Association Between *GABRA1* and Drinking Behaviors in the Collaborative Study on the Genetics of Alcoholism Sample

Danielle M. Dick, Jevon Plunkett, Leah Flury Wetherill, Xiaoling Xuei, Alison Goate, Victor Hesselbrock, Marc Schuckit, Raymond Crowe, Howard J. Edenberg, and Tatiana Foroud

Background: A wealth of literature supports the role of γ-aminobutyric acid (GABA) in neurobiological pathways contributing to alcohol dependence and related phenotypes. Animal studies have consistently tied rodent homologs of the $GABA_A$ receptor genes on human chromosome 5q to alcohol-related behaviors; however, human studies have produced mixed results. Family-based association analyses previously conducted in the Collaborative Study on the Genetics of Alcoholism (COGA) sample yielded no evidence of association with *Diagnostic and Statistical Manual of Mental Disorder*—fourth edition (DSM-IV) alcohol dependence and these genes. As a follow-up to that study, we examined several alcohol-related behaviors in the COGA sample as follows: (1) a broader definition of alcohol dependence, including DSM-III-R symptoms and Feighner criteria (referred to as COGA alcohol dependence); (2) withdrawal; (3) history of alcohol-induced blackouts; (4) level of response to alcohol; (5) age of onset of regular drinking; and (6) age at first drunkenness.

Methods: Family-based association tests were conducted, using multiple single-nucleotide polymorphisms (SNPs) in each of the 4 $GABA_A$ receptor genes on chromosome 5q.

Results: In *GABRA1*, we found evidence of association with several of the drinking behavior phenotypes, including COGA alcohol dependence, history of blackouts, age at first drunkenness, and level of response to alcohol. We did not find consistent evidence of association with the remaining genes and any of the phenotypes.

Conclusions: We found evidence for association between *GABRA1* and COGA alcohol dependence, history of blackouts, age at first drunkenness, and level of response to alcohol. These analyses suggest that efforts to characterize genetic contributions to alcohol dependence may benefit by examining alcohol-related behaviors in addition to clinical alcohol dependence diagnoses.

Key Words: GABA Receptor Genes, Chromosome 5, Alcohol Dependence, Association Analyses, Drinking Behavior.

A WEALTH OF literature supports the role of γ-aminobutyric acid (GABA) in neurobiological pathways contributing to alcohol dependence and related phenotypes. GABA, the principal inhibitory neurotransmitter in the human central nervous system, is thought to play a central role in the behavioral effects of alcohol, such as motor incoordination, sedation, tolerance, and withdrawal (Grobin et al., 1998). $GABA_A$ receptor antagonists reduce some ethanol-induced behaviors and decrease alcohol drinking, whereas agonists can relieve alcohol withdrawal symptoms (Parsian and Cloninger, 1997). Furthermore, innate differences in the GABA system have been observed between lines of selectively bred rodents for alcohol traits, such as the alcohol preferring/nonpreferring (P/NP) (McBride and Li, 1998), high alcohol drinking/low alcohol drinking (HAD/LAD) (McBride and Li, 1998), and the high initial sensitivity to ethanol/low initial sensitivity to ethanol (HAS/LAS) rat lines (Liu and Dietrich 1998).

The cluster of $GABA_A$ receptor genes on human chromosome 5q, which includes *GABRA1*, *GABRA6*, *GABRB2*, and *GABRG2*, is of particular interest. Animal studies have consistently tied rodent homologs of these

From Washington University, St. Louis Missouri (DMD, JP, AG); the Indiana University School of Medicine, Indianapolis, Indiana (LFW, XX, HJE, TF); the University of Connecticut, Farmington, Connecticut (VH); the University of California, San Diego, California (MS); and the University of Iowa, Iowa City, Iowa (RC).

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Reprint requests: Danielle M. Dick, PhD, Department of Psychiatry, Washington University School of Medicine, 660 South Euclid Ave. Box 8134, St. Louis, MO 63110; Fax: 314-286-2213; E-mail: dickd@wustl.edu

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genes to alcohol-related behaviors. Evidence from knock-out, gene expression, and between-strain comparisons in rodents supports a link between the gene cluster and acute and chronic alcohol withdrawal (Boehm et al., 2004; Buck and Hood, 1998; Kralic et al., 2005; Reily and Buck, 2000), level of response to alcohol (Hanchar et al., 2005; Loh and Ball, 2000; Sander et al., 1999; Sanna et al., 2003), and other related behaviors, such as alcohol preference and the drug's hypnotic effects (Boehm et al., 2004). Moreover, quantitative trait loci (QTL) mapping in rodents has implicated the homologs of *GABRA1*, *GABRA6*, *GABRB2*, and *GABRG2* in alcohol-related behaviors, such as severity of alcohol withdrawal (Bergeson et al., 2003; Buck et al., 1997).

However, despite the wealth of positive findings in rodents, there have been mixed results in human studies of the 5q $GABA_A$ receptor gene cluster. A positive association has been found by various studies between the gene cluster and alcohol dependence (Loh and Ball, 2000; Loh et al., 1999; Radel et al., 1999; Sander et al., 1999; Schuckit et al., 1999, 2004). Yet, other studies have found no association between these genes and alcohol dependence (Dick et al., 2005; Hu et al., 2005; Loh et al., 1999; Parsian and Cloninger, 1997; Sander et al., 1999; Song et al., 2003). These studies have used a variety of different populations and analytic methods. Additionally, several different phenotypes have been studied, most notably alcohol dependence and alcohol dependence comorbid with antisocial behavior. However, there has been no consistency in results by population, method, or phenotype.

Linkage analyses conducted in the Collaborative Study of the Genetics of Alcoholism (COGA) sample found no significant linkage with alcohol dependence to the region on chromosome 5 containing the $GABA_A$ receptor genes (Reich et al., 1998). In addition, the COGA group has previously reported family-based association analyses yielding no evidence of association with *Diagnostic and Statistical Manual of Mental Disorder*—fourth edition (DSM-IV) alcohol dependence and the 4 $GABA_A$ receptor genes on chromosome 5 (Dick et al., 2005). However, there was linkage on chromosome 5 close to the *GABRA1* receptor gene in the COGA sample to Beta 2 EEG waves, an endophenotype related to alcoholism (Ghosh et al., 2003). Moreover, much of the literature supporting the involvement of chromosome 5 $GABA_A$ receptor genes in alcohol dependence comes from animal studies in which alcohol-related behaviors, rather than clinical alcohol dependence, per se, were studied. Accordingly, we chose to follow-up on our previous study (Dick et al., 2005) by examining the potential association between the $GABA_A$ receptor genes on chromosome 5 and additional alcohol-related phenotypes in the COGA sample. Specifically, we examined the following:

(1) A broader definition of alcohol dependence (referred to as COGA alcohol dependence in other publications), which uses DSM-III-R and Feighner criteria (Reich et al., 1998).
(2) *Withdrawal*. Several knock-out, gene expression, between-strain comparisons, and QTL linkage studies in rodents supporting the involvement of chromosome 5 $GABA_A$ receptor genes have used withdrawal as an outcome measure (as detailed above).
(3) *Alcohol-induced blackouts*. A history of alcohol-induced blackouts is significantly associated with lifetime alcohol dependence diagnosis and has been shown to have approximately 50% heritability (Nelson et al., 2004). Furthermore, $GABA_A$ receptor agonists can induce periods of anterograde amnesia (Nelson et al., 2004), making the chromosome 5 $GABA_A$ receptor genes appealing candidate genes for involvement in this phenotype.
(4) *Level of response to alcohol*. Offspring of alcoholic individuals have been shown to have a reduced response to alcohol compared with controls, suggesting that level of response to alcohol may serve as a marker of a genetic predisposition toward alcohol dependence (Schuckit et al., 1999). Animal studies strongly suggest that low-level response to alcohol is correlated with variation in this gene cluster (see above). Additionally, the Pro385Ser polymorphism in GABRA6 previously has been tied to reduced reaction to alcohol and benzodiazepines in humans (Hu et al., 2005; Iwata et al., 1999; Schuckit et al., 1999). Moreover, a genomewide scan in humans for genes related to low-level response to alcohol showed moderate linkage (LOD = 1.2) to the 5q gene cluster's chromosomal region (Wilhelmsen et al., 2003).
(5) *Age of onset of regular drinking*. Grant et al. (2001) found that the likelihood of meeting criteria for alcohol dependence was significantly associated with the age at which an individual began drinking regularly, with the odds of alcohol dependence decreasing at least 5% for every year that regular drinking was delayed. It may also be of relevance that age at first drink has been shown to be a familial trait that increases the risk for alcohol dependence and that the association with alcohol dependence of this related trait may be genetically mediated (McGue et al., 2001; Prescott and Kendler, 1999). In the COGA sample, only age at onset of regular drinking was assessed, and this is the phenotype analyzed here.
(6) *Age at first drunkenness*. Hingson et al. (2003) found that the age at which college students were first drunk was highly correlated with alcohol dependence; college students surveyed who had first been drunk before 13 years old were 3.1 times more likely to meet criteria for alcohol dependence than their peers who were first drunk at 19 years old or later.

Accordingly, this paper reports family-based association results between multiple SNPs tested in each of the 4 genes

located in the chromosome 5 cluster of $GABA_A$ receptor genes, with the above alcohol-related phenotypes in the COGA sample.

MATERIALS AND METHODS

Sample

COGA is a multisite project, in which families were collected at 6 centers across the United States: Indiana University, State University of New York Health Science Center, University of Connecticut, University of Iowa, University of California in San Diego, and Washington University in St. Louis. Probands identified through inpatient or outpatient alcohol treatment programs at these 6 sites were invited to participate if they had a sufficiently large family (usually sibships >3 with parents available), with 2 or more members in a COGA catchment area (Reich, 1996). The institutional review boards of all participating institutions approved the study. A total of 1,227 families of alcohol-dependent probands were recruited for the first stage of the study. All individuals were administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview (Bucholz et al., 1994; Hesselbrock et al., 1999). Multiplex alcoholic individual families that were not bilineal and had at least 2 affected first-degree relatives in addition to the proband were invited to participate in the more intensive stage of the study, which included obtaining blood for genetic analyses. Second-degree and third-degree relatives in the families were assessed when they were considered to be informative for the genetic linkage studies. A total of 2,282 individuals from 262 multiplex alcoholic families are available for genetic analyses. Of the 262 families, 219 were Caucasian families, 35 were African American families, and 7 were of another race. We ran analyses both in the full sample and in the sample limited to Caucasian individuals. Here, we report the data from the Caucasian-only subset for ease of interpretation, as patterns of LD and allele frequencies often differ between the races. However, we note that the results were largely unchanged in the full sample (available upon request from the authors).

Phenotypes

COGA Alcohol Dependence. Individuals were diagnosed with COGA alcohol dependence if they met criteria for both DSM-III-R (American Psychiatric Association, 1987) and Feighner criteria for alcoholism (Reich et al., 1998). One thousand sixty-five of 2,200 individuals in the genotyped sample (48.4%) were diagnosed with COGA alcohol dependence. This measure correlates strongly with DSM-IV alcohol dependence ($r = 0.79$); however, COGA alcoholism is a broader, somewhat less severe phenotype, as fewer individuals are diagnosed as being alcohol dependent using the DSM-IV definition (909/2,200, 41%) than the COGA definition.

Withdrawal. Withdrawal was evaluated using a tally of an individual's affirmative responses to questions in the SSAGA on whether he or she had experienced various somatic symptoms after stopping or reducing drinking. These questions were phrased as follows: "Did you have the shakes (hands trembling)?"; "Were you unable to sleep?"; "Did you feel anxious or depressed?"; "Did you sweat?"; "Did your heart beat fast?"; "Did you have nausea/vomiting?"; "Did you feel physically weak?"; "Did you have headaches?"; "Did you hear or see things that weren't there?"(Bucholz et al., 1994). Tally scores ranged from 0 to 8, with a median of 1.2 symptoms.

Blackouts. Alcohol-induced blackout was evaluated by participants' responses to the SSAGA question: "Have you ever had blackouts when you didn't pass out while drinking, that is, you drank enough so that the next day you couldn't remember things you had said or done?" (Bucholz et al., 1994). Fifty-three percent of respondents reported a history of blackouts.

Level of response to alcohol. The intensity of reaction to alcohol was evaluated by averaging self-report data on number of drinks needed to achieve a certain response for the first 5 times an individual drank, as measured by the Self-Rating of the Effects (SRE) of Alcohol Questionnaire (Schuckit et al., 1997). Scores on the SRE ranged from 1 to 32.5, with a median score of 3.3.

Drinking initiation variables. We examined the age at which an individual began drinking regularly and the age at first drunkenness, obtained from responses to the following SSAGA questions: "At what age did you begin to drink regularly—that is, drinking at least once a month for 6 months or more?"; "How old were you when you were first drunk, that is, your speech was slurred or you were unsteady on your feet?" (Bucholz et al., 1994). Reported age at which an individual began drinking regularly ranged from 6 to 60, with the median age being 18. Similarly, age at first drunkenness ranged from 6 to 60, with the median age being 16.

Table 1 contains the correlations between each of the phenotypes. Because males and females evidenced mean differences in SRE, age at initiation of regular drinking, and age at first drunkenness, gender-residualized scores were used in genetic analyses.

DNA Analyses

Single-nucleotide polymorphisms were chosen across the genes from public databases and locations determined from annotations in the NCBI human genome assembly or by BLASTing the sequence against the human genome assembly. Single-nucleotide polymorphisms were genotyped on approximately 80 unrelated individuals from the Coriell Caucasian and African American samples to determine allele frequencies. Genotyping was performed with a modified single-nucleotide extension reaction, with allele detection by mass spectroscopy (Sequenom MassArray system, Sequenom, San Diego, CA). All genotypic data were checked for Mendelian inheritance of

Table 1. Correlations Between Traits Studied Using Data From the Genetic Sample ($n = 2,200$)

	COGA alcohol dependence	History of blackouts	Withdrawal	Level of response to alcohol	Age onset regular drinking	Age at first drunkenness
COGA alcohol dependence		$r = 0.56$	$r = 0.51$	$r = 0.25$	$r = 0.27$	$r = 0.32$
History of blackouts			$r = 0.38$	$r = 0.16$	$r = 0.24$	$r = 0.27$
Withdrawal				$r = 0.22$	$r = -0.17$	$r = -0.21$
Level of response to alcohol					$r = -0.14$	$r = -0.14$
Age onset regular drinking						$r = 0.62$

COGA, Collaborative Study on the Genetics of Alcoholism.

marker alleles with the USERM13 (Boehnke, 1991) option of the MENDEL linkage computer programs, which was then used to estimate marker allele frequencies. Forty-five SNPs were genotyped in the chromosome 5 $GABA_A$ gene cluster: 13 in *GABRB2*, 5 in *GABRA6*, 16 in *GABRA1*, and 11 in *GABRG2*. The average heterozygosity of the SNPs was 0.36. These SNPs, the gene in which they are located, their chromosomal position and heterozygosity are shown in Table 2. Sixteen of these SNPs are newly genotyped since the publication of our previous negative report with DSM-IV alcohol dependence (Dick et al., 2005) to provide more thorough gene coverage; 6 of these new SNPs were in *GABRB2* and 10 were in *GABRA1*.

Statistical Analyses

Multiplex families of alcoholic individuals were used in tests of association between each of the SNPs and each of the phenotypes studied: alcohol dependence, withdrawal, occurrence of blackouts, initial sensitivity to alcohol, age began drinking regularly, and age at first drunkenness. To test for association with the qualitative measures, COGA alcohol dependence and history of blackouts, the pedigree disequilibrium test (PDT; Martin et al., 2000) was performed, using the PDT program. The PDT uses all available trios (2 parents plus child) in a family as well as discordant siblings. This test produces 2 statistics: the "PDT-ave," which averages the association

Table 2. Association Between SNPs in the Chromosome 5 $GABA_A$ Receptor Gene Cluster and the Qualitative Phenotypes: (1) COGA Alcohol Dependence and (2) History of Blackouts[+]

				COGA alcohol dependence		Occurrence of blackouts	
SNP ID	GENE	Chromosome position[b]	Heterozygosity	PDT sum	PDT ave	PDT sum	PDT ave
rs1592752	GABRB2	159880572	0.500	0.507	0.468	0.953	0.568
rs253041	GABRB2	160656576	0.224	0.890	0.862	0.892	0.627
rs252957	GABRB2	160672421	0.489	0.895	0.607	0.864	0.342
rs252944	GABRB2	160690915	0.246	0.890	0.258	0.917	0.366
rs194072	GABRB2	160691233	0.246	0.871	0.203	0.896	0.266
rs967771	GABRB2	160694752	0.328	0.551	0.873	0.851	0.276
rs2303055	GABRB2	160696194	0.056	0.317	0.317	0.366	0.311
rs1159170	GABRB2	160740311	0.192	0.896	0.448	0.496	0.482
rs2962398	GABRB2	160754972	0.479	0.457	0.492	0.983	0.588
rs1363698	GABRB2	160759968	0.199	0.948	0.473	0.547	0.664
rs2962393	GABRB2	160766449	0.198	1.000	0.487	0.626	0.709
rs3923773	GABRB2	160803080	0.475	0.494	0.522	0.840	0.580
rs2066949	GABRB2	160890538	0.416	0.607	0.865	0.278	0.566
rs1992646	GABRA6	161043839	0.496	0.279	0.174	0.489	0.224
rs3811995	GABRA6	161045271	0.497	0.266	0.315	0.617	0.221
rs3811992	GABRA6	161049520	0.497	0.202	0.233	0.610	0.354
rs3811991	GABRA6	161060987	0.472	0.164	0.072	0.252	0.064
rs3219151	GABRA6	161061492	0.494	0.341	0.342	0.744	0.367
rs4478357[a]	gabra6/gabra1	161204427	0.499	**0.034**	0.344	0.125	0.126
rs4608967	GABRA1	161207991	0.205	0.133	0.347	0.218	0.221
rs6883877[a]	GABRA1	161210916	0.082	0.903	0.630	0.453	0.120
rs1129647	GABRA1	161213823	0.377	0.933	0.976	0.548	0.986
rs4263535[a]	GABRA1	161217407	0.330	**0.014**	0.088	**0.026**	0.060
rs4605831[a]	GABRA1	161223378	0.334	0.657	0.727	0.675	0.400
rs6556562	GABRA1	161238287	0.487	**0.011**	0.105	**0.044**	**0.026**
rs11135172	GABRA1	161242330	0.337	0.823	0.772	0.803	0.356
rs1350372	GABRA1	161246703	0.488	**0.027**	0.143	**0.036**	**0.021**
rs1037715[a]	GABRA1	161248347	0.220	0.589	0.927	0.409	0.926
rs1026447	GABRA1	161250348	0.333	0.425	0.163	0.492	0.207
rs980791[a]	GABRA1	161251014	0.459	**0.011**	**0.017**	0.089	**0.007**
rs1157122[a]	GABRA1	161251892	0.241	**0.029**	**0.023**	0.094	0.067
rs2279020	GABRA1	161255467	0.460	**0.033**	0.070	0.096	**0.015**
rs2290732	GABRA1	161257476	0.475	0.120	0.222	0.087	**0.036**
rs998754	GABRA1	161258512	0.474	**0.031**	0.253	0.063	**0.046**
rs2268583	GABRG2	161428430	0.158	0.166	0.370	0.721	0.889
rs2268582	GABRG2	161447793	0.244	0.844	0.730	0.139	0.211
rs211017	GABRG2	161455533	0.278	0.848	0.892	0.445	0.164
rs211037	GABRG2	161460858	0.392	0.908	0.566	0.887	0.858
rs210991	GABRG2	161466026	0.307	0.258	0.806	0.468	0.482
rs210983	GABRG2	161471712	0.420	0.937	0.708	0.592	0.662
rs989694	GABRG2	161497059	0.500	0.135	0.085	0.648	0.579
rs211015	GABRG2	161508231	0.477	0.133	0.238	0.529	0.579
rs211014	GABRG2	161508996	0.378	0.862	0.570	0.108	0.217
rs211013	GABRG2	161512019	0.499	0.059	0.064	0.534	0.420
rs418210	GABRG2	161513561	0.384	0.821	0.322	0.109	0.432

[+]Significant *p*-values at $p < 0.05$ indicated in bold. *p*-Values are not corrected for multiple tests.
[a]Haplotype tagging SNPs in *GABRA1*.
[b]According to NCBI build 124.
SNP, single-nucleotide polymorphism; COGA, Collaborative Study on the Genetics of Alcoholism; $GABA_A$, γ-aminobutyric $acid_A$.

Table 3. Association Between SNPs in the Chromosome 5 $GABA_A$ Receptor Gene Cluster and the Quantitative Phenotypes: (1) Withdrawal, (2) Level of Response to Alcohol, (3) Age of Onset of Regular Drinking, and (4) Age at First Drunkenness[+]

				Withdrawal	Initial sensitivity	Age began drinking regularly	Age at first drunkenness
SNP ID	GENE	Chromosomal position[b]	Heterozygosity	QPDT *p*-value	QPDT *p*-value	QPDT *p*-value	QPDT *p*-value
rs1592752	GABRB2	159880572	0.50	0.300	0.384	0.037	0.350
rs253041	GABRB2	160656576	0.22	0.988	0.825	0.562	0.538
rs252957	GABRB2	160672421	0.49	0.619	0.259	0.108	0.468
rs252944	GABRB2	160690915	0.25	0.162	0.999	0.270	0.482
rs194072	GABRB2	160691233	0.25	0.289	0.236	0.463	0.519
rs967771	GABRB2	160694752	0.33	0.327	0.243	0.148	0.373
rs2303055	GABRB2	160696194	0.06	0.044	0.330	0.873	0.977
rs1159170	GABRB2	160740311	0.19	0.942	0.057	0.546	0.533
rs2962398	GABRB2	160754972	0.48	0.930	0.200	0.189	0.693
rs1363698	GABRB2	160759968	0.20	0.902	0.052	0.506	0.465
rs2962393	GABRB2	160766449	0.20	0.831	0.048	0.617	0.550
rs3923773	GABRB2	160803080	0.48	0.959	0.168	0.218	0.885
rs2066949	GABRB2	160890538	0.42	0.655	0.812	0.994	0.318
rs1992646	GABRA6	161043839	0.50	0.975	0.857	0.636	0.445
rs3811995	GABRA6	161045271	0.50	0.546	0.472	0.882	0.296
rs3811992	GABRA6	161049520	0.50	0.360	0.444	0.843	0.291
rs3811991	GABRA6	161060987	0.47	0.063	0.467	0.350	0.081
rs3219151	GABRA6	161061492	0.49	0.432	0.451	0.839	0.181
rs4478357[a]	gabra6/gabra1	161204427	0.50	0.656	0.247	0.623	0.828
rs4608967	GABRA1	161207991	0.20	0.224	0.752	0.056	**0.007**
rs6883877[a]	GABRA1	161210916	0.08	0.693	0.092	0.255	0.759
rs1129647	GABRA1	161213823	0.38	0.977	0.130	0.195	0.171
rs4263535[a]	GABRA1	161217407	0.33	0.144	0.686	**0.046**	**0.008**
rs4605831[a]	GABRA1	161223378	0.33	0.594	0.236	0.845	0.731
rs6556562	GABRA1	161238287	0.49	0.193	0.320	0.203	0.077
rs11135172	GABRA1	161242330	0.34	0.649	0.206	0.760	0.415
rs1350372	GABRA1	161246703	0.49	0.183	0.333	0.190	0.093
rs1037715[a]	GABRA1	161248347	0.22	0.817	**0.008**	0.504	0.204
rs1026447	GABRA1	161250348	0.33	0.475	0.074	0.292	0.661
rs980791[a]	GABRA1	161251014	0.46	0.198	0.355	0.154	0.069
rs1157122[a]	GABRA1	161251892	0.24	0.196	0.349	0.459	0.154
rs2279020	GABRA1	161255467	0.46	0.447	0.349	0.183	0.154
rs2290732	GABRA1	161257476	0.47	0.231	0.347	0.187	0.090
rs998754	GABRA1	161258512	0.47	0.680	0.219	0.402	0.212
rs2268583	GABRG2	161428430	0.16	0.741	0.475	0.084	0.109
rs2268582	GABRG2	161447793	0.24	0.059	0.640	0.356	0.347
rs211017	GABRG2	161455533	0.28	0.840	0.933	0.793	0.427
rs211037	GABRG2	161460858	0.39	0.737	0.282	0.611	0.422
rs210991	GABRG2	161466026	0.31	0.901	0.808	0.159	**0.025**
rs210983	GABRG2	161471712	0.42	0.676	0.838	0.478	0.251
rs989694	GABRG2	161497059	0.50	0.646	0.543	0.224	0.895
rs211015	GABRG2	161508231	0.48	0.630	0.584	**0.026**	0.615
rs211014	GABRG2	161508996	0.38	0.341	0.250	0.381	0.246
rs211013	GABRG2	161512019	0.50	0.329	0.745	0.489	0.998
rs418210	GABRG2	161513561	0.38	0.401	0.569	0.115	0.865

[+]Significant *p*-values at $p < 0.05$ indicated in bold. *p*-Values are not corrected for multiple tests.
[a]Haplotype tagging SNPs in *GABRA1*.
[b]According to NCBI build 124.
SNP, single-nucleotide polymorphism; $GABA_A$, γ-aminobutyric $acid_A$.

statistic over all families, and the ''PDT-sum,'' which gives greater weight to larger families with more informative trios and discordant siblings (Martin et al., 2000). We expect heterogeneity in our sample, whereby not every gene will necessarily have a detectable impact on alcohol dependence/related phenotypes in all families in the sample. By chance, depending on whether larger or smaller families are associated with the gene under study, this will determine whether association would be detected using the sum or average statistic. This could be particularly influential in the COGA sample where there are a number of large families in the sample, which could strongly influence our ability to detect association. We routinely compute both statistics as we expect genetic heterogeneity and it is impossible to know a priori what subset of families will show association.

Quantitative traits, withdrawal, level of response to alcohol, age of onset of regular drinking, and age at first drunkenness, were evaluated with the Quantitative PDT (QPDT) using the QPDTPHASE program contained in the UNPHASED software suite (Dudbridge, 2003). This program is an extension of the PDT developed for the analysis of quantitative traits (Zhang et al., 2001). The QPDT test produces a single *p*-value that estimates the significance of the test statistic (Zhang et al., 2001). Haplotype association tests were performed using the PDTPHASE (for qualitative phenotypes) and QPDTPHASE (for quantitative phenotypes) programs contained in the UNPHASED software suite (Dudbridge, 2003). Sliding window haplotype analyses were computed using tag SNPs, as indicated by (Nyholt, 2004).

Linkage disequilibrium (LD) was evaluated between markers in each gene using the program GOLD (Abecasis and Cookson, 2000).

The COGA families of Caucasian descent were used to calculate LD. GOLD uses haplotype input from Simwalk2 (Sobel and Lange, 1996) and produces pairwise disequilibrium measures for all markers entered into the analysis. The extent of LD was measured using Lewontin's-standardized disequilibrium coefficient (D') and delta-squared.

RESULTS

SNP Association

Table 2 presents results for all genotyped SNPs in *GABRA1*, *GABRA6*, *GABRB2*, and *GABRG2* with the 2 qualitative phenotypes analyzed, COGA alcohol dependence, and history of blackouts. *p*-Values are listed in the table for both PDT-sum and -average statistics for the qualitative traits. Eight SNPs in *GABRA1* showed association ($p<0.05$) with COGA alcohol dependence and 7 SNPs in *GABRA1* showed association at $p<0.05$ with history of blackouts. We did not find any association with the remaining genes (*GABRA6*, *GABRB2*, and *GABRG2*) with COGA alcohol dependence or history of blackouts.

Table 3 presents *p*-values from the QPDTPHASE for all SNPs with each of the quantitative phenotypes: withdrawal, level of response to alcohol, age of onset of regular drinking, and age at first drunkenness. Two SNPs in *GABRA1* showed significant association ($p = 0.007$, 0.008) with age at first drunkenness. However, we did not find any association between the remaining genes (*GABRA6*, *GABRB2*, and *GABRG2*) and age at first drunkenness. One SNP in *GABRA1* (rs1037715) showed a significant association ($p = 0.008$) with level of response to alcohol. No consistent association was seen between withdrawal or age of onset of regular drinking and any of the genes.

LD

We used information about LD in the region to further interpret our association results. Table 4 presents LD for the SNPs located in *GABRA1*, the gene yielding evidence of association with multiple phenotypes in the analyses reported here. Linkage disequilibrium for other genes in the cluster is reported in Dick et al. (2005). In *GABRA1*, D' was fairly high between markers (average $D' = 0.80$). δ^2 was considerably lower, indicating that many of the SNPs are contributing additional, nonredundant information for association because of differences in allele frequencies between the SNPs (average delta-squared = 0.28).

We note that the *p*-values reported in Tables 2 and 3 are uncorrected for multiple testing. Correcting for multiple testing in genetic analyses is complicated by the fact that the SNPs are not independent. Accordingly, we performed a Nyholt adjustment to our data (Nyholt, 2004) by conducting a spectral decomposition on the pairwise LD matrix for the 16 SNPs across *GABRA1*. We computed the effective number of SNPs (M_{eff}) based on the

Table 4. Linkage Disequilibrium (LD) Table for *GABRA1*

										D'								
delta^2	rs4478357	rs4608967	rs6883877	rs1129647	rs4263535	rs4605831	rs6556562	rs11135172	rs1350372	rs1037715	rs1026447	rs980791	rs1157122	rs2279020	rs2290732	rs998754
rs4478357		0.87	0.93	0.79	0.78	0.81	0.81	0.80	0.81	0.78	0.78	0.71	0.71	0.69	0.80	0.71
rs4608967	0.11		0.95	0.83	0.87	0.92	0.84	0.69	0.85	0.60	0.84	0.79	0.86	0.82	0.85	0.78
rs6883877	0.02	0.17		0.50	0.85	0.81	0.93	0.62	1.00	0.67	0.61	0.81	1.00	0.80	0.93	0.79
rs1129647	0.33	0.02	0.002		0.90	0.87	0.65	0.85	0.65	0.82	0.86	0.65	0.84	0.61	0.68	0.26
rs4263535	0.16	0.43	0.08	0.05		0.86	0.86	0.79	0.88	0.71	0.83	0.37	0.87	0.43	0.66	0.56
rs4605831	0.28	0.02	0.003	0.61	0.04		0.88	0.89	0.87	0.85	0.84	0.84	0.85	0.83	0.88	0.83
rs6556562	0.57	0.12	0.03	0.26	0.22	0.39		0.87	0.90	0.90	0.83	0.80	0.89	0.81	0.88	0.79
rs11135172	0.28	0.01	0.002	0.59	0.03	0.79	0.38		0.83	0.88	0.88	0.81	0.85	0.85	0.87	0.83
rs1350372	0.57	0.12	0.03	0.26	0.23	0.37	0.82	0.34		0.89	0.85	0.81	0.85	0.80	0.87	0.81
rs1037715	0.13	0.005	0.001	0.28	0.01	0.38	0.20	0.39	0.20		0.86	0.79	0.66	0.85	0.88	0.81
rs1026447	0.25	0.02	0.002	0.59	0.03	0.70	0.34	0.75	0.35	0.38		0.81	0.88	0.83	0.86	0.79
rs980791	0.36	0.13	0.03	0.30	0.05	0.41	0.53	0.38	0.55	0.19	0.38		0.86	0.88	0.80	0.88
rs1157122	0.08	0.67	0.16	0.03	0.49	0.02	0.15	0.02	0.14	0.007	0.02	0.17		0.87	0.86	0.81
rs2279020	0.34	0.14	0.02	0.27	0.07	0.41	0.55	0.43	0.54	0.22	0.40	0.77	0.17		0.80	0.89
rs2290732	0.50	0.14	0.03	0.31	0.14	0.43	0.69	0.42	0.68	0.22	0.40	0.60	0.15	0.60		0.82
rs998754	0.41	0.11	0.02	0.63	0.10	0.36	0.58	0.36	0.62	0.18	0.33	0.70	0.13	0.71	0.64	

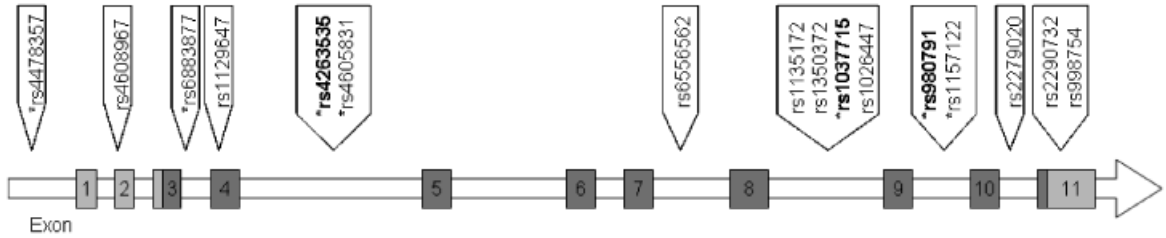

Fig. 1. Location of single-nucleotide polymorphisms (SNPs) flanking and within the *GABRA1* gene on chromosome 5. Single-nucleotide polymorphisms indicated with an * are tagging SNPs. Bold tagSNPs yielded the most significant *p*-values in association tests. Dark gray boxes represent coding sequences, light gray boxes represent exons encoding untranslated sequences, and the white bars represent intronic sequences.

observed eigenvalue variance, using the updated method of Li and Ji (2005). $M_{\text{eff}} = 7$. The 7 SNPs that contributed the most to each rotated factor are indicated with an [a] in Tables 2 and 3. These flagged SNPs can be considered "haplotype tagging SNPs" (Nyholt, 2004). With $M_{\text{eff}} = 7$, the modified significance threshold required across *GABRA1* would be $p = 0.007$. The tagSNP rs980791 yielded a *p*-value of 0.008 for history of blackouts. The tagSNP rs4263535 yielded a *p*-value of 0.008 with age at first drunkenness, and another SNP in *GABRA1* yielded $p = 0.007$. The tagSNP rs1037715 yields a *p*-value of 0.008 for level of response to alcohol. Figure 1 shows the location of all genotyped SNPs across *GABRA1*, with rs4263535, rs980791, and rs1037715 indicated in bold.

Haplotype Analyses

Sliding-window haplotype analyses were conducted using 3 SNP windows across the 7 haplotype tagging SNPs. Table 5 presents the results of haplotype association tests for the 4 phenotypes that showed association with *GABRA1*: COGA alcohol dependence, history of blackouts, age at first drunkenness, and SRE. The global *p*-values from haplotype analyses were significant at $p < 0.01$ for all 4 phenotypes.

DISCUSSION

We have tested for association, using family-based methods, between the chromosome 5 $GABA_A$ receptor genes and several alcohol-related phenotypes, as a follow-up to our previous paper reporting no association with these genes and DSM-IV alcohol dependence in the COGA sample. We tested a broader definition of alcohol dependence ("COGA alcoholism"), incorporating DSM-III-R and Feighner criteria; a tally of withdrawal symptoms; history of blackouts; level of response to alcohol; age of onset of regular drinking; and age at first drunkenness. In addition, we have genotyped 16 additional SNPs since the publication of our previous paper to provide more complete coverage of the genes in the region. We used family-based designs, tested multiple SNPs in each gene, and used patterns of LD to interpret association results.

The results indicate an association between *GABRA1* and several of these alcohol-related phenotypes. The SNP rs980791 was associated with a history of blackouts ($p = 0.007$) and COGA alcohol dependence ($p = 0.01$). Another tagSNP rs4263535 yielded the most significant single SNP association ($p = 0.008$) for age at first drunkenness, but the most significant haplotype with this phenotype included rs980791. Another tagSNP rs1037715 also yielded significant evidence of association with level of response to alcohol. Our data are consistent with *GABRA1* being a gene of small effect in relation to the phenotypes with which we find evidence of association. For example, the overtransmission rate at the SNP rs980791 was 1.1 for both COGA alcohol dependence and the experience of blackouts. The SNP rs980791 was one included in the original publication on the chromosome 5 $GABA_A$ receptor genes and DSM-IV alcohol dependence, and it was not significantly associated with DSM-IV diagnoses. The other SNPs in *GABRA1* associ-

Table 5. *p*-Values From Sliding Window Haplotype Analyses Across Tagging SNPs in GABRA1, With Each of the Phenotypes Yielding Evidence of Association With Individual SNPs

	COGA AD		Blackouts		QPDT	
Sliding window haplotype	PDT-Ave	PDT-Sum	PDT-Ave	PDT-Sum	Age first drunk	SRE
rs4478357-rs6883877-rs4263535	0.3130	0.0541	0.1713	0.1459	0.2695	0.0738
rs6883877-rs4263535-rs4605831	0.3072	0.0587	0.0487	**0.0469**	0.1009	**0.0361**
rs4263535-rs4605831-rs1037715	0.2522	**0.0391**	0.0504	**0.0368**	**0.0459**	**0.0059**
rs4605831-rs1037715-rs980791	**0.0051**	**0.0160**	**0.0021**	0.1282	**0.0049**	**0.0425**
rs1037715-rs980791-rs1157122	**0.0096**	0.0709	**0.0084**	0.1325	**0.0112**	0.1574

Note: *p*-values < 0.05 indicated in bold. *p*-Values are not corrected for multiple tests.

SNP, single-nucleotide polymorphism; COGA, Collaborative Study on the Genetics of Alcoholism; SRE, Self-Rating of the Effects of Alcohol; PDT, pedigree disequilibrium test.

ated with the drinking-related phenotypes reported here were not genotyped as part of the previous (negative) paper by our group (Dick et al., 2005); however, these SNPs also show no association with DSM-IV alcohol dependence (results available upon request), further supporting the conclusions of that paper. No other genes showed consistent evidence of association with these phenotypes in our sample. In addition, the other phenotypes studied, withdrawal and age of onset of regular drinking, lacked consistent evidence of association with *GABRA1* or the other genes in the cluster. However, we note that a small number of SNPs in the surrounding genes showed sporadic evidence of association with various phenotypes; because LD was low between these SNPs and neighboring SNPs, we cannot rule out the possibility that there are additional genetic variants in these genes that may be associated with alcohol-related traits.

Previous human studies of the chromosome 5 $GABA_A$ receptor gene cluster have examined alcohol dependence almost exclusively. Positive findings for association between alcohol dependence and *GABRA6* (Loh et al., 1999; Radel et al., 2005; Sander et al., 1999; Schuckit et al., 1999), *GABRB2* (Loh et al., 1999; Radel et al., 2005), and *GABRG2* (Loh and Ball, 2000; Radel et al. 1999) have previously been reported; however, to date, no association has been found with *GABRA1* and alcohol dependence diagnoses. An equal number of studies have found negative evidence for association between alcohol dependence and 1 or more of the chromosome 5 $GABA_A$ receptor genes (Dick et al., 2005; Hsu et al., 1998; Loh et al., 1999; Loh and Ball, 2000; Parsian and Cloninger, 1997; Sander et al., 1999; Song et al., 2003). Few studies have examined alcohol-related behaviors other than alcohol dependence with these genes in humans; to the authors' knowledge, only level of response to alcohol has been studied to date. Schuckit et al. (1999) found evidence for association between the Pro385Ser polymorphism in *GABRA6* and reaction to alcohol; however, an extension of that population revealed no such association (Hu et al., 2005). These investigations differed from our study by using a case–control study design, alcohol challenges to measure reaction, and fewer subjects. Furthermore, in our study, we did not examine the Pro385Ser polymorphism directly, but instead looked at SNPs across *GABRA6* and the rest of the gene cluster, in the context of LD patterns. We did not find an association with *GABRA6*, *GABRG2*, or *GABRB2* with level of response to alcohol, supporting the findings of Hu et al. (2005).

Most of the evidence for a relationship between the chromosome 5 $GABA_A$ receptor gene cluster and alcohol-related phenotypes has come from animal studies, which have examined alcohol-related traits, such as withdrawal and level of response to alcohol. The current findings support such efforts to link these genes with alcohol-related phenomena rather than DSM alcohol dependence diagnoses, per se. Although animal studies guided our choice of traits to analyze, these characteristics were operationalized in a different manner than in animal studies. For example, while alcohol withdrawal in animals is typically defined by handling-induced seizures (Buck et al., 1997), in this study, the definition used a tally of somatic symptoms from the DSM. Similarly, while level of response to alcohol in animals is measured by the duration of the loss of righting reflex, here the response used self-reports on number of drinks needed to achieve an effect the first 5 times an individual drank alcohol. Such differences in phenotype definitions may explain the lack of support for an association between withdrawal or level of response to alcohol with several of the chromosome 5 $GABA_A$ receptor genes in the COGA sample, despite a wealth of evidence in the animal literature to the contrary.

Our findings should be interpreted within the context of several limitations. Only the association between the tagSNP rs980791 and blackouts met the threshold for significance after adjusting for multiple testing ($p = 0.007$). However, we note that tagSNPs in *GABRA1* also yielded evidence of association at $p = 0.008$ with age at first drunkenness and initial sensitivity and that multiple SNPs across *GABRA1* yielded evidence of association with COGA alcohol dependence at $p = 0.01$. We think these findings are of importance because there continues to be debate about whether correction methods remain overly conservative; it has been our experience that the genetic findings that have been detected in the COGA project and, importantly, that have replicated across multiple independent studies (e.g., the association between *GABRA2* and alcohol dependence), would not have met traditional significance levels after correction for multiple testing. Accordingly, we believe that the consistent association observed across multiple SNPs in *GABRA1* with multiple alcohol-related phenotypes provides evidence that this gene may be involved in patterns of alcohol use and susceptibility to develop problems; ultimately, replication in independent samples will be necessary to evaluate the potential role of *GABRA1*. In addition, we note that the SNPs yielding evidence of association here are intronic (as shown in Fig. 1). It is unknown how *GABRA1* may be involved in biological differences that translate into differences in alcohol use and related phenotypes. It is unclear whether the SNPs implicated here are involved in risk or whether they are in LD with the actual variant(s) in *GABRA1* that causes differences in susceptibility (perhaps a more likely possibility as SNPs across multiple intronic regions show association). Finally, we note that the COGA families are not representative of the general population; they have a high density of alcoholism and high rate of ASPD compared with the general population. Also, the majority of families are Caucasian, with a smaller subset of African American families; consequently, racial groups other than Caucasian have not been adequately tested.

In conclusion, these results support an association between SNPs located in *GABRA1* and COGA alcohol dependence, a history of blackouts, the age at first drunkenness, and level of response to alcohol. Future studies should be carried out to further examine the role of *GABRA1* and other candidate genes in alcohol-related behaviors, which may replicate findings from animal studies better than studies only examining alcohol dependence as a clinical disorder.

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