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Evidence for Genes on Chromosome 2 Contributing to Alcohol Dependence With Conduct Disorder and Suicide Attempts

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Twin studies provide strong evidence that there is a shared genetic liability that predisposes to a number of different psychiatric outcomes related to behavioral disinhibition. Further, alcohol dependence comorbid with other disinhibitory disorders is particularly heritable. Chromosome 2p14-2q14.3 has been linked to multiple psychiatric conditions related to behavioral undercontrol. In the Collaborative Study on the Genetics of Alcoholism (COGA), we previously reported linkage to this region with alcohol dependence (AD), suicide attempts (SUI), and conduct disorder (CD). In this study, we follow-up on these previous reports of linkage by combining the phenotypes in analyses that jointly consider the presence of multiple conditions. Linkage analyses of the combined phenotype of AD with CD or SUI results in a maximum LOD score of 5.4 in this region. In addition to this primary linkage peak, independent samples have reported linkage to other alcohol-related phenotypes across chromosome 2. Accordingly, we followed-up these linkage signals by testing for association with SNPs across chromosome 2 in a case–control sample, in which a subset of the cases consisted of alcohol-dependent probands from the linkage sample. We find evidence of association with the combined AD with CD or SUI phenotype, with 23 genes surviving permutation testing. The number of associated genes across the chromosome may explain the persistent linkage findings reported on chromosome 2 across a number of independent studies of alcohol and disinhibitory phenotypes. Further, none of the genes were located directly under the primary COGA linkage peak, which has implications for association tests following-up linkage peaks.

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INTRODUCTION

Twin studies provide strong evidence of shared genetic liability across a number of different psychiatric conditions. For example, in a large study of the genetic architecture of the major common psychiatric and substance use disorders, Kendler et al. [2003], using data from the Virginia Twin Registry, demonstrated that there were two broad genetic factors: one that contributed to externalizing disorders (alcohol dependence, drug abuse/dependence, childhood conduct disorder, and adult antisocial behavior) and a second that contributed to internalizing disorders (major depression, generalized anxiety disorder and phobia). These findings have implications for gene identification efforts, as they suggest that some genes may not be specific to any one disorder, but rather, may predispose to a variety of psychiatric outcomes. Furthermore, individuals meeting a psychiatric diagnosis are often a heterogeneous group clinically. For example, in the case of alcohol dependence, affected individuals often vary on a number of important dimensions, including age of onset, course of illness, and the presence of comorbid conditions [Cloninger, 1987; Babor et al., 1992; Hesselbrock and Hesselbrock, 1994; Finn et al., 1997]. Evidence from twin and family studies suggests that alcohol dependence with comorbid disinhibitory disorders may represent a more heritable form of the disorder [Pickens et al., 1991, 1995; Johnson et al., 1996; Ohannessian et al., 2004] suggesting that comorbid, or combined, phenotypes may be particularly relevant for gene identification efforts.

Data from the Collaborative Study on the Genetics of Alcoholism (COGA) suggest that chromosome 2p14-2q14.3 may contain a gene (or genes) with pleiotropic effects on alcohol dependence and related psychiatric conditions. Initially, linkage was detected near the marker D2S379 (LOD = 3.0) with an alcohol dependence (AD) phenotype, defined as meeting diagnostic criteria according to the DSM-IIIR and Feighner classification systems [Foroud et al., 2000]. Subsequently, linkages to the same region were identified with the phenotypes of suicide attempts (SUI) [Hesselbrock et al., 2004] and conduct disorder (CD) [Dick et al., 2003]. These findings are robust, with replication reported in multiple independent samples: Suicide attempts were linked to this same region of chromosome 2 in pedigrees affected with early-onset major depression [Zubenko et al., 2004] and with bipolar disorder [Willour et al., 2007]. Linkage of conduct disorder to this region was replicated in the Irish Affected Sib Pair Study for Alcohol Dependence [Kendler et al., 2006a]. Further analysis of a subset of the COGA pedigrees on whom genome-wide SNP linkage data were produced for the Genetic Analysis Workshop 14 (GAW14) [Edenberg et al., 2005] suggested that the chromosome 2 linkage finding for alcohol dependence was one of the most robust linkage signals in the sample [Doan et al., 2005; Wang et al., 2005; Wiener et al., 2005]. Extension of the linkage markers available at the ends of the chromosomes in the GAW14 SNP set also suggested evidence for linkage peaks with alcohol dependence at the p and q ends of the chromosome, in addition to the primary centromeric linkage peak discussed here [Wang et al., 2005; Wiener et al., 2005; Agrawal et al., 2008]. Linkages to alcohol dependence [Wilhelmsen et al., 2005] and the related traits of alcohol withdrawal [Kuo et al., 2006] and smoking [Straub et al., 1999; Goode et al., 2003] have also been reported on chromosome 2 in independent samples. Although linkage to a comorbid habitual smoking and alcohol dependence phenotype has been reported to chromosome 2p in COGA [Bierut et al., 2004], the finding is largely due to the alcohol dependence phenotype.

The phenotypes of alcohol dependence, conduct disorder, and suicide attempts, which show linkage on chromosome 2, are all characterized by elements of impulsivity and behavioral undercontrol. Conduct disorder is a robust predictor of both concurrent and future alcohol problems [Crowley et al., 1998; Moss and Lynch, 2001; White et al., 2001]. Furthermore, numerous twin studies indicate that the overlap between childhood conduct disorder and adult alcohol dependence is largely due to shared genetic factors [Slutske et al., 1998; Krueger, 1999; Young et al., 2000; Kendler et al., 2003]. This common genetic liability is thought to be a predisposition toward behavioral undercontrol/disinhibition, which can manifest as conduct disorder in childhood and alcohol dependence later in life [Slutske et al., 2002]. It has been demonstrated that *GABRA2*, a gene associated with alcohol dependence in adults [Edenberg et al., 2004; Lappalainen et al., 2005; Enoch, 2008; Soyka et al., 2008] is associated with CD symptoms and externalizing behavior in adolescents [Dick et al., 2006, 2009], providing evidence that variations in one gene can manifest as different conditions at different stages of the life cycle. Electrophysiological endophenotypes, which are thought to index genetic vulnerability to psychiatric phenotypes, are also shared across substance use disorders and conduct disorder [Iacono et al., 1999; Porjesz et al., 2005]. For example, a reduced P3 event-related potential amplitude has been found among adolescents with both substance use disorders and externalizing disorders [Iacono et al., 2002]. Suicide attempts are also considerably elevated in individuals with alcohol dependence and conduct disorder [Kessler et al., 1999]. A recent study by Conner et al. [2009], found that proactive aggression (unemotional aggression executed for reward) is associated with both suicide attempts and suicidal ideation among inpatient substance abuse patients. Furthermore, in the COGA adult sample, unplanned suicide attempts among persons with alcohol dependence are associated with externalizing behaviors, including antisocial behavior and alcohol-related aggression [Conner et al., 2007]. Suicidal behavior is influenced by genetic factors [Bondy et al., 2006], and it is thought that the predisposition to suicidal behavior reflects a heritable liability to personality traits such as impulsivity and aggression [Baud, 2005]. To the extent that these characteristics also predispose to substance use and externalizing problems, it is reasonable to hypothesize that suicidal behavior may represent another manifestation of an underlying predisposition toward behavioral disinhibition. Longitudinal studies have found that behavioral disinhibition measured in childhood/adolescence predicts both the development of substance use disorders *and* a propensity toward suicidal behavior in young adulthood [Tarter et al., 2004].

Additional follow-up of the linkage signals observed across the three phenotypes in COGA suggested that it was not simply the same individuals contributing to the results across the phenotypes; for example, only half of the individuals who had attempted suicide also had alcohol dependence, and only 25% of the individuals with

alcohol dependence have childhood conduct disorder. To the extent that these phenotypes represent an underlying liability to behavioral disinhibition, considering these phenotypes jointly in linkage analyses, rather than analyzing each individually, should enhance the power to detect linkage if there is a gene (or genes) in the region that is predisposing to this broad constellation of psychiatric outcomes. Further, to the extent that AD characterized by suicide attempts and conduct disorder may represent a more homogeneous and heritable form of the disorder, considering a combined phenotype would also increase our power to detect linkage.

Here we report a series of linkage and association analyses in the COGA sample to follow up the individual reports of linkage to alcohol dependence, conduct disorder, and suicide attempts previously reported on chromosome 2. First, we conducted linkage analyses that jointly consider the previously linked phenotypes in the COGA sample. We conducted these analyses using the high risk COGA family-based sample, in which both microsatellite [Reich, 1996] and SNP [Edenberg et al., 2005] linkage panels are available. Secondly, we tested for association with SNPs across chromosome 2 in a case–control sample, which included a subset of the alcohol-dependent probands from the linkage sample.

METHODS

Sample

COGA is a multi-site project, with the goal of identifying genes contributing to alcoholism and related phenotypes. Probands were identified through inpatient or outpatient alcohol treatment programs at six sites around the United States and were invited to participate if they had a sufficiently large family (usually sibships >3 with parents available) with two or more members in a COGA catchment area [Begleiter et al., 1995]. The institutional review boards of all participating centers approved the study. Written consent was obtained from all study participants. Additional details about the study have been published previously [Reich et al., 1998; Foroud et al., 2000; Edenberg et al., 2004]. All individuals aged 18 or older were interviewed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) [Bucholz et al., 1994; Hesselbrock et al., 1999]. Alcoholism was defined by the presence of a DSM-IIIR alcohol dependence diagnosis [Diagnostic and Statistical Manual of Mental Disorders: IIIR, 1987], plus definite alcoholism according to Feighner Criteria [1972]. The SSAGA makes a diagnosis of childhood conduct disorder according to DSMIII-R through retrospective report of behavioral problems evidenced before the age of 15; diagnoses required the presence of three or more symptoms. Suicide attempts were assessed using an item in the SSAGA that asks individuals if they have ever tried to kill themselves.

Molecular Methods and Analysis

Linkage samples and analyses. A microsatellite linkage scan was conducted on a sample of 2,282 individuals from 262 families densely affected with alcohol dependence [Reich et al., 1998; Foroud et al., 2000]. Genotyping for the microsatellite linkage scan was carried out in laboratories at Indiana University and Washington University in St. Louis using radioactive and fluorescence-based detection systems, as described previously [Reich et al., 1998; Foroud et al., 2000]. The current analyses are based on a map of 315 autosomal microsatellite markers with an average intermarker distance of 11.5 cM. Pedigrees were checked for non-Mendelian inheritance using the GeneMaster database and the programs CRIMAP [Green, 1990] and USERM13 [Boehnke, 1991]. Recombination-based marker maps were generated from the sample using CRIMAP. Maximum likelihood estimates of marker allele frequencies were computed from the data using USERM13. The majority of the sample (84%) was Caucasian; 13% was African American, and <3% reported mixed or other ethnicities. Alleles were coded separately for Caucasians and African Americans/others to take into account allele frequency differences between the populations.

In addition to the primary COGA linkage sample, a subset of the COGA linkage families were selected for additional genotyping as part of GAW14 [Edenberg et al., 2005]. A subset of 1,364 individuals from 143 families was genotyped on an Illumina panel of 4,596 SNPs intended as a linkage SNP panel. Parallel to the full COGA sample, the majority of the sample was Caucasian (83%), with 13% African American, and 4% other. Because linkage disequilibrium can produce spurious inflations in identity-by-descent (IBD) estimates and inflate information content as a result [Huang et al., 2004], we conducted linkage on a thinned panel of 1,717 SNPs, in which all SNPs with $r^2 \geq 0.1$ with any other SNP within 1 Mb were deleted [Agrawal et al., 2008]. The thinned map provided similar information content across the genome when compared to the full panel of SNPs [Hinrichs et al., 2005].

Non-parametric, multipoint methods of linkage analysis for affected sibling pairs were employed, first using the microsatellite linkage panel, and subsequently with the SNP linkage panel, using the program ASPEX (Hinds & Risch, 1999). The linkage analyses were performed using only those affected siblings with both parents genotyped (sib_ibd), which allows unambiguous estimation of IBD. This type of analysis results in greater accuracy in the estimate of marker allele sharing among affected siblings. Analyses were performed using all possible pairs of affected siblings [$n(n-1)/2$, where n = number of affected siblings in a nuclear family]. First, each of the phenotypes (alcohol dependence, conduct disorder, and suicide attempts) was analyzed individually, parallel to the previous reports (however, we note that the results are slightly different than the original publications, reflecting an updated genetic map). Subsequently, all three phenotypes were combined, such that affected status was defined by the presence of alcohol dependence (AD) or conduct disorder (CD) or suicide attempts (SUI). Finally, an analysis focused on only the subset of alcohol-dependent individuals who also have either conduct disorder or a suicide attempt (AD *with* CD or SUI) was conducted.

Association analyses. SNP data were available across chromosome 2, generated as part of the genome-wide association study (GWAS) of the COGA sample by the Center for Inherited Disease Research, using the Illumina HumanHap1M BeadChip platform, and are available through dbGaP. After all data cleaning, genome wide data were available for 1905 individuals [Edenberg et al., 2010]. All 1,205 cases met criteria for DSM-IV Alcohol

Dependence, as assessed by the SSAGA, and all 700 controls were screened against Alcohol Dependence and related substance use disorders. Three hundred twenty-four of the cases were drawn from families included in the primary COGA linkage sample. Males were overrepresented in the cases. Additional details about the COGA GWAS sample are available in Edenberg et al. [2010].

Because all cases genotyped in the GWAS sample were affected with AD, the association analyses focused on the combined AD with CD or SUI phenotype. Individuals were considered affected if they met criteria for AD and either CD or SUI, in addition to the primary AD case phenotype: 511 individuals (of the original 1,205 cases) met criteria for the AD with CD or SUI phenotype. A subset of individuals in the COGA GWAS sample (N = 321) were not assessed for suicide attempts; accordingly, when unaffected by conduct disorder, their combined "case" status was unknown, so 231 individuals were excluded from analyses for this reason. Additionally, 40 controls were excluded from analyses because of previous suicide attempts (N = 18) or the presence of a conduct disorder diagnosis (N = 22). Of the 511 AD cases who met criteria for the AD with either CD or SUI phenotype, 218 (42.7%) had a reported suicide attempt and 367 (71.8%) met criteria for conduct disorder; 74 individuals (14.5%) met criteria for both. 70.8% were male and 29.2% female. The age range was 18–74 (mean = 39.41). Of this group, 74.0% were European American (n = 378), 24.9% were African American (n = 127), and 1.2% were of another ethnicity (n = 6).

The program Plink [Purcell et al., 2007] (http://pngu.mgh.harvard.edu/purcell/plink/) was used to conduct all association tests. Logistic regression association analyses using an additive genetic model were conducted on the combined phenotype defined by the presence of AD *with* CD or SUI. The association analyses were run on each of the 82,562 SNPs genotyped across chromosome 2, using as covariates sex and ethnicity, as defined based on principal component-based analysis performed in PLINK to cluster the samples along with HapMap reference samples. Additional details are available in [Edenberg et al., 2010]. There were 1,134 genes across the chromosome. We used a gene-based strategy for association testing, an approach which can offer the advantage of identifying association to genes where multiple common variants may exist, rather than focusing on a single SNP [Moskvina et al., 2009]. In previous studies of AD in the COGA sample, we have frequently observed multiple signals across the gene [e.g., Wang et al., 2004; Edenberg et al., 2007; Dick et al., 2008; Wetherill et al., 2008]. The observation of multiple (not entirely correlated) signals across a gene enhances our confidence that an observed association is real.

Permutation tests were conducted for all genes yielding at least 1 *P*-value <0.001, as well as for genes which yielded at least two SNPs with *P*-values <0.01, with an $r^2 < 0.8$ between those SNPs. This approach captured genes with single associated SNPs or genes with converging evidence for association from multiple SNPs. To take into account the LD structure across SNPs in a gene, Plink begins with the most significant SNP in the gene, removes SNPs with $r^2 > 0.80$, and continues this process for all SNPs with $P < 0.05$ to obtain an independent group of associated SNPs. The *P*-value associated with the average test statistic across the remaining SNPs is compared across 10,000 permutation runs, yielding an empirical *P*-value for the gene based on the independent, significant SNPs observed in the gene.

RESULTS

Linkage Analyses

Table I shows the number of affected sibling pairs, LOD scores, and allele sharing for each of the phenotypes using the primary COGA microsatellite linkage panel and the SNP linkage panel available on a subset of the full COGA sample. Since LOD scores are influenced by sample size, and different numbers of sibling pairs were available for each of the phenotypes (and between the microsatellite and SNP linkage panels), allele sharing is provided in Table I to allow comparisons across the phenotypes and across the two sets of linkage analyses. Although the LOD scores differ across the microsatellite and SNP linkage panels (not surprising due to differences in sample sizes and methodology), the pattern of allele sharing observed using the microsatellite and SNP linkage panels is largely consistent. Of the individual phenotypes, CD and SUI yield higher rates of allele sharing than AD. The allele sharing for AD *with* CD or SUI was considerably elevated compared to the full AD analysis, and the AD *or* CD or SUI showed only a very small elevation in allele sharing. Further, the combined phenotype (AD *with* CD or SUI) showed elevated allele sharing compared to the broader AD *or* CD or SUI phenotype. This suggests, across both sets of analyses, that the phenotype of AD *with* CD or SUI represents a more genetically homogeneous subgroup influenced by gene(s) in the region. This was the phenotype used in subsequent association testing.

TABLE I. Results From Affected Sibling Pair Linkage Analyses Using Microsatellite Markers and SNP Linkage Panel

	Microsatellite markers				SNPs			
Phenotype	# Sibling pairs	Maximum LOD Score	Position (cM)	Allele-sharing (%)	# Sibling pairs	Maximum LOD Score	Position (cM)	Allele-sharing (%)
Alcohol dependence (AD)	797	2.9	114	55	384	1.03	117	57
Conduct disorder (CD)	113	2.4	117	64	76	3.7	102	86
Suicide attempts (SUI)	58	2.7	117	68	40	2.1	99	84
AD or CD or SUI	988	3.7	114	56	531	1.5	132	57
AD with CD or SUI	128	3.6	113	66	90	5.4	109	77

TABLE II. Genes on Chromosome 2 Yielding Evidence for Association at $P < 0.05$ After Permutation Testing

GENE symbol	Gene name	BP position	NSNP	NSIG	ISIG	EMP	Significant SNPs
NTSR2	Neurotensin receptor 2	11724667	3	2	2	0.0055	rs12612207|rs4669765
TRIB2	Tribbles homolog 2	12784955	12	3	3	0.0221	rs890069|rs10189072|rs17465002
PPM1G	Protein phosphatase 1G, magnesium-dependent, gamma isoform	27459602	18	1	1	0.0003	rs2384629
MEMO1	Mediator of cell motility 1	31964229	65	3	3	0.0216	s17011668|rs17011667|rs3769609
HAAO	3-Hydroxyanthranilate3,4-dioxygenase	42849352	13	7	6	0.0327	rs3755541|rs13027051|rs2374442|rs3816184|rs3816182|rs737148
MTIF2	Mitochondrial translational initiation factor 2	55345558	17	2	2	0.0171	rs6721728|rs6707902
CCDC139	Pseudouridylate synthase 10	61068848	33	5	5	0.0298	rs7564317|rs6708713|rs6715485|rs11691111|rs9309333
EHBP1	EH domain binding protein 1	62977118	146	22	10	0.0246	rs2710638|rs13006926|rs4671453|rs1123508|rs2018650|rs13027462|rs1468748|rs4671052|rs7557501|rs17432497
BUB1	Budding uninhibited by benzimidazoles 1 homolog	111135899	16	5	3	0.0240	rs7609252|rs13398617|rs12053209
TTL	Tubulin tyrosine ligase	112962482	25	7	7	0.0302	rs6726169|rs6718489|rs1561266|rs7570679|rs7578685|rs34179430|rs7559710
CKAP2L	Cytoskeleton associated protein 2-like	113230296	12	3	3	0.0244	rs6731822|rs10209160|rs7577241
MGAT5	Mannosyl (alpha-1,6-)-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase	134754387	96	4	4	0.0318	rs16830319|rs7564658|rs11887041|rs3791269
ARHGAP15	Rho GTPase activating protein 15	143831058	212	13	13	0.0344	rs6430025|rs10205708|rs6714864|rs11890035|rs2890714|rs10172663|rs13406291|rs17814868|rs17229439|rs16858636|rs11681284|rs10195682|rs67046 67
KIAA1189	Ermin, ERM-like protein	157883898	7	1	1	0.0006	rs17282140
COBLL1	COBL-like 1	165344115	63	13	13	0.0106	rs355895|rs355907|rs355865|rs355846|rs355868|rs355900|rs355844|rs6414069|rs355825|rs355810|rs6748091|rs355911|rs355849
FAM130A2	Cysteine–serine-rich nuclear protein 3	166211286	39	2	2	0.0290	rs6720974|rs17251144
LPR2	LiPocalin-related protein 2	169733586	118	10	9	0.0416	rs2239602|rs2302694|rs4140872|rs13417486|rs2075252|rs990626|rs2239591|rs2239596|rs1548936
CHN1	Chimerin 1	175484629	84	4	4	0.0438	rs16862927|rs1193623|rs1193630|rs2605285
PRKRA	Protein kinase, interferon-inducible double stranded RNA-dependent activator	179015447	8	2	2	0.0112	rs13427914|rs13392094

(*Continued*)

TABLE II. (*Continued*)

GENE symbol	Gene name	BP position	NSNP	NSIG	ISIG	EMP	Significant SNPs
PPP1R1C	Protein phosphatase 1, regulatory (inhibitor) subunit1C	182688766	59	6	5	0.0210	rs10451546|rs16822592|rs1882212|rs16822590|rs16867518
LOC402117	Von Willebrand factor C domain-containing protein 2-like	215092215	52	18	15	0.0204	rs10932539|rs9288497|rs10932540|rs7558283|rs6719667|rs13425618|rs4629153|rs12612233|rs4321367|rs13417343|rs4673830|rs6760280|rs11904475|rs6729204|rs6740246
IL8RA	Interleukin 8 receptor, alpha	218737177	11	3	3	0.0084	rs16858808|rs16858816|rs16858811
FARP2	FERM, RhoGEF and pleckstrin domain protein 2	241951484	43	8	6	0.0458	rs3771550|rs3771561|rs2240482|rs6723363|rs1476698|rs764081

The base pair position corresponds to the most significant SNP in each gene; NSNP, the total number of SNPs that were tested across the gene; NSIG, the total number of SNPs that yielded *P*-values <0.05 across the gene; ISIG, the number of independent SNPs yielding *P*-values <0.05 (as determined by Plink); EMP, the empirical *P*-value for the gene based on permutation tests.

Association Analyses of the Phenotype AD With CD or SUI

Sixty-one genes across chromosome 2 yielded at least one SNP with *P*-value <0.001, or at least two SNPs (with an $r^2 < 0.8$) with *P*-values <0.01, and were subjected to permutation testing. Twenty-three genes had an empirical $P < 0.05$. Table II shows the genes that yielded empirical *P*-values <0.05 in permutation tests. Table II lists the number of SNPs that were tested across each gene, the total number of SNPs that yielded *P*-values <0.05 across the gene, the number of independent SNPs yielding empirical *P*-values <0.05, the empirical *P*-value for the gene based on permutation tests, and the significant SNPs. The original *P*-values for those SNPs are presented in Supplemental Table I. In Figure 1, the approximate location of these genes is listed along the top of the LOD score graph (based on the microsatellite linkage analyses from the full COGA family sample) to illustrate roughly their chromosomal positions with respect to the linkage peak. Interestingly, none of these genes was located within a 1 LOD drop of the primary, centromeric linkage peak. Rather, those genes in closest proximity to the linkage peak were clustered on either side of the peak. In addition, consistent with previous reports of linkage across the chromosome, additional genes surviving permutation testing were located across chromosome 2.

DISCUSSION

In this article we follow-up on evidence for linkage on chromosome 2 with alcohol dependence [Foroud et al., 2000; Wilhelmsen et al., 2005], conduct disorder [Dick et al., 2003; Kendler et al., 2006a], and suicide attempts [Hesselbrock et al., 2004; Zubenko et al., 2004; Willour et al., 2007] in the COGA sample, and independent samples [Zubenko et al., 2004; Kendler et al., 2006b; Willour et al., 2007]. These phenotypes have shown consistent linkage to the centromeric region of chromosome 2 across multiple samples. In addition, there have been linkages reported to alcohol and related phenotypes on the p and q arms of chromosome 2 [Straub et al., 1999; Goode et al., 2003; Wang et al., 2005; Wiener et al., 2005; Wilhelmsen et al., 2005; Kuo et al., 2006; Agrawal et al., 2008]. We find that the phenotype of alcohol dependence with either conduct disorder or suicide attempts results in a maximum LOD score of 5.4 in the centromeric region of chromosome 2. Although the joint phenotype of AD or CD or SUI produced a similar LOD score in the microsatellite linkage analyses, the allele sharing was considerably lower, and in the SNP linkage scan, the LOD score was considerably lower. One hypothesis for this pattern of results is that limiting analyses to the AD individuals *with* CD or SUI yielded a more homogeneous group with a more heritable, behaviorally disinhibited phenotype. Not only is AD more narrowly defined by this joint phenotype, so might be SUI. By requiring at least some of the suicide attempts to be comorbid with alcohol problems, we may have largely retained "externalizing" attempts and eliminated attempts that reflected planned, internalizing-based behavior. The subset of AD individuals in the association sample who also met criteria for conduct disorder or a suicide attempt were a more severe subset of the cases by a number of criteria: they were more likely to meet criteria for an illicit drug dependence diagnosis (77.1% vs. 51.5%, $P < 0.001$), had

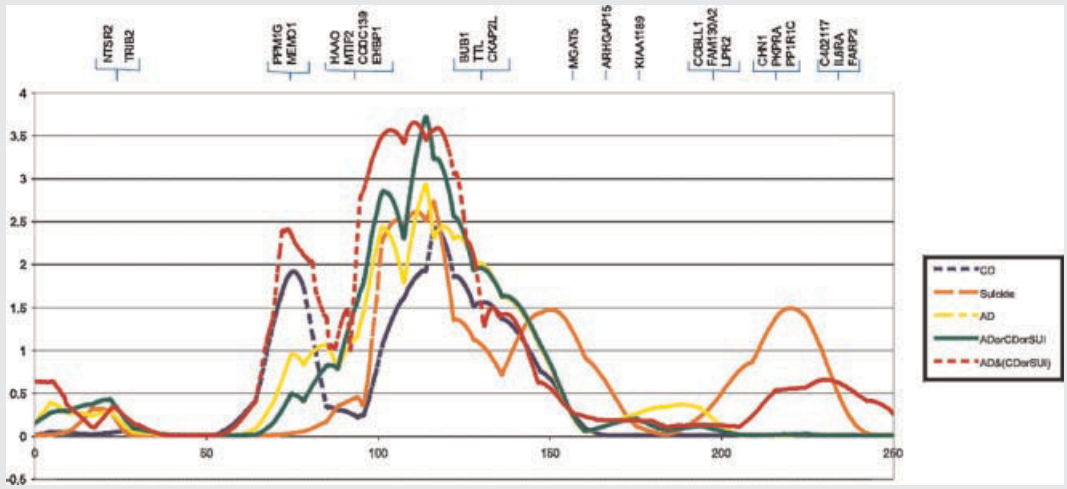

FIG. 1. Lod score graphs for each of the phenotypes using the microsatellite linkage panel across chromosome 2. Genes with empirical P<0.05 are shown according to their approximate position along the top of the graph. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

a higher mean number of illicit drug dependence symptoms (12.10, SD = 10.10 in AD cases with CD/SUI; 6.53, SD = 7.74 in other AD cases; $P < 0.001$), had a younger age of onset of AD (23.86, SD = 7.37 in AD cases with CD/SUI; 28.12, SD = 9.08 in other AD cases; $P < 0.001$), were more likely to meet criteria for alcohol withdrawal (59.3% vs. 51.3%, $P = 0.01$), and had a slightly higher mean symptom count for alcohol dependence symptoms (5.72 vs. 5.27; $P < 0.001$). Several of the previous reports of associated genes in the COGA sample have been driven by the more severe, comorbid AD individuals in the sample [Edenberg et al., 2006; Dick et al., 2007; Edenberg et al., 2007; Wetherill et al., 2008].

The availability of SNP data across chromosome 2 as part of the GWAS panel allowed us the opportunity to follow-up the chromosome 2 findings without having to a priori define a targeted region. We previously conducted a systematic SNP screen of LD-tagging SNPs across a 2 LOD support interval bracketing a linkage peak on chromosome 7 in the COGA sample [Dick et al., 2008]. In that systematic screen, strong evidence for association was detected with only a single gene (*ACN9*), a surprising result since we know that linkage is not a powerful technique for detecting genes of small effect, and we hypothesize that linkage peaks that are detected for complex traits represent the involvement of multiple genes in the region, an idea that has been supported in following up other linkage peaks in the COGA sample, for example on chromosome 4 [Edenberg et al., 2004, 2006, 2007]. In fact, several other genes have been detected in the vicinity of the chromosome 7 peak in COGA [Hinrichs et al., 2006; Wang et al., 2004, 2007], though these were located outside the 2 LOD support interval. This pattern of results may also reflect the fact that the localization of linkage peaks is known to be imprecise [Roberts et al., 1999]. Accordingly, we made the decision here to take advantage of the existent data across chromosome 2 to test for association. This allowed us to evaluate the location(s) of associated genes with respect to the primary linkage peak. It also allowed us to address the imprecise nature of linkage peaks, the hypothesis of the involvement of multiple genes in the region (some of which may be missed by narrowly targeting the region just around the peak), and the evidence for linkage to alcohol dependence-related phenotypes across chromosome 2, suggesting there may be relevant susceptibility genes at multiple locations across the chromosome.

The results did prove to be instructive about the localization of linkage peaks with respect to associated genes. Despite the presence of a strong, narrow linkage peak in our sample, none of the genes that passed permutation testing for significance were located with a 1 LOD—or 2 LOD—support interval for the primary, centromeric linkage peak with the AD with CD or SUI phenotype. Although simulations have previously demonstrated that the location of linkage peaks can vary substantially from the position of the underlying variant(s) [Roberts et al., 1999], the empirical results from this study clearly underscore the danger of focusing narrowly on follow-up of linkage regions as defined by the location of the peak. These findings appear to illustrate the hypothetical "worst case scenario" whereby clusters of associated genes are located on either side of the linkage peak, contributing to a peak location in the middle. Because linkage is a within-family test, whereas association is a between-family test, it is also possible that genetic heterogeneity between families could contribute to a detectable linkage signal for which association tests would not be able to detect the underlying genetic variants. This is another possible explanation for the failure to identify any genes directly under the linkage peak. Systematic follow-up across the entirety of chromosome 2 also yields significant evidence of association with multiple other genes that appear to map loosely to the more distal linkage peaks that have been reported on the p and q arms of the chromosome. A preponderance of genes involved in alcohol dependence and related traits across the chromosome likely contributes to the consistent implication of this chromosome in linkage scans.

The original *P*-values were more significant than the empirical *P*-values (compare Table II and Supplemental Table I) because the permutation tests took into account the multiple SNPs tested across

the genes. Most of the genes that yielded empirical $P < 0.05$ had multiple independent significant SNPs in the gene, a criteria which COGA has routinely used to bolster confidence in significantly associated genes. However, we note that none of the SNPs would have passed a stringent Bonferroni correction for all SNPs tested across the chromosome, which would have required a *P*-value of 6×10^{-7}. Because our study was not completely atheoretical, but rather, we targeted this region of the genome and this particular phenotype based on evidence of linkage in the region, we believe that a gene-based permutation strategy represents a more appropriate means of evaluating significance than the overly conservative Bonferroni correction. However, follow-up in independent samples will ultimately be necessary to evaluate the role of the genes implicated in this study.

None of the genes identified here is currently associated with a large literature involving substance dependence or disinhibitory behavior. One exception may be the gene *NTSR2* that codes for neurotensin receptor 2, a protein that belongs to the G protein-coupled receptor family that activates a phosphatidylinositol–calcium second messenger system. There are previous reports that neurotensin exerts complex effects on the mesolimbic dopamine system that alter motivation and contribute to neuroadaptations associated with psychostimulant drug administration [Garlow et al., 2006; Reynolds et al., 2006]. However, before formulating hypotheses about the potential biological effects of the associated genes, replication in independent samples will be critical.

In conclusion, follow-up of previous independent reports of linkage on chromosome 2 in the COGA sample with alcohol dependence, conduct disorder, and suicide attempts results in a maximal LOD score of 5.4 in the sample with the phenotype of AD with CD or SUI. A systematic screen of SNPs across chromosome 2 with the comorbid AD with CD or SUI phenotype yields evidence of association with 23 genes located across chromosome 2, likely contributing to the preponderance of reported linkages with alcohol dependence and related phenotypes across chromosome 2. These genes may represent novel genes associated with phenotypes resulting from behavioral undercontrol. Confirmation in independent samples will be the next step.

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