Published in final edited form as:

Alcohol. 2016 September ; 55: 43–50. doi:10.1016/j.alcohol.2015.10.005.

Test for association of common variants in *GRM7* with alcohol consumption

Whitney E. Melroy-Greif^{a,b,1}, Csaba Vadasz^c, Helen M. Kamens^d, Matthew B. McQueen^a, Robin P. Corley^b, Michael C. Stallings^b, Christian J. Hopfer^b, Kenneth S. Krauter^e, Sandra A. Brown^f, John K. Hewitt^b, and Marissa A. Ehringer^{a,b}

^aDepartment of Integrative Physiology, University of Colorado, Boulder, CO 80309 USA

^bInstitute for Behavioral Genetics, University of Colorado, Boulder, CO 80309 USA

^cNathan Kline Institute for Psychiatric Research, New York University School of Medicine, Orangeburg, NY 10962 USA

^dDepartment of Biobehavioral Health, The Pennsylvania State University, University Park, PA 16802 USA

^eDepartment of Molecular and Cellular Biology, University of Colorado, Boulder, CO 80309 USA

^fDepartment of Psychology and Psychiatry, University of California, San Diego, CA 92093 USA

Abstract

Recent work using a mouse model has identified the glutamate metabotropic receptor 7 (*Grm7*) gene as a strong candidate gene for alcohol consumption. Although there has been some work examining the effect of human glutamate metabotropic receptor 7 (*GRM7*) polymorphisms on human substance use disorders, the majority of the work has focused on other psychiatric disorders such as ADHD, major depressive disorder, schizophrenia, bipolar disorder, panic disorder, and autism spectrum disorders. The current study aimed to evaluate evidence for association between *GRM7* and alcohol behaviors in humans using a single nucleotide polymorphism (SNP) approach, as well as a gene-based approach. Using 1,803 non-Hispanic European Americans (EAs) (source: the Colorado Center on Antisocial Drug Dependence [CADD]) and 1,049 EA subjects from an independent replication sample (source: the Genetics of Antisocial Drug Dependence [GADD]), two SNPs in *GRM7* were examined for possible association with alcohol consumption using two family-based association tests implemented in FBAT and QTDT. Rs3749380 was suggestively associated with alcohol consumption in the CADD sample ($p = 0.010$) with the minor T allele conferring risk. There was no evidence for association in the GADD sample. A gene-based test using four Genome-Wide Association Studies (GWAS) revealed no association between variation in *GRM7* and alcohol consumption. This study had

Corresponding Author: Marissa Ehringer, Ph.D., Department of Integrative Physiology, Institute for Behavioral Genetics, University of Colorado Boulder, 447 UCB, Boulder, CO 80309-0447, **Telephone:** +1 303 492 1463, **Fax:** +1 303 492 8063, Marissa.Ehringer@colorado.edu.

¹Present address: Department of Molecular and Cellular Neuroscience, The Scripps Research Institute, La Jolla, CA 92037 USA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

several limitations: the SNPs chosen likely do not tag expression quantitative trait loci; a human alcohol consumption phenotype was used, complicating the interpretation with respect to rodent studies that found evidence for a *cis*-regulatory link between alcohol preference and *Grm7*; and only common SNPs imputed in all four datasets were included in the gene-based test. These limitations highlight the fact that rare variants, some potentially important common signals in the gene, and regions farther upstream were not examined.

Keywords

alcohol consumption; family-based test; gene test; glutamate metabotropic receptor 7; human genetics

Introduction

There is strong evidence that alcohol consumption (AC) phenotypes are more heritable in adults than in adolescents, with estimates of heritability ranging from 31–51% (Hansell et al., 2008). Furthermore, studies have indicated that the same genetic influences are acting on both alcohol dependence (AD) and AC (Kendler, Myers, Dick, & Prescott, 2010), suggesting that heavy AC in adolescence can lend itself to the development of alcohol abuse and potentially AD in adulthood. While Genome-Wide Association Studies (GWAS) have identified several genes associated with AC (Baik, Cho, Kim, Han, & Shin, 2011; Chen et al., 2012; Heath et al., 2011; Kapoor et al., 2013; Pan et al., 2013; Pei et al., 2012; Quillen et al., 2014; Schumann et al., 2011), these associations often have small effect, indicating that AC is a complex problem influenced by numerous genes, many of which are likely still unidentified.

Recent work has identified the mouse glutamate metabotropic receptor 7 (*Grm7*) as a candidate gene for AC (Vadasz et al., 2007). This receptor affects alcohol drinking patterns and neurological processes. *Grm7* knockout mice show increased AC, while mice overexpressing this gene drink less alcohol. Furthermore, there are significant differences in *Grm7* mRNA abundance between congenic and background strains in addiction-related brain regions. In this experiment, the congenic strain contained a small segment of the inbred BALB/cJ mouse strain chromosome 6 (on which *Grm7* resides) on a C57BL/6By background strain. Comparison between the congenic and background strains revealed that congenic strains consumed less alcohol than the background strains, providing further evidence that *Grm7* affects alcohol consumption (Gyetvai et al., 2011). Moreover, viral-mediated knockdown of *Grm7* in the nucleus accumbens (NAc), a region strongly implicated in drug reward, was also shown to increase ethanol consumption and preference in rats (Bahi, 2013).

Pharmacological interventions in rodent models have also supported a role for *Grm7* in the rewarding effects of drugs. Administration of the *Grm7* agonist AMN082 (Mitsukawa et al., 2005) was shown to decrease gamma aminobutyric acid (GABA) and increase glutamate levels in the NAc (Li, Gardner, & Xi, 2008). The same agonist was later shown to decrease cocaine self-administration and cocaine-induced enrichment of electrical brain-stimulation reward in rats, suggesting that *Grm7* is involved in cocaine reinforcement (Li et al., 2009). In

addition, AMN082 was shown to decrease ethanol consumption and preference in rats (Bahi, Fizia, Dietz, Gasparini, & Flor, 2012), and subsequent work has shown that although AMN082 had no effect on extinction of ethanol-induced conditioned place preference, it reduced ethanol-induced conditioned place preference reinstatement in mice (Bahi, 2012). This suggests that *Gm7* activation can lessen the reinstatement of the rewarding effects of alcohol (Bahi, 2012). These studies provide strong evidence for *Gm7* as a potential target for alcohol cessation therapies.

Although there has been work examining the effect of human glutamate metabotropic receptor 7 (*GRM7*) polymorphisms on human substance use disorders, the majority of the work has focused on psychiatric disorders (Saus et al., 2010), including bipolar disorder (Alliey-Rodriguez et al., 2011), schizophrenia (Ganda et al., 2009; Ohtsuki et al., 2008; Shibata et al., 2009), major depressive disorder (Breen et al., 2011; Hamilton, 2011; Muglia et al., 2010; Pergadia et al., 2011; Shyn et al., 2011), panic disorder (Otowa et al., 2009), autism spectrum disorders (Yang & Pan, 2013), and ADHD (Elia et al., 2011; Park et al., 2013). Although not genome-wide significant, studies have found evidence for an association between *GRM7* and nicotine dependence and cessation success (Uhl et al., 2010), and alcoholism (Li et al., 2011). Furthermore, *GRM7* was identified as a leading edge gene in a Gene Set Enrichment analysis analyzing subjects' level of response to alcohol (Joslyn, Ravindranathan, Brush, Schuckit, & White, 2010). Two single nucleotide polymorphisms (SNPs) in *GRM7* were chosen in the present study to test for association with AC: rs3749380, based on previous associations with schizophrenia (Ohtsuki et al., 2008), and rs1485175 as a negative control.

The goal of the current study was two-fold: first, to follow up on previous studies of *GRM7* by examining two common SNPs in *GRM7* for association with AC in two independent samples; and second, to use a gene-based approach to test all available common SNPs in *GRM7* for association with AC. *GRM7* is roughly 833 kb in length (from the UCSC genome browser, <http://genome.ucsc.edu/>) (Kent et al., 2002). The expanse of the gene makes a comprehensive examination of which genetic signals may be associated with alcohol behaviors difficult. For that reason, the initial SNP analysis was followed up using a more extensive approach by investigating all common variants available in existing dbGAP datasets.

Materials and methods

Samples

For the single SNP analyses, two independent samples were used. The discovery sample consisted of 1,803 non-Hispanic European American (EA) subjects drawn from the Colorado Center on Antisocial Drug Dependence (CADD). Currently in its third wave of data collection, the CADD is a longitudinal study. It consists of four separate samples. The Family Study (FS) is a sample of clinical adolescent probands, ascertained while in treatment for antisocial drug dependence, their family members, and a matched set of control families (Stallings et al., 2003, 2005). The Colorado Adoption Project (CAP) (Petrill, Plomin, DeFries, & Hewitt, 2003), the Colorado Longitudinal Twin Study (CLTS) (Rhea, Gross, Haberstick, & Corley, 2006), and the Colorado Community Twin Study (CCTS)

(Rhea et al., 2006) are community unselected samples that were used in this study for phenotypic standardization (see below). Only subjects from the FS were included in this molecular genetic study. Ascertainment of this sample is described as follows. Clinical probands were recruited and selected from individuals who had consecutive admissions to treatment facilities in the Denver area between February 1993 and June 2001. Control subjects were recruited from the community and matched to the clinical probands based on age, gender, ethnicity, and zip code. All family members living in the same household as the proband were asked to participate in the study, which created family-based data. To increase similarity between this study and findings by Vadasz et al. (Vadasz et al., 2007), the most recent wave of data collection, representing probands past the age of initiation, was used. If the subject had not been assessed at wave 3, data from wave 2 were used, and if there were no data from wave 2, wave 1 data were used. Specifically, the work by Vadasz and colleagues identifying *Grm7* as a candidate gene for AC utilized adult mice (Vadasz et al., 2007). The first wave of data collection from the CADD recruited adolescents aged 13–19; each subsequent wave of data collection began roughly 5–7 years after the previous one. Thus, using the most recent wave of data collection ensured that most subjects included in this analysis were adults. When assessed, buccal cell DNA was collected from subjects who provided informed voluntary consent. All recruitment, assessment, and DNA collection procedures were approved by the University of Colorado's IRB and are in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Families of EA subjects from the Genetics of Antisocial Drug Dependence (GADD) were assessed as previously described (Kamens et al., 2013), to provide an independent replication sample. One thousand forty-nine subjects were used in the analysis. The GADD sample is a high-risk sample; probands aged 14–19 years old, from Denver, CO, and San Diego, CA, were identified from treatment programs, involvement with the criminal justice system, or special schools. Probands were required to meet at least one of the criteria for both a substance use disorder (other than nicotine dependence) and conduct disorder. Siblings of the proband, as well as one or both biological parents, were recruited. The GADD is a longitudinal study in its second wave of data collection; as in the CADD, data from wave 2 were preferentially used, and data from wave 1 were included if the subject did not have wave 2 data. DNA was acquired with consent, using either buccal cells or blood. All procedures are in accordance with the Helsinki Declaration of 1975, as revised in 1983. The University of California and the University of Colorado IRBs approved all subject recruitment, assessment, and DNA collection procedures.

For the gene-based test, data for EAs from four GWAS were obtained: The Study of Addiction: Genetics and Environment (SAGE) (Bierut et al., 2010); genome-wide association study of alcohol use and alcohol use disorder in Australian twin families (OZ-ALC) (Grant et al., 2007; Knopik et al., 2004; Saccone et al., 2007); the Multi-Ethnic Study of Atherosclerosis (MESA) (Bild et al., 2002); and a GWAS comprised of unrelated EA subjects from the CADD and GADD samples (CADD-GADD) (Derringer et al., 2015). As mentioned above, the CADD and GADD samples include probands targeted for drug use, their families, and matched-control families, as well as community twin samples. MESA is a community-based sample, whereas SAGE and OZ-ALC were ascertained based on substance dependence. All GWAS datasets were imputed to Phase 1 of the 1,000 Genomes

data and were cleaned using standard quality-control procedures. These included removing variants with low imputation accuracy (<0.9), low minor allele frequency ($MAF < 0.01$), and variants out of Hardy-Weinberg equilibrium (HWE, $p < 0.001$).

SNP selection

For the single SNP analysis, two SNPs were selected based on MAF and location; rs3749380 and rs1485175 are common SNPs ($MAF > 5\%$), have been previously examined in GWAS, and code for synonymous changes in exons 1 and 8, respectively. Additional SNPs could not be examined due to budgetary constraints. Preliminary examination of the haplotype structure of *GRM7* in Hapmap (<http://hapmap.ncbi.nlm.nih.gov/>) using Haploview (Barrett, Fry, Maller, & Daly, 2005) revealed that these two SNPs were not in linkage disequilibrium (LD) at $r^2 = 0.0$. Although rs3749380 did not tag any other SNPs in the region, rs1485175 was indicated to tag eight other SNPs in the gene.

For the gene-based test, SNPs were annotated to *GRM7* using the hg19 build. Variants 20 kb upstream and downstream of the gene were included, as previous evidence has suggested most genetic variation lies in this region (Veyrieras et al., 2008). Only variants present in all four imputed GWAS were included in the final analysis.

Genotyping

SNP genotyping for rs3749380 and rs1485175 was performed using the TaqMan® assays for allelic discrimination according to manufacturer's instructions (Applied Biosystems, Foster City, CA). Two thousand four hundred and ninety-six subjects from CADD and 3,072 subjects from GADD were genotyped. Polymerase Chain Reaction (PCR) reactions were performed with the Biomek® 3000 Laboratory Automation Workstation (Beckman Coulter, Inc., Brea, CA) and the Dual 384-Well GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA). A 7900 Real-Time PCR System (Applied Biosystems, Foster City, CA) was used to analyze the amplified plates. Taking into account all the SNPs that have been previously genotyped in the CADD (33) and GADD (12), SNP genotypes for subjects with overall call rates $<90\%$ were excluded. Three hundred and eighty-four samples were genotyped twice for each SNP to determine concordance between replicate reactions; the percent of discordant calls for SNPs in the CADD and GADD, respectively, were as follows: rs3749380 (0.78%, 0.00%), rs1485175 (1.04%, 0.52%). Genotype clusters from the amplified plates were auto-called by the Applied Biosystems TaqMan® Genotyper software (Applied Biosystems, Foster City, CA). Genotype calls were also visually examined by two independent lab personnel to confirm calls. Final calls were determined when both laboratory personnel agreed; genotypic data were excluded if they did not agree. Mendelian errors were identified using FBAT (Rabinowitz & Laird, 2000). If Mendelian errors were detected, the SNP genotype for that family was removed. Using previous SNPs genotyped in the CADD sample (in the $\alpha 4$ [$N = 10$] and $\beta 2$ [$N = 10$] subunits of the nicotinic acetylcholine receptor genes), three families in the CADD sample were identified as having Mendelian errors in three or more SNPs in both genes; the SNP genotypes for these families were also removed. Using Haploview (Barrett et al., 2005), pairwise linkage disequilibrium (LD) (r^2), MAF, and HWE were evaluated.

Statistical analysis

Phenotypic descriptive

For the single SNP analysis, the phenotype examined was AC, defined here as the maximum number of drinks consumed in 24 h during one's lifetime, as assessed with the Composite International Diagnostic Interview – Substance Abuse Module (CIDI-SAM) (Cottler & Keating, 1990). Only phenotypic data for subjects who had previously used alcohol were included; in other words, if a subject had never had a drink of alcohol they were excluded from the analysis. AC was standardized based on the distribution of AC in the CADD community sample (CAP, CLTS, and CCTS samples, described above). A linear regression was performed using Statistical Analysis System (SAS) 9.3 software (SAS Institute Inc., Cary, NC) to determine residuals from sex, age, and age-squared. The coefficients from the CADD community sample were applied to the CADD clinical sample (i.e., Z scores of clinical subjects were expressed as deviations from the means of the community samples), as well as the GADD sample.

Although AC was defined differently in each GWAS obtained from dbGAP, all attempts were made to harmonize the phenotype between samples. As the CADD-GADD GWAS is longitudinal, phenotypes were collected the same as for the SNP study; data were preferentially drawn from wave 3, from wave 2 if the subject was not assessed at wave 3, and from wave 1 if the subject did not have data at either later wave. In the CADD-GADD, as well as SAGE and OZ-ALC samples, AC was defined as the maximum number of drinks consumed in 24 h during one's lifetime and assessed with the CIDI-SAM (Cottler & Keating, 1990) and versions of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994), respectively. All subjects from these samples were included since information about whether each subject had tried alcohol was not always available. AC was defined in MESA as the largest number of drinks consumed in 24 h during the previous month in current drinkers (non-current drinkers were not asked the question). AC was log-transformed in order to normalize the phenotype. Age and sex, as well as the first five principal components to control for ancestry, were used as covariates in the analysis. As the SAGE sample is composed of three sub-studies with slightly different ascertainment, study was also included as a covariate.

Statistical genetics analysis

Rs3749380 and rs1485175 were tested for association using an additive genetic model in a family-based association test performed in FBAT (Rabinowitz & Laird, 2000). FBAT builds on the original Transmission Disequilibrium Test (TDT) (Spielman, McGinnis, & Ewens, 1993), where alleles transmitted to affected offspring are compared to the expected distribution of alleles among offspring under Mendel's law of segregation and conditioning. For results with $p < 0.05$, correction for multiple testing was implemented using the Min p test in FBAT, which accounts for correlation between SNPs. This test provides a p value for the most significant result based on the number of SNPs tested; if this p value is less than 0.05, the result is considered significant after correction for multiple SNP testing at $p < 0.05$. In order to contrast findings using a different family-based test, analyses were repeated using the within-family association and linkage test implemented in Quantitative Transmission

Disequilibrium Test (QTD) (Abecasis, Cardon, & Cookson, 2000). Correction for multiple testing was implemented in QTD, where the output shows the risk allele and Bonferroni significance level for the most significant association based on the number of SNPs tested. While both tests examine the transmission of alleles within a family, thus controlling for population stratification, QTD uses regression-based framework to test for association where the phenotype is the outcome. Conversely, FBAT uses the genotype as the outcome; using the additive model, the outcome is allele count for each offspring.

The gene-based test was carried out using two analytical approaches. Joint Association of Genetic Variants (JAG; available at <http://ctglab.nl/software/jag/>) (Lips, Kooyman, de Leeuw, & Posthuma, 2015) uses raw data and invokes PLINK (Purcell et al., 2007) to run a genome-wide association on each SNP in the gene. By summing the $-\log_{10}$ of each SNP p value, a multivariate test statistic is created. Permutations of the phenotype are run and summed as described above. The empirical p value for each gene (P_{emp}) is the number of times the sum of the $-\log_{10}$ of the permuted p value exceeds or equals the sum of the $-\log_{10}(p \text{ value})$ from the genome-wide association. The second analytical approach used to confirm findings from the JAG analyses was the set-test implemented in PLINK (Purcell et al., 2007). Briefly, for each gene, PLINK determines which SNPs are in LD above a certain threshold ($r^2 = 0.5$), performs a standard SNP regression, and selects up to N “independent” SNPs with $p < 0.05$. From this subset, the statistic for each gene is calculated as the mean of these SNP statistics. Keeping LD between SNPs constant, the phenotype is permuted. The empirical p value (P_{emp}) is the number of times the permuted gene statistic exceeds the original statistic for that gene. The P_{emp} for each sample from each JAG and PLINK analysis were combined using the weighted Z-score method (Whitlock, 2005).

Results

SNP analysis

The phenotypic characteristics between the CADD and GADD samples were comparable (Table 1). Although the GADD sample is somewhat younger than the CADD sample, AC means and distributions were comparable between the clinical samples.

The minor alleles and MAF for rs3749380 and rs1485175 were similar between each sample (Table 2). The discordant genotype rate was lower for both SNPs in the GADD than in the CADD sample; this may be because many of the DNA samples for the GADD were drawn from blood, as opposed to saliva as in the CADD. DNA samples from blood generally have higher genotyping success (Philibert, Zadorozhnyaya, Beach, & Brody, 2008), although it is not clear that this would necessarily result in a lower error rate. However, consistent with these differential error rates in the two samples, the genotype for rs3749380 was removed for 22 and 8 families in the CADD and GADD, respectively, and in 24 and 8 families for rs1485175 in the CADD and GADD, respectively, after Mendelian errors were detected in these families. There were no significant deviations from the HWE at $p < 0.05$. Furthermore, both SNPs represent independent signals in *GRM7* and were not correlated ($r^2 = 0$).

In the CADD sample, rs3749380 was significantly associated with AC at $p = 0.010$ and $p = 0.002$ using FBAT and QTD, respectively (Table 3). These associations survived correction

for multiple testing with the Min p test p value of 0.020 for FBAT, and Bonferroni significance level of 0.031 in QTDT. The T allele was the risk allele. However, neither rs3749380 nor rs1485175 were associated with AC in EAs from the GADD (Table 3). Results using QTDT confirmed the lack of findings with EAs in the GADD sample.

Gene-based test

Phenotypic characteristics for the samples utilized in the gene-based test are given in Table 4. For the analysis, a random subset of unrelated subjects was selected from the OZ-ALC study. The OZ-ALC and SAGE samples had expectedly high scores for AC since these samples were ascertained based on alcohol and other drug use. The community-based MESA sample had low AC reporting. Finally, the CADD-GADD GWAS sample showed slightly higher AC values than MESA but markedly lower values than those samples ascertained on alcohol and drug use only.

Nine hundred and two SNPs in *GRM7* were tested for association with AC using a gene-based test. Results indicated that variation in *GRM7* is not associated with AC with combined $p = 0.743$ using the JAG test (Table 5) and combined $p = 0.812$ using the PLINK test (Table 6). The correlation between the JAG and PLINK p values for each study was high ($r = 0.76$). Locus zoom plots (Pruim et al., 2010) showing the $-\log_{10}(p \text{ value})$ of each SNP tested in the region for each dataset are provided in Supplementary Figs. 1, 2, 3, and 4.

Discussion

The goal of this study was to evaluate the evidence for association of *GRM7* with AC, based on findings that *Grm7* is a candidate gene for AC in a mouse model (Vadasz et al., 2007). To our knowledge, this is one of the first studies to examine both individual polymorphisms in *GRM7* as well as to perform a gene-based test for association with AC. Although one prior study has identified a differentially inherited SNP module (a collaboration of SNPs contributing to the disease synergistically that is differentially inherited in affected and unaffected sibpairs) in *GRM7* in alcoholics, the two SNPs in the module were in introns and not in LD with the SNPs examined in the present study (Li et al., 2011). Our gene-based test is an important extension of this work; it represents an independent study because none of the four previously studied SNPs were included in our gene-based test as none were present in all four imputed GWAS utilized.

Consistent with our hypothesis based on these two SNPs selected from the schizophrenia study (Ohtsuki et al., 2008), the T allele of rs3749380 was associated with AC in the CADD sample using FBAT and QTDT, while the negative control SNP rs1485175 showed no evidence for association. Neither SNP was associated with the phenotype in the GADD sample. This discrepancy could be due to lack of power to detect true associations, or undetected differences between the CADD and GADD samples. The GADD sample did not include any control families, so this difference in study design may be reflected in differential transmission effects. Since this SNP has not been examined in any previous drug behavior studies, its role in alcohol behaviors cannot be compared to others; however, the T allele of rs3749380 was nominally associated with schizophrenia in two independent Japanese populations, and was shown to have lower promoter activity than the C allele using

a luciferase assay (Ohtsuki et al., 2008). In addition, this SNP has been associated with panic disorder in a Japanese population (Otowa et al., 2009).

A gene-based test was run to assess whether collapsing variants of potentially small effect and testing them together may provide evidence for association between *GRM7* and AC. Two gene-based tests were run on each of four imputed GWAS datasets and the *p* values from each analysis were combined; no association was found between *GRM7* and AC using either analytical method. Using JAG, the effective number of SNPs tested was calculated, a measure that takes LD into account to estimate how many overall signals were tested. In the MESA, OZ-ALC, and SAGE samples 16 signals were included; in the CADD-GADD sample, 15 signals were included. This difference is likely caused by the samples having different underlying LD structure. Notably, the two SNPs previously examined were not included in the gene-based test; rs3749380 is not in LD with any of the GWAS SNPs, and rs1485175 passed quality control procedures after imputation in the CADD-GADD dataset alone. Although the gene-based test encompasses more genetic variation than single SNP studies, the exclusion of rs3749380 and rs1485175 highlights the limitation of focusing only on current GWAS to perform human genetic studies and boosts the importance of single SNP studies.

While two analytical methods were used for both the SNP analysis and gene-based test and results were consistent, there are some differences between the two methods worth noting. In the single-SNP analyses, both FBAT and the within-family QTDT association test control for population stratification because they use a family-based approach. However, the QTDT test employs a regression-based approach where the phenotype is the outcome, which may explain the slightly different results obtained using the two methods. Under a normally distributed phenotype with no stratification, QTDT can be more powerful. Nevertheless, for testing a continuous trait in the entire sample with a phenotype of mean 0, heritability of 0.1 and frequency of 0.3, both tests can have roughly 0.75 power (Lange, DeMeo, & Laird, 2002). For the gene-based tests, JAG and PLINK consolidate SNP associations throughout a gene, but JAG takes into account every SNP and performs best when there are many signals in the gene contributing to risk for the phenotype. Conversely, PLINK selects only the most significant signals to contribute to the test statistic and therefore performs better with a gene with a few very strong signals.

Although the present study presents primarily negative findings, it is important to consider the limitations when interpreting this study. First, in context of comparison to the mouse genetics model on which this study was based, it is important to recognize that it is not possible to directly model the human AC phenotype in rodents, so differences in phenotypes across species limit direct comparisons. In humans, the phenotypic measure of AC has poor distributional properties, and duration was not taken into account. Future studies may benefit from imposing a duration limit or averaging typical and peak drinking levels to get a smoother distribution. Furthermore, the mouse studies found evidence for a *cis*-regulatory link between alcohol preference and *Grm7*, but the SNPs examined in the present study were necessarily limited by what is available currently and are unlikely to represent expression quantitative trait loci. Likewise, only common variants imputed in all four datasets were included in the gene-based test, which excluded many potentially important common

variants and rare variants in the gene. Finally, we opted to include only variants 20 kb upstream and downstream of *GRM7*, based on a study suggesting most genetic variation lies in this region (Veyrieras et al., 2008). However, separate work has shown that genes several hundred kb from an associated SNP may be functionally relevant (Loos et al., 2008). This study also did not consider gene by SNP interactions, SNP by SNP interactions, or SNPs capable of identifying an expression quantitative trait locus. With regard to study design, there may be low power to detect and replicate genetic SNP associations of very small effect in the CADD and GADD samples, which could lead to false negatives. In addition, the multiple testing correction only adjusted for the number of SNPs tested, not the number of samples tested. There might exist unidentified ascertainment or sample characteristic differences between the CADD and GADD samples, or the GWAS samples utilized. In the CADD and GADD samples, selective attrition could result in bias, particularly if subjects who did not return at wave 2 or 3 failed to do so because of incarceration or confinement in a treatment facility for drug use.

Despite these limitations, there are several strengths of our study that help mitigate some of these limitations. In particular, the use of a family-based design protects against population stratification, and so should minimize false positive associations. Furthermore, our SNP analysis included an important replication sample that was recruited and assessed in a manner similar to the first sample. Although it is likely that neither of the two SNPs examined is a causal variant, a gene-based test was used to try to cover most of the variation in the gene. However, we only analyzed SNPs that were common between all four imputed GWAS (in order to combine *p* values across datasets), potentially ignoring important signals. Despite this limitation, the gene-based test represents an effort to characterize all common variation in *GRM7* in relation to alcohol behaviors using currently available datasets. The use of several samples resulted in a large overall sample size. Lastly, SNP-set based approaches can increase power to detect genetic loci with individually small effects by consolidating SNP associations (Purcell et al., 2009; Wang, Jia, Wolfinger, Chen, & Zhao, 2011).

Although this study focused on *GRM7*, there are several other glutamate metabotropic receptors implicated in alcohol behaviors that warrant discussion. Activation of glutamate metabotropic receptor 5 (*Grm5*) has direct excitatory effects and potentiates N-methyl-D-aspartate receptor currents (Awad, Hubert, Smith, Levey, & Conn, 2000). In addition, *Grm5* antagonists have been shown to decrease ethanol seeking and relapse (Bäckström, Bachteler, Koch, Hyttiä, & Spanagel, 2004) and self-administration (Cowen, Djouma, & Lawrence, 2005; Schroeder, Overstreet, & Hodge, 2005) in rats, as well as in mice (Hodge et al., 2006). This is the opposite effect seen with *Grm7*, where overexpression leads to decreased ethanol consumption and knocking out the gene results in increased ethanol consumption (Gyetvai et al., 2011). Although the association was not replicated, *GRM5* was also associated with AD in a study of adolescents assessed for risky drinking (Schumann et al., 2008). In addition, several SNPs in glutamate metabotropic receptor 8 (*GRM8*) have been associated with AD (Chen et al., 2009). Recently, a stop codon in the glutamate metabotropic receptor 2 (*Grm2*) gene was linked to increased AC and preference in rats. Pharmacological blockade of the receptor further increased AC in rats, and *Grm2* knockout mice displayed elevated AC (Zhou et al., 2013), paralleling the effect seen with *Grm7* knockout mice (Gyetvai et al., 2011).

While this provides evidence that *Grm2* mediates alcohol behaviors, the total loss of function of *Grm2* seen in rodent models is most likely rare in human populations. Regardless, *GRM5*, *GRM8*, and *GRM2* are interesting candidates for alcohol treatment (Holmes, Spanagel, & Krystal, 2013; Joslyn et al., 2010).

In conclusion, this work provides preliminary evidence for an association between rs3749380 and AC in EAs. However, this association did not replicate in an independent sample and a gene-based test did not support an association between *GRM7* and AC. Importantly, the gene-based test did not include rs3749380 or rs1485175. This emphasizes the importance of single SNP analyses, as current GWAS datasets often lack potentially important signals. The availability of an independent replication sample and publically available GWAS on dbGaP, as well as the use of two different analytical tools, allowed us to weigh findings in the context of their reproducibility across samples and between analyses. Although our results do not provide evidence for association between AC and *GRM7*, there are many genetic factors in/around the gene that have not been investigated fully. As more genotyping data become available it will be important to revisit this question. As mentioned above, *Grm5*, *GRM8*, and *Grm2* have also been implicated in AC (Awad et al., 2000; Bäckström et al., 2004; Chen et al., 2009; Cowen et al., 2005; Hodge et al., 2006; Schroeder et al., 2005; Zhou et al., 2013), suggesting that the glutamate system is a likely target for alcohol cessation therapies, which emphasizes the importance of continued investigation of these genes in alcohol behaviors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by AA017889, AA017449, AA013525, DA011015, DA012845, and DA017637. We would like to thank Esther Lips for her invaluable help and assistance running JAG and performing the meta-analysis. The MESA, OZ-ALC, and SAGE data were obtained through dbGaP.

References

- Abecasis GR, Cardon LR, Cookson WO. A general test of association for quantitative traits in nuclear families. *American Journal of Human Genetics*. 2000; 66:279–292. [PubMed: 10631157]
- Alliey-Rodriguez N, Zhang D, Badner JA, Lahey BB, Zhang X, Dinwiddie S, et al. Genome-wide association study of personality traits in bipolar patients. *Psychiatric Genetics*. 2011; 21:190–194. [PubMed: 21368711]
- Awad H, Hubert GW, Smith Y, Levey AI, Conn PJ. Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus. *The Journal of Neuroscience*. 2000; 20:7871–7879. [PubMed: 11050106]
- Bäckström P, Bachteler D, Koch S, Hyytiä P, Spanagel R. mGluR5 antagonist MPEP reduces ethanol-seeking and relapse behavior. *Neuropsychopharmacology*. 2004; 29:921–928. [PubMed: 14735132]
- Bahi A. The selective metabotropic glutamate receptor 7 allosteric agonist AMN082 prevents reinstatement of extinguished ethanol-induced conditioned place preference in mice. *Pharmacology, Biochemistry, and Behavior*. 2012; 101:193–200.
- Bahi A. Viral-mediated knockdown of mGluR7 in the nucleus accumbens mediates excessive alcohol drinking and increased ethanol-elicited conditioned place preference in rats. *Neuropsychopharmacology*. 2013; 38:2109–2119. [PubMed: 22781839]

- Bahi A, Fizia K, Dietz M, Gasparini F, Flor PJ. Pharmacological modulation of mGluR7 with AMN082 and MMPiP exerts specific influences on alcohol consumption and preference in rats. *Addiction Biology*. 2012; 17:235–247. [PubMed: 21392179]
- Baik I, Cho NH, Kim SH, Han BG, Shin C. Genome-wide association studies identify genetic loci related to alcohol consumption in Korean men. *The American Journal of Clinical Nutrition*. 2011; 93:809–816. [PubMed: 21270382]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–265. [PubMed: 15297300]
- Bierut LJ, Agrawal A, Bucholz KK, Doheny KF, Laurie C, Pugh E, et al. A genome-wide association study of alcohol dependence. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:5082–5087. [PubMed: 20202923]
- Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. *American Journal of Epidemiology*. 2002; 156:871–881. [PubMed: 12397006]
- Breen G, Webb BT, Butler AW, van den Oord EJ, Tozzi F, Craddock N, et al. A genome-wide significant linkage for severe depression on chromosome 3: the depression network study. *The American Journal of Psychiatry*. 2011; 168:840–847. [PubMed: 21572164]
- Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger II Jr, et al. A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *Journal of Studies on Alcohol*. 1994; 55:149–158. [PubMed: 8189735]
- Chen AC, Tang Y, Rangaswamy M, Wang JC, Almasy L, Foroud T, et al. Association of single nucleotide polymorphisms in a glutamate receptor gene (GRM8) with theta power of event-related oscillations and alcohol dependence. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*. 2009; 150B:359–368.
- Chen XD, Xiong DH, Yang TL, Pei YF, Guo YF, Li J, et al. ANKRD7 and CYTL1 are novel risk genes for alcohol drinking behavior. *Chinese Medical Journal (Engl)*. 2012; 125:1127–1134.
- Cottler LB, Keating SK. Operationalization of alcohol and drug dependence criteria by means of a structured interview. *Recent Developments in Alcoholism*. 1990; 8:69–83. [PubMed: 2333396]
- Cowen MS, Djouma E, Lawrence AJ. The metabotropic glutamate 5 receptor antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine reduces ethanol self-administration in multiple strains of alcohol-preferring rats and regulates olfactory glutamatergic systems. *The Journal of Pharmacology and Experimental Therapeutics*. 2005; 315:590–600. [PubMed: 16014750]
- Derringer J, Corley RP, Haberstick BC, Young SE, Demmitt BA, Howrigan DP, et al. Genome-Wide Association Study of Behavioral Disinhibition in a Selected Adolescent Sample. *Behavior Genetics*. 2015; 45:375–381. [PubMed: 25637581]
- Elia J, Glessner JT, Wang K, Takahashi N, Shtir CJ, Hadley D, et al. Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder. *Nature Genetics*. 2011; 44:78–84. [PubMed: 22138692]
- Ganda C, Schwab SG, Amir N, Heriani H, Irmansyah I, Kusumawardhani A, et al. A family-based association study of DNA sequence variants in GRM7 with schizophrenia in an Indonesian population. *The International Journal of Neuropsychopharmacology*. 2009; 12:1283–1289. [PubMed: 19638256]
- Grant JD, Heath AC, Bucholz KK, Madden PA, Agrawal A, Statham DJ, et al. Spousal concordance for alcohol dependence: evidence for assortative mating or spousal interaction effects? *Alcoholism: Clinical and Experimental Research*. 2007; 31:717–728.
- Gyetyvai B, Simonyi A, Oros M, Saito M, Smiley J, Vadász C. mGluR7 genetics and alcohol: intersection yields clues for addiction. *Neurochemical Research*. 2011; 36:1087–1100. [PubMed: 21448595]
- Hamilton SP. A new lead from genetic studies in depressed siblings: assessing studies of chromosome 3. *The American Journal of Psychiatry*. 2011; 168:783–789. [PubMed: 21813496]
- Hansell NK, Agrawal A, Whitfield JB, Morley KI, Zhu G, Lind PA, et al. Long-term stability and heritability of telephone interview measures of alcohol consumption and dependence. *Twin Research and Human Genetics*. 2008; 11:287–305. [PubMed: 18498207]

- Heath AC, Whitfield JB, Martin NG, Pergadia ML, Goate AM, Lind PA, et al. A quantitative-trait genome-wide association study of alcoholism risk in the community: findings and implications. *Biological Psychiatry*. 2011; 70:513–518. [PubMed: 21529783]
- Hodge CW, Miles MF, Sharko AC, Stevenson RA, Hillmann JR, Lepoutre V, et al. The mGluR5 antagonist MPEP selectively inhibits the onset and maintenance of ethanol self-administration in C57BL/6J mice. *Psychopharmacology (Berl)*. 2006; 183:429–438. [PubMed: 16292590]
- Holmes A, Spanagel R, Krystal JH. Glutamatergic targets for new alcohol medications. *Psychopharmacology (Berl)*. 2013; 229:539–554. [PubMed: 23995381]
- Joslyn G, Ravindranathan A, Brush G, Schuckit M, White RL. Human variation in alcohol response is influenced by variation in neuronal signaling genes. *Alcoholism: Clinical and Experimental Research*. 2010; 34:800–812.
- Kamens HM, Corley RP, McQueen MB, Stallings MC, Hopfer CJ, Crowley TJ, et al. Nominal association with CHRNA4 variants and nicotine dependence. *Genes, Brain, and Behavior*. 2013; 12:297–304.
- Kapoor M, Wang JC, Wetherill L, Le N, Bertelsen S, Hinrichs AL, et al. A meta-analysis of two genome-wide association studies to identify novel loci for maximum number of alcoholic drinks. *Human Genetics*. 2013; 132:1141–1151. [PubMed: 23743675]
- Kendler KS, Myers J, Dick D, Prescott CA. The relationship between genetic influences on alcohol dependence and on patterns of alcohol consumption. *Alcoholism: Clinical and Experimental Research*. 2010; 34:1058–1065.
- Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. *Genome Research*. 2002; 12:996–1006. [PubMed: 12045153]
- Knopik VS, Heath AC, Madden PA, Bucholz KK, Slutske WS, Nelson EC, et al. Genetic effects on alcohol dependence risk: re-evaluating the importance of psychiatric and other heritable risk factors. *Psychological Medicine*. 2004; 34:1519–1530. [PubMed: 15724882]
- Lange C, DeMeo DL, Laird NM. Power and design considerations for a general class of family-based association tests: quantitative traits. *American Journal of Human Genetics*. 2002; 71:1330–1341. [PubMed: 12454799]
- Li C, Li Y, Xu J, Lv J, Ma Y, Shao T, et al. Disease-driven detection of differential inherited SNP modules from SNP network. *Gene*. 2011; 489:119–129. [PubMed: 21920414]
- Li X, Gardner EL, Xi ZX. The metabotropic glutamate receptor 7 (mGluR7) allosteric agonist AMN082 modulates nucleus accumbens GABA and glutamate, but not dopamine, in rats. *Neuropharmacology*. 2008; 54:542–551. [PubMed: 18155073]
- Li X, Li J, Peng XQ, Spiller K, Gardner EL, Xi ZX. Metabotropic glutamate receptor 7 modulates the rewarding effects of cocaine in rats: involvement of a ventral pallidal GABAergic mechanism. *Neuropsychopharmacology*. 2009; 34:1783–1796. [PubMed: 19158667]
- Lips ES, Kooyman M, de Leeuw C, Posthuma D. JAG: A Computational Tool to Evaluate the Role of Gene-Sets in Complex Traits. *Genes (Basel)*. 2015; 6:238–251. [PubMed: 26110313]
- Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nature Genetics*. 2008; 40:768–775. [PubMed: 18454148]
- Mitsukawa K, Yamamoto R, Ofner S, Nozulak J, Pescott O, Lukic S, et al. A selective metabotropic glutamate receptor 7 agonist: activation of receptor signaling via an allosteric site modulates stress parameters in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:18712–18717. [PubMed: 16339898]
- Muglia P, Tozzi F, Galwey NW, Francks C, Upmanyu R, Kong XQ, et al. Genome-wide association study of recurrent major depressive disorder in two European case-control cohorts. *Molecular Psychiatry*. 2010; 15:589–601. [PubMed: 19107115]
- Ohtsuki T, Koga M, Ishiguro H, Horiuchi Y, Arai M, Niizato K, et al. A polymorphism of the metabotropic glutamate receptor mGluR7 (GRM7) gene is associated with schizophrenia. *Schizophrenia Research*. 2008; 101:9–16. [PubMed: 18329248]
- Otowa T, Yoshida E, Sugaya N, Yasuda S, Nishimura Y, Inoue K, et al. Genome-wide association study of panic disorder in the Japanese population. *Journal of Human Genetics*. 2009; 54:122–126. [PubMed: 19165232]

- Pan Y, Luo X, Liu X, Wu LY, Zhang Q, Wang L, et al. Genome-wide association studies of maximum number of drinks. *Journal of Psychiatric Research*. 2013; 47:1717–1724. [PubMed: 23953852]
- Park S, Jung SW, Kim BN, Cho SC, Shin MS, Kim JW, et al. Association between the GRM7 rs3792452 polymorphism and attention deficit hyperactivity disorder in a Korean sample. *Behavioral and Brain Functions*. 2013; 9:1. [PubMed: 23295062]
- Pei YF, Zhang L, Yang TL, Han Y, Hai R, Ran S, et al. Genome-wide association study of copy number variants suggests LTBP1 and FGD4 are important for alcohol drinking. *PLoS One*. 2012; 7:e30860. [PubMed: 22295116]
- Pergadia ML, Glowinski AL, Wray NR, Agrawal A, Saccone SF, Loukola A, et al. A 3p26-3p25 genetic linkage finding for DSM-IV major depression in heavy smoking families. *The American Journal of Psychiatry*. 2011; 168:848–852. [PubMed: 21572167]
- Petrill, SA.; Plomin, R.; DeFries, JC.; Hewitt, JK. *Nature, Nurture, and the Transition to Early Adolescence*. Oxford University Press; 2003.
- Philibert RA, Zadorozhnyaya O, Beach SR, Brody GH. Comparison of the genotyping results using DNA obtained from blood and saliva. *Psychiatric Genetics*. 2008; 18:275–281. [PubMed: 19018232]
- Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010; 26:2336–2337. [PubMed: 20634204]
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*. 2007; 81:559–575. [PubMed: 17701901]
- Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009; 460:748–752. [PubMed: 19571811]
- Quillen EE, Chen XD, Almasy L, Yang F, He H, Li X, et al. ALDH2 is associated to alcohol dependence and is the major genetic determinant of “daily maximum drinks” in a GWAS study of an isolated rural Chinese sample. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*. 2014; 165B:103–110.
- Rabinowitz D, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Human Heredity*. 2000; 50:211–223. [PubMed: 10782012]
- Rhea SA, Gross AA, Haberstick BC, Corley RP. Colorado Twin Registry. *Twin Research and Human Genetics*. 2006; 9:941–949. [PubMed: 17254434]
- Saccone SF, Pergadia ML, Loukola A, Broms U, Montgomery GW, Wang JC, et al. Genetic linkage to chromosome 22q12 for a heavy-smoking quantitative trait in two independent samples. *American Journal of Human Genetics*. 2007; 80:856–866. [PubMed: 17436240]
- Saus E, Brunet A, Armengol L, Alonso P, Crespo JM, Fernández-Aranda F, et al. Comprehensive copy number variant (CNV) analysis of neuronal pathways genes in psychiatric disorders identifies rare variants within patients. *Journal of Psychiatric Research*. 2010; 44:971–978. [PubMed: 20398908]
- Schroeder JP, Overstreet DH, Hodge CW. The mGluR5 antagonist MPEP decreases operant ethanol self-administration during maintenance and after repeated alcohol deprivations in alcohol-preferring (P) rats. *Psychopharmacology (Berl)*. 2005; 179:262–270. [PubMed: 15717208]
- Schumann G, Coin LJ, Lourdasamy A, Charoen P, Berger KH, Stacey D, et al. Genome-wide association and genetic functional studies identify autism susceptibility candidate 2 gene (AUTS2) in the regulation of alcohol consumption. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:7119–7124. [PubMed: 21471458]
- Schumann G, Johann M, Frank J, Preuss U, Dahmen N, Laucht M, et al. Systematic analysis of glutamatergic neurotransmission genes in alcohol dependence and adolescent risky drinking behavior. *Archives of General Psychiatry*. 2008; 65:826–838. [PubMed: 18606955]
- Shibata H, Tani A, Chikuhara T, Kikuta R, Sakai M, Ninomiya H, et al. Association study of polymorphisms in the group III metabotropic glutamate receptor genes, GRM4 and GRM7, with schizophrenia. *Psychiatry Research*. 2009; 167:88–96. [PubMed: 19351574]

- Shyn SI, Shi J, Kraft JB, Potash JB, Knowles JA, Weissman MM, et al. Novel loci for major depression identified by genome-wide association study of Sequenced Treatment Alternatives to Relieve Depression and meta-analysis of three studies. *Molecular Psychiatry*. 2011; 16:202–215. [PubMed: 20038947]
- Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *American Journal of Human Genetics*. 1993; 52:506–516. [PubMed: 8447318]
- Stallings MC, Corley RP, Dennehey B, Hewitt JK, Krauter KS, Lessem JM, et al. A genome-wide search for quantitative trait Loci that influence antisocial drug dependence in adolescence. *Archives of General Psychiatry*. 2005; 62:1042–1051. [PubMed: 16143736]
- Stallings MC, Corley RP, Hewitt JK, Krauter KS, Lessem JM, Mikulich SK, et al. A genome-wide search for quantitative trait loci influencing substance dependence vulnerability in adolescence. *Drug and Alcohol Dependence*. 2003; 70:295–307. [PubMed: 12757967]
- Uhl GR, Drgon T, Johnson C, Walther D, David SP, Aveyard P, et al. Genome-wide association for smoking cessation success: participants in the Patch in Practice trial of nicotine replacement. *Pharmacogenomics*. 2010; 11:357–367. [PubMed: 20235792]
- Vadasz C, Saito M, Gyetvai BM, Oros M, Szakall I, Kovacs KM, et al. Glutamate receptor metabotropic 7 is cis-regulated in the mouse brain and modulates alcohol drinking. *Genomics*. 2007; 90:690–702. [PubMed: 17936574]
- Veyrieras JB, Kudaravalli S, Kim SY, Dermitzakis ET, Gilad Y, Stephens M, et al. High-resolution mapping of expression-QTLs yields insight into human gene regulation. *PLoS Genetics*. 2008; 4:e1000214. [PubMed: 18846210]
- Wang L, Jia P, Wolfinger RD, Chen X, Zhao Z. Gene set analysis of genome-wide association studies: methodological issues and perspectives. *Genomics*. 2011; 98:1–8. [PubMed: 21565265]
- Whitlock MC. Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach. *Journal of Evolutionary Biology*. 2005; 18:1368–1373. [PubMed: 16135132]
- Yang Y, Pan C. Role of metabotropic glutamate receptor 7 in autism spectrum disorders: a pilot study. *Life Sciences*. 2013; 92:149–153. [PubMed: 23201551]
- Zhou Z, Karlsson C, Liang T, Xiong W, Kimura M, Tapocik JD, et al. Loss of metabotropic glutamate receptor 2 escalates alcohol consumption. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:16963–16968. [PubMed: 24082084]

Table 1

Demographic characteristics of the CADD and GADD samples

Sample	N	Age ^a	AC ^b
CADD			
Community			
Adolescents	349	24.4 ±5.3	2.9 ±4.2
Adults	399	48.3±6.3	2.9±4.1
Clinical			
Adolescents	596	24.4±6.2	6.3±8.8
Adults	458	46.4 ±9.0	5.4 ±6.7
GADD			
Clinical			
Adolescents	843	21.4±4.1	5.6±6.8
Adults	206	45.4 ±6.0	7.8 ±4.5

^aMean ± standard deviation^bAlcohol consumption; mean ± standard deviation

Table 2
Genotypic characteristics of the two SNPs examined in the CADD and GADD samples

Sample	SNP	Location ^a	Alleles ^b	MAF ^c	HWE ^d
CADD	rs3749380	6903047	C/T	0.40	0.36
	rs1485175	7620539	C/T	0.45	0.88
GADD	rs3749380	6903047	C/T	0.38	0.74
	rs1485175	7620539	C/T	0.45	0.19

^aOn chromosome 3, from the UCSC genome browser

^bMajor/minor allele

^cMinor allele frequency

^dHardy-Weinberg equilibrium *p* value

Table 3

Results from the FBAT and QTDT analyses in each sample for AC

FBAT					QTDT		
Sample	SNP	Risk allele	Z score ^a	p value	# Fam ^b	SNP	p value # Probands ^c
CADD	rs3749380	T	2.574	0.010 *	115	rs3749380	0.002 ** 247
	rs1485175	C	0.667	0.505	123	rs1485175	0.722 261
GADD	rs3749380	C	1.499	0.134	133	rs3749380	0.705 73
	rs1485175	C	1.311	0.190	133	rs1485175	0.804 59

^aA positive z score indicates the allele is over-transmitted and is interpreted as the risk allele.

^bThe number of informative families included in the analysis

^cThe number of informative probands included in the QTDT test

* Significant after correction for multiple testing at $p = 0.020$

** Overall Bonferroni significance level for this association is $p = 0.031$

Table 4

Phenotypic characteristics of the samples utilized in the gene-based test

Study	N	Age ^d	% Male	AC (raw) ^b	AC (log) ^c
CADD-GADD	1031	23.81±4.25	70.71%	5.17±6.91	0.72±0.32
MESA	897	62.51±10.16	54.18%	2.63±2.33	0.62±0.19
OZ-AIC	2000	44.03±9.53	50.25%	18.79±14.41	1.21±0.32
SAGE	2247	38.19±9.60	44.68%	19.47±18.87	1.21±0.32

^aMean ± standard deviation
^bRaw values for AC; mean ± standard deviation
^cLog10 transformed AC used in the analysis; mean ± standard deviation

Table 5

Gene-based test results from the JAG analysis

Study	sumlogReal ^a	P _{emp}	var(Perms) ^b	mean(Perms) ^c	nEff ^d	Combined <i>p</i> value
CADD-GADD	321.51	0.750	9921.65	391.92	15	0.743
MESA	275.26	0.916	9441.08	392.15	16	
OZ-ALC	333.77	0.692	9567.68	390.33	16	
SAGE	410.65	0.365	9405.54	391.37	16	

^aThe test statistic ($\Sigma\text{-log10(P)}$) of the original data

^bThe variance of the distribution of the test statistic from the permutations

^cThe mean of the test statistics from the permutations

^dThe effective number of SNPs

Gene-based test results from the PLINK analysis. Additional information on LD structure is provided in the supplementary information.

Table 6

Study	nSIG ^a	iSIG ^b	P _{emp}	SNP ^c			p value	Combined p value
				SNP	Minor allele	β		
CADD-GADD	16	5	0.601	rs3804849 ^d	G	0.040	0.007	
				rs4054135 ^d	C	0.035	0.014	
				rs1356268	G	−0.075	0.026	
				rs3804826 ^e	A	0.028	0.044	
				rs9844393	G	0.036	0.048	
MESA	2	2	0.809	rs9828691	G	0.029	0.024	
				rs7611549	A	0.018	0.036	
OZ-ALC	8	2	0.852	rs162773 ^e	C	−0.019	0.020	0.812
				rs3804861	T	0.022	0.050	
SAGE	71	8	0.462	rs9872244 ^f	G	0.029	0.004	
				rs28437102 ^f	G	0.036	0.006	
				rs9813021	A	0.041	0.016	
				rs28378912	A	0.019	0.021	
				rs17693618	G	0.030	0.027	
				rs7634808	G	0.019	0.032	
				rs7634694	G	0.017	0.043	
				rs1356268	G	−0.043	0.048	

^aTotal number of SNPs below p value threshold ($p < 0.05$)

^bNumber of significant SNPs also passing LD criteria ($r^2 > 0.5$)

^cList of significant SNPs passing LD criteria and SNP statistics from the PLINK test

^dIn LD at $r^2 > 0.5$ from CEU 1,000 Genomes Phase 1 data

^eIn LD at $r^2 = 0.816$ from CEU 1,000 Genomes Phase 1 data

^fIn LD at $r^2 > 0.5$ from CEU 1,000 Genomes Phase 1 data