

Genome-Wide Association Study of Alcohol Dependence Implicates a Region on Chromosome 11

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Background: Alcohol dependence is a complex disease, and although linkage and candidate gene studies have identified several genes associated with the risk for alcoholism, these explain only a portion of the risk.

Methods: We carried out a genome-wide association study (GWAS) on a case-control sample drawn from the families in the Collaborative Study on the Genetics of Alcoholism. The cases all met diagnostic criteria for alcohol dependence according to the *Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition*; controls all consumed alcohol but were not dependent on alcohol or illicit drugs. To prioritize among the strongest candidates, we genotyped most of the top 199 single nucleotide polymorphisms (SNPs) ($p \leq 2.1 \times 10^{-4}$) in a sample of alcohol-dependent families and performed pedigree-based association analysis. We also examined whether the genes harboring the top SNPs were expressed in human brain or were differentially expressed in the presence of ethanol in lymphoblastoid cells.

Results: Although no single SNP met genome-wide criteria for significance, there were several clusters of SNPs that provided mutual support. Combining evidence from the case-control study, the follow-up in families, and gene expression provided strongest support for the association of a cluster of genes on chromosome 11 (*SLC22A18*, *PHLDA2*, *NAP1L4*, *SNORA54*, *CARS*, and *OSBPL5*) with alcohol dependence. Several SNPs nominated as candidates in earlier GWAS studies replicated in ours, including *CPE*, *DNASE2B*, *SLC10A2*, *ARL6IP5*, *ID4*, *GATA4*, *SYNE1*, and *ADCY3*.

Conclusions: We have identified several promising associations that warrant further examination in independent samples.

Key Words: Alcohol Dependence, Genome-Wide Association Study, Case-Control Study, Family Study, Gene Expression.

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ALCOHOL DEPENDENCE (ALCOHOLISM) is a major health and social issue affecting 4 to 5% of the United States population at any given time (Li et al., 2007), with a lifetime prevalence of 12.5% (Hasin et al., 2007). Alcohol dependence is characterized by serious problems in multiple domains. Family, twin, and adoption studies have consistently demonstrated a substantial genetic contribution to disease etiology (Kendler et al., 1994; McGue, 1999; Nurnberger et al., 2004; Pickens et al., 1991). Alcohol dependence is a complex disease in which both genetic and environmental factors affect susceptibility.

Strategies for identifying genes in which variations contribute to the risk for alcohol dependence have employed linkage analysis or candidate gene approaches (Edenberg and Foroud, 2006). These methods have led to the identification of several genes associated with alcohol dependence, including *GABRA2* (Covault et al., 2004; Drgon et al., 2006; Edenberg et al., 2004; Enoch et al., 2006, 2008; Fehr et al., 2006; Lappalainen

et al., 2005; Lind et al., 2008; Soyka et al., 2008), *ADH4* (Edenberg and Foroud, 2006; Edenberg et al., 2006; Guindalini et al., 2005; Kuo et al., 2008; Luo et al., 2005b, 2006), *GABRG3* (Dick et al., 2004), *CHRM2* (Luo et al., 2005a; Wang et al., 2004), *NFKB1* (Edenberg et al., 2008b), *OPRK1* (Edenberg et al., 2008a; Xuei et al., 2006), *PDYN* (Xuei et al., 2006), *NPY2R* (Wetherill et al., 2008), *ANKKI/DRD2* (Dick et al., 2007b); *CHRNA5* (Wang et al., 2009); *GRM8* (Chen et al., 2009), *TACR3* (Foroud et al., 2008), and *GABRR2* (Xuei et al., 2010). However, the effect of variation in each of these genes is small, and much of the genetic contribution to the risk for alcoholism remains to be discovered.

We completed a genome-wide association study (GWAS) to identify genes contributing to alcohol dependence, defined according to *Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV)* criteria (American Psychiatric Association, 1994). Both the alcohol-dependent cases and the controls have completed a rigorous clinical evaluation, with controls being drinkers who are free from alcohol dependence or abuse by several diagnostic classification systems (DSM-III-R, DSM-IV, ICD-10) (American Psychiatric Association, 1987, 1994; World Health Organization, 1993), and also free of dependence on illicit drugs. To prioritize among the most promising single nucleotide polymorphisms (SNPs) identified from the GWAS, we genotyped the top SNPs in a sample of alcohol-dependent families and performed family-based association analysis, and tested whether they fell within or near genes whose expression is affected by alcohol exposure and were expressed in brain.

MATERIALS AND METHODS

Ascertainment of Subjects

Alcohol-dependent probands were ascertained through alcohol treatment programs and evaluated at 7 centers in the United States: Indiana University, State University of New York Health Science Center Brooklyn, University of Connecticut, University of Iowa, University of California/San Diego, Washington University/St Louis, and Howard University as part of the Collaborative Study on the Genetics of Alcoholism (Begleiter et al., 1995; Edenberg, 2002; Foroud et al., 2000; Reich et al., 1998). The institutional review boards of all participating institutions approved the study. After providing informed consent, the probands and their relatives were administered a validated poly-diagnostic instrument, the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994; Hesselbrock et al., 1999), which allows assessment of alcohol dependence under several diagnostic systems. Details of the ascertainment and assessment have previously been published (Begleiter et al., 1995; Foroud et al., 2000; Reich et al., 1998) and are available at zork.wustl.edu/niaaa/coga_instruments/resources.html.

The same 7 centers also recruited community probands through driver's license records, random mailings to employees and students at a university, and attendees at medical and dental clinics. After providing informed consent, the community probands and their relatives were administered the same SSAGA interview as the alcohol-dependent probands and their families.

Genome-Wide Association Study

Case-Control Sample Selection. For the GWAS, a sample of genetically unrelated cases and controls were selected from this pool

of alcohol-dependent and community-ascertained families. Cases were all alcohol dependent by DSM-IV criteria at some time during their lives and were selected from the families ascertained through alcohol-dependent probands. The selected controls were required to have consumed alcohol but not to have a diagnosis of alcohol abuse, dependence or harmful use by any of the 4 diagnostic systems assessed in the SSAGA (Feighner, DSM-III-R, DSM-IV, ICD-10) at any time in their lives. Because there might be shared genetic vulnerability with other substance use disorders, the selected controls also could not meet criteria for DSM-III-R or DSM-IV diagnoses of abuse or dependence on cocaine, marijuana, opioids, sedatives, or stimulants. Controls were selected from both the community-recruited families and the families recruited through an alcohol-dependent proband, but could not share a known common ancestor with a case. Controls older than 25 years were preferentially selected, to increase the probability that they were beyond the peak age of risk for developing alcohol dependence. A subset of the COGA participants were assessed on multiple occasions; if a subject was assessed more than once, only one whose diagnosis or lack thereof was consistent across time was selected.

Microarray Genotyping, Quality Assessment, and Statistical Analysis. Genotyping was performed by the Center for Inherited Disease Research (CIDR). DNA sources included blood ($n = 1453$) and lymphoblastoid cell lines (LCL, $n = 492$). Genotyping was performed using the Illumina Infinium II assay protocol (Gunderson et al., 2006) with hybridization to Illumina HumanHap1M Bead-Chips (Illumina, San Diego, CA), which contain 1,199,187 markers with a mean spacing of 2.4 kb. Allele cluster definitions for each SNP were determined using Illumina BeadStudio Genotyping Module version 3.1.14 and the combined intensity data from 96% of study samples. The resulting cluster definition file was used on all study samples to determine genotype calls and quality scores. Genotype calls were made when a genotype yielded a quality score (Gencall value) of 0.15 or higher. The final raw dataset released by CIDR to the investigators and to dbGaP contained 1,041,465 SNPs for 1,945 unique DNA samples from case and control subjects. Twenty-seven samples were removed because of poor sample quality before release of the dataset on dbGaP (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap>; Accession number: phs000125.v1.p1). Blind duplicate reproducibility was 99.97%.

Starting with the dbGaP data, additional quality control measures were applied to both the samples and the SNPs. Samples having genotypes for at least 98% of the SNPs were considered for inclusion in analyses. These samples were rigorously checked for cryptic relatedness, population stratification, and related issues. Thirteen additional samples were removed from further analyses because of poor sample quality ($n = 4$) or cryptic relatedness among subjects ($n = 9$). A principal component-based analysis was performed in PLINK (Purcell et al., 2007) to cluster these samples along with HapMap reference samples (CEU, YRI, CHB, and JPT) to assign the study subjects to groups of predominantly European and African ancestry. The final European American (EA) sample included 847 alcohol-dependent cases and 552 controls ($n = 1,399$ individuals). The African American (AA) sample contained 345 cases and 140 controls ($n = 485$ individuals). The remaining 21 individuals did not cluster with either of the 2 samples and were not analyzed.

SNPs with a call rate of 98% or greater in the EAs ($n = 1,015,550$) were included and subjected to further quality control. From these, SNPs were removed if the minor allele frequency was less than 0.01 in the combined case and control dataset ($n = 161,048$) or if among those with minor allele frequency ≥ 0.01 there was significant deviation from Hardy Weinberg equilibrium ($p < 10^{-4}$) in the controls ($n = 1,127$). The final dataset for analysis of EAs consisted of 853,375 SNPs that passed all quality control measures. Similar quality control thresholds were applied to the 1,010,230 SNPs with a call rate of 98% or greater in the AAs. This

resulted in removal of 68,600 SNPs with a minor allele frequency below 0.01 in the combined AA case and control sample and 332 SNPs that demonstrated significant deviation from Hardy Weinberg equilibrium in AA controls. The final dataset for the AA sample consisted of 941,298 SNPs. Positions of all SNPs are from dbSNP build 130, genome build 36.3.

Given the limited number of non-EA subjects in the sample, and that self-defined ethnicity carries nongenetic as well as genetic differences, we focused our primary analyses on the larger EA subsample. Logistic regression covarying for gender was employed to test for the association of each SNP with alcohol dependence, using an additive model. Odds ratios and *p* values were computed to assess the strength of the association. All analyses were performed using PLINK (Purcell et al., 2007).

Previously, stronger association results were often obtained when the alcohol-dependent cases were limited to those with greater correlates of disease severity, such as earlier age of onset, comorbid drug dependence, or higher likelihood of conduct disorder symptoms (Agrawal et al., 2006; Dick et al., 2007a; Edenberg et al., 2008b; Foroud et al., 2008). We performed secondary analyses to identify potential associations with early-onset alcohol dependence. A median split of our sample (Table 1) was performed and those alcohol-dependent cases with an onset of 22 years or younger (*n* = 454) were classified as having earlier onset of alcohol dependence. Logistic regression using the additive model with sex as a covariate was employed to test for association between the genotyped SNPs and the phenotype of early-onset alcohol dependence.

Family-Based Replication Study

Family Sample. Previously, a set of 262 genetically informative, multiplex alcohol dependence pedigrees, each with at least 3 first degree relatives who met lifetime criteria for both DSM-III-R alcohol dependence (American Psychiatric Association, 1987) and Feighner definite alcoholism (Feighner et al., 1972) were selected from the larger COGA sample and used in linkage and family-based association studies (Edenberg and Foroud, 2006; Edenberg et al., 2004; Foroud et al., 2000; Reich et al., 1998); 59 additional trios were also genotyped. After reviewing the results from the primary analysis of DSM-IV alcohol dependence, the 199 SNPs with the smallest *p* values for association ($p \leq 2.1 \times 10^{-4}$) were selected for genotyping in this family-based sample. Genotypes were completed on 180 of these SNPs; the others did not design into assays or did not provide useable data.

Some members of these families, primarily probands, were included in the GWAS case control sample. There were 426 individuals included in the EA GWAS sample (326 cases; 100 controls) that were also a member of the families used for replication. The analytic methods applied to detect association in the case control and family-

based tests of association are statistically independent (Abecasis et al., 2000; Vogel et al., 2009; Zuo et al., 2009).

Genotyping, Quality Assessment, and Statistical Analysis. Genotyping assays were designed for the Sequenom MassArray system (Sequenom, San Diego, CA) using MassArray Assay Design Software. Genotyping used iPLEX Gold assays (Sequenom), with alleles discriminated by mass spectrometry. Assays were tested on 2 independent groups, 40 unrelated EA individuals and 40 unrelated AA individuals obtained from Coriell Cell Repositories. SNPs that were not in Hardy Weinberg equilibrium in both groups were not genotyped in the COGA families. All SNPs were tested for Mendelian inheritance using the program PEDCHECK (O'Connell and Weeks, 1998).

Family-based association analyses were performed using the pedigree disequilibrium test (PDT) (Martin et al., 2000) as implemented in the program UNPHASED (version 2.404) (Dudbridge, 2003). Affected individuals were defined as those meeting criteria for DSM-IV alcohol dependence. The PDT utilizes data from all available trios in a family, as well as discordant sibships. We report results using the PDT average option, which weights each family equally in computing the overall test statistic. Analysis was also performed classifying as affected only those DSM-IV alcohol dependence cases who also met criteria for early onset, defined as with the case control sample, as onset by age 22 or younger. After examining results in the family sample, 5 additional SNPs (cSNPs or SNPs in 3' UTRs) from regions with evidence for replication were selected for genotyping in the family sample.

Gene Expression Analyses

In a separate study, we are analyzing the effects of ethanol exposure on gene expression in immortalized LCLs. Details of the gene expression studies will be reported separately; here, we note whether there was an effect of alcohol exposure on the expression of any of our top candidate genes. Briefly, 42 LCLs from subjects in the COGA study were either treated for 24 hour with 75 mM ethanol or left untreated. The RNA was extracted and purified, and aliquots were analyzed on Affymetrix GeneChip® Human Genome U133 Plus 2.0 microarrays following standard procedures. Results were analyzed by ANOVA with factors of treatment, phenotype (cells from alcoholics or controls), and sex (Edenberg et al., in preparation). False discovery rates (FDR) were calculated using the Storey *q* value package (Storey and Tibshirani, 2003).

Gene expression was assayed using standard Affymetrix procedures on samples from 9 different brain regions (prefrontal cortex, cerebral cortex, thalamus, visual cortex, hippocampus, amygdala, caudate, putamen, and cerebellum) of 4 individuals (1 each male

Table 1. Demographics of the GWAS Sample

	Alcohol-dependent cases		Controls	
	EA	AA	EA	AA
Number	847	345	552	140
Gender (% female)	30.3	35.9	71.6	71.4
Alcohol dependent	847	345	0	0
Age at onset of dependence, median (25%ile, 75%ile)	22 (18, 27)	25 (20, 31)	NA*	NA*
Age at last interview, median (25%ile, 75%ile)	NA*	NA*	47 (38, 55)	39 (32, 49)
Marijuana dependent (%)	34.7	33.9	0	0
Cocaine dependent (%)	35.1	56.2	0	0
Dependent on other illicit substances (opioids, stimulants, sedatives) (%)	29.5	13	0	0

EA, European American; AA, African American.

*Not applicable.

alcoholic, female alcoholic, male control, female control), obtained from the National Institute on Alcohol Abuse and Alcoholism (NIAAA)-supported brain bank at the Tissue Resource Center located in the Neuropathology Unit of the Department of Pathology, University of Sydney, Australia. Hybridization was to GeneChip® Human Gene 1.0 ST Arrays. Genes were presumed expressed in brain if the average expression level of the 4 arrays from any region was higher than the average expression level of the negative probe sets on the arrays (Edenberg et al., in preparation). Genes from the expression studies were matched to genes from the SNP microarrays by matching gene symbols based on Affymetrix annotations.

RESULTS

Association Analysis in the GWAS Case–Control Sample

The final EA case–control analytic sample included 1399 individuals. Cases all met lifetime DSM-IV criteria for alcohol dependence; many were also dependent upon other drugs (Table 1). Controls all had consumed alcohol, but were not affected by alcohol or drug abuse or dependence; most were well past the primary age of risk for alcohol or illicit drug disorders (Table 1). Thus the controls provide a strong phenotypic contrast to the cases.

Association of the SNPs with alcohol dependence was carried out employing an additive model, with sex as a covariate. The λ value (inflation factor) after these adjustments was 1.049; the Q–Q plot for this analysis is shown in Fig. 1. None of the SNPs analyzed met conventional criteria for genome-wide significance. However, eleven SNPs had p values $< 10^{-5}$, and a total of 93 SNPs had p values $\leq 10^{-4}$ (Table S1 shows data from all SNPs with $p \leq 10^{-3}$). There were many genes or

regions in which surrounding SNPs provided additional support for association (Table S1). A region of chromosome 11 (Fig. 2A) had many SNPs among those with lowest p values.

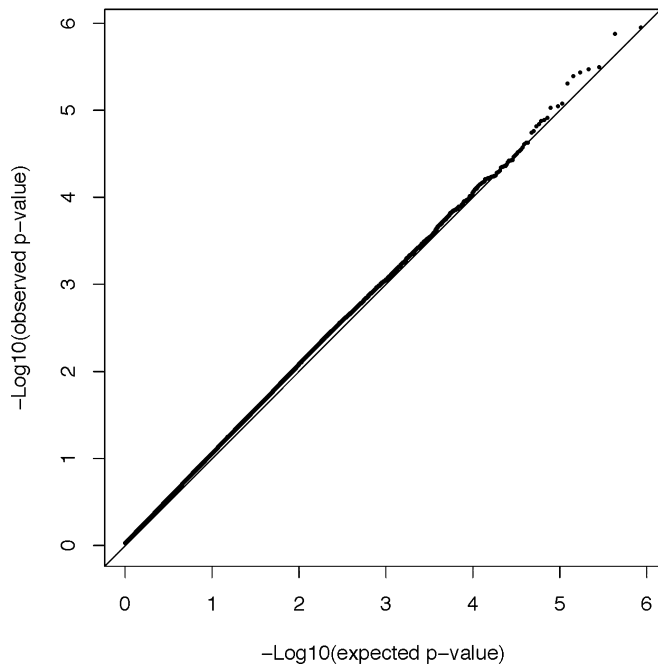


Fig. 1. Q–Q plot for association analysis of the alcohol dependence phenotype. Observed log p values (black dots) and expected log p values (line) are plotted versus the expected log p value (x-axis).

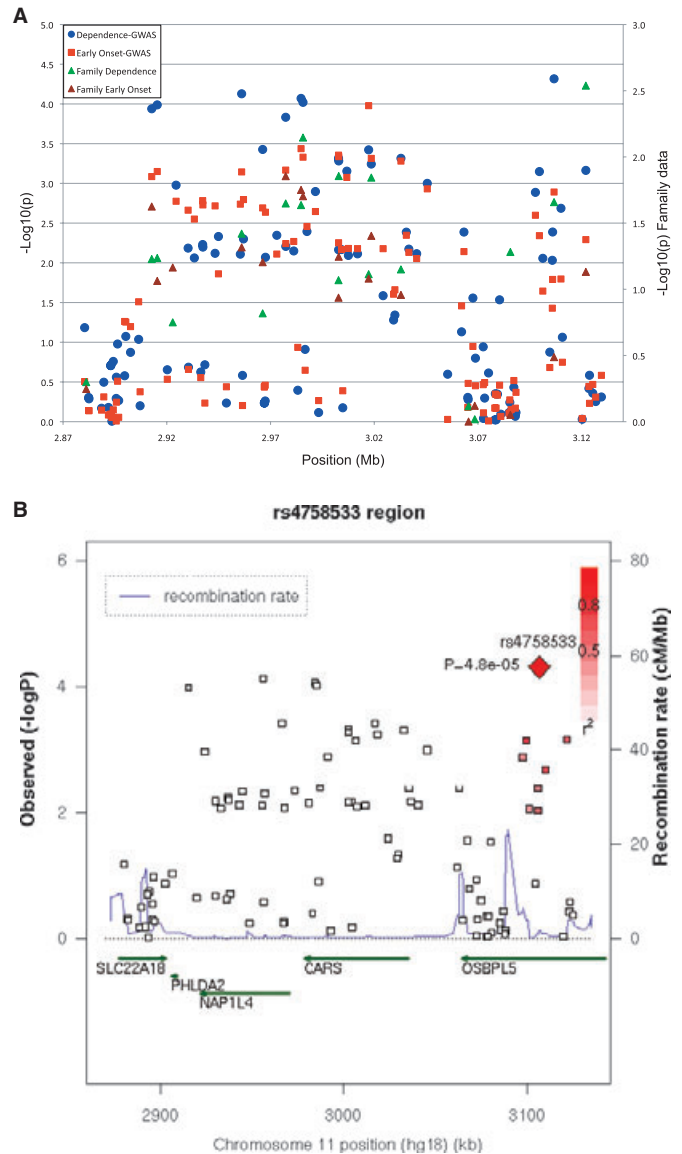


Fig. 2. Chromosome 11 region with supporting evidence for association. (A) GWAS data from primary analysis and analysis of early onset and data from family analysis. All single nucleotide polymorphisms (SNPs) are plotted as $-\log_{10}(p$ value) versus position along the chromosome; circles = GWAS alcohol dependence, squares are GWAS early onset; for GWAS data, the scale is on the left. Family data are also presented for those SNPs tested; green triangles are alcohol dependence, brown triangles are early onset; for the family data, the scale is on the right. (B) LD between the SNP with lowest p value and other SNPs in the region, depicted in a SNAP plot (Johnson et al., 2008). Along the x-axis is the physical position in the region (in kb) with known genes shown in their orientation. The left Y-axis denotes the association test result as $-\log(p$ value) corresponding to squares in the figure. The red diamond identifies the primary SNP result labeled with an rs# and p value. The darkness of the squares indicate the pairwise linkage disequilibrium with the primary SNP; the scale for this is shown in the top right corner. r^2 values were obtained from this study using Haploview (Barrett et al., 2005). The right Y-axis indicates the recombination rate, obtained from the available HapMap data in the CEPH Caucasians and shown within the figure by the solid blue line.

This was not because of a single SNP in high linkage disequilibrium (LD) with other SNPs; including the most significant SNP (rs4758533) as a covariate in the logistic regression model decreased the evidence of association with several SNPs near the right end but did not substantially alter the evidence of association with most of the SNPs in the region (see also Fig. 2B, which shows that most of the significant SNPs in the chromosome 11 region are not in high LD with rs4758533).

Secondary analysis tested for association within the subset of cases that met criteria for early onset of alcohol dependence (≤ 22 years; $n = 454$). No SNP met genome-wide significance. The SNPs with the smallest p values in the 2 analyses substantially overlap: 25 of the 100 SNPs with $p \leq 10^{-4}$ for alcohol dependence also had $p \leq 10^{-4}$ for early-onset alcohol dependence, despite the smaller sample size (Table S1). Notably, the odds ratios for the early-onset phenotype and the dependence phenotype are very similar: of the top 199 SNPs for dependence, 166 had odds ratios for both phenotypes within ± 0.10 of each other.

Data from the AA case control sample provided nominal evidence of association for DSM-IV alcohol dependence for 6 SNPs among the top 985 from European Americans; these were in *TMEM132C*, *EPHA7*, *OPA3*, *KCNMA1* (encoding a potassium large conductance calcium-activated channel important in neuronal excitability), *DMRTA2* and *SPTA1* (Table S1). *EPHA7* encodes ephrin receptor A7; ephrin receptors are tyrosine kinases implicated in the development of the nervous system. A secondary analysis of the smaller AA sample showed a different set of SNPs that provided evidence for association with alcohol dependence (Table S2). Among the 790 SNPs at $p \leq 10^{-3}$, 41 were nominally significant in the EA group; prominent among them were 7 SNPs (several, but not all, in high LD) in the region of *SELL* and *SELE*, 2 selectins (adhesion/homing receptors), 4 in *LOC91431*, encoding the protein prematurely terminated mRNA decay factor-like, and SNPs in *PPARG* (peroxisome proliferator-activated receptor gamma), *CTNN2* (catenin, alpha 2). Also of note is a SNP between the leptin receptor (*LEPR*) and *PDE4B*, a cAMP-specific phosphodiesterase that has been implicated in schizophrenia and bipolar disorder.

Results of Family-Based Association Analysis

Ten of the 180 SNPs genotyped in the sample of 262 genetically informative, alcohol-dependent families provided nominally significant ($p \leq 0.05$) evidence of association with alcohol dependence (Tables 2 and S1); 8 of these were also nominally significant for the early-onset phenotype. Five of these SNPs lie in 1 region of chromosome 11 (Fig. 2A), which contained *CARS* (all 3 tested SNPs replicated), *OSBPL5*, *NAP1L4*. The other SNPs were in *BBX*, *SLC9A8*, *OPA3*, *TOX2*, and near *CD53*. Several additional SNPs near other clusters of significant SNPs were also genotyped and also provided evidence for association (Table S1), including

additional SNPs in and near *CARS*, *OSBPL5*, *SLC9A8*, *SLC2A14*, *SOX5*, *COL8A1*, and *ADCK1*.

Fifteen of the SNPs with the smallest p values for alcohol dependence ($p \leq 2.1 \times 10^{-4}$) provided nominal evidence of association in the family sample with the early-onset alcohol dependence phenotype. In addition to the SNPs noted earlier, these included *SLC37A3*, *KCNMA1*, *CDH8*, *ZNF608*, and *API5*. A few candidate genes in which a SNP had $p \leq 2.1 \times 10^{-4}$ in the GWAS or cluster of SNPs with $p \leq 10^{-3}$ were also tested in families, and significant support was found for *CAT* and *GRIN2C* (Table S1).

Expression of the Genes Containing the More Significant SNPs

We also tested whether the genes associated with alcohol dependence were differentially affected by ethanol exposure in LCLs. Forty of the SNPs listed in Table 2 were in genes expressed in brain and also significantly affected by ethanol in LCLs. One of the SNPs in *CARS*, rs729662 (synonymous, Pro706 in exon 19), was ranked 80 in the GWAS and was also significant in the family data (Table S1).

DISCUSSION

Although the overall number of subjects was modest for a GWAS, the sample studied here had extensive phenotypic characterization and a strong contrast in substance dependence between affected and unaffected subjects. The affected subjects all met DSM-IV criteria for alcohol dependence, whereas the controls did not meet criteria for alcohol dependence or abuse or harmful use, nor were they dependent on other illicit drugs. Many of the affected subjects (56.5%) were also dependent upon an illicit drug; while it is possible that risk for a more general substance dependence contributed to the association signal, testing for this was not judged to be feasible, given the sample size and reduction in power that would result. None of the SNPs tested met conventional criteria for genome-wide significance, probably because of the modest size of the sample. Therefore, it was important to use other lines of evidence to prioritize potential candidates. Regions in which several SNPs provided evidence for association were found. Evidence of association, with similar estimates of effect size (odds ratios), was found for many SNPs when the sample of alcohol-dependent individuals was dichotomized and only those with early-onset alcohol dependence were defined as cases. Early onset generally marks a group of more severe cases, often with comorbid psychiatric problems. Analyses of this subgroup have yielded smaller p values, despite the reduced sample size, in some previous studies (Agrawal et al., 2006; Dick et al., 2007a; Edenberg et al., 2008b; Foroud et al., 2008). We assessed segregation within alcohol-dependent families as a means to prioritize among our SNP results. We also used gene expression data as additional lines of support, testing whether a gene in or nearest the SNP was expressed in human brain samples. We tested

Table 2. SNPs and Genes With Supporting Evidence

SNP	CHR	Position	Gene within 15 kb	European American case-control				Family sample (PDT)				Gene expression				African American case-control		
				AD (p value)	AD OR	Early onset (p value)	Early onset AD OR	AD (p value)	Early onset AD (p value)	Expr in brain	FOLD Et/C	FDR	MAF	AD (p value)	AD OR			
				MAF	AD OR	AD (p value)	AD OR	AD (p value)	AD OR	Yes	Yes	Yes	Yes	Yes	Yes	Yes		
rs3010931	1	12,606,911	DHRS3	3.9E-04	0.5	1.9E-03	0.5	0.04	3.9E-04	0.5	1.9E-03	0.5	1.22	2.9E-03	0.25	3.7E-01	1.2	
rs343819	1	111,144,013		1.5E-04	0.7	8.0E-06	0.6	0.27	1.5E-04	0.7	8.0E-06	0.6	1.04	1.8E-03	0.25	9.4E-01	1.0	
rs6540681	1	209,560,196	RCOR3 TRAF5	6.8E-04	0.7	1.2E-02	0.7	0.16	6.8E-04	0.7	1.2E-02	0.7	-1.28	5.6E-04	0.17	6.0E-01	0.9	
rs12471136	2	72,642,825	SEC15L2	8.0E-04	1.6	4.3E-03	1.6	0.11	8.0E-04	1.6	4.3E-03	1.6	1.34	7.7E-16	0.21	8.4E-01	1.0	
rs13410476	2	161,118,788		1.2E-04	0.7	3.1E-03	0.7	0.27	1.2E-04	0.7	3.1E-03	0.7	1.21	1.6E-07	0.40	4.0E-01	1.1	
rs16849209	2	213,319,466	OXTR	5.0E-04	1.4	9.3E-03	1.4	0.23	5.0E-04	1.4	9.3E-03	1.4	-1.20	3.9E-02	0.27	5.8E-01	0.9	
rs237899	3	8,783,515	FHIT	2.2E-04	1.4	1.1E-03	1.4	0.37	2.2E-04	1.4	1.1E-03	1.4	1.36	1.8E-15	0.36	1.2E-01	0.8	
rs1735460	3	60,666,645		3.7E-05	0.3	3.4E-05	0.2	0.03	3.7E-05	0.3	3.4E-05	0.2	1.25	3.2E-07	0.08	5.5E-01	1.2	
rs2700667	3	100,568,496		4.0E-04	1.4	7.8E-03	1.3	0.27	4.0E-04	1.4	7.8E-03	1.3	No	0.43	0.43	3.1E-01	1.2	
rs2700586	3	100,577,480		3.5E-04	1.4	7.0E-03	1.3	0.27	3.5E-04	1.4	7.0E-03	1.3	No	0.43	0.43	2.7E-01	1.2	
rs2200019	3	100,578,586		4.1E-04	1.4	8.3E-03	1.3	0.30	4.1E-04	1.4	8.3E-03	1.3	No	0.32	0.32	9.7E-01	1.0	
rs2700648	3	100,590,373		8.2E-04	1.4	1.2E-02	1.3	0.26	8.2E-04	1.4	1.2E-02	1.3	No	0.44	0.44	6.3E-01	0.9	
rs10511260	3	108,715,787	BBX	3.4E-06	0.6	4.7E-04	0.7	0.29	3.4E-06	0.6	4.7E-04	0.7	Yes	1.13	8.5E-05	0.06	9.6E-01	1.0
rs11918092	3	135,996,046	EPHB1	9.7E-04	0.7	1.3E-03	0.6	0.13	9.7E-04	0.7	1.3E-03	0.6	Yes	1.21	8.6E-18	0.45	3.2E-01	1.2
rs4384980	3	183,941,763		3.2E-04	0.7	1.1E-03	0.7	0.42	3.2E-04	0.7	1.1E-03	0.7	Yes	1.22	8.9E-11	0.46	6.3E-01	0.9
rs10029539	4	31,401,050		3.1E-04	0.7	2.1E-02	0.8	0.49	3.1E-04	0.7	2.1E-02	0.8	Yes	1.52	1.1E-16	0.21	6.6E-01	1.1
rs6881283	5	91,091,413		5.6E-05	1.5	1.1E-04	1.6	0.24	5.6E-05	1.5	1.1E-04	1.6	Yes	1.52	1.1E-16	0.07	8.7E-01	1.0
rs9327332	5	123,917,480		1.4E-04	1.6	2.1E-03	1.5	0.16	1.4E-04	1.6	2.1E-03	1.5	Yes	1.25	1.2E-14	0.14	6.1E-01	1.1
rs4388209	5	125,786,272	GRAMD3	4.7E-04	0.7	1.1E-03	0.7	0.29	4.7E-04	0.7	1.1E-03	0.7	Yes	1.31	1.3E-14	0.25	6.0E-01	1.1
rs1883339	5	132,642,677		2.7E-04	0.5	2.6E-03	0.5	0.05	2.7E-04	0.5	2.6E-03	0.5	Yes	1.39	2.4E-07	0.01	1.9E-01	2.9
rs1982389	7	37,693,476		3.2E-04	0.5	3.2E-03	0.5	0.05	3.2E-04	0.5	3.2E-03	0.5	Yes	1.39	2.4E-07	0.30	1.9E-01	1.2
rs4728699	7	87,006,685	ABCY1	9.9E-04	0.5	1.6E-02	0.6	0.04	9.9E-04	0.5	1.6E-02	0.6	Yes	1.39	2.4E-07	0.01	1.9E-01	2.9
rs6955063	7	139,723,897	SLC37A3	4.0E-05	0.6	1.5E-03	0.6	0.10	4.0E-05	0.6	1.5E-03	0.6	Yes	1.39	2.4E-07	0.50	1.9E-01	1.2
rs17637986	8	6,621,112		5.5E-05	0.6	5.2E-05	0.5	0.09	5.5E-05	0.6	5.2E-05	0.5	Yes	1.39	2.4E-07	0.57	1.9E-01	1.2
rs2094372	9	77,186,279		1.1E-03	1.4	1.9E-03	1.4	0.25	1.1E-03	1.4	1.9E-03	1.4	Yes	-1.31	4.9E-14	0.03	7.6E-01	1.2
rs17527069	10	10,317,351	PRTFDC1	5.1E-04	0.7	1.7E-03	0.7	0.21	5.1E-04	0.7	1.7E-03	0.7	Yes	-1.30	1.8E-05	0.15	4.6E-01	1.2
rs7078551	10	25,214,732		6.1E-04	1.4	2.7E-03	1.4	0.32	6.1E-04	1.4	2.7E-03	1.4	Yes	-1.35	7.5E-11	0.03	4.8E-01	0.8
rs12219105	10	78,843,546	KCNMA1	1.5E-04	0.6	9.7E-05	0.6	0.14	1.5E-04	0.6	9.7E-05	0.6	Yes	-1.21	1.3E-07	0.25	3.1E-01	0.8
rs1963335	10	121,401,851	RPS8P4 BAG3	1.3E-04	1.7	1.9E-02	1.5	0.10	1.3E-04	1.7	1.9E-02	1.5	Yes	1.39	1.1E-06	0.13	5.3E-01	0.9
rs2583442	11	2,912,742	SLC22A18	1.2E-04	0.7	8.2E-04	0.7	0.30	1.2E-04	0.7	8.2E-04	0.7	Yes	1.14	4.2E-02	0.09	3.3E-01	0.8
			PHLDA2															
rs8505	11	2,922,869	NAP1L4-3' UTR	7.4E-05	0.7	7.2E-04	0.7	0.18	7.4E-05	0.7	7.2E-04	0.7	Yes	-1.08	9.2E-06	0.15	1.4E-01	0.7
rs3814964	11	2,956,083	NAP1L4					0.31					Yes					
			SNORA54										Yes					
rs4758621	11	2,966,216	NAP1L4 CARS	3.7E-04	0.7	2.0E-03	0.7	0.32	3.7E-04	0.7	2.0E-03	0.7	Yes		0.09	3.0E-01	0.8	
rs12805661	11	2,977,316	NAP1L4 CARS	1.5E-04	0.7	6.8E-04	0.7	0.29	1.5E-04	0.7	6.8E-04	0.7	Yes		0.09	3.0E-01	0.8	
rs729662	11	2,984,716	NAP1L4 CARS	8.5E-05	0.7	3.7E-04	0.7	0.29	8.5E-05	0.7	3.7E-04	0.7	Yes	1.03	5.0E-02	0.09	3.2E-01	0.8
rs7481584	11	2,985,665	CARS	9.5E-05	0.7	4.7E-04	0.7	0.29	9.5E-05	0.7	4.7E-04	0.7	Yes		0.13	1.2E-01	0.7	
rs377765	11	3,002,874	CARS	5.2E-04	0.7	4.6E-04	0.7	0.41	5.2E-04	0.7	4.6E-04	0.7	Yes		0.12	4.6E-01	0.8	
rs369461	11	3,018,568	CARS	5.7E-04	0.7	4.8E-04	0.7	0.40	5.7E-04	0.7	4.8E-04	0.7	Yes		0.10	2.8E-01	0.8	
rs2277301	11	3,068,442	OSBPL5-cSNP					0.96										
rs3741350	11	3,085,603	OSBPL5-cSNP					0.05										
rs4758533	11	3,106,662	OSBPL5	4.8E-05	0.7	1.3E-03	0.7	0.21	4.8E-05	0.7	1.3E-03	0.7	Yes		0.14	1.4E-01	0.7	
rs4468331	11	3,121,988	OSBPL5	6.9E-04	0.7	5.1E-03	0.7	0.20	6.9E-04	0.7	5.1E-03	0.7	Yes		0.16	2.3E-01	1.3	
rs11032702	11	34,424,699	CAT	5.2E-04	0.5	2.9E-03	0.5	0.05	5.2E-04	0.5	2.9E-03	0.5	Yes	1.08	7.3E-05	0.31	7.8E-01	1.0
rs12366192	11	42,261,639		1.3E-04	0.6	7.5E-03	0.7	0.12	1.3E-04	0.6	7.5E-03	0.7	Yes		0.16	9.6E-02	0.7	
rs10502172	11	112,704,356	TTC12	7.0E-04	0.7	2.1E-04	0.7	0.48	7.0E-04	0.7	2.1E-04	0.7	Yes	-1.25	3.9E-03	0.10	5.3E-01	0.9

Table 2. (Continued)

SNP	CHR	Position	Gene within 15 kb	European American case-control				Family sample (PDT)			Gene expression			African American case-control		
				MAF	AD (p value)	AD OR	Early onset AD (p value)	Early onset AD OR	AD (p value)	Early onset AD (p value)	Expr in brain	FOLD E/C	FDR	MAF	AD (p value)	AD OR
rs7963376	12	796,509	<i>WNK1</i> <i>LOC647502</i>	0.48	5.5E-04	0.7	8.7E-03	0.8			Yes	1.30	5.0E-05	0.42	4.4E-01	0.9
rs7965203	12	7,891,603	<i>SLC2A14</i>	0.49	4.5E-04	1.4	3.7E-04	1.4	0.035	0.022	Yes	-1.10	5.6E-04	0.46	1.8E-01	0.8
rs11612319	12	7,908,192	<i>SLC2A14</i> <i>NANOGP1</i>	0.32	6.6E-05	0.7	4.4E-05	0.6	0.55	0.033	Yes	-1.10	5.6E-04	0.13	6.0E-01	1.1
rs550338	12	24,403,304	<i>SOX5</i>	0.24	2.2E-04	0.7	8.9E-05	0.6	0.04	0.13	Yes	1.25	1.2E-14	0.29	9.7E-01	1.0
rs7311374	12	27,851,172	<i>KLHDC5</i>	0.14	3.9E-04	0.7	5.4E-03	0.7	0.52	0.83	Yes	1.33	1.7E-03	0.42	9.3E-01	1.0
rs1144962	12	67,621,252	<i>CPM</i>	0.35	1.6E-04	0.7	9.0E-04	0.7			Yes	1.28	2.0E-02	0.29	6.5E-02	0.7
rs1195812	12	67,627,292	<i>CPM</i>	0.34	5.3E-04	0.7	2.5E-03	0.7			Yes	1.28	2.0E-02	0.12	9.4E-01	1.0
rs10847023	12	125,010,473	<i>RPL22P19</i>	0.16	2.3E-04	1.6	8.4E-05	1.7	0.05	0.20	Yes	-1.22	3.8E-04	0.36	6.6E-01	0.9
rs10483889	14	77,627,258		0.19	3.4E-04	0.7	8.3E-04	0.6	0.04	0.46	Yes	1.22	1.8E-09	0.27	9.0E-01	1.0
rs7164726	15	57,502,958	<i>FAM81A</i>	0.12	6.0E-04	0.6	5.3E-04	0.6			Yes	1.37	1.1E-09	0.29	6.7E-01	1.1
rs7175167	15	68,312,713		0.16	3.1E-04	0.7	3.8E-03	0.7	0.15	0.031	Yes	1.22	1.8E-09	0.41	1.2E-01	0.8
rs1909766	16	59,618,891		0.15	9.9E-05	1.6	1.4E-03	1.6	0.38	0.48	Yes	1.37	1.1E-09	0.29	6.7E-01	1.1
rs1037260	17	68,668,860	<i>LOC390811</i> <i>SSTR2</i>	0.06	6.5E-05	2.3	4.3E-03	1.9			Yes					
rs1568447	17	70,348,607	<i>TMEM104</i> <i>GRIN2C</i>	0.38	7.1E-04	0.7	2.6E-03	0.7	0.38	0.0087	Yes			0.36	7.5E-01	1.0
rs11652088	17	70,351,427	<i>TMEM104</i> <i>GRIN2C</i>	0.38	4.4E-04	0.7	1.3E-03	0.7	0.33	0.023	Yes			0.31	2.2E-01	1.2
rs532241	18	10,130,629		0.45	7.2E-04	0.7	2.9E-03	0.7			Yes	-1.47	8.1E-10	0.42	5.3E-01	0.9
rs7245090	18	54,786,303	<i>ZNF532</i>	0.02	1.3E-04	0.3	2.6E-03	0.4	0.46	0.70	Yes	-1.21	8.2E-03	0.22	7.3E-01	1.1
rs7242186	18	66,132,841	<i>SOC56</i>	0.25	8.8E-04	1.4	1.4E-03	1.5			Yes	1.25	9.7E-05	0.33	6.7E-01	0.9
rs2353203	19	16,960,980	<i>CPAMD8</i>	0.44	3.8E-04	1.4	1.6E-03	1.4			Yes	-1.37	4.1E-03	0.30	6.5E-02	0.7
rs8111589	19	50,726,398	<i>VASP OPA3</i>	0.44	1.5E-04	1.4	8.3E-03	1.3	0.028	0.018	Yes			0.48	1.6E-02	0.7
rs11696193	20	41,958,904		0.26	6.2E-05	0.7	4.4E-04	0.7	0.030	0.010	Yes			0.29	3.0E-01	0.8
rs8118160	20	47,868,043	<i>SLC9A8</i>	0.50	2.0E-04	0.7	1.7E-03	0.7	0.01	0.09	Yes			0.24	2.8E-01	1.2
rs4809760	20	47,888,078	<i>SLC9A8</i>	0.43	2.5E-04	1.4	1.4E-03	1.4	0.02	0.18	Yes			0.43	6.4E-01	1.1
rs2281223	20	47,917,648	<i>SLC9A8</i>	0.42	2.2E-04	1.4	1.4E-03	1.4	0.02	0.18	Yes	-1.15	1.3E-04	0.45	6.1E-01	1.1
rs1996017	20	51,356,045	<i>TSHZ2</i>	0.36	4.6E-04	1.4	4.5E-03	1.3	0.16	0.049	Yes			0.17	2.0E-01	1.3
rs11704468	22	46,037,678		0.07	6.8E-04	0.6	4.1E-03	0.6			Yes	-1.37	1.3E-10	0.02	7.6E-02	0.4

Single nucleotide polymorphisms (SNPs) with evidence for alcohol dependence in the GWAS that either had support from the family-based analyses ($p \leq 0.05$) or evidence for differential expression in the presence of ethanol (≥ 1.2 -fold change, with FDR ≤ 0.05) are listed by position along the genome based upon dbSNP build 130 and genome build 36.3. Genes within 15 kb of the SNP are shown (blank means no gene is within 15 kb). MAF, minor allele frequency. AD p value is the p value for association with alcohol dependence in the GWAS EA sample; AD OR is the odds ratio for this association. Early onset AD p value is the p value for association with alcohol dependence in the GWAS with affected status defined as having an onset of alcohol dependence at or below the median (22 years); Early onset AD OR is the odds ratio for this association. Family sample [pedigree disequilibrium test (PDT)] AD and Early onset AD use these same phenotypic definitions but are analyzed by PDT in the families; bold indicates $p \leq 0.05$. Expression in brain: Yes, reliably detected in at least 1 of 9 brain regions; no, not reliably detected; blank, no data; FOLD E/C, ratio of gene expression in immortalized lymphoblastoid cell lines treated with ethanol for 24 hours compared to untreated cells; FDR, false discovery rate for this ratio, calculated according to Storey and Tibshirani (Storey and Tibshirani, 2003). MAF and p value for alcohol dependence in the smaller African American sample of the GWAS are also presented for comparison.

whether the expression of the gene in LCLs was altered by ethanol exposure; 80% of the genes differentially expressed in LCLs after ethanol exposure are also expressed in at least 1 of the 9 brain regions we analyzed (Edenberg et al., in preparation).

Overall, the convergence of evidence supports a region on chromosome 11 as the strongest candidate. It contained many of the top SNPs from the case control sample, and several SNPs genotyped in the family sample support the association of this region with alcohol dependence and the early onset of dependence (Table 2; Fig. 2A,B; Table S1). This region contains 6 genes: *SLC22A18* (solute carrier family 22, member 18, a poly-specific organic cation transporter), *PHLDA2* (pleckstrin homology-like domain, family A, member 2, important in regulating placental growth), *NAPIL4* (nucleosome assembly protein 1-like 4, a likely histone chaperone), *CARS* (cysteinyl-tRNA synthetase), *OSBPL5* (oxysterol-binding protein-like protein 5, an intracellular lipid receptor that interacts with retinoic acid and estradiol), and *SNORA54* (small nucleolar RNA, H/ACA box 54). The 4 genes that were assayed (*PHLDA2*, *NAPIL4*, *CARS*, *OSBPL5*) are expressed in the human brain. In LCLs, expression of *SLC22A18* and *PHLDA2* are increased 9 to 14% by ethanol exposure (FDR < 0.05), while expression of *NAPIL4* is decreased 8% by ethanol (FDR < 10^{-5} ; Edenberg et al., in preparation). These lines of evidence suggest that 1 or more of these genes is a good candidate for affecting the risk for alcoholism. Although not previously considered candidates for affecting the risk for alcoholism, several of these genes have functions that might relate to alcoholism, such as growth regulation, cation transport, and lipid signaling. Careful dissection of a quantitative trait locus (QTL) hotspot on mouse distal chromosome 1 (Mozhui et al., 2008) that includes QTLs affecting alcohol dependence (Buck et al., 2002), alcohol withdrawal (Crabbe, 1996), and alcohol-induced locomotor activity (Downing et al., 2003) showed that genes located in the QTL had a *trans* effect on expression in nervous tissue of a set of aminoacyl tRNA synthetases (Mozhui et al., 2008). As noted by Mozhui and colleagues (2008), the nervous system depends heavily on finely tuned protein metabolism in dendrites and axons (Chang et al., 2006; Malgaroli et al., 2006). Thus, *CARS* is a reasonable candidate.

One of the top-ranked SNPs was in the promoter region of *BBX* (Table 2); this SNP was also significant in the family sample, for both alcohol dependence and early onset (Table 2). Several additional SNPs just upstream of *BBX* were associated with both phenotypes in the GWAS (Table S1). *BBX* is widely expressed in human tissues and encodes the human homolog of *Drosophila* Bobbysox, an HMG-BOX transcription factor. The potential role of *BBX* in affecting risk for alcohol dependence is likely to be complex; *BBX* mRNA levels are themselves significantly increased by alcohol exposure (13% increase in lymphoblastoid cell lines, FDR = 9×10^{-5} ; Table 2). A SNP in intron 1 of *KCNMA1* provided evidence in subjects of both European and African ancestry, and in the family sample (Table 2),

KCNMA1 is expressed in brain, and encodes potassium large conductance calcium-activated channel, subfamily M, alpha member 1, a protein important in controlling neuronal excitability.

We examined results in our case control sample for genes that were previously associated with alcoholism in our family-based sample. Among the best-supported findings was the *ANKKI/DRD2* region (Dick et al., 2007b), in which 24 SNPs were nominally associated with dependence and 22 with early onset; SNPs extending through *TTC12* were also significant, supporting results from Yang and colleagues (2007). Three GWAS SNPs in *NFKB1* were nominally associated with early-onset alcoholism, as were more extending upstream, consistent with our previous work (Edenberg et al., 2008). Thirty-two SNPs in *GRM8* (Chen et al., 2009), encoding metabotropic glutamate receptor 8, were associated with early onset of dependence. There was also nominal support for *PDYN* (Xuei et al., 2006), *CHRNA3* and *CHRNA5* (Wang et al., 2009), and (among AA only) *CHRM2* (Wang et al., 2004). In the *ADH* gene cluster (Edenberg et al., 2006), SNPs in *ADH4* were not significant in this GWAS, although 15 SNPs (particularly in the region between *ADH1B* and *ADH1A*) were. Genes encoding GABA_A receptors continued to provide evidence for association with alcoholism. A SNP in *GABRR2* was among those with the smallest *p* value in this GWAS (6×10^{-5}); that SNP was not itself significant in the earlier family study, although other SNPs in the gene were (Xuei et al., 2010). Although *GABRA2* has been associated with alcohol dependence (Edenberg et al., 2004) in many samples (Bauer et al., 2007; Covault et al., 2004; Drgon et al., 2006; Enoch et al., 2006, 2008; Fehr et al., 2006; Lappalainen et al., 2005; Soyka et al., 2008), there was no evidence in this case-control GWAS, although 5 SNPs in *GABRG1* were nominally associated with alcoholism and 11 with early onset (cf Covault et al., 2008). Six SNPs in *GABRG3* (Dick et al., 2004) were associated with alcohol dependence in the EA sample, and 17 SNPs in the much smaller AA sample. In *GABRA1* (Dick et al., 2006), we found 3 SNPs associated with alcohol dependence and 4 with early onset. Many SNPs in and near *GABRG2* were also associated with dependence (and early onset). Thus, overall, there continues to be evidence that variations in GABA_A receptors affect alcohol dependence.

Several SNPs nominated as candidates in earlier GWAS studies replicated in ours. Johnson and colleagues (2006) reported 51 regions, containing 181 SNPs, that provided the strongest evidence for association in a GWAS of alcohol dependence using pooled samples of 120 cases and 160 controls drawn from the COGA study (we do not know the overlap with the present study). Among the 68 of those SNPs for which we had data, 4 provided evidence of replication (*p* < 0.02; Table 3). *CPE* encodes carboxypeptidase E, present in the central nervous system (Lynch et al., 1990), which catalyzes an important step in the processing of peptide hormones and neurotransmitters (Hook et al., 2008). A pair of closely linked SNPs in *DNASE2B*, encoding

Table 3. Replication of SNPs Detected in a Pooling Study

SNP	Chr	Position	GENE	Alcohol dependence (<i>p</i> value)	Alcohol dependence odds ratio	Early onset alcohol dependence (<i>p</i> value)	Early onset alcohol dependence odds ratio	
rs1506700	1	84,649,319	<i>DNASE2B</i>	0.007	0.80	0.001	0.73	DLAD DNase II-like acid DNase
rs3121147	1	84,649,571	<i>DNASE2B</i>	0.009	0.80	0.001	0.73	DLAD DNase II-like acid DNase
rs6729553	2	84,763,346	<i>DNAH6</i>	0.302	0.90	0.050	0.79	Dynein, axonemal, heavy chain 6
rs1370687	4	166,609,805	<i>CPE</i>	0.001	1.36	0.027	1.27	CPE carboxypeptidase E
rs1927384	13	101,943,751	<i>FGF14</i>	0.188	0.86	0.037	0.75	Between FGF14 and TPP2
rs279929	13	102,538,720	<i>SLC10A2</i>	0.016	1.26	0.015	1.31	Flank SLC10A2 solute carrier family 10 (sodium/bile acid cotransporter family), member 2
rs35164	16	63,532,702	<i>CDH11</i>	0.314	0.91	0.022	0.78	Flank CDH11 cadherin 11, type 2

Single nucleotide polymorphisms (SNPs) from Johnson and colleagues (2006) that provided evidence for association ($p \leq 0.05$) in the present GWAS. Alcohol dependence is the *p* value for association in the GWAS European American sample. Early onset alcohol dependence is the *p* value for the GWAS with affected status defined as having an onset of alcohol dependence at or below the median (22 years). Bold, $p \leq 0.05$.

deoxyribonuclease II beta, provided evidence for replication, and other SNPs in that region provided further support. *SLC10A2*, which encodes a sodium/bile acid cotransporter, also replicated; expression of *SLC10A2* is regulated in part by retinol (Neimark et al., 2004). Three other SNPs replicated when the subgroup with early-onset alcoholism was analyzed: rs35164 just downstream of *CDH11* (a type II classical cadherin), rs1927384, which lies between *FGF14* (fibroblast growth factor 14) and *TPP2* (tripeptidyl peptidase II), and rs6729553 in *DNAH6* (axonemal dynein heavy chain 6, a microtubule-associated motor protein important in retrograde axonal organelles (Schnapp and Reese, 1989)).

Treutlein and colleagues (2009) recently reported results from a GWAS of German alcoholics. Their cases were male alcoholics who had been hospitalized for treatment or prevention of severe withdrawal. We had data for 114 of their top 140 SNPs (121 at $p < 10^{-4}$ in their study and 19 they nominated from rodent studies); 14 were significant in our primary analysis of alcohol dependence; 11 of these were also significant in our smaller subset of early-onset alcoholics (Table 4). Only one of these, rs13273672 in *GATA4*, was among the 15 SNPs for which Treutlein et al. reported confirmation in their follow-up (Treutlein et al., 2009). Among the SNPs for which we had replication, 6 have the same risk allele; these lie in or near *ARL6IP5*, *ID4*, *GATA4*, *SYNE1*, *ADCY3*, and *PRKCA*; 3 of these (*GATA4*, *ADCY3*, *SYNE1*) were among the top 6 with our early-onset phenotype. These are all good candidate genes; 2 regulate transcription (*ID4*, *GATA4*), 2 (*ADCY3*, *PRKCA*) regulate important second messenger systems, one (*ARL6IP5*) inhibits the glutamate transporter EAAC1, and one (*SYNE1*) is associated with autosomal recessive spinocerebellar ataxia 8. *PRKCA* expression is lower in the nucleus accumbens of alcohol-preferring P rats after operant ethanol self-administration (Rodd et al., 2008) and is reduced by chronic alcohol in vertebrae of Sprague–Dawley rats after chronic binge exposure to ethanol (Himes et al., 2008). We have confirming evidence for 3 of these (*PRKCA*, *ADCY3*, *ARL6IP5*) in our African American sample.

A recently submitted manuscript (Bierut et al., 2010) describes results from the Study of Addiction: Genetics and Environment Consortium, which has performed a GWAS using the phenotype of alcohol dependence in a sample of cases and controls ascertained through 3 different studies (dbGaP accession phs000092.v1.p1). One of the 3 studies was COGA, with 612 EA alcohol-dependent individuals and 413 EA controls included in both our analysis and the SAGE analyses. Two other studies also provided EA cases: one recruited cases with nicotine dependence through a population screening design ($n = 343$ alcohol-dependent EA cases) and the other recruited cases with cocaine dependence through treatment centers ($n = 278$ alcohol-dependent EA cases). While 52% of EA COGA subjects also reported another substance dependence, the inclusion of cases in the SAGE analysis recruited for different primary diagnoses will likely introduce a number of novel genes contributing to alcohol dependence and another comorbid condition, either nicotine dependence or cocaine dependence. For this reason, while we might hope for some commonality between the results from the COGA and SAGE studies because of the overlapping samples, it is not surprising that in practice results of GWAS from each study are quite different.

Overall, although we did not detect any SNP that met genome-wide significance, we have assembled several different lines of evidence to prioritize SNPs and genes from among the results for further study. In a multi-stage follow-up to the GWAS, we: a) analyzed a set of top SNPs by analyzing transmission in a family sample, which provided additional support for some of the SNPs; b) determined which among the top SNPs were expressed in human brain; and c) determined which top SNPs are affected by exposure to alcohol in human LCLs. The convergence of evidence from the GWAS, family-based association analyses and the response of genes to ethanol exposure provides a set of interesting candidate genes for further analyses in larger samples.

Table 4. Replication of SNPs Detected in a GWAS of German Alcoholics

SNP	Chr	Location (bp)	Gene	Treutlein et al.				COGA results – EA				COGA results – AA			
				Risk allele	p value	Dependence (p value)	Risk allele	OR	Early onset p value	Dependence (p value)	OR	MAF	Gene name		
rs420033	1	111,097,197	KCNA3	A	2.7E-05	9.6E-03	0.8	G	7.7E-03	0.7	0.26	6.6E-01	1.2	0.06	Potassium voltage-gated channel, shaker-related subfamily, member 3
rs343802	1	111,118,652	CD53	T	9.0E-05	1.8E-03	0.7	C	1.4E-03	0.7	0.24	7.9E-01	1.1	0.06	CD53 molecule
rs17799872	2	24,898,461	ADCY3	A	2.1E-03	4.4E-02	1.4	A	1.9E-02	1.5	0.08	3.1E-03	3.1	0.07	Adenylylate cyclase 3
rs11706542	3	28,303,587	CMC1	T	4.3E-05	2.0E-02	0.8	C	4.1E-02	0.8	0.28	6.8E-01	0.9	0.12	COX assembly mitochondrial protein homolog
rs1606388	3	28,309,831	CMC1	T	5.2E-05	1.5E-02	0.8	G	3.2E-02	0.8	0.28	2.0E-01	0.7	0.08	COX assembly mitochondrial protein homolog
rs4680758	3	28,576,547	ZCWPW2	A	4.7E-05	2.5E-02	0.8	C	6.0E-02	0.8	0.25	5.1E-01	0.9	0.10	ADP-ribosylation-like factor 6 interacting protein 5
rs6549184	3	69,232,967	ARL6IP5	A	7.6E-05	1.6E-02	1.7	A	6.4E-02	1.6	0.04	1.1E-04	2.1	0.25	Inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
rs12527834	6	19,929,760	ID4	G	3.7E-05	2.1E-02	1.4	G	7.7E-02	1.4	0.10	2.7E-01	1.5	0.04	Spectrin repeat containing, nuclear envelope 1
rs214959	6	152,744,514	SYNE1	A	4.7E-05	2.8E-02	1.2	A	1.8E-02	1.3	0.50	9.3E-01	1.0	0.12	ATP-binding cassette, subfamily B (MDR/TAP), member 5
rs2188594	7	20,645,811	ABCB5	A	9.3E-05	6.9E-03	0.7	G	2.2E-02	0.7	0.18	4.6E-01	0.9	0.49	ATP-binding cassette, subfamily B (MDR/TAP), member 5
rs6958596	7	20,661,260	ABCB5	T	1.4E-03	4.6E-03	0.7	C	1.7E-02	0.7	0.16	2.6E-01	0.8	0.21	ATP-binding cassette, subfamily B (MDR/TAP), member 5
rs13273672*	8	11,649,790	GATA4	C	2.2E-03	2.2E-02	1.2	C	1.6E-03	1.4	0.31	2.9E-01	0.8	0.35	GATA binding protein 4
rs753708	13	100,219,370	TMTC4	C	7.9E-05	3.1E-02	0.7	A	2.5E-02	0.6	0.07	3.2E-01	1.3	0.12	transmembrane and tetra-trico-peptide repeat containing 4
rs12603061	17	62,242,660	PRKCA	A	7.8E-05	1.2E-01	1.1	A	4.7E-02	1.2	0.48	3.3E-02	0.7	0.46	Protein kinase C, alpha
rs8082983	18	55,306,698	CCBE1	G	9.0E-05	1.7E-02	0.8	A	2.3E-02	0.8	0.35	2.1E-01	0.8	0.49	Collagen and calcium binding EGF domains 1
rs11702690	21	42,280,737	ZNF295	G	2.0E-05	7.8E-02	1.3	G	4.6E-02	1.4	0.08	8.6E-01	0.9	0.02	Zinc finger protein 295

Single nucleotide polymorphisms (SNPs) from Treutlein and colleagues (2009) that provided evidence for association ($p \leq 0.05$) in the present GWAS. Results from Treutlein are shown for comparison with the present GWAS in EA and AA. AA, African American; EA, European American; OR, odds ratio; MAF, minor allele frequency. Bold SNPs have the same risk allele.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. SNPs Associated With Alcohol Dependence in European Americans ($p \leq 10^{-3}$).

Table S2. SNPs Associated With Alcohol Dependence in African Americans ($p \leq 10^{-3}$).

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