Association of Single-Nucleotide Polymorphisms in a Metabotropic Glutamate Receptor GRM3 Gene Subunit to Alcohol-Dependent Male Subjects

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Abstract — Aims: The purpose of this study was to investigate the association between the metabotropic glutamate receptor 3 (GRM3) subunit gene and alcohol dependence by the single-nucleotide polymorphisms (SNPs). Methods: Two hundred and forty-eight male alcohol-dependent patients and 235 male control subjects were recruited. Ten SNPs in the GRM3 region were studied, and genotyping of SNPs was performed by ligase detection reactions. Results: We found highly significant differences in allele and genotype frequencies of rs6465084 between the alcohol-dependent and control group, with the greater frequency of A allele of SNP rs6465084 in alcohol-dependent group. We also found significant differences of haplotype frequencies in five combinations (including TAATATT, CAGTATT, TCGTATT, CAATAGC, TAATATC) in the linkage disequilibrium constructed by seven SNPs between the groups. Conclusion: Our results supplied the first evidence that the polymorphism of GRM3 gene associates with the morbidity of alcohol dependence in human being, which may support a new potential target for alcoholism treatment.

INTRODUCTION

Alcohol dependence and abuse are among the most costly health problems in the world, and constitute a complex disease resulting from interplay among polygenic, sociocultural and environmental factors. It is characterized by impaired ability to control intake and intense desire for alcohol, despite related environmental factors. It is characterized by impaired ability resulting from interplay among polygenic, sociocultural and health problems in the world, and constitute a complex disease Alcohol dependence and abuse are among the most costly.

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the DSM-IV criteria for alcohol dependence and consumed >70 g of pure ethanol daily for at least 3 years. Two hundred and thirty-five male control subjects were recruited from the Medical Examination Centre of 1st Affiliated Hospital of Harbin Medical University. If control subjects drank alcohol, they were very mild social drinkers, with a mean of 4 g to a maximum of 12 g pure ethanol per day (one subject), and they were carefully screened for any past or present alcohol- or drug-related problems. All subjects were Han Chinese in origin between the ages of 18 and 60 years at the time of enrollment. Participants were excluded from the study for any concomitant psychiatric disorder, antisocial personality disorder, organic liver failure, or medical or neurological illness or trauma that would affect the CNS. This study was approved by the university’s Ethics Committee, and all subjects provided written informed consent.

Data collected on alcohol consumption included duration of alcohol dependence, daily alcohol intake, number of drinks in the prior month and drinks per month in the past year. Daily alcohol intake was estimated by the averaging alcohol consumption over 1 week in the past 30 days. Pure alcohol intake was computed in g/day. Duration of alcohol dependence was computed as the difference between current age and age of onset for alcohol dependence.

**Genotyping**

We selected SNPs in the GRM3 region from the reports of Fuji et al. (2003), Martí et al. (2002) and Chen et al. (2005) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), and examined allele frequencies in 20 samples from healthy Chinese. After evaluating 14 SNPs, we selected 10 of them for further genotyping (rs274622(C/T), rs2282958(G/C), rs724226(A/G), rs917071(C/T), rs757656(A/C), rs6465084(A/G), rs2237562(C/T), rs1468412(A/T), rs2299225(G/T), rs1989796(C/T)] with minority allele frequencies over 10% (Supplementary Table S1). SNP rs274622 is located in the promoter region, rs2282958 in the untranslated region and the others in introns, with five combinations including TAATATT (Supplementary Fig. S1; Taillon-Miller et al., 2000). Statistical analysis for the haplotype association was carried out as previous studies (Fujii et al., 2003; Chen et al., 2005). The differences of characters between the groups were evaluated using the unpaired t-test. For all of the tests conducted, the criterion for significance was set at $P < 0.05$. All calculations were carried out using the SPSS 13.0 software.

**RESULTS**

**Characters of subjects**

The average age of alcohol-dependent subjects was 46.04 ± 6.50 years and the length of education was 14.23 ± 2.75 years. Mean duration of alcohol dependence was 17.31 ± 6.21 years. Mean daily alcohol intake of alcoholic subjects was 162.47 ± 69.17 g, mean monthly intake over the prior month before the sampling was 4409.39 ± 1943.87 g and mean monthly intake over the prior year was 4875.02 ± 2075.02 g. The mean age of control subjects was 46.28 ± 5.78 years and the duration of education was 13.06 ± 3.18 years. We have examined associations between age and the 10 SNPs across all the cases and controls. Subjects in different groups differ significantly in age or education, and the drinking habit in alcohol dependence group remained stable.

**SNP association analysis**

None of the 10 SNPs’ genotype frequencies showed any significant deviations from Hardy–Weinberg equilibrium in either the alcoholic subjects or the controls. The analyses of allele frequencies for each single SNP are presented in Table 1. A highly significant difference in allele frequencies was shown with SNP rs6465084 ($P < 0.001$, OR = 3.063, 95% CI = 2.055–4.567), and the frequency of A allele was greater in alcohol-dependent group (92.9%) than in control group (81.8%). In addition, there was also a significant difference in genotype frequencies of SNP rs6465084 ($P < 0.001$, OR = 3.249, 95% CI = 2.094–5.039). No statistical differences of allele frequencies were found among other SNPs in GRM3 gene between the groups.

**Haplotype analysis**

LD between each pair of all the SNPs is presented in both Fig. 1 and Table 2. We observed significant LD (D' > 0.7) with the combinations of rs917071- rs757656- rs2237562- rs1468412- rs2299225- rs1989796 in this analysis. As none of the SNPs are in complete LD, 17 pairwise haplotypes were constructed by the seven SNPs and the frequencies of each haplotype were evaluated (Table 3). Significant differences of haplotype frequencies were observed with five combinations including TAATATT ($\chi^2 = 12.701$, $P < 0.001$), CAGTATT ($\chi^2 = 14.854$, $P < 0.001$), TCCTATT ($\chi^2 = 10.710$, $P = 0.001$), CAATAGC ($\chi^2 = 6.542$, $P = 0.011$),
Our study supplied the first evidence to establish the association between alcohol dependence and polymorphism in GRM3 gene in human being. The frequency of A allele of SNP rs6465084 was significantly higher in alcohol-dependent group than in control group. Moreover, significant differences of haplotype frequencies were observed in five combinations (including TAATATT, CAGTATT, TCGTATT, CAATAGC, TAATATC) in the LD constructed by seven SNPs between the alcohol-dependent and control group.

Alcohol dependence is found to be related to dysregulation of glutamate transmission in the brain (Melendez et al., 2005; Vengeliene et al., 2008). Changes in glutamate receptor mGluR function are related to substance addiction, and regarded as promising targets for drug abuse treatment (Kenny and Markou, 2004; Heilig and Egli, 2006; Gass and Olive, 2008). In the past, Glutamate Decarboxylase Gene SNPs in alcohol-dependent patients have been conducted, and 3 of 10 SNPs were demonstrated to have significant differences in allele frequencies in glutamic acid decarboxylase in 140 alcoholism patients (GAD1, but not GAD2; Loh et al., 2006), and TAATATC ($\chi^2 = 4.205, P = 0.040$) between the alcohol-dependent and control group (Table 3).

**DISCUSSION**

Our study supplied the first evidence to establish the association between alcohol dependence and polymorphism in GRM3 gene in human being. The frequency of A allele of SNP rs6465084 was significantly higher in alcohol-dependent group than in control group. Moreover, significant differences of haplotype frequencies were observed in five combinations (including TAATATT, CAGTATT, TCGTATT, CAATAGC, TAATATC) in the LD constructed by seven SNPs between the alcohol-dependent and control group.

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GRM3 works through G protein-coupled mechanisms to reduce excitatory postsynaptic potentials (Ishida et al., 1992; Robbe et al., 2002). A study that applied three tagging SNPs in GRM2 and one SNP (rs6465084) in GRM3 have conducted a genetic association analysis of case-control samples in the Japanese population. In their test, rs6465084 was the only marker in GRM3 that has been associated with Japanese major depressive disorder patients in the allele-wise analysis (Tsunoka et al., 2009). The finding that a selective GRM2/3 agonist (LY379268) was effective in reducing ethanol self-administration and stress-induced reinstatement following dependence-inducing ethanol exposure further implies the possibility of treatment target potential for GRM2/3 receptors in alcohol addiction (Bäckström and Hyytiä, 2005; Rood et al., 2006; Zhao et al., 2006; Siddhura et al., 2010). GRM2/3 agonists may therefore be valuable for clinical management of alcoholism.

However, there was still lack of direct evidence to associate GRM3 with alcoholic dependence in clinic. Recently, our laboratory provided evidence of glutamate system dysfunction in the prefrontal functional abnormalities seen in alcohol dependence (Xia et al., 2012). We have demonstrated that certain GRM3 SNP genotypes (the A/A genotype of rs6465084 and the T allele of rs1468412) lowered brain N-acetylaspartate concentrations and executive function skills in active alcoholics (Xia et al., 2012). Our work supplied the first evident that the polymorphism of GRM3 gene associates with the morbidity of alcohol dependence in human being, which may support a new potential target for alcoholism treatment.

New wave of pharmacological treatment in alcoholism comprised of compounds based on animal model studies, and the research on these models revealed a short list of sufficient preclinical validation to merit clinical development. These include the cannabinoid CB1 receptor, receptors modulating glutamatergic transmission (mGlur2, 3 and 5), and receptors for stress-related neuropeptides corticotropin-releasing factor, neuropeptide Y and nociceptin (Heilig and Egli, 2006). Worst et al. (2005) also studied the differences in normative cortical gene expression between rat strains genetically selected for alcohol self-administration preference, AA (Alko, alcohol) and P (Indiana, preferring), or avoidance, ANA (Alko, nonalcohol) and NP (Indiana, nonpreferring). They found that the GRM3 mRNA levels were down-regulated in AA compared with ANA lines. This finding supported the association of GRM3 with alcohol dependence not only in genetic susceptibility but also in treatment aimed at affecting glutamatergic neurotransmission in CNS.

Based on above findings, the association between variation in GRM3 and alcohol dependence reported here warrants further research, including replicating genetic analyses to clarify the meaning of rs6465084. In particular, we wish to study other genetic polymorphisms both upstream and downstream of SNP rs11542313 located in exon 3 of GAD67 gene region was the only one of the ten SNPs to be confirmed to associate with the condition of alcohol dependence in 107 subjects (Terranova et al., 2010). As a member of mGlus-II family, the metatropic glutamate receptor 5 and 8 (GRM5 and GRM8) have been extensively studied. Genotyping 7 SNPs in 1057 subjects associated with alcohol dependence for 3 SNPs (rs2832407 in intron 9, rs917071, rs724226) have conducted a genetic association analysis of case-control samples. Genotyping in 1057 subjects associated with alcohol dependence for 3 SNPs (rs2832407 in intron 9, rs917071, rs724226) have conducted a genetic association analysis of case-control samples. Genotyping in 1057 subjects associated with alcohol dependence for 3 SNPs (rs2832407 in intron 9, rs917071, rs724226) have conducted a genetic association analysis of case-control samples.
rs6465084 and SNPs, to see whether a LD exists with the SNP of interest, and whether it interferes with splicing and/or GRM3 mRNA stability. Further findings, related to gene expression or functional alteration of GRM3 in animals study, may support a potential pharmacogenetic effect of this variation in alcohol dependence subjects.

SUPPLEMENTARY MATERIAL

Supplementary material is available at Alcohol and Alcoholism online.

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Conflict of interest statement. None declared.

REFERENCES


Xia Y, Ma D, Hu J et al. (2012) Effect of metabotropic glutamate receptor 3 genotype on N-acetylaspartate levels and neurocognition in non-smoking, active alcoholics. *Behav Brain Funct* 8:42.