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A genome wide association study of alcohol dependence symptom counts in extended pedigrees identifies *C15orf53*

Jen-Chyong Wang, PhD[1,*], **Tatiana Foroud, PhD**[2,*], **Anthony L Hinrichs, PhD**[1,*], **Nhung XH Le, MS**[1], **Sarah Bertelsen, MA**[1], **John P Budde, BA**[1], **Oscar Harari, PhD**[1], **Daniel L Koller, PhD**[2], **Leah Wetherill, MS**[2], **Arpana Agrawal, PhD**[1], **Laura Almasy, PhD**[5], **Andrew I Brooks, PhD**[6], **Kathleen Bucholz, PhD**[1], **Danielle Dick, PhD**[7], **Victor Hesselbrock, PhD**[8], **Eric O Johnson, PhD**[9], **Sun Kang, PhD**[10], **Manav Kapoor, PhD**[1], **John Kramer, PhD**[11], **Samuel Kuperman, MD**[12], **Pamela AF Madden, PhD**[1], **Niklas Manz, PhD**[10], **Nicholas G Martin, PhD**[13], **Jeanette N McClintick, PhD**[3], **Grant W Montgomery, PhD**[13], **John I Nurnberger Jr, MD, PhD**[4], **Madhavi Rangaswamy, PhD**[10], **John Rice, PhD**[1], **Marc Schuckit, MD**[14], **Jay A Tischfield, PhD**[5], **John B Whitfield, PhD**[13], **Xiaoling Xuei, PhD**[3], **Bernice Porjesz, PhD**[10], **Andrew C Heath, DPhil**[1], **Howard J Edenberg, PhD**[2,3], **Laura J Bierut, MD**[1], and **Alison M Goate, DPhil**[1,**]

[1]Department of Psychiatry, Washington University School of Medicine, Saint Louis, MO

[2]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN

[3]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN

[4]Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN

[5]Department of Genetics, Texas Biomedical Research Institute, San Antonio, TX

[6]Department of Genetics, Rutgers University, Piscataway, NJ

[7]Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA

[8]Department of Psychiatry, University of Connecticut Health Center, Farmington, CT

[9]Division of Health, Social and Economic Research, Research Triangle Institute International, Research Triangle Park, NC

[10]Henri Begleiter Neurodynamics Laboratory, Department of Psychiatry and Behavioral Sciences, SUNY Downstate Medical Center, Brooklyn, NY

[11]Department of Psychiatry, University of Iowa College of Medicine, Iowa City, IA

[12]Division of Child Psychiatry, University of Iowa Hospitals, Iowa City, IA

[13]Queensland Institute of Medical Research, Queensland, Australia

[14]Department of Psychiatry, University of California-San Diego, La Jolla, CA

[**]Correspondence: Alison M. Goate, Dept. of Psychiatry, B8134, Washington University School of Medicine, 660 S. Euclid Ave, St. Louis, MO 63110, goatea@psychiatry.wustl.edu, tel. 314-362-8691, fax. 314-747-2544.
[*]These authors contributed equally

Conflicts of Interest

Drs. LJ Bierut, AM Goate, AJ Hinrichs, J Rice and JC Wang are listed as inventors on the patent “Markers for Addiction” (US 20070258898) covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction.

Supplementary information is available at *Molecular Psychiatry*’s website

Abstract

Several studies have identified genes associated with alcohol use disorders, but the variation in each of these genes explains only a small portion of the genetic vulnerability. The goal of the present study was to perform a genome-wide association study (GWAS) in extended families from the Collaborative Study on the Genetics of Alcoholism (COGA) to identify novel genes affecting risk for alcohol dependence. To maximize the power of the extended family design we used a quantitative endophenotype, measured in all individuals: number of alcohol dependence symptoms endorsed (symptom count). Secondary analyses were performed to determine if the single nucleotide polymorphisms (SNPs) associated with symptom count were also associated with the dichotomous phenotype, DSM-IV alcohol dependence. This family-based GWAS identified SNPs in *C15orf53* that are strongly associated with DSM-IV alcohol (p=4.5×10^{-8}, inflation corrected p=9.4×10^{-7}). Results with DSM-IV alcohol dependence in the regions of interest support our findings with symptom count, though the associations were less significant. Attempted replications of the most promising association results were conducted in two independent samples: non-overlapping subjects from the Study of Addiction: Genes and Environment (SAGE) and the Australian twin-family study of alcohol use disorders (OZALC). Nominal association of *C15orf53* with symptom count was observed in SAGE. The variant that showed strongest association with symptom count, rs12912251 and its highly correlated variants (D′=1, r^2≥ 0.95), has previously been associated with risk for bipolar disorder.

Keywords

DSM-IV alcohol dependence symptoms; Family-based GWAS; *C15orf53*; Quantitative traits

Introduction

Alcohol use disorders (AUDs) are among the most common and costly public health problems throughout the world[1]. Family and twin studies have provided evidence for a genetic predisposition toward AUDs[2,3], with genetic factors accounting for approximately 40% to 60% of the total variance in risk for alcohol dependence (AD)[3–8].

A variety of study designs have been employed to identify genes influencing the vulnerability to AD. Genome-wide association studies (GWAS) are a potentially more comprehensive way to study a complex, common disease like AD where we have little knowledge of disease pathophysiology. Several GWAS have sought to identify variants associated with the risk for AD using case-control designs, including treatment seeking subjects with AD[9], individuals selected from densely affected families with AD[10], a case-control series drawn from treatment and community-based samples from several diseases[11], subjects ascertained from large unselected sibships as well as individuals selected for heavier alcohol use[12]. GWAS using quantitative traits derived from alcohol consumption and AD symptomatology have also been examined in a population-based sample[13] and an Australian sample of related individuals[12]. Results thus far have identified interesting candidate genes for AD, although the overlap of the top genetic signals across studies has been limited.

The Collaborative Study of the Genetics of Alcoholism (COGA) provides another opportunity to examine genes associated with problematic alcohol use. COGA is a multi-site, longitudinal study established to identify vulnerability genes for AD by recruiting multiplex alcohol dependent families as well as representative families from the community[14–18]. In the current analysis, we performed a family-based GWAS in large multi-generational families severely affected by AD. These families likely represent a subgroup enriched for AD susceptibility alleles.

The power to identify genes contributing to the risk for disease may be increased through the analysis of quantitative endophenotypes highly correlated with that disorder but measurable in all individuals. Rather than focus on the presence or absence of AD, we used the number of AD symptoms as our primary phenotype. Some research has indicated that AD may be better captured with a symptom count rather than with a dichotomous diagnosis[19–21]. Evidence from twin studies has shown that two quantitative measures, dependence symptoms and alcohol consumption are highly correlated with alcohol dependence and index closely the risk for alcohol dependence[22,23]. Symptom counts can be computed for any drinker, including older adolescents who are just beginning to use alcohol but may not fulfill criteria for AD, thus allowing us to use more of our sample in the analysis and increase the power to detect association. Since most other studies on alcoholism have used a dichotomous diagnosis, DSM-IV alcohol dependence, we analyzed the regions of interest identified in the symptom count analysis to evaluate if similar findings emerge.

Material and Methods

COGA Study Subjects

Following approval of institutional review boards at all participating institutions, AD probands were recruited through alcohol treatment programs and administered a validated poly-diagnostic instrument, the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA), to assess AD[14,16,18,19,24]. Individuals below the age of 18 were administered an adolescent version of the SSAGA. The same assessment approach, used for all probands and their relatives, was repeated at several-year intervals for a large number of individuals.

The goal of this study was to identify genetic variants associated with alcohol-related phenotypes. All families were reviewed and the genetically most informative subset of COGA families that could be used for analyses of a variety of alcohol-related phenotypes, including DSM-IV symptom count (SC), were selected for a family-based GWAS. Prioritization in selecting subjects for analysis was based on a higher number of AD family members, the number of relatives who supplied DNA, as well as the number of family members with another key COGA phenotype, electrophysiology measures. To reduce the heterogeneity in the sample, only families that were primarily of Caucasian descent were selected for genotyping, yielding a total of 2322 subjects from 118 extended families who were genotyped. The resulting dataset includes multi-generational families affected by AUDs with an average of 20 subjects per family (Figure S1). After genotype quality control and cleaning, correcting pedigree inconsistencies, and processing the phenotypes (see below), 2010 genotyped individuals were included in the subsequent analyses. Full details on the genotype cleaning are included in the supplementary information.

Phenotype

We computed the symptom count (SC) using the seven lifetime diagnostic criteria for DSM-IV alcohol dependence (AD). This measure, with a value ranging from 0 to 7, was available on all individuals with an adolescent or adult SSAGA who had reported ever consuming alcohol. When longitudinal data were available, we used the maximum number of symptoms endorsed at any interview. Individuals who were younger than 15 years at the most recent interview were excluded from the analysis because SC in this population is likely to be non-representative of adult cohorts. Of the 2010 subjects =15yr who drank, 622 did not report any of the 7 symptoms of AD, and 765 had 3 or more such symptoms. The distribution of SC is shown in Figure 1.

DSM-IV AD was used as a secondary phenotype, to enable comparison with other studies in the literature. Given the wide age range of the subjects included in the analysis and the fact

that many had not passed through the age of risk for an alcohol use disorder (AUD), the following algorithm was developed to reach the final diagnosis after considering all evaluations. Individuals aged 15 years or older who met DSM-IV criteria at any evaluation were classified as alcohol dependent. Individuals 23 years and older who drank but did not meet criteria for AD on any adult SSAGA were classified as unaffected. Individuals who did not consume alcohol, were under the age of 15 years at all evaluations, or were age 15 through 22 years old and did not meet DSM-IV criteria, were classified as unknown and were removed from subsequent analyses – this avoids ascribing an unaffected status to an adolescent or young adult who may be at high genetic risk but not yet past the peak period of vulnerability. There were 684 subjects in the 118 extended families who met criteria for AD using either an adult or adolescent SSAGA (Table S1). Among 1638 remaining individuals, 964 were classified as unaffected. The average number of individuals in a family diagnosed with AD was 5.9 (Figure S2).

Genotype

Genotyping was performed at the Genome Technology Access Center at Washington University School of Medicine in St. Louis (http://gtac.wustl.edu/) using the Illumina Human OmniExpress array 12.VI (Illumina, San Diego, CA, USA). We also included in the analysis genotypes for subjects (n=275) from these 118 families who were genotyped in a previous case-control GWAS using the Illumina 1M array[10]. For quality control purposes, 51 of the 275 subjects were genotyped again on the Illumina Human OmniExpress array. Imputed data were obtained using the program BEAGLE[25]. A detailed description of imputation and subsequent data cleaning is included in supplementary information.

Statistical Analysis

A total of 707,557 autosomal SNPs passed quality control. Given their limited power to detect association, SNPs with a minor allele frequency below 5% (n=115,872) were excluded from further analysis. Thus association analysis was performed with the remaining 591,685 SNPs, giving a Bonferronic corrected threshold for genome-wide significance of $p=8.45\times10^{-8}$.

We first tested the effect of covariates on our phenotypes: SC and DSM-IV AD. As expected, gender was a highly significant predictor of SC and DSM-IV AD, and was included as a covariate in all analyses. We identified cohort effects and therefore divided subjects into 4 cohorts based on their year of birth (< 1930, 1930 – 1949, 1950 – 1969, and ≥ 1970). For SC, the age-squared parameter was still significant after cohort effect was included, and the final model therefore included gender, age, age-squared and cohort. For DSM-IV AD, the age-squared parameter was not a significant factor after considering cohort and was omitted. The first principal component from the EIGENSTRAT analysis (pc1), while not statistically significant, was still included in all analyses to reduce the risk of false positive associations due to population stratification.

In this sample, the SC phenotype best fit a negative binomial distribution, which was identified by applying PROC COUNTREG and PROC SGPLOT in SAS (http://support.sas.com/rnd/app/da/glimmix.html). By specifying a negative binomial distribution and a logarithmic link function, we parametrically modeled the observed trait distribution and included relevant covariates described above. Association with SC was analyzed for each SNP using a dose-effect model (number of minor alleles present in each individual) as implemented in PROC GLIMMIX from SAS. To control for relatedness, the test was placed in a general linear mixed model (GLMM) framework[26] using an independent working correlation matrix where each family was a separate cluster.

Inflation of *p* values was revealed by preliminary examination of the quantile-quantile (Q-Q) plot (Figure S3). The genomic inflation factor (GIF), calculated by computing the median of the χ^2 statistics divided by the median of the central χ^2 distribution with df =1, was 1.25. In order to control for this inflation, we used the method of the Genomic Control[27] with a λ of 1.25. In particular, we re-computed the level of association with each marker by dividing the observed χ^2 with inflation factor λ of 1.25. We verified that these new, inflation corrected *p* values had a GIF of 1, indicating no further inflation.

The analyses of AD were conducted using the Genome-Wide Association Analyses with Family Data package[28]. A logistic regression model was employed with gender, age and cohort included as covariates, and a log additive model for each SNP was tested for association. The generalized estimating equation (GEE) framework was used to control for relatedness. No inflation of *p* values was observed (λ = 1.05).

Replication samples

The Study of Addiction: Genetics and Environment (SAGE) sample—The SAGE sample is a case-control series selected from three large, complementary datasets: Collaborative Study on the Genetics of Alcoholism, Family Study of Cocaine Dependence, and Collaborative Genetics Study of Nicotine Dependence. After removing 129 individuals in SAGE who were also part of the 118 extended families in the primary analysis, data from 2647 subjects of European descent were used to replicate promising associations ($p<0.0001$) identified in the COGA sample. Detailed characteristics of this sample and the genotyping platform were described in Bierut et al.[11]. Imputed dosage data were obtained using the same method as described in supplementary information. The distribution of SC was similar to that of the COGA sample. We used PROC GLIMMIX in SAS to test the association of individual SNPs with SC including age, age-squared, gender, nicotine dependence, cocaine dependence, and pc1 as covariates. We used the GEE framework described above to analyze the association with AD.

The Australian Twin-family Study of Alcohol Use Disorder (OZALC) Sample—The twins in this study were initially ascertained through the Australian Twin Registry, followed by cascading recruitment of non-twin siblings, parents, adult offspring and spouses[12]. Data from 6166 subjects of European descent were used for replication analysis with the SC. Detailed characteristics of this sample and genotyping platform were described in Heath et al.[12]. Imputed dosage data were obtained using MACH (http://www.sph.umich.edu/csg/abecasis/MACH). The association of individual SNPs with SC was performed using PROC GLIMMIX from SAS. Age and gender were included as covariates for association analysis. The GEE model described above was used for the association analysis with AD.

Results

Association with symptom count

Results for the entire genome are summarized in the Manhattan plot (Figure 2). After correcting for inflated, 72 SNPs of 591,685 genotyped autosomal SNPs tested showed evidence of association with symptom count with inflation corrected *p* values $<10^{-4}$ (Table S2). None of these 72 SNPs reached genome-wide significance. Among these top signals, we identified 7 chromosomal loci containing 3 or more SNPs within 50 kb of each other that show association with SC (Table 1).

The strongest association was detected with rs12903120 ($p=5.45\times10^{-8}$, inflation corrected $p=1.09\times10^{-6}$) in an uncharacterized gene, *C15orf53* on chromosome 15q14. Two other

genotyped SNPs, rs12916379 and rs2132157, that are highly correlated with rs12903120 (D′=1, r^2=1 in HapMap EUR reference sample) also showed strong association with SC (with inflation corrected $p=2.79\times10^{-6}$ and $p=3.02\times10^{-6}$, respectively) (Table S3). Rs12916379 is located in the 3′ un-translated region of *C15orf53* (Figure 3). Using imputed genotypes, 15 additional SNPs in *C15orf53* gene region also showed suggestive evidence of association (inflation corrected $p\leq1.0\times10^{-5}$) with SC (Table S3). Three of these 15 SNPs have stronger association (inflation corrected $9.4\times10^{-7}\leq p\leq9.7\times10^{-7}$) with SC than the genotyped SNP rs12903120. A non-synonymous coding SNP in *C15orf53* that is highly correlated with rs12903120 (rs7165988; r^2=0.95, D′=1 in HapMap EUR reference sample) is associated with SC at an inflation corrected $p=1.7\times10^{-6}$.

Association with DSM-IV alcohol dependence in the regions of interest

We tested whether SNPs in the 7 chromosome regions associated with SC were also associated with DSM-IV AD. Our association analysis using a GEE model found none of the genotyped SNPs in these regions attained genome-wide significance with DSM-IV AD. By comparing the associations for SC with DSM-IV AD, we observed a less significant association with the dichotomous diagnostic trait than the association with SC (Table 1). However, the effect sizes between the two phenotypes were highly correlated (Figure 4).

Replication of association in SAGE and OZALC studies

Seventy-two SNPs that associated with SC (with corrected $p<10^{-4}$) in the COGA families were tested in SAGE. Based on a prior hypothesis (our initial results from GWAS) for each SNP regarding the direction of effect, we found 5/72 SNPs showing nominal association with the same direction of effect for SC ($0.007\leq p\leq0.05$). However, none of these SNPs is significant after correcting for multiple testing. We then tested the replication of the 18 SNPs in *C15orf53* that showed suggestive evidence of association with SC in COGA sample (inflation corrected $p=1.0\times10^{-5}$). Using imputed data, 8 of these 18 SNPs showed nominal association with SC in SAGE sample ($p\leq0.03$) (Table S3). These 18 SNPs all lie within a single LD bin (r^2=0.8, D′≥0.9), so the data shown in Table S3 reflects a single statistical test.

Sixty-nine of the 72 SNPs associated with SC (with inflation corrected $p<10^{-4}$) in the COGA families were tested in OZALC. None of these SNPs showed association with SC in this sample (Table S2). Association of SNPs in the *C15orf53* gene region (rs2132157, rs12916379 and rs12903120, with p=0.03, p=0.03, and p=0.05, respectively) was observed with DSM-IV AD but not with SC (data not shown).

DISCUSSION

We conducted a family-based GWAS and identified genome-wide significant association ($p=5.4\times10^{-8}$) between SC and SNPs in chromosome 15q14. However, the Q-Q plot for the COGA family-based sample suggests genomic inflation factor of 1.25. One possible explanation is the presence of polygenic inheritance[29]. The SC trait is likely due to many loci of small effect. Because of this and the high LD in the dense map, it is possible that there are relatively few genomic regions free of loci with small effect moderately inflating GIF. A second possibility is that the inflation may be due to the presence of very large families in our sample. Since our association test controls for relatedness by treating each family as a separate cluster, this may be insufficient in families where distantly related individuals should not be clustered into the same class. After correcting for the genomic inflation factor of 1.25 using the genomic controls method, none of the SNPs associated with SC reached genome-wide significance.

Most GWAS studies do not employ family-based data; thus, there are inherent challenges in estimating the power of the sample to detect particular effect sizes. However, the implementation of a correlation matrix subdivided by family clusters to control for relatedness among the families pares symptom count analysis to an association test similar to a case-control study, as do the generalized estimating equations (GEE) used to analyze alcohol dependence. Both methods provide similar power estimates based on simulation studies[30,31]. For this sample, we estimated power using Quanto[32]. These extended pedigrees have 70% power to detect an effect size of 1.1 for symptom count when the minor allele frequency is between 0.10 and 0.30.

The strongest association (inflation corrected $9.4\times10^{-7}\leq p\leq 9.8\times10^{-6}$) was detected with a group of 18 highly correlated variants ($r^2\geq=0.95$) within and flanking *C15orf53*. Interestingly, recent GWAS data has reported consistent evidence showing that variation in a region close to *C15orf53* influences susceptibility for bipolar disorder [33–36]. SNPs that influenced bipolar disorder susceptibility (rs12912251, rs2172835, and rs12899449) also show strong association with SC (inflation corrected $p=9.4\times10^{-7}$, $p=3.1\times10^{-6}$, and $p=1.26\times10^{-5}$, respectively) in COGA families severely affected by AD. The alleles that are associated with reduced risk for BD are also associated with lower dependence symptom counts. We did not detect association between symptom counts and other variants reported by the Psychiatric GWAS Consortium Bipolar Disorder Working Group (2011) to be strongly associated with bipolar disorder. This suggests a specific effect of this gene on risk for bipolar disorder and alcohol dependence rather than a more general shared underlying liability to both disorders. Studies of psychiatric disorders have shown that bipolar disorder and alcoholism commonly co-occur[37–39], and that individuals with bipolar disorder have a greater likelihood of AUDs than the general population[40]. Approximately 46% of subjects with bipolar disorder type I have AUDs, while 39% of subjects with a less severe form of mania, bipolar disorder II, have an AUDs[37–39]. The association of *C15orf53* with SC detected in this study suggests the possibility of a specific genetic link between bipolar disorder and alcoholism.

Two of the imputed SNPs showing strong association with SC are located within the exons of *C15orf53* (Figure 3). *C15orf53* encodes an uncharacterized protein of 179 amino acids with homology to uncharacterized proteins in other species including chimpanzee, gibbon and orangutan. Rs7165988 in exon 1 results in a non-synonymous coding change of valine to leucine at codon 3. This substitution is predicted to be possibly damaging by POLYPHEN[41]. Rs12916379 is located within the 3′ UTR of *C15orf53* and could influence transcript stability. Our preliminary data showed that *C15orf53* mRNA expression is detectable in 9 brain regions tested though the expression level is low. The method of this assay is included in the supplementary information.

There are 5 additional candidate genes located in chromosomal regions showing association with SC (Table 1). Among these, *SLC9A9* encodes a sodium and hydrogen exchanger in chromosome 3q24, and is of particular interest as it has been linked to tobacco smoking initiation, a behavior highly comorbid with alcohol use[42]. Studies have repeatedly shown evidence of association between *SLC9A9* and attention deficit hyperactivity disorder (ADHD)[43–45]. Because inattention is a predictor of smoking initiation[46] and ADHD and smoking are co-transmitted through families more often than expected by chance[47], it is possible *SLC9A9* influences both smoking and inattention. The association of *SLC9A9* with SC identified in this study suggests a genetic connection between ADHD, smoking and alcoholism. Our preliminary data showed that *SLC9A9* mRNA expression is detectable in human frontal cortex. Total mRNA expression in brain tissues derived from alcoholics is 1.09 fold higher than in the brain tissues derived from non-alcoholic subjects. Another gene of interest, *CXCR7* in chromosome 2q37.3, encodes C-X-C chemokine receptor type 7, a

member of the G-protein coupled receptor family. Our preliminary assay with human frontal cortices showed *CXCR7* total mRNA expression is 1.33 fold higher in alcoholics, compared to the expression level in non-alcoholic subjects. The method of these assays is included in the supplementary information.

To date, four previous case-control GWAS analyses of AUDs have not provided evidence of association that reached genome-wide significance with AD or quantitative traits [9–12]. In this study our approach to identifying genetic risk factors for alcohol problems focused primarily on a quantitative measure of alcohol symptom count, rather than an AUD diagnosis. Our SC measure was based on 7 DSM-IV AD criteria and deliberately excluded the 4 criteria associated with DSM-IV alcohol abuse. We crafted this measure to allow the most straightforward comparisons between findings for SC and findings for diagnosis of dependence. Dimensional dependence measures such as SC are more powerful than dichotomized phenotypic measures (i.e. DSM-IV AD) for detecting risk factors, especially in samples containing adolescent subjects. In the present study, we compared chromosomal regions that showed strong associations with both SC and DSM-IV AD and consistently observed a stronger relationship for genetic variants with SC than with DSM-IV AD (Table 1, Figure 4); the effect sizes and directions for both phenotypes were relatively consistent.

Although we are encouraged by our findings, we recognize that there are limitations to our study. Among our top candidate genes, we only detected nominal association for *C15orf53* and SC in SAGE. Several reasons could possibly explain this limited replication. First, the power to replicate findings of small effect across studies in samples of the size used in this study is low. Second, in contrast to the COGA sample, neither the SAGE nor the OZALC samples were ascertained from large families severely affected by AUDs. It is possible that severely affected families have a concentration of genetic variants that influence risk for alcoholism and that may have less effect on alcoholism in the general population. A coordinated evaluation including many more families severely affected by alcoholism is necessary to confirm our findings.

In summary, our family-based GWAS identified SNPs in the gene *C15orf53* that showed suggestive evidence of association with DSM-IV alcohol dependence symptom count. Interestingly, SNPs in this gene have previously been associated with risk for bipolar disorder in other GWAS and suggest there may be some common genetic factors contributing to both disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Collaborative Study on the Genetics of Alcoholism (COGA)

COGA, Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes ten different centers: University of Connecticut (V. Hesselbrock); Indiana University (H.J. Edenberg, J. Nurnberger Jr., T. Foroud); University of Iowa (S. Kuperman, J. Kramer); SUNY Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, A. Goate, J. Rice, K. Bucholz); University of California at San Diego (M. Schuckit); Rutgers University (J. Tischfield); Southwest Foundation (L. Almasy), Howard University (R. Taylor) and Virginia Commonwealth University (D. Dick). A. Parsian and M. Reilly are the NIAAA Staff Collaborators. We continue to be inspired by our memories of Henri Begleiter and Theodore Reich, founding PI and Co-PI of COGA, and also owe a debt of gratitude to other past organizers of COGA, including Ting-Kai Li, currently a consultant with COGA, P. Michael Conneally, Raymond Crowe, and Wendy Reich, for their critical contributions. This national collaborative study is supported by NIH Grant U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA).

The Study of Addiction: Genetics and Environment (SAGE)

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The Australian Twin-family Study of Alcohol Use Disorder (OZALC) Sample

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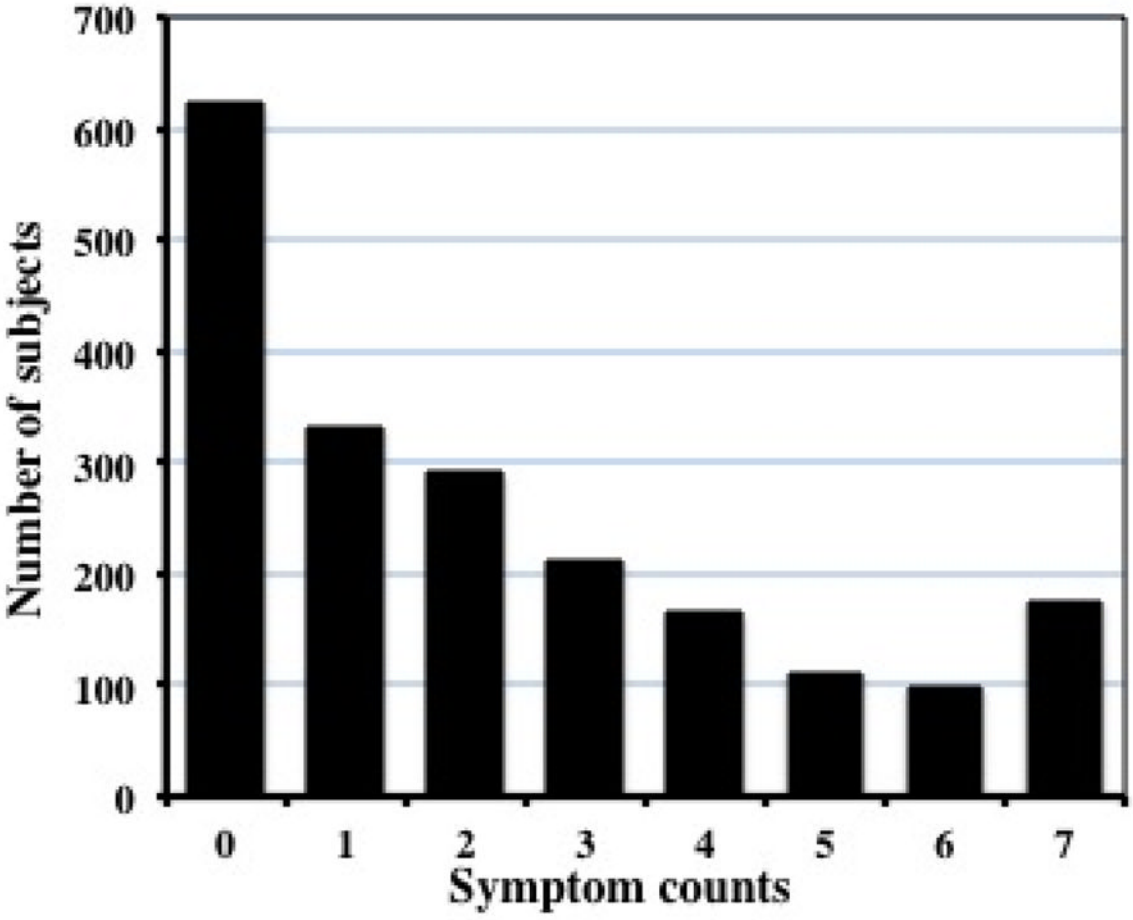

Figure 1. Distribution of DSM_IV alcohol dependence symptom count in the genotyped GWAS sample

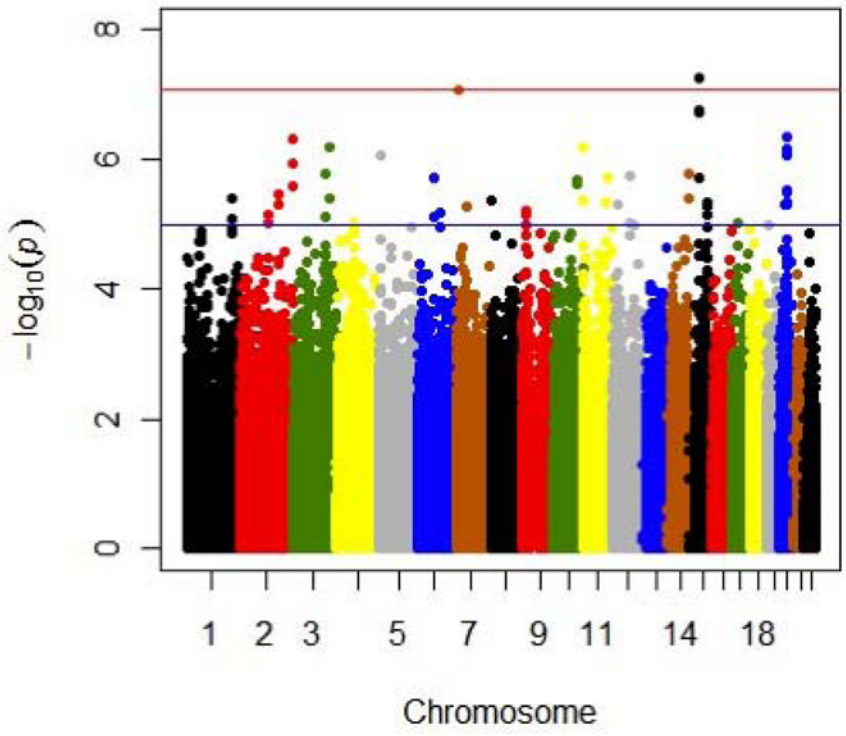

Figure 2. Manhattan plot of genome-wide association results for DSM-IV alcohol dependence symptom count using negative binomial analysis

−log10 values shown here were not corrected for inflation factor.

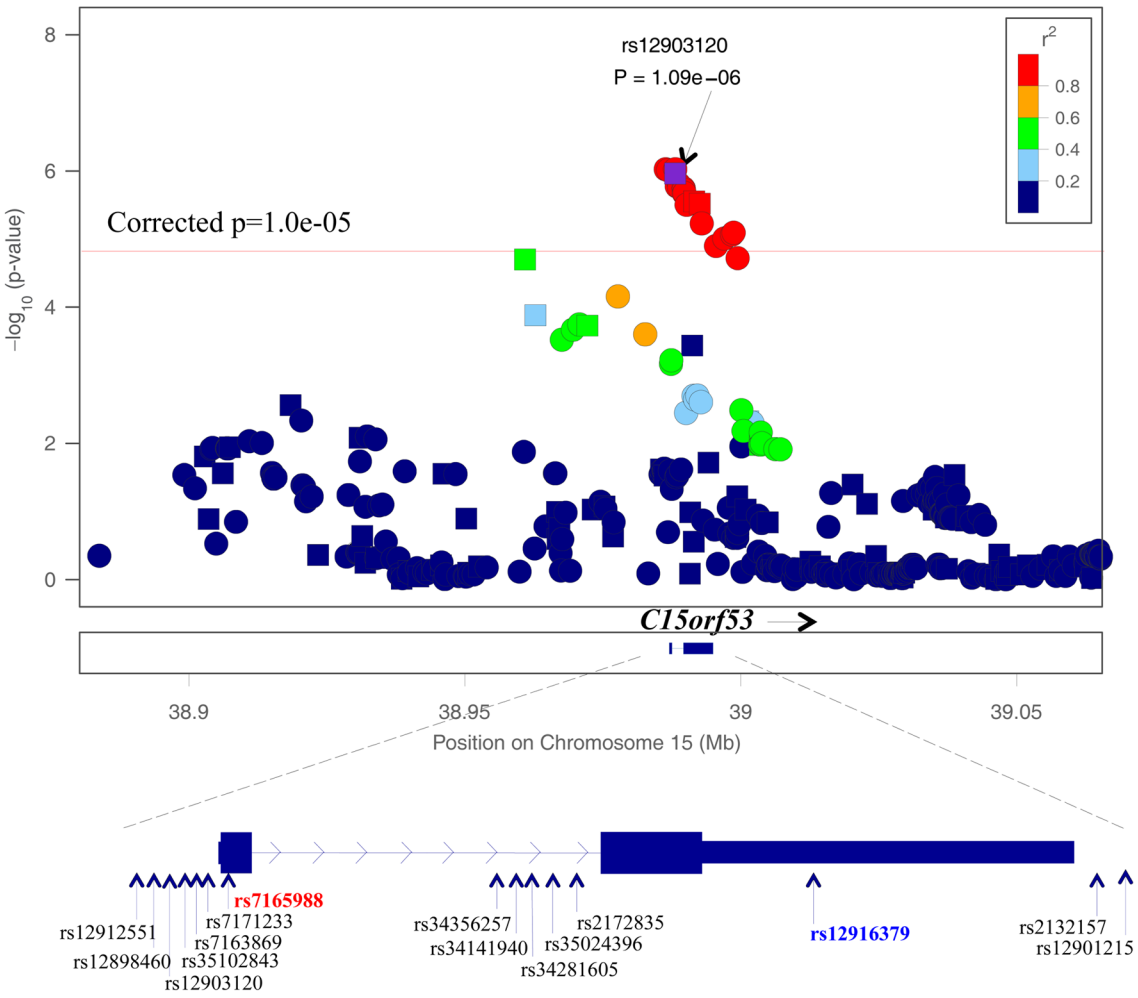

Figure 3. Plot of chromosome 15q14 association with DSM_IV alcohol dependence symptom count

Squares represent genotyped SNPs; Circles represent imputed SNPs. SNP rs7165988 (in red) is a non-synonymous coding variant. SNP rs12916379 (in blue) is in 3′un-translated region of *C15orf53*.

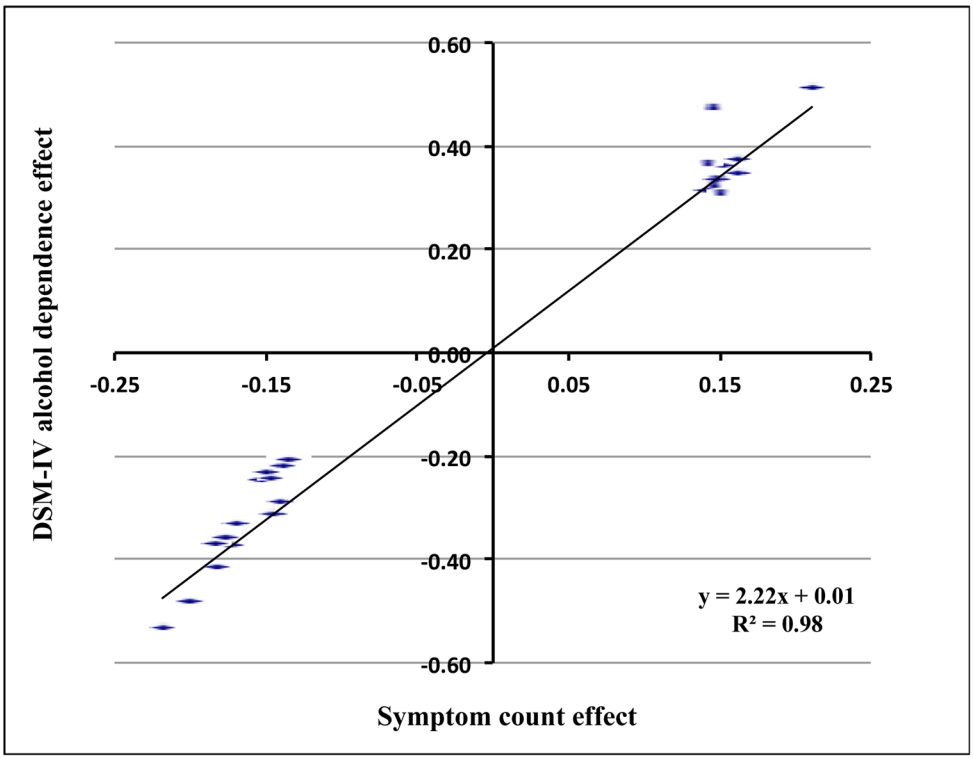

Figure 4. Correlation between symptom count effect and DSM-IV alcohol dependence effect in COGA sample

Table 1

Chromosome regions containing 3 or more SNPs within 50 kb of each other that are associated with symptom count at inflation corrected p<0.0001 and the comparison of association between alcohol dependence symptom count and DSM-IV alcohol dependence in these regions.

					Alcohol dependence symptom count			DSM-IV alcohol dependence	
Chromosome location	**SNP**	**Position (hg19)**	**Gene/Transcript**	**Gene/Transcript position**	**Effect**	**p value**	**Corrected p value**	**Effect**	**p value**
1q32.3	rs612414	212602176	NENF	212606229---212619721	−0.15	1.07E-05	7.85E-05	−0.26	2.25E-03
	rs583058	212610755	NENF		−0.13	1.42E-05	9.92E-05	−0.23	9.44E-04
	rs4804	212619339	NENF		−0.14	8.36E-06	6.44E-05	−0.24	7.15E-04
	rs483954	212620214	NENF		−0.15	3.90E-06	3.47E-05	−0.27	6.30E-04
2q37.3	rs896543	237509207	CXCR7	237478380---237490997	−0.22	4.75E-07	6.29E-06	−0.56	4.35E-06
	rs6431476	237517937	CXCR7		−0.20	1.14E-06	1.28E-05	−0.51	3.92E-06
	rs7594454	237537935	CXCR7		−0.18	2.60E-06	2.49E-05	−0.44	3.05E-05
3q24	rs7431637	143049769	SLC9A9	142984064---143567373	−0.14	7.58E-06	5.95E-05	−0.31	3.60E-04
	rs10446322	143068250	SLC9A9		−0.15	1.73E-06	1.80E-05	−0.33	5.77E-05
	rs868702	143085345	SLC9A9		0.15	1.73E-06	1.79E-05	0.34	2.58E-05
4q21.21	rs12513014	81061422	intergenic		0.16	1.38E-05	9.70E-05	0.35	1.02E-03
	rs13102102	81073672	intergenic		0.14	1.11E-05	8.12E-05	0.35	1.37E-05
	rs13138779	81087073	intergenic		0.21	9.75E-06	7.30E-05	0.49	7.69E-04
9p22.2	rs10963462	18130036	intergenic		−0.18	6.22E-06	5.07E-05	−0.40	1.18E-05
	rs763976	18134914	intergenic		−0.17	1.00E-05	7.46E-05	−0.40	7.40E-06
	rs12006002	18166899	intergenic		0.15	7.24E-06	5.73E-05	0.45	4.87E-07
15q14	rs7168475	38960882	C15orf53	38988799---38992239	−0.18	1.98E-06	2.00E-05	−0.39	4.66E-04
	rs12903120	38988097	C15orf53		−0.18	5.45E-08	1.09E-06	−0.38	7.62E-06
	rs12916379	38991520	C15orf53		−0.17	1.74E-07	2.79E-06	−0.36	4.06E-05
	rs2132157	38992547	C15orf53		−0.17	1.92E-07	3.02E-06	−0.36	4.35E-05
15q24.2	rs2029519	75415962	intergenic		0.14	1.07E-05	7.87E-05	0.29	1.60E-03
	rs4479194	75422131	intergenic		0.15	5.27E-06	4.43E-05	0.30	1.02E-03
	rs7172677	75424593	intergenic		0.15	7.15E-06	5.68E-05	0.32	5.35E-04

					Alcohol dependence symptom count			DSM-IV alcohol dependence	
Chromosome location	**SNP**	**Position (hg19)**	**Gene/Transcript**	**Gene/Transcript position**	**Effect**	**p value**	**Corrected p value**	**Effect**	**p value**
20q11.22	rs6060124	33536897	GSS	33516236---33543601	0.16	4.41E-07	5.92E-06	0.33	2.03E-04
	rs6088664	33551100	MYH7B	33543704---33590240	−0.15	3.23E-06	2.98E-05	−0.27	1.91E-03
	rs6579204	33553677	MYH7B		0.15	4.54E-06	3.93E-05	0.29	1.25E-03