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Association study of polymorphisms in *N*-methyl-D-aspartate receptor 2B subunits (GRIN2B) gene with Korean alcoholism

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Abstract

The *N*-methyl-D-aspartate (NMDA) receptor 2B gene (*GRIN2B*) was studied as a candidate gene of alcoholism. This study aimed to investigate the association between each of the three *GRIN2B* polymorphisms (rs1806201, rs1805247, and rs1805502) and alcoholism. This study included 206 alcoholic patients and 189 unrelated control subjects of Korean origin. Associations between genotype, allele, and haplotype frequencies of the polymorphisms and alcoholism were investigated. The genotype frequencies of rs1806201 and the haplotype analysis of SNPs in this study show significantly differences between the case and controls. These findings suggest new candidate SNPs in *GRIN2B* for studying the genetic susceptibility to alcoholism.

Keywords: Alcoholism; *N*-Methyl-D-aspartate (NMDA) receptor 2B gene (*NR2B*; *GRIN2B*); Polymorphism; Haplotype; Association

1. Introduction

NMDA receptor antagonism has been thought an important mechanism for ethanol action. It is believed that alcohol dependence, development of tolerance to alcohol, and the alcohol withdrawal syndrome are mediated via *N*-methyl-D-aspartate (NMDA) receptors (Tsai and Coyle, 1998; Krystal et al., 2003). Up-regulation of the NMDA receptor by chronic ethanol exposure can explain development of tolerance and hyperexcitable state by abrupt withdrawal (Dodd et al., 2000; Henniger et al., 2003).

The NMDA receptor is a heteromeric subunit complex and the sensitivity of NMDA receptors to ethanol varies regionally. The diversity of NMDA receptor sensitivity is believed to result, at least in part, from the heterogeneity of the receptor subunit composition (Fink and Gothert, 1996). Several studies suggest that chronic ethanol exposure induce changes of NMDA receptor subunit composition (Gulya et al., 1991). NMDA receptor 2B subunit (NR2B; GRIN2B) confers a high sensitivity to ethanol induced inhibition (Masood et al., 1994). The weak NMDA receptor antagonist acamprosate has been used for relapse prevention in alcohol dependence (Rammes et al., 2001). This anti-craving action of acamprosate is thought to be mediated by interaction with a polyamine site particularly on the GRIN2B and inhibiting NMDA receptor mediated hyperexcitability (Williams et al., 1994). And the selective NR2B-containing NMDA receptor antagonist, ifenprodil had suppressed ethanol withdrawal signs in mice (Narita et al., 2000).

Although it shows polygenic inheritance, alcoholism is a disorder with strong genetic influences (Tyndale, 2003). Recent molecular genetic studies have linked several genes to alcoholism, including the gene for the NMDA receptor subunit. Wernicke et al. (2003) reported association of alcoholism with a single nucleotide polymorphism (SNP) in the NMDA receptor 2B gene variant (rs1806201) for the first time. However, several

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studies which were tested at the same polymorphic site with other Caucasian samples had revealed conflicting results (Schumann et al., 2003; Tadic et al., 2005).

In this study, we selected three SNPs (rs1806201, rs1805247, and 1805502) of *GRIN2B* and investigated with 206 alcoholic patients and 189 healthy control subjects of Korean origin. Two SNPs (rs1805247, and 1805502) had not been tested in alcoholism in previous studies.

2. Materials and methods

2.1. Subjects

The controls comprised 189 apparently healthy unrelated Korean males who visited a public health center for evaluating their health status; they did not have psychiatric and physical diseases or a personal or familial history of psychiatric or neurological illness, and their mean age was 40.1 years (range: 23–69 years). Most controls were nondrinkers and some were occasional light drinkers, as revealed by AUDIT-K, which is a questionnaire for selecting alcohol dependence in Korean social environments. The alcoholic patients comprised 206 unrelated males, who were hospitalized and diagnosed according to *Diagnostic and Statistical Manuals of Mental Disorders* (4th ed.) (American Psychiatric Association, 1994) criteria by trained psychiatrists; the mean age of the patients was 46.1 years (range: 28–66 years). All alcoholic patients and controls participating in the study signed a written consent form approved by an IRB in Eulji University.

2.2. Genotyping

Blood was collected by venipuncture from affected individuals and their parents and stored in EDTA Vacutainer tubes at −70 °C. DNA was extracted using a G-spin Genomic DNA Extraction Kit (Intron, Deajeon, Korea). We selected for the three *GRIN2B* polymorphisms (rs1806201, rs1805247, and 1805502) were performed as previously described (Ohtsuki et al., 2001). The information of primers and restriction enzymes used for genotyping of SNPs were given in Table 1. PCR was performed in 40 μl reaction volume containing 50 mM tris, pH 8.3, 16 mM $(NH_4)_2SO_4$, 1.75 mM $MgCl_2$, 2.5 mM each dNTP, 2 U *HiPi* thermostable DNA polymerase (Elpis biotech Inc. Daejeon, Korea) and 0.6 μg genomic DNA, 10 pmol of specific primers. The amplification was performed in thermo-cycler, PCR MBS 0.2G block (Thermo Electron Corporation, USA). The amplified products were electrophoresed on 2% agarose gel in a 0.5 × TBE running buffer, visualized with ethidium bromide for size estimation. Each PCR products were incubated with 1 U specific restriction enzymes during 20 h. The restricted fragments were electrophoresed on 3.5% agarose gel in 0.5 × TBE running buffer, visualized with ethidium bromide.

2.3. Statistics

Statistical procedures were carried out using SAS (Ver 8.01, SAS Inc.). The χ^2-test was performed to test allelic, genotypic differences between the two groups. Hardy–Weinberg equilibrium and linkage disequilibrium were tested in each group. Haplotype frequencies were estimated and differences in haplotype distribution were tested by using SNPAlyze 5.0.4 (Dynacom, Japan). The normalized linkage disequilibrium coefficient D' and the squared correlation coefficient r^2 were also calculated by SNPAlyze. $p < 0.05$ was considered to be statistically significant.

3. Results

The frequencies of the T allele of rs1806201, rs1805247, and rs1805502 were 0.50, 0.78, and 0.79 in the controls and 0.47, 0.79, and 0.79 in the alcoholic patients, respectively. The genotype distribution did not deviate from Hardy–Weinberg equilibrium in either alcoholic patients or controls for all three polymorphisms. For rs1806201 polymorphism, comparison of genotype distribution between controls and alcoholic patients detected significant differences ($\chi^2 = 6.2956$, d.f. = 2, $p = 0.0429$), but not allele frequencies. After Bonferroni adjustment, this result was no longer statistically significant. For rs1805247 and rs1805502, genotype distribution and allele frequencies did not differ between alcoholic patients and controls (Table 2).

The result of all haplotypes analyses, inclusive of all the studied three SNPs, showed a statistically significant difference in haplotype frequencies between alcoholic patients and controls ($\chi^2 = 50.544$, d.f. = 7, $p < 0.001$). Some haplotype

Table 1
Detailed information of the PCR-RFLP analysis for the SNPs

SNP	Primer sequence (5′ → 3′)	Product (bp)	RFLP	Allele (bp)	
rs1806201	taacctgtccctccattctt; ctgcggcggtgctctgagat	248	*Aci*I	T (181/59/3/5)	C (92/89/59/3/5)
rs1805247	cggacatcaccaccacaaca; tgaaagccctggggtttttg	320	*Nco*I	C (320)	T (202/118)
rs1805502	cccccaaaactgattacaac; tgttaagtgaagggagcatc	353	*Aci*I	T (353)	C (239/114)

Table 2
Genotype and allele frequencies of GRIN2B gene polymorphisms in Korean

	rs1806201					rs1805247					rs1805502				
	Genotype			Allele		Genotype			Allele		Genotype			Allele	
	C/C	C/T	T/T	C	T	T/T	T/C	C/C	T	C	T/T	T/C	C/C	T	C
Control ($n = 187$)	0.28	0.44	0.28	0.50	0.50	0.62	0.30	0.07	0.78	0.22	.0.61	0.37	0.02	0.79	0.21
Alcoholism ($n = 206$)	0.25	0.56	0.19	0.53	0.47	0.60	0.37	0.03	0.79	0.21	0.60	0.37	0.02	0.79	0.21
χ^2	6.2956			0.5535		5.3413			0.1465		0.0518			0.0277	
p-Value	0.0429			0.4569		0.7019			0.0692		0.9744			0.8679	
Odd ratio (95% confidence interval)				0.8994 (0.6801–1.1893)					0.9362 (0.6681–1.3119)					0.9713 (0.6889–1.3694)	