A Pilot Study for Discovering Candidate Genes of Chromosome 18q21 in Methamphetamine Abusers: Case-control Association Study

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Objective: It was previously suggested that the malic enzyme 2 (ME2) as the candidate gene for psychosis in fine mapping of chromosome 18q21. Chromosome 18q21 is also one of the possible regions that can contribute to addiction.

Methods: We performed a pilot study for discovering candidate gene of chromosome 18q21 in the methamphetamine abusers for elucidating the candidate gene for methamphetamine addiction leading to psychosis. We have selected 30 unrelated controls (16 males, 14 females; age=59.8±10.4) and 37 male methamphetamine abusers (age=43.3±7.8). We analyzed 20 single nucleotide polymorphisms (SNPs) of 7 neuronal genes in chromosome 18q21 for DNA samples that was checked for the data quality and genotype error. The association between the case–control status and each individual SNP was measured using multiple logistic regression models (adjusting for age and sex as covariates). And we controlled false discovery rate (FDR) to deal with multiple testing problem.

Results: We found 3 significant SNPs of 2 genes in chromosome 18q21 (p-value <0.05; adjusting for age as covariate) in methamphetamine abusers compared to controls. We also found 2 significant SNPs of 1 gene (p-value <0.05; adjusting for age and sex as covariates) (rs3794899, rs3794901:MAPK4). Two SNPs in MAPK4 gene were significant in both statistical groups.

Conclusion: MAPK4, the gene for mitogen-activated protein kinase 4, is one of the final 6 candidate genes including ME2 in 18q12-21 in our previous finemapping for psychosis. Our results suggest that MAPK4 can be a candidate gene that contribute to the methamphetamine addiction leading to psychosis.

KEY WORDS: Substance-related disorders; Methamphetamine; Psychotic disorders; Malic enzyme 2; Mapk4; Comorbidity.

INTRODUCTION

Psychotic disorders, including schizophrenia and bipolar disorder, are debilitating mental illnesses that lead to progressive deterioration in the social and occupational functioning of individuals,1,2 and increased economic burden of the society.3,4 Methamphetamine, a central nervous system stimulant, is the most commonly abused drug in Korea as well as other parts of East Asia.5-7 Methamphetamine abuse is a significant public health concern not only in Korea, but also in other parts of the world.5,8 In humans, abuse of methamphetamine leads to serious psychiatric conditions,9-13 including a variety of psychotic disorders.

Studies suggested that genetic factors contribute to the development of these psychotic disorders.14,15 Although bipolar disorder and schizophrenia were considered as distinct illnesses, recent studies have shown that these disorders are genetically overlapping.15,22,23 Linkage studies identified wide regions containing a number of genes, and required subsequent analyses to examine the association of specific genes to the disease phenotypes.23 Previous studies conducted in the Costa Rican population have shown evidence of linkage disequilibrium between markers within the 18q21 region of both these psychiatric phenotypes: severe bipolar disorder and schizophrenia.22,24 Further analyses revealed that psychotic symptomatology (hallucinations, delusions, disorganized thought, disorganized behavior) was the relevant phenotype showing association to the 18q21 region, as individuals, both with and without a history of mania, showed evidence of linkage disequilibrium to this region.22,23 Psychotic symptoms...
are a core feature of both schizophrenia and schizoaffective disorder, and are frequently observed during acute phases of bipolar disorder.

Development of new molecular genetic techniques has propelled advanced studies aimed at understanding the genetic basis of drug abuse. It is possible that personality factor mediates intermediately in the genetic contributions to drug abuse. Classical genetic studies documented the contributions of a highly complex set of genetic factors to the abuse of multiple addictive substances. Human psychopathology of psychosis emerges as anxiety, a state of mental disequilibrium. Men become depressed when their anxieties are not resolved. Psychopathologically, psychosis and mania are deviations from reality in which, depressed patients fall, and addiction is the final defense beyond psychosis, thereby leading to comorbidity. The comorbidity of psychosis and addiction is very clear from a psychopathologic perspective. Clinically, addictive symptoms are prevalent among patients with schizophrenia and bipolar disorder. The close interrelationship between psyche and soma has been studied. The co-morbidity of psychosis and addiction needs to be analyzed genetically from the perspectives of several broad processes, including personality, memory, and cognition, for understanding the mechanisms underlying the psychiatric symptoms. The genetic regulation of these processes may explain the methamphetamine addiction leading to psychosis.

Genetics is commonly used for explaining the biological basis of psychiatric disorders. Fine mapping of chromosome 18 revealed that malic enzyme 2 (ME2) is a candidate gene for psychosis. In an effort to identify the genes within the 18q21 that may be contributing to the development of psychotic disorders in the Costa Rican population, we performed additional fine mapping with microsatellite markers by utilizing a sample that allowed us to establish the association between psychotic disorders and this region of the chromosome. Marker D18S474, located at 71.32 centimorgan (cM) from the pter (sex-averaged distance, Marshfield Genetic Map), had shown evidence of linkage disequilibrium (LD) with all the four psychotic phenotypes tested (psychotic disorders with and without a history of mania, Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) Schizophrenia, or Schizoaffective Disorder). For the follow-up study, we focused on the 5 cM region surrounding D18S474. This region (from D18S450 to D18S484) contains 21 known genes, many of which are expressed in the central nervous system. To determine if any of these genes were relevant to mania or psychosis, we analyzed mRNA expression patterns in bipolar versus control post-mortem brains, and reviewed previous studies that examined altered gene expression patterns in schizophrenia and bipolar disorder. Further, single nucleotide polymorphism (SNP) analyses were performed to test the association between a specific gene within this region and the phenotypes of psychosis and mania. Based on the results of our genetic mapping and gene expression, we report that ME2, a mitochondrial enzyme involved in energy metabolism in neurons, is associated with a spectrum of psychotic disorders, including schizophrenia, schizoaffective disorder, and bipolar disorder.

A number of studies suggested that chromosome 18 contains several regions that likely harbor candidate genes contributing to the susceptibility to develop addiction. However, data from genome-wide association studies in humans and mice suggested a larger role for “higher order” pharmacogenomics in the molecular genetics of addiction. For instance, it is possible that overlapping genetic vulnerabilities influences the probability for developing dependence on a variety of addictive substances. We performed a pilot study for fine mapping of chromosome 18q21 of methamphetamine abusers for identifying the candidate genes responsible for the methamphetamine addiction leading to psychosis.

METHODS

We have selected 30 unrelated controls (16 males, 14 females; age=59.8±10.4 years) and 37 male methamphetamine abusers (age=43.3±7.8 years). We analyzed 56 SNPs of 18 neuronal genes in chromosome 18 for DNA samples that was checked for the data quality and genotype error. The association between the case-control status and each individual SNP was measured using multiple logistic regression models (adjusting for age and sex as covariates). And we controlled false discovery rate (FDR) to deal with multiple testing problem.

Sample Collection

All subjects were recruited in accordance with the principles of the Declaration of Helsinki and with approval from the Institutional Review Boards of the Bugok National Hospital in Korea. Each subject was interviewed by a psychiatrist who was blind to the previous history of the subject, using the Diagnostic Interview for Genetic Studies (DIGS). In addition, information on each subject was gathered through an interview with a family...
Fig. 1. Linkage disequilibrium (LD) analyses of selected 7 positionally only relevant genes which was focused in 18q21 fine mapping research. Twenty single nucleotide polymorphisms (SNPs) genotyped in our fine mapping for methamphetamine abusers and controls are also represented. Haplotype block pattern constructed by the Haploview program (Barrett et al. 2005) is shown. The number in each cell represents the LD. Parameter D’ (×100), blank cells mean D’=1. Each cell is painted with graduate color relative to the strength of LD between markers, which is defined by both D’ value and confidence bounds on D’. SNPs are indicated by a SNP ID number (rs number).

member, using the Family Interview for Genetic Studies (FIGS).32) Medical records (inpatient and outpatient) were also abstracted. All affected subjects (i.e., those given formal psychiatric diagnoses for the present study) were diagnosed using a best estimate diagnostic process, as described by Walss-Bass et al.22) The process arrives at a lifetime consensus diagnosis or diagnosis using the DSM-IV.

Design for reference data
A study was performed in 30 normal Korean subjects. The general inclusion criterion was that subjects should be normal and belong to the age groups specified in the sample. Subjects with conditions suspected of affecting any biases were excluded from the study. Subjects were also excluded if they had been receiving corticoid, estrogen, androgen, T3 (triiodothyronine)-T4 (thyroxine) therapy or diphenylhydantoin, vitamin D, bisphosphonate, calcitonin, fluoride, thiazide diuretics, or barbiturates for more than 6 months, as all these drugs affect any biases. Since it was impossible to obtain a population-based register for technical and legal reasons, the selection of control subjects meeting the inclusion/exclusion criteria was made from volunteers (students, hospital workers, patients’ relatives, etc.) attending the hospital.

Experimental design
The subjects of the study included inpatients at the Addiction Inpatient Unit of the Bugok National Hospital in Korea, which has an established protocol for evaluation, treatment, and follow-up of patients with drug abuse. We identified 37 patients that fulfilled DSM-IV diagnostic criteria of methamphetamine abuse or dependence.39) The patients also satisfied the following criteria: (1) duration of methamphetamine abuse longer than 1 year, and its frequency more than 100 times and (2) absence of concomitant diseases that might affect any biases. Factors that may influence any biases were also recorded. We could not include female patients under environmental circum-
Genotyping

DNA from 30 control persons and 37 patients, which were affected with methamphetamine abuse, was genotyped using 56 SNPs of 18 neuronal genes in chromosome 18 (fluorescently labeled markers of the highest heterozygosity available within chromosome 18). These markers spanned the region of chromosome 18, functionally relevant to neuropsychiatric disorders. We have first searched for functionally relevant candidate genes to the comorbidity of psychosis and addiction. And then we finally selected 56 SNPs of 18 neuronal genes in chromosome 18. The genotyping process was carried out as described previously in Lee et al. by laboratory personnel who were blinded to phenotype data. Molecular genetic studies are represented as two major studies (linkage study and association study). Linkage study progress in the concept of linkage equilibrium between gene and so close marker (e.g., microsatellite) and association study progress in the concept of LD between gene and so very, very close marker (e.g., SNP). Earlier studies had included microsatellites in my previous fine mapping association analyses. But in this pilot study for discovering candidate genes rather than candidate regions, we chose to focus on SNPs, which are more effective markers than microsatellites for detecting association due to strong LD with disease causing genetic variants as Korean population may have diverged many generations previously.

Standard PCR was performed using the ABI 877 automated thermocycler (Applied Biosystems Inc., Foster City, CA, USA) or the PE 9700 PCR instrument (Applied Biosystems Inc.). Amplified fragments were analyzed on the 7900HT Sequence Detection System (Applied Biosystems Inc.) for SNPs. Genotypes were assigned using GeneScan, Genotyper, and SDS software (Applied Biosystems Inc.). Each genotype was scored separately by two individuals, who were blind to diagnosis of the subjects. Genotype scores were compared using a software program implemented in Microsoft Excel (Microsoft Inc., Redmond, WA, USA), discrepancies were discussed with review of the original gels, and final genotypes agreed upon. Genotypes were checked for violations of Mendelian inheritance by PEDSYS program INFER (Southwest Foundation for Biomedical Research, San Antonio, TX, USA). Genotypes for two SNPs were discarded from the statistical analyses due to recurrent Mendelian discrepancies.

Fine Mapping Analyses

In the fine mapping stage (where 20 SNPs markers were tested), we selected 20 SNPs of 7 neuronal genes in chromosome 18q21. We screened NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/projects/mapview/maps.cgi?taxid=9606&chr=18) for exploring candidate genes in chromosome 18q21 for the methamphetamine addiction

| SNP name   | Nearest gene | Position on chromosome 18(bp) | Location of SNP with relation to nearest gene | Allele | Allele frequency |
|------------|--------------|-------------------------------|----------------------------------------------|--------|-----------------|
| rs125555   | MBD1         | 46054177                      | Coding NS                                   | N/G    | 0.211           |
| rs140686   | MBD1         | 46057352                      | Coding A/G                                  | 0.083  |
| rs1893490  | MAPK4        | 46449799                      | Intron T/C                                  | 0.083  |
| rs3892158  | MAPK4        | 46454011                      | Intron A/C                                  | 0.442  |
| rs3752088  | MAPK4        | 46490259                      | Intron A/C                                  | 0.108  |
| rs3794899  | MAPK4        | 46500101                      | Intron T/C                                  | 0.125  |
| rs3794901  | MAPK4        | 46505052                      | Intron A/G                                  | 0.108  |
| rs3752087  | MAPK4        | 46444438                      | Coding A/G                                  | 0.488  |
| rs2276186  | MRO          | 46681813                      | Coding A/G                                  | 0.487  |
| rs2256059  | MRO          | 46656201                      | Intron T/C                                  | 0.483  |
| rs2849233  | MRO          | 46595551                      | Coding T/C                                  | 0.408  |
| rs4940019  | MRO          | 46687201                      | Coding C/G                                  | 0.492  |
| rs2586770  | MRO          | 46597648                      | Intron T/C                                  | 0.417  |
| rs16952692 | ME2          | 46693267                      | Coding C/C                                  | 0.017  |
| rs685533   | ME2          | 46699801                      | Intron A/G                                  | 0.400  |
| rs12277    | ME2          | 46727751                      | Untranslated 3' region A/G                  | 0.408  |
| rs620698   | ELAC1        | 46763146                      | Intron A/T                                  | 0.108  |
| rs8065092  | SMA4         | 46835599                      | Intron A/G                                  | 0.417  |
| rs2509617  | SMA4         | 46857401                      | Intron T/C                                  | 0.067  |
| rs8098933  | MEXC         | 46971268                      | Intron A/G                                  | 0.408  |

SNP, single nucleotide polymorphism; NS, not-specified.
leading to psychosis. Finally selected 7 neuronal genes were MBD1, MAPK4, MRO, ME2, ELAC1, SMAD4, MEX3C. They were chosen based on functional and positional relevance to neuropsychiatric disorders. Twenty SNPs were genotyped and analyzed from those 7 genes.

SNPs selection (Table 1)

We have first searched possible candidate genes functionally relevant to neuropsychiatric disorders in chromosome 18q21 from the NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/projects/mapview/maps.cgi?taxid=9606&chr=18), which was referenced from positional relevance in my previous fine mapping analyses. Finally genotyped SNPs in selected neuronal genes were chosen from previous literatures and availability in Illumina Customized Sentrix Array Matrix genotyping_v6A with Standard Illumina procedures using Illumina BeadStation 500G array scanner in the NCBI ENTREZ SNP (http://www.ncbi.nlm.nih.gov/sites/entrez).

Case-control association tests

Case-control association tests were performed for the phenotype of “methamphetamine abuse”. All other subjects were classified as unknown controls for purposes of the analyses. For testing of association, we performed analyses for all 56 SNPs, using the phenotype of methamphetamine abuse.

Additional analyses

We have set analyses into four forms. The first set was the original case-control analysis and the second set was the original case-control analysis adjusted by male sex because cases are composed of only males. Sequential analyses, adjusted by age, were performed because the age difference between two groups was significant. The third set was the original case-control analysis adjusted by age. Finally, the fourth set was the original case-control analysis adjusted by age and sex.

Statistical Analysis

We calculated genotype frequencies for each individual polymorphism and evaluated Hardy-Weinberg equilibrium to check the data quality and genotype error. Chi-square test statistic is used to compare the observed numbers of each genotype with those expected for the population following chi-square distribution with a one degree of freedom.41

The association between the case-control status and each individual SNP, measured by the odds ratio and its corresponding 95% confidence interval using multiple logistic regression models (adjusting for sex and age as covariates). All analyses were performed assuming a dominant, recessive, additive, allelic effect for each polymorphism. In the dominant model, both the heterozygous variant and the rare homozygous variant were combined. In the recessive model, the variant was defined as only the rare homozygous genotype and in the additive model, each genotype variant has the same effect and in allele model, rare allele variant has effect. The likelihood ratio test was used to test the effect of each SNP at the 5% significant level.

And we controlled FDR to deal with multiple testing problem.42 We used the Benjamini and Hochberg method to control FDR.

All data were processed and analyzed by the R software, version 2.6.2. (Institute for Statistics and Mathematics, Wirtschaftsuniversität Wien, Vienna, Austria; http://www.r-project.org).

| Characteristic | Methamphetamine abusers (n=37) | Controls (n=30) | p value |
|----------------|-------------------------------|----------------|---------|
| **Demographic** |                               |                |         |
| Gender         |                               |                |         |
| Male           | 37 (100)                      | 16 (53)        | 0.000*  |
| Female         | -                             | 14 (47)        |         |
| Age (year)     | 43.3±7.8                      | 59.8±10.4      | 0.000*  |
| < 40           | 14 (38)                       | 1 (3)          |         |
| 40-50          | 15 (40)                       | 7 (23)         |         |
| >50            | 8 (22)                        | 22 (74)        |         |
| Marital status |                               |                |         |
| Unmarried      | 10 (27)                       | -              |         |
| Married        | 12 (32.4)                     | -              |         |
| Divorced       | 15 (40.6)                     | -              |         |
| Occupation     |                               |                |         |
| Unemployed     | 9 (24.3)                      | -              |         |
| Employed       | 28 (75.6)                     | -              |         |
| **Clinical**   |                               |                |         |
| Duration of use (year) |                 |                |         |
| ≤10            | 10 (27)                       | -              |         |
| 10-20          | 15 (40.6)                     | -              |         |
| >20            | 12 (32.4)                     | -              |         |
| Total dose (g) |                               |                |         |
| <200           | 15 (40.6)                     | -              |         |
| 201-400        | 13 (35.1)                     | -              |         |
| 401-600        | 4 (10.8)                      | -              |         |
| >600           | 5 (13.5)                      | -              |         |
| Number for arrests |                           |                |         |
| ≤2             | 12 (32.4)                     | -              |         |
| 3-4            | 13 (35.1)                     | -              |         |
| 5-6            | 7 (19)                        | -              |         |
| >6             | 5 (13.5)                      | -              |         |

Values are presented as number (%) or mean±standard deviation.
*p<0.001.
RESULTS

We found 3 significant SNPs of 2 genes in chromosome 18q21 (p-value < 0.05; adjusting for age as covariate) in methamphetamine abusers compared to controls (rs3794899, rs3794901:MAPK4; rs2849233:MRO). We also found 2 significant SNPs of 1 gene (p-value<0.05; adjusting for age and sex as covariates) (rs3794899, rs3794901:MAPK4). Two SNPs in MAPK4 gene were significant in both statistical groups.

Demographic and Clinical Characteristics of Patients of Methamphetamine Abuse (Table 2)

Demographic and clinical characteristics of the study patients are listed in Table 2. The duration and total dose of methamphetamine abuse varied widely among patients. The mean duration of methamphetamine abuse was 14.35±1.10 years (mean±standard error [SE]), and the mean total dose of methamphetamine that had been consumed during the period of drug abuse was 326.46±40.94 g (mean±SE), estimated solely on the basis of the patients’ statement and uncorrected for purity of drug. We compared the height, weight, and body mass index of methamphetamine abusers with those of the Korean male population from data obtained from Korean Statistical Information Service (KOSIS, 2004). There were no significant differences between our study and control groups for these parameters.

SNP Fine Mapping Analyses

Set 1 (original case-control association): We found 1 significant SNP of one gene in chromosome 18q21 (p-value < 0.05) in methamphetamine abusers compared to controls (rs2276186:MRO) (Table 3).

Set 2 (original case-control association adjusted by sex): We also found 1 significant SNP of one gene (p-value<0.05; adjusting for sex as covariates) (rs3794899:MAPK4) (Table 4).

Set 3 (original case-control association adjusted by age): We found 3 significant SNP of 2 genes in chromosome 18q21 (p-value < 0.05; adjusting for age as covariate) in methamphetamine abusers compared to controls (rs3794899, rs3794901:MAPK4; rs2849233:MRO) (Table 5).

Set 4 (original case-control association adjusted by age and sex): We also found 2 significant SNPs of one gene (p-value < 0.05; adjusting for age and sex as covariates) (rs3794899, rs3794901:MAPK4; rs2849233:MRO) (Table 6).

SNPs in 2 genes (MAPK4, MRO) of 7 genes were significantly associated with methamphetamine abuse in all four sets. Two SNPs in MAPK4 gene were significantly associated with methamphetamine abuse in final set 4 ad-
justed by age and sex. The MAPK4 gene lies between D18S473 and D18S474, finally concentrated region in our previous fine mapping analysis for psychosis and mania.27) Given the very basic mechanism for protein kinase of this gene, we focused on this gene.

LD analyses of selected 7 positionally only relevant genes which was focused in 18q21 fine mapping research was performed. Twenty SNPs genotyped in our fine mapping for methamphetamine abusers and controls are also represented (Fig. 1).

DISCUSSION

The results of fine mapping study presented here are in line with our earlier findings regarding psychosis and mania.22,27) Our findings suggest that MAPK4 gene could be associated with methamphetamine abuse. Therefore, this gene could have the possibility of being involved in the processes of methamphetamine abuse leading to psychosis in our samples of Korean population.

The MAPK4 gene that encodes mitogen-activated protein kinase 4 was one of the final 6 candidate genes in our previous fine mapping studies of 18q12-21 in our previous fine mapping for psychosis, although not directly associated.27) Our results suggest that MAPK4 is a candidate gene contributing to the methamphetamine addiction leading to psychosis.

Our previous findings27) (fine mapping of a chromosomal locus associated with psychosis and mania, and mRNA expression analyses in post-mortem brain) suggested that a specific mitochondrial enzyme, ME2, likely contributed to the expression of a spectrum of phenotypes ranging from schizophrenia to bipolar disorder. ME2 is a mitochondrial enzyme involved in neuronal glucose metabolism and in the synthesis of 2 key neurotransmitters, namely, GABA and glutamate.30) These findings are consistent with the reports that mitochondrial enzyme dysfunction plays a role in the pathophysiology of psychiatric disorders.44-49) Previous studies conducted in the Central Valley of Costa Rica (CVCR; one using samples of severe bipolar disorder, the other using subjects primarily diagnosed with schizophrenia) showed evidences for the existence of LD between the phenotypes of schizophrenia and bipolar disorders and the 18q21,22,50) 2.41 cM region (2.7 Mb) spanning from D18S450 to D18S474. Additionally, three separate linkage studies have pointed to a genetic predisposition locus in the general region for SC,51) BP,52) or both BP and SC.53) Maziade et al.53) showed evidence of linkage in a combined sample of SC and BP at the marker D18S472, which is approximately the same position as the marker D18S474 used in the present study.

In our earlier report,27) we described the fine mapping analyses for the 18q21 locus in the CVCR. Microsatellite analyses narrowed the area of strongest association of the psychosis phenotype to the region from 45.74 Mb (D18S473) to 46.94 Mb (D18S474). There are 11 known genes within this 1.20-Mb region: MYO5B (45.60 Mb-45.98 Mb), FLJ32743 (46.01 Mb-46.05 Mb), MBD1 (46.05 Mb-46.06 Mb), CXXC1 (46.06 Mb-46.07 Mb), C18orf24 (46.16 Mb-46.17 Mb), LOC390853 (46.24 Mb-46.34 Mb), MAPK4 (46.44 Mb-46.51 Mb), MRO (46.58 Mb-46.60 Mb), ME2 (46.66 Mb-46.73 Mb), ELAC1 (46.75 Mb-46.77 Mb), and SMAD4 (46.81 Mb-46.86 Mb). All of the genes in this region are of special interest because of their known functions and/or their relationship (association) with neuropsychiatric disorders. MBD1 is known to be associated with Rett syndrome and autism spectrum disorders.54,55) The ME2 gene is shown to be associated with idiopathic generalized epilepsy; the strongest association was seen with variants in the promoter region area of this gene.56) In the present study, the SMAD4 gene, which contains a single SNP, showed the strongest association to psychosis, and therefore is a candidate gene for schizophrenia and bipolar disorder.57) SMAD4 have been shown to be involved in neuronal proliferation and differentiation.58)

Genotyping evidence showed direct association of haplotypes containing SNPs within the ME2 and SMAD4 genes (hcv2752066-hcv7457521-hcv22274384) and the phenotype of psychosis. An individual SNP (hcv22274383) that is physically outside of the ME2 gene, but in strong LD with the ME2 gene, also showed significant association to the

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Table 6. Significant SNPs results in the original case-control association adjusted by age and sex (set 4)

| SNP name     | Chr | Gene symbol | Location | Dominant | Recessive | Additive | Allelic |
|--------------|-----|-------------|----------|----------|-----------|----------|---------|
| rs3794899    | 18  | MAPK4       | Intron   | 0.0338   | 0.3319    | 0.0470   | -       |
| rs3794901    | 18  | MAPK4       | Intron   | 0.0355   | 0.3319    | 0.0496   | -       |

SNP, single nucleotide polymorphism.
Candidate Genes of Chromosome 18q21 in Methamphetamine Abusers

The mitogen-activated protein kinase 4 encoded by MAPK4 gene is a member of the mitogen-activated protein kinase family. Growth factor receptor tyrosine kinases activate mitogen-activated protein kinases, which then translocate into the nucleus where they phosphorylate nuclear targets. A wide variety of extracellular stimuli activates MAPK pathways leading to cell proliferation, differentiation, growth, and migration. A typical MAPK cascade involves a three-kinase module architecture by which a signal from an upstream kinase is transmitted to a downstream kinase by sequential phosphorylation. Four well-defined groups of MAPKs are known (ERK1/2, c-Jun N-terminal kinases, p38s, and ERK5), but additional members, including ERK3, ERK4 (p63 MAPK, ERK3-related, ERK3, MAPK4, Prkm4), and ERK8, have been identified. ERK4 (p63 MAPK) was described in humans, soon after ERK1, ERK2, and ERK3 were identified. Among MAPKs, ERK4 is most closely related to ERK3, displaying 62% overall amino acid sequence identity and 73% within the predicted kinase domain. Both ERK4 and ERK3 lack the highly conserved activation loop ("a-loop") motif TXY between the kinase subdomains VII and VIII, but possess a segmented sequence at this position. Even the APE motif of subdomain VIII, which is highly conserved in other MAPKs is replaced by an SPR motif in ERK4 and ERK3. ERK4 and ERK3 carry long C-terminal extensions. Human Erk4 was mapped on chromosome 1q12-21, and a cDNA for the rat homolog rMNK2 has been isolated. Activators and relevant substrates of ERK4 remain elusive, and the enzymatic activities of the atypical ERKs have not been defined. Initially, the MAPK-activated protein kinase MK5, also known as p38-regulated -activated kinase (PRAK), was described as a member of the MK family and a downstream target of p38. MK5 easily interacts with and is activated by ERK3.

Given this very basic mechanism for the cellular neuro-physiological cascades, the MAPK4 gene could influence the intracellular signaling pathways leading to the co-morbidity of psychosis and addiction.

Whole genome SNP association studies suggested that many candidate "cell adhesion" genes located on chromosome 18 are involved in the molecular genetics of addiction. The CHST9, OSBPL1A, IMPACT, DOK6, HRH4, ZNF407, KIAA1713, and CCDC102B genes were identified by the whole genome SNP association studies of 420 unrelated European-American substance abusers versus 320 control European-Americans, and 560 unrelated African-American substance abusers versus 360 African-American controls.

Our study has several limitations. One limitation of the current study is the relatively low statistical significance of association (global p values showing association of methamphetamine abuse to SNPs within or in strong LD with the MAPK4 gene were between 0.0496 and 0.0119). Therefore, our findings need to be confirmed using a larger sample from Korea or other population. The second limitation is that, the individual SNPs that were shown to be associated with psychosis in our previous fine mapping were within ME2 gene (not the MAPK4 gene). Third, the control subjects in our samples were never exposed to methamphetamine. It is very critical, but very difficult to recruit sufficient number of very age and sex matched control in case-control association analysis. Therefore, we additionally analyzed case-control association with adjustment by age and sex for these unmatched controls for the results to be negative (rs1990162) although not re-
crucial for the recruitment of matched control in this study due to the very many limitations. Also we are now designing for recruiting matched control. Meanwhile there are some opinions among researchers that it is sufficient to do case-control association study without age-sex adjustment in spite of possible population stratification by covariate or confounder. Finally, denser genotyping, sequencing, and functional studies are needed to confirm whether the Mapk4 gene or other genes in tight LD with ME2, contributes to the comorbidity of psychosis and addiction in the Korean and other populations, which could be very over-inclusive with many limitations including very small number of uncotted subjects.

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