permutation test option as provided in the haplotype-wise analysis to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1000 iterations (random permutations). Power calculation was performed using a genetic power calculator [20]. The significance level for all statistical tests was 0.05.

3. RESULTS

The LD structure of PROK2 from the HapMap database samples can be seen our previous paper [21]. Genotype frequencies of all SNPs were in HWE. We did not detect between all tagging SNPs and METH dependence in the Japanese population in the allele /genotype-wise (Table 1) or haplotype-wise analysis (P haplotype= 0.145). In addition, we found no association between four tagging SNPs and METH-induced psychosis patients in the allele /genotype-wise (Table 1) or haplotype-wise analysis (P haplotype= 0.225).

In the power analysis, we obtained more than 80% power for the detection of association when we set the genotype relative risk at 1.67-1.76 in METH dependence for PROK2 under a multiplicative model of inheritance.

4. DISCUSSION

We did not find an association between PROK2 and Japanese METH dependence and METH-induced psychosis patients. Therefore, we reasoned that PROK2 may not play an important role in the pathophysiology of METH dependence and METH-induced psychosis in the Japanese population. However, because our samples are small, we considered that there is a possibility of statistical error in these results.

We hypothesized that mood disorders and drug addiction have common susceptibility genes. For example, we detected the associations between prokineticin 2 receptor (PROKR2) and not only mood disorders including MDD and BP but also METH dependence and METH-induced psychosis in the Japanese population [11, 21]. Recently, Mohawk and colleagues reported that mice which were arrhythmic due to a lack of circadian clock genes showed circadian locomotor rhythms when treated with methamphetamine [22]. If patients with drug addiction disorders had disrupted circadian rhythms, it is possible that taking methamphetamine helps to restore circadian rhythms of taking into methamphetamine to keep to circadian rhythm. From this evidence, although we detected no association between PROK2 and METH dependence and METH-induced psychosis, we consider that it will be necessary to conduct further investigation of the relationship between circadian clock genes and METH dependence and METH-induced psychosis.

A few points of caution should be mentioned with respect to our results. Firstly, the lack of association may be due to small sample size. Ideal samples for this study are METH use disorder samples with and without dependence and psychosis. Because we had only a few METH use disorder sam-
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Table 1. Association Analysis of PROK2 with Methamphetamine Dependence and Methamphetamine-Induced Psychosis

| SNP ID* | Phenotype* | MAF* | N   | Genotype Distributiona | P-Value |
|---------|------------|------|-----|------------------------|---------|
|         |            |      |     | M/M | M/m | m/m | HWE* | Genotype | Allele |
| SNP1    | rs1316780  | Controls | 0.388 | 232 | 87 | 110 | 35 | 0.183 |
| T>A     | 5’flanking region | METH dependence | 0.347 | 215 | 88 | 105 | 22 | 0.251 | 0.295 | 0.200 |
|         |            | METH-induced psychosis | 0.345 | 197 | 82 | 94 | 21 | 0.436 | 0.357 | 0.196 |
| SNP2    | rs10865660 | Controls | 0.358 | 232 | 93 | 112 | 27 | 0.441 |
| A>G     | intron2    | METH dependence | 0.312 | 215 | 99 | 98 | 18 | 0.360 | 0.320 | 0.144 |
|         |            | METH-induced psychosis | 0.358 | 197 | 89 | 91 | 17 | 0.352 | 0.430 | 0.212 |
| SNP3    | rs3796224  | Controls | 0.183 | 232 | 157 | 65 | 10 | 0.331 |
| G>A     | intron2    | METH dependence | 0.174 | 215 | 148 | 59 | 8  | 0.490 | 0.936 | 0.733 |
|         |            | METH-induced psychosis | 0.183 | 197 | 137 | 52 | 8  | 0.288 | 0.917 | 0.686 |
| SNP4    | rs1374913  | Controls | 0.394 | 232 | 80 | 106 | 29 | 0.512 | 0.917 | 0.682 |
| T>G     | 3’flanking region | METH dependence | 0.381 | 215 | 73 | 98 | 26 | 0.440 | 0.900 | 0.682 |
|         |            | METH-induced psychosis | 0.381 | 197 | 73 | 98 | 26 | 0.440 | 0.900 | 0.682 |

a major allele>minor allele
b METH; methamphetamine
c MAF: minor allele frequency
d M: major allele, m: minor allele
e Hardy-Weinberg equilibrium

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