Further Analyses of Genetic Association Between *GRM8* and Alcohol Dependence Symptoms Among Young Adults

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ABSTRACT. Objective: The gene *GRM8,* a metabotropic glutamate receptor, has emerged as a gene of interest for its possible role in the development of alcohol dependence, with evidence of association with an electrophysiological endophenotype and level of response to alcohol as well as suggestive evidence of association with alcohol dependence. **Method:** The present study further investigated the association between *GRM8* and alcohol dependence symptom counts among young adults using a new sample of individuals collected as part of the prospective sample (ages 18–26 years; $N = 842$) from the Collaborative Study on the Genetics of Alcoholism (COGA). **Results:** Two single-nucleotide polymorphisms were significantly associated with alcohol dependence in European Americans using the Nyholt corrected p value of .007: rs886003 ($\beta = -.212$, $p = .0002$) and rs17862325 ($\beta = -.234$, $p < .0001$), but not in African Americans, likely because of the lower power to detect association in this group. **Conclusions:** These results further implicate the role of glutamate receptor genes such as *GRM8* in the development of alcohol dependence. (*J. Stud. Alcohol Drugs, 76,* 414–418, 2015)

ALCOHOL DEPENDENCE IS a common disorder, with a lifetime prevalence of 12.5% (Hasin et al., 2007). It is a leading cause of morbidity and premature death (Caces et al., 1995; Campbell et al., 1995). Research using both twin studies and adoption studies has shown that the heritability of alcohol dependence is on the order of 50%–60% (Kendler et al., 1994; Reich et al., 1998).

However, alcohol dependence is a complex disease with both genetic and environmental contributions. As such, it does not follow a simple pattern of inheritance, making difficult the identification of genes that affect risk (Edenberg et al., 2010). Accordingly, a combination of strategies has been used, including candidate gene and genome-wide approaches as well as the use of endophenotypes, to attempt to identify the specific genes involved (Agrawal et al., 2012).

GRM8 has emerged as a gene of interest with respect to a possible role in the development of alcohol dependence (Chen et al., 2009; Edenberg et al., 2010; Joslyn et al., 2010). *GRM8* is a metabotropic glutamate receptor that maps to chromosome 7q31.3-q32, spanning over 800 kb, and is composed of 11 exons. It has been proposed that glutamate transmission and the neuroadaptive changes involved with it are key molecular determinants of craving and relapse (Cornish & Kalivas, 2000). It is suggested that alcohol relapse behavior is mediated by a hyperglutamergic state (Tsai & Coyle, 1998). Thus, genes involved in regulating glutamate transmission are plausible biological candidates for involvement in the development of alcohol dependence.

The potential role of *GRM8* in alcohol dependence was first suggested by Chen and colleagues (2009), who demonstrated that *GRM8* was associated with the endophenotype of theta oscillations. Theta oscillations are reduced in alcohol-dependent individuals (Jones et al., 2006) and in the adolescent offspring of alcoholics (Rangaswamy et al., 2007), suggesting that they may be a biological marker of the predisposition to alcohol dependence.

Using a sample of 1,049 individuals from 209 families recruited as part of the Collaborative Study on the Genetics of Alcoholism (COGA), Chen and colleagues (2009) found three single-nucleotide polymorphisms (SNPs) in intron 6 of *GRM8* (rs1361995, rs10487457, rs10487459) that were significantly associated with theta oscillations and also with

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alcohol dependence. The theta power peak is located in the fronto-central brain region (Karakas et al., 2000), and theta rhythms have been shown to be involved in attention, recognition memory, and episodic retrieval (Klimesch et al., 2001). The glutamatergic system is thought to be involved in theta oscillations (Frodl-Bauch et al., 1999). These results suggest that *GRM8* may play a role in the modulation of event-related theta oscillations during information processing, which in turn may be associated with an increased vulnerability for the development of alcohol dependence (Chen et al., 2009).

More recently, Joslyn and colleagues (2010) found that variation in alcohol response is influenced by variation in glutamate signaling genes, including *GRM8.* Alcohol response is defined as the subjective experience of feeling high and physiological and behavioral responses associated with blood alcohol concentrations. Individual variation in level of response to alcohol, particularly early in a drinking career, has been shown to be a risk factor for the development of alcohol dependence and is also associated with alcohol dependence in families (Schuckit & Smith, 2000; Schuckit et al., 2007).

In a recent investigation, 173 functionally related sets of genes (i.e., genes that share a common biological function) were identified and analyzed. Many of these genes were found to contribute to the variation in the level of response to alcohol. The genes that were associated with variations in the level of response to alcohol showed enrichment for genes that were also involved in glutamate signaling, suggesting that this type of signaling may modulate the effects of alcohol (Joslyn et al., 2010).

Finally, Edenberg and colleagues (2010), in a genome-wide association study, found that 32 SNPs in *GRM8* were nominally associated with early onset of alcohol dependence. The aim of the present study is to further investigate the association between *GRM8* and alcohol dependence symptoms using a new sample of individuals collected from COGA.

Method

Sample

COGA is a multisite project with participating centers around the United States, whose primary goal is to identify genes and phenotypes that contribute to alcohol dependence. Probands were recruited from inpatient or outpatient alcohol treatment programs at six centers across the United States. Requirements for the probands' participation in the study included having a sibship greater than three, having parents available, and having two or more of these family members within the COGA catchment area. Comparison families not selected for alcohol dependence were used as controls. Approval was obtained from all institutional boards, and written consent was obtained from all participants. For additional details about COGA, see Reich et al., 1998.

Table 1. Demographics of study participants

Variable	European Americans (n = 602)	African Americans (n = 240)
Age at survey, *M* (*SD*)	19.79 (1.46)	19.83 (1.49)
Sex, *n*		
Male	271	118
Female	331	122
No. of alcohol dependence symptoms		
0	315	164
1	132	39
2	82	24
3	33	5
4	13	3
5	14	2
6	7	2
7	4	0
Comorbid disorders, *n*		
Conduct disorder	16	3
Antisocial personality disorder	25	7

Note: No. = number.

Data for the present analysis were acquired through the Prospective Study of the COGA sample. All participants had at least one parent who was interviewed in a previous phase of COGA and came from either control families or families with a history of alcohol dependence. There was no overlap between our participants and the COGA participants used in the Chen et al. (2009) study. Recruitment of these participants began in December 2004 and is ongoing. Follow-up assessments are administered every 2 years. The present analyses use the data from the initial (baseline) assessments.

To test for association with adult alcohol dependence symptoms, we limited our sample to individuals who were age 18 and older (ages 18–26; N = 842). No other exclusionary criteria were applied. The mean age at interview for European Americans (n = 602) was 19.79 years (*SD* = 1.46). For African Americans (n = 240), the mean age was 19.83 years (*SD* = 1.49). Additional demographic information, including sex, number of alcohol dependence symptoms, and number of comorbid disorders, is presented in Table 1.

Psychiatric interview

All participants were administered the adult version of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994; Hesselbrock et al., 1999). The SSAGA is a semi-structured interview that assesses, among other psychiatric disorders, symptoms of alcohol dependence as outlined in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV; American Psychiatric Association, 1994).

Table 2. Associations with DSM-IV alcohol dependence symptom counts by population

SNP	MAF	β	*p*
European Americans (*n* = 602)			
rs2299456	0.15	-.11	.35
rs2299459	0.21	-.17	.10
rs1361995	0.36	-.12	.07
rs4731334	0.11	-.15	.14
rs886003	0.23	-.31	<.001*
rs17862325	0.29	-.34	<.001*
rs2237807	0.33	.03	.65
rs17866960	0.03	-.06	.71
African Americans (*n* = 240)			
rs2299456	0.22	.14	.23
rs2299459	0.34	.08	.33
rs1361995	0.35	-.07	.34
rs4731334	0.09	-.05	.72
rs886003	0.03	-.23	.27
rs17862325	0.14	-.08	.55
rs2237807	0.37	-.11	.15
rs17866960	0.11	.21	.20

Notes: DSM-IV = *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition;* SNP = single-nucleotide polymorphism; MAF: minor allele frequencies. Significant *p* values are indicated with an asterisk (*).

Genotyping

Genotyping was performed using Sequenom MassARRAY (www.sequenom.com) or OpenArray Technologies (www.lifetechnologies.com). For Sequenom genotyping, polymerase chain reaction primers were designed with Sequenom MassARRAY Assay Designer software. Genotype spectra were analyzed with the Sequenom SpectroTYPER software Version 3.4. OpenArray genotyping is a multiplex TaqMan assay platform. We used OpenArray Genotyping Plate Configurator to design assays. Arrays were scanned on the OpenArray NT imager, and genotypes were called using the OpenArray SNP Genotyping analysis software.

Eight SNPs were genotyped across *GRM8:* rs2299456, rs2299459, rs1361995, rs4731334, rs886003, rs17862325, rs2237807, and rs17866960. These SNPs were selected as tag SNPs for *GRM8* based on previous evidence of association between the gene and event-related oscillations (Chen et al., 2009). All of these SNPs except rs17862325 were successfully genotyped with the Sequenom platform. The SNP rs17862325 was genotyped using the OpenArray platform. All SNPs were in Hardy–Weinberg equilibrium. Table 2 shows the minor allele frequencies (MAFs) in European Americans and African Americans.

Analyses

Analyses were performed separately for European Americans and African Americans because of their different MAFs and linkage disequilibrium (LD) patterns (see Figures 1 and 2 for the LD patterns). The Statistical Analysis System (SAS) was used to run linear regression analyses using alcohol dependence symptom counts as the dependent variable. An additive genetic model was used with the survey option, which can be used for complex survey sample designs and to control for relatedness. Covariates included age and sex.

Results

Table 2 presents the beta values and *p* values from the association tests with the eight SNPs from the *GRM8* gene and DSM-IV alcohol dependence symptom counts. Using the web-based software SNPSpD (Nyholt, 2004), the Nyholt correction was performed, which is a multiple testing correction across the SNPs. This correction takes into account the number of SNPs genotyped and the LD structure between them. Based on this test, the significance of the results was evaluated based on an adjusted significance value of $p = .007$ for both European Americans and African Americans. After we corrected for multiple testing, two SNPs were significantly associated with DSM-IV alcohol dependence for European Americans: rs886003 ($\beta = -.212$, $p = .0002$) and rs17862325 ($\beta = -.234$, $p < .0001$). The mean number of alcohol dependence symptoms is 2.48 and 2.55 for rs886003 and rs17862325, respectively. None of the SNPs were significant for African Americans.

Discussion

In this study, we investigated the association of tag SNPs across the *GRM8* gene with a DSM-IV alcohol dependence symptom count. Adding to previous research showing associations between *GRM8* and alcohol dependence diagnosis (Chen et al., 2009), response to alcohol (Joslyn et al., 2010), and an early onset of alcohol dependence (Edenberg et al., 2010), it was found that two SNPs, rs886003 and rs17862325, were significantly associated with alcohol dependence symptom counts in a young adult sample of European Americans. None of the SNPs were found to be significantly associated with alcohol dependence symptoms in the African American sample. However, this may be attributable to the much smaller sample size ($n = 240$) compared with the European American sample size ($n = 602$).

We ran power analyses in the African American sample using the program Quanto (Gauderman, 2002) and assumed an additive genetic model, a *p* value of .007, and an allele frequency of .23 and .29, respectively, reflecting the allele frequencies of the SNPs that were significant in the European American sample. The power to detect a genetic variant accounting for ~2% of the variance, which is the effect size for these alleles estimated in the European American sample, was .030 and .041 for rs886003 and rs17862325, respectively.

Accordingly, power was very low in the African American sample. The corresponding power figures in the European American sample were .65 for rs886003 and .87 for

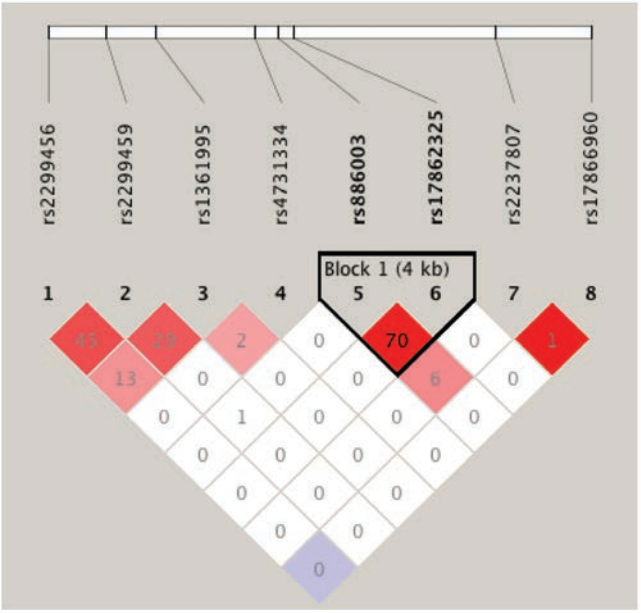

FIGURE 1. Linkage disequilibrium (r^2) among single-nucleotide polymorphisms in European Americans

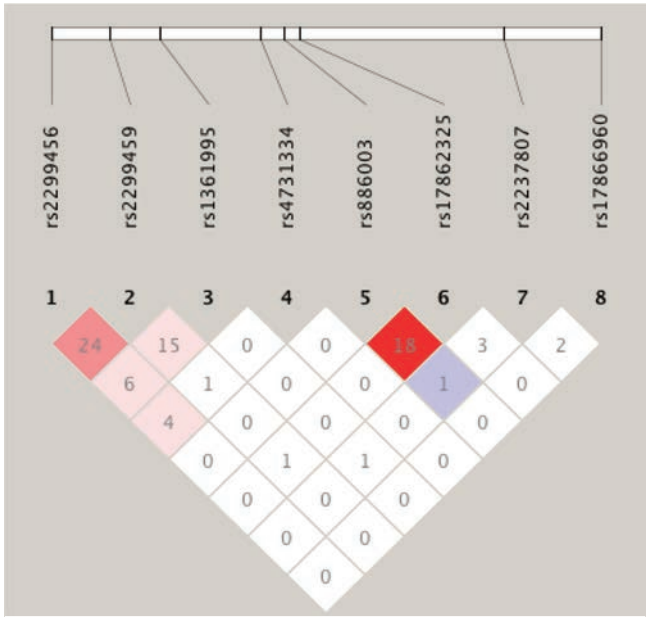

FIGURE 2. Linkage disequilibrium (r^2) among single-nucleotide polymorphisms in African Americans

rs17862325. The beta values were in the same direction in the African American sample as in the European American sample, indicating that the effect was in the same direction for both groups. Therefore, the lack of significant findings in the African American sample may be attributable to very low power. We also conducted a post hoc analysis to test for gender differences and found no significant differences (results available on request).

The two SNPs with significant associations in the present study were correlated (r^2 = .70 and D' = .97), indicating that the signals provided by each SNP were correlated but not entirely redundant (as determined by HapMap genome browser release #28, genome build 36). These SNPs were in low LD with one of the SNPs (rs886004) from the study conducted by Joslyn and colleagues (2010; rs886003: r^2 = .15 and D' = 1; rs17862325: r^2 = .18 and D' = 1). All three of these SNPs (the two associated in our sample and the correlated SNP from the Joslyn et al. study) were located in intron 1 of *GRM8*.

Although Joslyn and colleagues (2010) found 12 SNPs from *GRM8* to be associated with subjective responses to alcohol, the other 11 SNPs were not in LD with ours. Previous reports (Chen et al., 2009; Edenberg et al., 2010; Joslyn et al., 2010) found significant associations that spanned the length of the gene across 7 introns. Only one of the SNPs genotyped in our sample directly overlapped with the SNPs previously genotyped in those studies: rs4731334 was significantly associated with early-onset alcohol dependence in the Edenberg et al. (2010) study and showed a parallel (although nonsignificant) trend toward association with alcohol dependence symptom counts in our sample of adult European Americans.

To conclude, the present study found two SNPS of the *GRM8* gene to be significantly associated with alcohol dependence symptoms in an adult sample of European Americans from the COGA prospective study. Identification of genes related to the development of alcohol dependence will be important to understand the basic etiology of alcohol use disorders, which could have important implications for prevention, intervention, and medication development.

These results add to the literature suggesting that genes involved in the modulation of glutamate, such as *GRM8,* may also be involved in the development of alcohol dependence in addition to playing a role in alcohol-related endophenotypes (e.g., theta oscillations, alcohol response). Future studies will be aimed at investigating other genes involved in glutamate transmission.

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