Association of opioid receptor gene polymorphism with drinking severity and impulsivity of alcohol dependence

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Directed by Professor Chan-Hyung Kim

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ABSTRACT

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Background

Recent evidence has suggested that endogenous opioid system is implicated in the pathophysiology of alcohol dependence. On the basis of several studies that showed associations between opioid receptor gene variants and alcohol dependence, we aimed to examine the genetic influence of opioid receptors on the susceptibility to alcohol dependence and its clinical and psychological characteristics such as multidimensional impulsivity in a Korean population.

Methods

We genotyped three single nucleotide polymorphisms (SNP) (rs1799971, rs609148, rs648893) from μ-opioid receptor gene (*OPRM1*) and two SNPs (rs702764, rs6473797) from κ-opioid receptor gene (*OPRK1*) using the SNaPshot assay. Four SNPs were analyzed (rs1799971, rs609148, rs702764, rs6473797), because rs609148 and rs648893 were completely linked. The genotype distributions and haplotype frequencies were examined in 320 male patients with alcohol dependence and 329 age-matched male controls. To analyze the associations between opioid receptor gene variants and impulsivity in alcohol dependence, patients have undertaken the stop signal task (SST), delay discounting task (DDT), and balloon analog risk taking task (BART), which measure different aspects of impulsivity. In addition, we used several scales, including alcohol use disorders identification test (AUDIT),

obsessive-compulsive drinking scale (OCDS), alcohol dependence scale (ADS), to examine the influence of opioid receptor genes on clinical characteristics and psychological traits in patients with alcohol dependence.

Results

No significant difference in either genotype distributions or haplotype frequencies was found between patients with alcohol dependence and controls. For behavioral tasks measuring impulsivity, in *OPRK1* SNP rs6473797, compared with the patients with heterozygote genotype GA, the patients with homozygote genotype AA and GG had significantly longer stop signal reaction time. (p = 0.0086). In addition, rs6473797 was significantly related to the severity of alcohol dependence, as measured with AUDIT (p = 0.0041), OCDS (p = 0.0002), and ADS (p = 0.0013). A haplotype containing rs6473797 was also related to the scores on these scales (AUDIT: permutated p = 0.0081; OCDS: permutated p = 0.0027; ADS: permutated p = 0.0033).

Conclusions

These results support that genetic variations of opioid receptor may contribute to the symptom severity and impulsivity in patients with alcohol dependence.

Key words : alcohol dependence, opioid receptor genes, polymorphism, alcohol consumption, impulsivity

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I. INTRODUCTION

Alcohol use disorder (AUD) is a complex disorder involving multiple genetic and environmental factors. Based on studies involving twins and adoption, the hereditary component of alcohol dependence (AD) has been estimated to be 50–60%.[1]

As with most other drugs of abuse, the rewarding effects of ethanol are thought to be associated with increased synaptic dopamine (DA) accumulation within the nucleus accumbens. Although ethanol interacts with numerous neurotransmitter systems, its ability to increase mesolimbic DA release appears to depend on the integrity of the endogenous opioid system. The endogenous opioid system has been implicated in the development of alcohol dependence due to its prominent role in the central rewarding mechanism.[2] Existing studies suggest that the level of alcohol dependent activation in endogenous opioid transmission might be in part genetically determined.[3, 4]

Pharmacological blockage of the endogenous opioid system by opioid receptor antagonists prevents ethanol consumption.[5] Naltrexone, a drug approved by the U.S. Food and Drug Administration for the treatment of alcoholism, an opioid receptor antagonist, decreases alcohol drinking, although its effects may depend on single nucleotide polymorphisms (SNPs) of opioid receptor genes.[6-9]

Opioid receptors are part of the Rhodopsin family of G-protein coupled receptors (GPCRs), which activate downstream signaling through interactions with heterotrimeric G proteins. The three most common types are the μ-opioid receptor (MOR), δ-opioid receptor (DOR), and κ-opioid receptor (KOR), encoded by the μ-opioid receptor gene (*OPRM1*), δ-opioid receptor gene (*OPRD1*), and κ-opioid receptor gene (*OPRK1*), respectively.[10]

MOR is activated by both endomorphins and β-endorphin, a cleavage product of the proopio melanocortin precursor. Enkephalin and deltorphin have been shown to activate DOR, while the dynorphin class of peptides is specific for the KOR protein. Many of these peptides have some affinity for more than one receptor type.[10]

There is a hypothetical model of the opioid reward system. Stimulation of MORs in the ventral tegmentum of the midbrain or DORs in the nucleus accumbens leads to an increase of dopamine release, whereas stimulation of presynaptic KORs in the nucleus accumbens reduce dopamine release.[11, 12]

Among opioid receptor genes, *OPRM1* is the most intensively studied in drug dependence and alcoholism. Several polymorphisms in the *OPRM1* gene have been reported to be associated with alcohol dependence, rs1799971 being the most commonly reported. Studies conducted on the effect of the rs1799971 SNP on alcohol dependence have contradictory results.[13] In a recent meta-analysis of several different studies, researchers reported that the *OPRM1* rs1799971 variant does not appear to influence risk for substance dependence.[14] Additionally, other researchers have not been able to show any association between this genetic polymorphism, alcohol consumption, and states of alcohol dependence.[15-17] A report studying the Korean population suggests that G(Asp40, rs1799971) allele may be an important genetic factor in the etiology of alcohol dependence and the frequency of alcohol consumption.[18] However, another Korean study of the relationship between AUD and *OPRM1* reported inconsistent results.[19]

Like rs1799971, other *OPRM1* genes have been examined in association studies of alcohol dependence.[20] [21] Their findings are, however, insufficient. Moreover, there is no study based on Asian populations.

As mentioned above, until now, the μ-opioid receptor received the most attention in alcoholism research. In addition, research has suggested that a variant of *OPRM1* may be associated with the response to naltrexone treatment.[22] However, naltrexone also acts at the κ-opioid receptor and it has not been clear whether this effect of naltrexone is relevant to alcoholism treatment. A growing body of research in animals implicates the KOR in alcoholism.[23] Vasdasz et al. reported that *OPRK1* variants might contribute to a genetic predisposition to voluntary alcohol-drinking behavior in mice.[24] In addition, a number of studies investigated the association of *OPRK1* SNPs with alcoholism and other addictions.[9] SNP rs6473797 in intron 2 and several other SNPs were reported to be associated with alcoholism in an European American (EA) population.[25] Like MOR, the KOR is localized in several areas of the dopaminergic nigrostriatal and mesolimbic–mesocortical systems which are the sites of known actions for drugs of abuse.[26] However, unlike MOR ligands, the endogenous KOR agonists, dynorphins, decrease basal and drug induced dopamine levels in several areas of dopaminergic nigrostriatal and

mesolimbic–mesocortical system. They also inhibit morphine-withdrawal symptoms induced by naloxone precipitation or morphine discontinuation in morphine-dependent animals.[27] The KOR gene polymorphisms have been reported to contribute to voluntary alcohol-drinking behavior in experimental animals.[28] Although some studies have explored the association of some SNPs of KOR gene with drug dependence, no definite identification of a risk allele has been detected.[29, 30]

A recent family-based study in European Americans demonstrated that variations in *OPRK1*, the gene encoding the KOR, and in PDYN, which encodes its dynorphin ligand, were associated with alcohol dependence.[25] The relationship between *OPRK1* and alcoholism was confirmed by a second study, reporting that a haplotype of *OPRK1* was associated with alcohol dependence[31] although individual SNPs were not, it should be noted that Zhang et al. did not test any SNPs in intron 2, the region where association had earlier been reported.[31] However, an earlier study examining three coding SNPs in Taiwanese Han subjects found no association between *OPRK1* and alcohol dependence.[15] Thus, conflicting results on the role of the opioid receptor genes in AUD need to be clarified.

There is an extensive literature linking impulsivity to alcohol use and alcohol problems in human studies, as has been reviewed previously.[32-34] It is also well known that heavy alcohol use can trigger impulsive behavior.[35, 36] The conversion has also been reported: impulsivity as measured in prospective studies has been shown to predict the development of AUD,[37 38] and to mediate the relationship between parental substance use disorders and the eventual development of substance use disorders in offspring.[39] The fact that impulsivity is elevated in offspring who are at high risk for substance use disorders based on a parental history of substance use disorders suggests that impulsivity may be a reflection of a genetic vulnerability for substance use problems.[40] Together, these literatures indicate that impulsivity may be a general risk factor for a number of conditions broadly termed the externalizing spectrum, and that AUD may represent one specific manifestation of this spectrum.[40]

As regarding impulsivity, there is growing consensus that impulsivity is heterogeneous and should not be considered a unitary construct and should instead reflect a variety of behaviours and processes.[41] In laboratory-based research, and it has been repeatedly suggested that there are at least two components,[42-44] including behavioral disinhibition (impulsive action) and impulsive decision making (impulsive choice).

Behavioral disinhibition refers to the ability to control and suppress reward driven behavior or prepotent response.[43] Behavioral disinhibition has been measured with behavioral tasks including stop signal tasks (SST) and Go/No-Go. Recent studies show that a disinhibited state resulted in increased alcohol consumption in comparison to healthy controls.[45] However, the results are not consistent across all studies.[46, 47] Gubner et al. investigated the relationship between inhibition and

response to alcohol in 15 inbred strains of mice using a Go/No-Go task.[48] They concluded this form of impulsivity (disinhibition) was heritable. Impulsive decision making, in which individuals are oversensitive to immediate rewards rather than waiting for larger delayed rewards, is commonly measured using the delay discounting task (DDT). In this task, preference for a smaller, immediate reward over a larger, but delayed, reward is interpreted as increased impulsivity. Several studies show that heavy social drinkers and alcoholics demonstrate an increased delay discounting compared to controls.[49-51]

In addition to disinhibition and impulsive decision making, recent research suggests that risk-taking propensity may be another component.[47, 52] Risk-taking behaviors have some potential for danger while providing little opportunity to obtain some form of reward.[53] Some studies have shown that risk-taking maybe a distinct aspect of impulsivity that is associated with substance abuse and heavy drinking.[54-57] Fernie et al. reported risk taking, but not response inhibition or delay discounting, as a possible predictor of alcohol consumption in social drinkers,[47] while Courtney et al. reported a contrasting result.[58] In these studies, the Balloon analogue risk taking task (BART) is used for measuring risk taking behavior. In this task, participants inflate a virtual balloon with a small potential payout per pump. However, the balloon may burst at any time, resulting in a forfeiture of the money earned for that trial. BART is representative of real world risk taking, which is indexed by increased reward seeking in the face of greater potential loss.[59]

High impulsiveness and low deliberation scores were associated with significantly higher regional μ-opioid receptor concentrations and greater stress-induced endogenous opioid system activation.[60] Further, the *OPRM1* genotype was linked to differential response inhibition-related functional connectivity.[61] Moreover, *ORPM1* knockout mice were found to exhibit increased motor impulsivity on a nose poke task.[62]

In recent study, Pfeifer et al. examined the three way relationship between alcohol consumption, impulsivity and the *OPRM*1 A118G polymorphism among individuals who were not severely exposed to stress. They reported the positive correlation between urgency subscale of Urgency, Premeditation, Perseverance, Sensation seeking, Positive urgency (UPPS-P) impulsive behavior scale and a higher drinking frequency among individuals with *OPRM1* 118G.[63] These findings support the idea that opioid receptors may influence development of AUD and impulsive behaviors in AUD patients. However, Pfeifer et al.'s study was conducted using only self-reported impulsivity scale.

The aim of our study was to investigate the potential role of *OPRM1* and *OPRK1* in susceptibility to AUD, as well as on its clinical and psychological characteristics in a Korean population. That is, we examined 3 *OPRM1* SNPs (rs1799971, rs609148, rs648893) and 2 *OPRK1* SNPs (rs702764, rs6473797) in both a healthy control group and an AUD patient group.

We also evaluated the association between clinical severity, and *OPRM1* and *OPRK1* in AUD patients. The severity of each patient's alcoholism was determined by using AUDIT, obsessive compulsive drinking scale (OCDS) and alcohol dependence scale (ADS) scores. Lastly, we explored the association between impulsivity and *OPRM1* and *OPRK1*. Impulsivity was measured by objective behavior tasks in order to assess the three dimensions we suspect may contribute to alcoholism: impulsive decision making (DDT), behavior disinhibition (SST) and risk taking (BART). Based on the biological evidence noted above, we hypothesize that opioid polymorphisms are associated severity and impulsivity in AUD patients.

II. MATERIALS AND METHODS

1. Participants

The present study included 320 male alcoholics, aged 21-65 years, who were hospitalized at the 14 psychiatric hospitals throughout Korea. All the subjects were diagnosed by psychiatrists as having alcohol dependence according to the DSM-IV criteria, and had been abstinent for at least one week. All subjects scored above the cut-off score of 8 on the AUDIT, which is indicative of hazardous drinking.[64]

Exclusion criteria were as follows: (1) physical or mental illness that would interfere with task performance; (2) history of major psychiatric disorder; (3) history of other substance dependence in the last 6months; (4) a score of less than 26 on MMSE-K (Mini Mental State examination – Korea version).

Participants were paid for their participation and had given written informed consent according to the procedures approved by the Severance Hospital Review Board.

329 non-alcoholic male controls were enrolled from the Cardiovascular Genome Center at Yonsei University College of Medicine in Korea, between November 2000 and March 2011. They visited Severance Hospital, Yonsei University Health System, for health check-ups. They had not specific medical conditions.

2. Genotyping

The genotye was screened using single base primer extension assay from the ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to the manufacturer's recommendation.

The genomic DNA flanking the SNP of interest was amplified with PCR reaction with forward and reverse primer pairs and standard PCR reagents in 10 microliter reaction volume. The reagents contained 10ng of genomic DNA, 0.5pM of each oligonucleotide primer, 1 microliter of 10X PCR buffer, 250μM dNTP(2.5mM each) and 0.25 unit DiaStar Taq DNA Polymerase(5unit/μl) (SolGent co., Ltd. Daejeon, South Korea). The PCR reactions were carried out as follows: 10 minutes at 95℃ for 1 cycle, 30seconds at 95℃ for 35cycles, 1 minute at Tm℃, and 1 minute at 72℃ followed by 1 cycle for 10 minutes at 72. After amplification, the PCR products were each treated with 1 unit of shrimp alkaline phosphatase (SAP) (USB Corporation, Cleveland, OH, USA) and exonuclease I (USB Corporation, Cleveland, OH, USA) at 37℃ for 75 minutes and 72℃ for 15 minutes to purify the amplified products. One microliter of the purified amplification products

were added to a SNaPshot Multiplex Ready reaction mixture containing 0.15pmols of genotyping primer for primer extension reactions. The primer extension reaction was carried out for 25cycles at 96℃ for 10 seconds, 50℃ for 5 seconds, and 60℃ for 30 seconds. The reaction products were treated with 1 unit of SAP at 37℃ for 1 hour and 72℃ for 15 minutes to remove excess fluorescent dye terminators. One microliter of the final reaction samples containing the extension products were added to 9 microliters of Hi-Di formamide (ABI, Foster City, CA). The mixture was incubated at 95℃ for 5 min, followed by 5min on ice, and then analyzed by electrophoresis in ABI Prism 3730xl DNA analyzer. Analysis was carried out using Genemapper software (version 4.0; Applied Biosystems).

3. Questionnaires

A. The Alcohol Use Disorders Identification Test (AUDIT)[65, 66]

The AUDIT questionnaire consists of items regarding alcohol consumption and the resulting consequences of drinking. Scores on the AUDIT range between 0 and 40, with scores of 8 or above indicating hazardous alcohol use. In this study, the Korean-translated version of AUDIT was applied to all participants.

B. Obsessive Compulsive Drinking Scale (OCDS)[67]

This 14-item, self-administered instrument assesses the efforts and ability to resist thoughts of alcohol and the impulse to drink. The questions use descriptors based on numerical ratings ranging from 0 to 4, where higher scores indicate higher craving intensities.

C. Alcohol Dependence Scale (ADS)[68]

ADS consists of 25 questions developed by Skinner and Allen through factor analysis. This scale evaluates self-administered compulsive drinking, problematic drinking behavior, and alcohol withdrawal symptoms. The translated questionnaire utilized in this study was previously standardized by Lee et al. in Korea.[69]

4. Behavior Tasks

A. Action impulsivity: Stop Signal Task (SST)[70]

Response inhibition (action impulsivity) was assessed using the SST, which consists of 120 total trials. In each trial, participants were presented with the go stimulus (the letter ‘‘X’’ or ‘‘O’’) for 1,000 ms with the instructions to press the ‘Z’ key for an X and the ‘/’ key for an O as quickly and as accurately as possible (go trials). For stop trials (25% of trials), a go stimulus was followed by a stop signal (a loud beeping sounds) after a variable delay, which signaled participants to

withhold a response. The onset of the stop signal was varied by a tracking algorithm, in which the stop signal delay was initially 250 ms, but was decreased by 50 ms after a previous stop task failure and increased by 50 ms after a previous success. To yield reliable stop signal reaction time (SSRT), we used the following outlier criteria: (1) percent inhibition on stop trials less than 25% or greater than 75%, (2) percent go-response less than 60%, (3) percent go-errors greater than 10%, and (4) SSRT estimate that is negative or less than 50 ms.[71] The main dependent variable, SSRT, is a sensitive measure of response inhibition, and was extracted by the quantile method which does not require an assumption of 50% inhibition[70]. A longer SSRT reflects worse inhibitory control (slower inhibitory process). In this study, we used the Korean version of the SST.[72]

B. Choice impulsivity: Delay Discounting Task (DDT)[73]

Delay discounting (choice impulsivity) was assessed using a binary choice procedure. In each trial, the computer screen showed a series of choices between two virtual money rewards: an immediate smaller reward and a delayed larger reward. The delayed reward was fixed at 1,000,000 Korean Won, which is approximately 100 US dollars. At the first session, the amount of delay was held constant at 1 week, and the 26 immediate rewards were presented on the screen in descending order, one per each trial. In the next session, the sequence of immediate monetary rewards ascended in amount until the largest reward was presented, with a particular temporal delay. The next sessions were repeated with incrementally larger temporal delays of 1 week, 2 weeks, 1 month, 6 months, 1 year, 3 years, and 10 years. Within each session, the amount of the immediate monetary value that was preferred equivalently to the large delayed monetary value was defined as the point of subjective equivalence (i.e., an indifference point). Indifference points across the delays were calculated using the hyperbolic decay function, yielding k values reflecting the delay discounting rate[74]. Higher k values indicate higher sensitivity to delayed rewards or choice impulsivity. In this study, we used the Korean version of the DDT.[75]

C. Risk Taking: Balloon Analogue Risk Task (BART)[76]

During the BART, participants were required to press a button to inflate a series of 30 balloons. With each button click the balloon inflated and participants earned a monetary reward (50 Korean Won) for each pump.

This money was added in a temporary bank for that balloon. Participants were told that at some point the balloon would pop and they would lose all the money in the temporary bank. An exploding balloon was represented by an appropriate auditory effect and the visual of an exploding balloon on the computer. Participants were instructed that they could collect their earnings from the temporary account and move it to their permanent account at any point before the balloon exploded by pressing a button marked ‘‘Collect.’’ When a participant collected or popped a balloon, a new balloon appeared. Participants did not actually receive the money, but were instructed to imagine

that their earnings were real. Risk taking propensity was measured by calculating the adjusted mean pumps (AMP), the average number of inflations over the trials in which the balloons did not explode. A larger adjusted value represents a higher risk taking propensity. As the Korean version of the BART was not available, we used the original version of BART,[76] which was translated into Korean.

5. Statistical analyses

The statistical analyses were performed using descriptive statistics for the demographic variables.

The genotypes for rs609148 and rs648893 were completely linked. Thus, rs648893 was dropped from the analyses, and allele frequencies and association with the traits for rs609148 would be extended to rs648893.

Differences in the allelic distribution of the four SNPs were examined using χ^2 tests. Associations between each SNP genotype and alcohol dependence status were examined using age-adjusted multivariate logistic regression analyses. Linear regression models were used to evaluate associations between genotypes and various clinical measures of drinking severity, other clinical characteristics and behavioral tasks measuring impulsivity only in AUD subjects.

Odds ratios (OR) and the associated 95% confidence intervals (CIs) were estimated for theses variables. Single marker analyses were conducted using the R package SNPassoc.[77] We applied Bonferroni correction to adjust for multiple comparisons when examining the 4 SNPs. Therefore, we set the statistical significance level at $p < 0.0125$.

In the haplotype analyses, the pairwise linkage disequilibrium (LD) pattern of the *OPRM1* and *OPRK1* SNPs were estimated with Haploview v4.0 (http://www.broadinstitute.org/haploview/haploview)[78] and haplotype blocks were determined from the four gamete rule. The associations between *OPRK1* genes haplotype and alcohol dependence status or the clinical characteristics of AUD were examined using the 'haplo.score'function of the program 'haplo.stats' (http://cran.r-project.org/src/contrib/Descriptions/haplo.stats.html),[79] with adjustments for age. For haplotype analyses, permutation adjustments were performed (n = 10,000), and simulated $p<0.05$ was regarded as significant.

II. RESULTS

1. Sample characteristics

Demographic and clinical characteristics for participants are presented in Table 1. A total of 320 alcohol-dependent male patients underwent testing. The mean age of subjects was 48.61 ± 7.94 years. The mean AUDIT score was 27.05 ± 7.21, and all subjects scored above 8 on the AUDIT.

Table1. Sociodemographic and clinical characteristics of the study sample

Variable	AUD (*n* = 320)	Controls (*n* = 329)	*P* value
Age, years (range)	48.61 ± 7.94 (22–64)	56.01 ± 6.83 (40–67)	0.092
Male/Female	320/0	329/0	
Education, years	11.49 ± 3.1		
Age of first drinking	19.59 ± 7.35		
Number of admission	7.43 ± 11.59		
Onset age of AUD	31.01 ± 10.16		
AUDIT	27.05 ± 7.21		
OCDS	28.96 ± 7.37		
ACDS	46.31 ± 9.92		

AUD, alcohol use disorder; AUDIT, Alcohol Use Disorders Identification Test; OCDS, Obsessive Compulsive Drinking Scale; ADS, Alcohol Dependence Scale;

2. Hardy–Weinberg equilibrium (HWE) tests and haplotype blocks

None of the SNPs significantly deviated from the Hardy-Weinberg equilibrium in controls ($p > 0.01$), and minor allele frequencies were higher than 0.05.

LD analyses of our case and control subjects using the program Haploview showed that SNPs were located in haplotype block (Fig. 1). In haplotype analyses, one haplotype block was identified: block1 (rs702764-rs6473797).

OPRK1

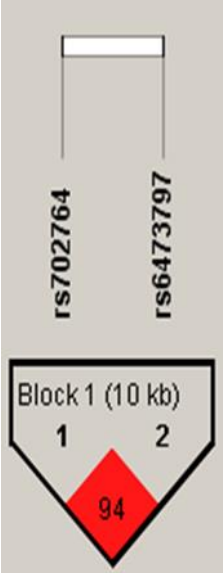

Figure1. Haplotype block estimated with markers that were examined in this study. Block1 (rs 702764, rs 6473797).

3. Association of SNPs and AUD

Comparison of allele or genotype distributions did not reveal significant differences between AUD cases and normal controls for all SNPs (Table 2). There were no significant difference in block1 haplotype between patients with AUD and controls (Table 3).

Table2. Distribution of genotype and allele frequencies of in the study sample.

SNP	Alleles			Genotypes						
	D/d[a]	AUD[b]	Control[b]	AUD[c]	Control[c]	OR_{cod} (95% CI); *P* value	OR_{dom} (95% CI); *P* value	OR_{rec} (95% CI); *P* value	OR_{ovd} (95% CI); *P* value	OR_{add} (95% CI); *P* value
rs1799971	A/G	0.399	0.363	125/144/59	131/146/43	0.93(0.43-1.56); 0.3094	0.99 (0.68-1.38); 0.8441	0.85 (0.43-1.12); 0.1360	1.09 (0.83-1.66); 0.3625	0.89 (0.70-1.14); 0.3625
rs609148	G/A	0.087	0.064	273/55/1	280/39/1	0.72 (0.02-6.86); 0.4721	0.88 (0.46-1.23); 0.2536	0.71 (0.02 -6.86); 0.5435	0.89 (0.47-1.26); 0.2936	0.75 (0.47-1.20) 0.2305
rs702764	A/G	0.066	0.064	286/41/1	281/35/3	1.45 (0.23-26.33); 0.6354	0.98 (0.55-1.55); 0.7576	1.76 (0.24-26.74); 0.4159	0.99 (0.51-1.48); 0.6037	0.98 (0.60-1.58) 0.9184
rs6473797	A/G	0.392	0.384	115/169/44	110/162/42	0.96 (0.53-1.64); 0.9645	0.98 (0.67-1.37); 0.8022	0.99 (0.57-1.60); 0.8688	0.99 (0.69-1.38); 0.8976	0.97 (0.74-1.25); 0.7902

AUD,alcohol-use disorder; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; cod: codminant; dom, dominant; rec, recessive; ovd, overdominant; add,log- additive.

[a]Lowercase d denotes the less frequent allele.

[b]Minor allele frequencies in indiviuals with AUD and controls.

[c]Number of genotypes in individuals with AUD and controls. Order of genotypes: DD/Dd/dd (d is the minor allele).

Table 3. Haplotype frequencies and association with AUD in case control study.

Haplotype		Hap-Freq[a]	Hap-Score[b]	Crude P[c]	Sim. P[d]
Block1[e]					
rs702764	rs6473797				
A	G	0.3235	-0.2420	0.8088	0.8151
G	G	0.0617	0.0129	0.9897	0.9903
A	A	0.6080	0.2620	0.7934	0.7937

[a] Hap-Freq, estimated frequency of the haplotype in the pool of all subjects; [b] Hap-Score, score for the haplotype; [c] asymptotic chi-square p-value (haplotype p); [d] simulated p-value; [e] global-stat=0.1019, df=3, p=0.9916, global simulation p=0.9916

4. Association of SNPs and severity of alcohol dependence

Rs6473797 was significantly related to the severity of alcohol dependence, as measured with AUDIT (p = 0.0041), OCDS (p = 0.0002), and ADS (p = 0.0013) (Table 4). In addition, a haplotype contain rs 6473797 was also related to the scores on severity scales. In block 1, A-G haplotype was significantly positively associated AUDIT (Hap-score =-2.6793, simulated [sim] p = 0.0071), OCDS (Hap-score =-3.6887 , simulated [sim] $p < 0.001$) and ADS (Hap-score =-3.1020, simulated [sim] p = 0.0018), additionally, in block 1, A-A haplotype was significantly positively associated AUDIT(Hap-score =2.7674, simulated [sim] p = 0.0081), OCDS (Hap-score =2.9426, simulated [sim] p = 0.0027) and ADS (Hap-score =2.9843, simulated [sim] p = 0.0033) (Table 5). However, other SNPs and other haplotypes were not related to the severity.

5. Association of SNPs and impulsivity in alcohol dependence

For SST, in *OPRK1* SNP rs6473797, compared with the patients with heterozygote genotype GA, the patients with homozygote genotype AA and GG had significantly longer stop signal reaction time. (p = 0.0086)) (Table 6). However, other SNPs and all haplotypes were not related to SST, DDT, or BART (Table 6, 7).

Table 4. Associations of *OPRM1* SNPs and *OPRK1* SNPs with clinical features of alcohol drinking. Significant values ($p < 0.01$) are highlighted in bold.

		AUDIT					OCDS					ADS				
		Pvalue[a] cod	*Pvalue* dom	*Pvalue* rec	*Pvalue* ovd.	*Pvalue* add	*Pvalue* cod	*Pvalue* dom	*Pvalue* rec	*Pvalue* ovd.	*Pvalue* add	*Pvalue* cod	*Pvalue* dom	*Pvalue* rec	*Pvalue* ovd.	*Pvalue* add
SNP	Alleses															
rs1799971	A/G	0.79	0.69	0.50	0.95	0.53	0.39	0.32	0.22	0.90	0.19	0.13	0.87	0.05	0.24	0.27
rs609148	G/A	0.50	0.77	0.28	0.63	0.91	0.22	0.55	0.13	0.39	0.74	0.13	0.53	0.07	0.35	0.75
rs702764	A/G	0.76	0.47	0.98	0.46	0.51	0.25	0.10	0.58	0.12	0.10	0.77	0.48	0.70	0.54	0.47
rs6473797	A/G	0.02	**0.007**	0.07	0.18	**0.0041**	**0.001**	**0.0002**	0.02	0.06	**0.000**	**0.004**	0.02	**0.004**	0.7	**0.0013**

AUDIT, Alcohol Use Disorders Identification Test; OCDS, Obsessive compulsive drinking scale; ADS, Alcohol Dependence Scale; cod, codominant; dom, dominant; rec, recessive; ovd, overdominant; add,log- additive age

Table 5. Haplotype frequencies and association with clinical features of alcohol drinking in AUD patients. Significant values ($p < 0.05$) are highlighted in bold.

		AUDIT				OCDS				ADS			
Haplotype		Hap-Freq[a]	Hap-Score[b]	Crude P[c]	Sim. P[d]	Hap-Freq[a]	Hap-Score[b]	Crude P[c]	Sim. P[d]	Hap-Freq[a]	Hap-Score[b]	Crude P[c]	Sim. P[d]
Block1													
rs702764	rs6473797												
A	G	0.6120	-2.6793	**0.0074**	**0.0071**	0.6120	-3.6887	**0.0002**	**0.000**	0.6120	-3.1020	**0.0020**	**0.0018**
G	G	0.0620	0.7688	0.4420	0.4318	0.0620	1.6865	0.917	0.0906	0.0320	0.8363	0.4030	0.4052
A	A	0.3209	2.7674	**0.0057**	**0.0081**	0.3209	2.9426	**0.0003**	**0.0027**	0.3209	2.9843	**0.0028**	**0.0033**
global-stat		11.1774				13.7041				10.8325			
df		3				3				3			
p		0.0108				0.0033				0.0127			
global simulation p		0.0087				0.0027				0.0121			

AUDIT, Alcohol Use Disorders Identification Test; OCDS, Obsessive compulsive drinking scale; ADS, Alcohol Dependence Scale; [a] Hap-Freq, estimated frequency of the haplotype in the pool of all subjects; [b] Hap-Score, score for the haplotype; [c] asymptotic chi-square p-value (haplotype p); [d] simulated p-value

Table 6. Associations of *OPRM1* SNPs and *OPRK1* SNPs with impulsive behavior tasks in AUD patients. Significant values ($p < 0.0125$) are highlighted in bold.

		SSRT					BART					DDT				
		Pvalue[a] cod	*Pvalue* dom	*Pvalue* rec	*Pvalue* ovd.	*Pvalue* add	*Pvalue* cod	*Pvalue* dom	*Pvalue* rec	*Pvalue* ovd.	*Pvalue* add	*Pvalue* cod	*Pvalue* dom	*Pvalue* rec	*Pvalue* ovd.	*Pvalue* add
SNP	Alleses															
rs1799971	A/G	0.06	0.03	0.93	0.03	0.14	0.14	0.60	0.10	0.10	0.65	0.97	0.98	0.82	0.85	0.92
rs609148	G/A	0.84	0.64	0.66	0.69	0.60	0.78	0.62	0.68	0.57	0.69	0.39	0.19	0.84	0.17	0.22
rs702764	A/G	0.97	0.90	0.85	0.85	0.94	0.82	0.62	0.61	0.71	0.57	0.40	0.17	0.72	0.19	0.17
rs6473797	A/G	0.02	0.15	0.06	**0.008**	0.27	0.39	0.17	0.72	0.27	0.24	0.92	0.72	0.77	0.88	0.69

SSRT, stop signal reaction time; BART, balloon analogue risk task; DDT, delay discounting task; cod, codominant; dom, dominant; rec, recessive; ovd, overdominant; add,log- additive

Table 7. Haplotype frequencies and association with impulsive behavior tasks in AUD patients. Significant values ($p < 0.05$) are highlighted in bold.

		SSRT				BART				DDT			
Haplotype		Hap-Freq[a]	Hap-Score[b]	Crude P[c]	Sim. P[d]	Hap-Freq[a]	Hap-Score[b]	Crude P[c]	Sim. P[d]	Hap-Freq[a]	Hap-Score[b]	Crude P[c]	Sim. P[d]
Block1													
rs702764	rs6473797												
A	G	0.3201	-0.1189	0.9053	0.9038	0.6226	-1.1712	0.2415	0.2485	0.3197	-1.0850	0.2779	0.2765
G	G	0.0584	0.1034	0.9177	0.9125	0.0574	-0.4803	0.6311	0.6338	0.6200	0.3021	0.7626	0.7643
A	A	0.6157	0.1431	0.8862	0.8873	0.3140	1.4612	0.1440	0.1479	0.0545	1.2234	0.2212	0.2211
global-stat		0.2068				2.3421				2.6664			
df		3				3				3			
p		0.9765				0.5045				0.4460			
global simulation p		0.9598				0.5067				0.4234			

SSRT, stop signal reaction time; BART, balloon analogue risk task; DDT, delay discounting task; [a] Hap-Freq, estimated frequency of the haplotype in the pool of all subjects; [b] Hap-Score, score for the haplotype; [c] asymptotic chi-square p-value (haplotype p); [d] simulated p-value

IV. DISCUSSION

This study examined the genetic association between the genes of opioid system (*OPRM1* and *OPRK1*) and clinical characteristics of AUD in a Korean population. In case control study, the two groups, AUD patients and controls, were not significantly different in their genotype or haplotype distribution.

The most investigated *OPRM1* polymorphism in association with AUD is rs 1799971. This genetic variant has been linked to alcohol and opioid addiction in individuals from EA descent. Our study did not reveal any statistically significant associations between AUD and the A118G (rs 1799971) polymorphism ($P > 0.05$). The finding is consistent with a number of previous studies.[15, 19, 21, 80-82] However, our results were not consistent with a previous study based on a Korean population by Kim et al.,[18] which suggested that the functional polymorphism (A118G) of the *OPRM1* may be an important genetic factor in the etiology of alcohol dependence and the frequency of alcohol consumption[18]. In our study, there was no evidence of the association between clinical characteristics, including impulsivity and polymorphisms of *OPRM1*. Additionally, previous studies have reported significant association[83, 84] between the *OPRM1* 118G-allele and alcohol dependence. In contrast, some studies have reported a significant association between the the *OPRM* 118A-allele and alcohol dependence.[17, 85-88] However, there was no significant difference in distribution frequency of either *OPRM1* 118G-allele or *OPRM* 118A-allele between AUD and healthy control in our study.

Interestingly, Chen et al. suggested that inconsistent results for the *OPRM1* SNP rs1799971 with substance addictions may be due to influence of ethnicity, since meta-analysis found association between the *OPRM1* SNP rs1799971 and alcoholism in Asian, but not in Caucasian populations, which did not accorded with our results in Korean population.

In addition, we found no evidence of association between AUD and another intronic SNP (rs 609148). A large case-control study showed that the rs609148 and was associated with a higher likelihood to develop alcohol addiction.[21] In that study of European-Americans, three intronic SNPs (rs 4954591, rs 609148, rs 648893) were associated with alcohol dependence and there was a positive association between rs 459491, rs 609148, rs 648893 of *OPRM1* and alcohol or drug dependence. But this report studied European Americans populations and was not replicated in a Taiwanese population.[15] Loh et al. reported no significant difference in either allele or genotype frequency of *OPRM1* in a Taiwanese population.[15] Association was also found between two *OPRM1* haplotype blocks including rs 1799971, rs609148 and 648893 and alcohol dependence in Caucasians.[21]

These contrasting results may be due to differences of ethnicity and the severity of alcohol problem. Our result about OPRM1 SNPs suggest the possibility of no association between *OPRM1* and development of AUD.

Another explanation of these contrasting results of the relationship of *OPRM1* with AUD is the possibility of interactions between *OPRM1* and other genotypes in AUD. A recent study on alcohol

consumption and subjective responses to alcohol in 127 young and healthy social drinkers demonstrated an epistatic interaction between *DAT1* (dopamine transporter gene) and *OPRM1* SNP rs 1799971.[89] The magnitude of subjective responses for *OPRM1* 118G-allele carriers was dependent on which *DAT1* VNTR (variable number tandem repeats) was also present.[89] There is possibility of effects of *OPRM1* polymorphism on alcohol consumption is not independent and associated with other polymorphisms.

Additionally, we discovered that *OPRM1* polymorphisms were not associated with clinical features including impulsivity in AUD patients. Our results were not accord with above mentioned Pfeifer et al.'s study, which reported higher drinking frequency among the *OPRM* 118G-allele carriers was linked with higher urgency and perseveration subscores of impulsivity.[63] The discrepancy between Pfeifer et al.'s results and the findings of our study may be dependent on the difference of sample papulations. The participants in the Pfeifer et al. study were non-treatment drinkers recruited from local community. In contrast, our study examined abstinent hospitalized patients with AUD.

We also found no association between *OPRK1* SNPs and AUD in the case control study, although in the limited study of AUD subjects, rs6473797 was found to be related significantly to the severity of alcohol dependence, as measured with AUDIT ($p = 0.0041$), OCDS ($p = 0.0002$), and ADS ($p = 0.0013$). In the haplotype-based association tests, the haplotype block 1 containing the two SNPs (rs 702764 and rs6473797) was also found to be related to the scores on these scales in AUD patients. The A-G haplotype and the A-A haplotype was associated with the AUD severity in AUD patients. These results suggest that *OPRK1* is not related to the development of AUD, but may be related to the severity of alcohol problem in AUD patients. In particular, with respect to *OPRK1* haplotype, haplotype including A allele of rs 702764 may be related to the severity of alcohol problem in AUD patients.

Although the role of MOR has been characterized, the contribution of KOR is less clear. KOR, like MOR, has influence on formation of addictive behavior through modulation of dopaminergic tone.[90] Our negative results for the *OPRK1* SNP rs6473797 are in accordance with the negative results of a study that reported no association with alcoholism in a Taiwanese sample.[15] By contrast, a large family-based study that included 219 multiplex alcohol dependent families of EA origin reported an association between 2 *OPRK1* SNPs in intron 2 (including rs6473797 investigated also in our study) and alcoholism.[25] Another study in EA population found an association between three other *OPRK1* SNPs (outside of intron 2) and alcohol or cocaine dependence.[31] So, it is possible that we were unable to detect an association of selected *OPRK1* SNPs with alcoholism due to the ethnic differences and/or modest sample size.

Additionally, studies of animals implicates the KOR in alcoholism.[24, 91] KORs are positioned to modulate multiple neurotransmitter systems within motivational and emotional circuitry that have been implicated in the etiology of numerous neuropsychiatric disorders.[92, 93] Therefore, stimulation of the KOR, which occurs with alcohol intake, could produce unpleasant and adverse effects. This receptor is hypothesized to play a role in alcohol dependence by promoting negative reinforcement processes and rewarding effect. To put it briefly, during development of alcohol dependence, the KOR system becomes

overstimulated, producing negative motional/affective states which then may drive organisms to excessively seek and use alcohol to alleviate those symptoms.[94-96] This theory may be in line with our result which show that *OPRK1* is associated with severe drinking pattern in alcohol dependent patients.

Interestingly, with respect to impulsivity, *OPRK1* rs 6473797 SNP was significantly associated with SSRT among computerized impulsive tasks, while other *OPRM1* and *OPRK1* SNPs were not associated every impulsive tasks. In a recent study, KOR expressions were found to be dysregulated within prefrontal brain circuitry associated with decision-making and impulse control in alcohol dependent humans and rodents, and have been shown to modify multiple neurotransmitter systems associated with impulse-control disorders.[97] In that study, the results demonstrated a dissociable effect of KOR agonist on impulsive phenotypes related to intolerance to delay or response inhibition, with selective effects in the SSRT. Furthermore, the pro-impulsive effects of KOR activation were rescued by pretreatment with the KOR antagonist nor-binaltorphimine.[97] Another recent study reports that KOR activation can regulate impulsive phenotypes, an effect that was shown to be specific to response inhibition and that supports contemporary assertions that the SSRT paradigm has predictive validity for an alcohol-dependent state.[98] Taken together with our study, which shows that *OPRK1* may be related with behavioral disinhibition (impulsive action), *OPRK1* is hypothesized to play a role in disinhibitory drinking behavior in AUD patients, at least in part.

In comparison to the positive association results of *OPRM1* and *OPRK1* from earlier studies, the present study does not provide new evidence of the association of these two receptor genes with AUD. Moreover, although there are some positive findings on the association between *OPRK1* and clinical symptoms in an AUD subject, we could not explore how *OPRK1* polymorphisms, alcohol consumption, or impulsivity interact in AUD patients. So the association between *OPRK1*, and drinking status and impulsive subtype needs to be evaluated in future studies.

Our study has several limitations. First, it is a cross-sectional examination of alcohol-dependent patients. Thus, further research is needed to examine long term change in clinical feature in these participants. A second limitation is the fact that the drinking history of the subjects was investigated on the basis of the charts or the patient's memory. Finally, many of our participants were taking various psychiatric medications including benzodiazepines when they were tested, which may have had confounding effects on our results

V. CONCLUSION

Our results suggest the possibility *OPRK1* reflects the severity and/or some aspect of impulsivity in AUD. Consequently further assessing the association of *OPRK1* on the clinical characteristics of AUD may be useful for improving the treatment of alcohol use disorder.

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ABSTRACT(IN KOREAN)

알코올 의존증에서 음주심각도 및 충동성과 아편계 수용체 유전자의 관련성

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황 성 식

최근의 다양한 연구들은 알코올 의존증의 병리에 있어 내인성 아편계가 연관되어있음을 보여주고 있다. 또한 아편계 수용체 유전자 변이와 알코올 의존증사이에 유의미한 관련이 있다는 다수의 연구들도 보고되고 있다.

본 연구에서는 한국인을 대상으로 하여 알코올 의존증의, 발병과 임상적 특징에 관여하는 아편계 수용체의 영향을 알아보고자 하였다.

본 연구에서는 μ 아편계 수용체 유전자 (*OPRM1*, μ -opioid receptor gene)의 3개의 단일염기다형성 (SNP, single nucleotide polymorphism)들 (rs1799971, rs609148, rs648893)과 κ 아편계 수용체 유전자 (*OPRK1*, κ-opioid receptor gene)의 2개의 단일염기다형성들 (rs702764, rs6473797)을 SNaPshot assay를 이용하여 유전자 분석을 하였다. 입원상태의 남성 알코올 환자 320명과 연령대를 맞춘 정상대조군 329명 남성을 대상으로 하였고 혈액에서 유전자를 추출하여 유전자형 분포와 일배체형 빈도를 파악하였다.

알코올 의존증 환자군에서는 임상적 특징을 알기위해 유전자형에 따른 alcohol use disorders identification test (AUDIT), obsessive-compulsive drinking scale (OCDS), alcohol dependence scale (ADS), 점수를 분석하였다. 또한 환자군에서 객관적이고 다양한 충동요인을 파악하기 위해 멈춤신호과제 (Stop-Signal Task, SST), 지연할인과제 (Delayed Discounting Task, DDT) 그리고 풍선유사위험감수과제 (Balloon Analogue Risk Tasking task, BART)를 시행하여 다양한 충동양상을 알아보고자 하였다.

본 연구에서 알코올 의존증 환자군과 정상대조군 사이에서 유전자형에 따른 의미있는 상관관계를 발견할 수 없었다. 환자군만을 대상으로 하였을 때 *OPRK1* SNP

rs6473797의 AA와 GG 동형접합체 유전자형이 GA 이형접합체 유전자형과 비교해서 SST의 정지신호반응시간 (SSRT, stop signal reaction time)의 유의미한 지연이 있었으며, rs6473797 단일염기다형성과 rs6473797을 포함하는 일배체형 모두에서 AUDIT, OCDS 그리고 ADS로 측정한 알코올 의존의 심각도와 상관관계가 있었다.

이러한 결과는 아편계 수용체의 유전적 변이가 알코올 의존증의 증상 심각도 및 충동성에 관여할 가능성을 제시하는 것이다.

핵심되는 말 : 알코올 의존증, 아편계 수용체 유전자, 유전자 다형성, 음주심각도, 충동성