0145-6008/04/2801-0004$03.00/0
ALCOHOLISM: CLINICAL AND EXPERIMENTAL RESEARCH

Vol. 28, No. 1
January 2004

Association of *GABRG3* With Alcohol Dependence

Danielle M. Dick, Howard J. Edenberg, Xiaoling Xuei, Alison Goate, Sam Kuperman, Marc Schuckit, Raymond Crowe, Tom L. Smith, Bernice Porjesz, Henri Begleiter, and Tatiana Foroud

Background: Evidence from human, animal, and in vitro cell models suggests that γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the human central nervous system, is involved in many of the neurochemical pathways that affect alcohol use, abuse, and dependence. Both linkage and association to the region on chromosome 15q that contains a cluster of $GABA_A$ receptor genes have previously been reported, but the role of these genes in alcoholism remains inconclusive.

Methods: We conducted family-based association analyses by using a large sample of multiplex alcoholic families collected as part of the Collaborative Study on the Genetics of Alcoholism, to test for an association between alcohol dependence and the $GABA_A$ receptor genes clustered on chromosome 15q. Multiple single-nucleotide polymorphisms were tested in each of the three chromosome 15q $GABA_A$ receptor genes: *GABRA5*, *GABRB3*, and *GABRG3*.

Results: Using both classic trio-based analyses and extended-family analyses, we found consistent evidence of association between alcohol dependence and *GABRG3*. Nearly all single-nucleotide polymorphisms across the gene yielded evidence of association, and haplotype analyses were highly significant. No consistent evidence of association was observed with either *GABRA5* or *GABRB3*, nor was there evidence for parent-of-origin effects with any of the genes.

Conclusions: These analyses suggest that *GABRG3* may be involved in the risk for alcohol dependence. These findings support the theory that the predisposition to alcoholism may be inherited as a general state of central nervous system disinhibition/hyperexcitability that results from an altered responsiveness to GABA.

Key Words: GABA, Alcohol Dependence, Genetic Analysis, COGA.

EVIDENCE FROM ANIMAL, human, and in vitro cell models suggests that γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the human central nervous system, is involved in many of the neurochemical pathways that affect alcohol use and related disorders. GABA is involved in several of the behavioral effects of alcohol, including motor incoordination, anxiolysis, sedation, withdrawal signs, and ethanol preference (Buck, 1996; Grobin et al., 1998). $GABA_A$ receptor agonists tend to potentiate the behavioral effects of alcohol, whereas $GABA_A$ receptor antagonists attenuate these effects. $GABA_A$ receptors have also been implicated in ethanol tolerance and dependence (Grobin et al., 1998). It is not clear exactly how GABA reception is involved in these actions of ethanol (Grobin et al., 1998).

Most of the $GABA_A$ receptor genes are organized into clusters. Chromosome 4p contains the genes *GABRA2*, *GABRA4*, *GABRB1*, and *GABRG1*; chromosome 5q contains *GABRA1*, *GABRA6*, *GABRB2*, and *GABRG2*; and chromosome 15q contains *GABRA5*, *GABRB3*, and *GABRG3* (LocusLink; National Center for Biotechnology Information). This study explored the relationship between the chromosome 15 $GABA_A$ receptor gene cluster and alcohol dependence. This cluster has not been studied as extensively as the clusters on chromosome 4 (Edenberg et al., 2002; Parsian and Zhang, 1999; Porjesz et al., 2002) and chromosome 5 [reviewed in Dick and Foroud (2003)], although evidence is accumulating to suggest that the $GABA_A$ receptor genes on chromosome 15 may be involved in alcohol dependence and related phenotypes. Microsatellite markers in *GABRA5* and *GABRB3* were previously tested for association by using parent-offspring trios selected from the Collaborative Study on the Genetics of Alcoholism (COGA) sample (Song et al., 2003). Modest evidence of association between *GABRA5* and alcohol dependence, as defined by the International Classification of Diseases, 10th revision, was found when the sample was limited to Caucasians. On the basis of evidence from a study demonstrating that *GABRB3*, *GABRA5*, and *GABRG3* were expressed only from the paternal alleles in

From the Indiana University School of Medicine, Indianapolis, Indiana (DMD, HJE, XX, TF); Washington University, St. Louis, Missouri (AG); University of Iowa, Iowa City, Iowa (SK, RC); University of California, San Diego, California (MS, TLS); and State University of New York, Brooklyn, New York (BP, HB).

Received for publication June 12, 2003; accepted October 1, 2003.

This national collaborative study is supported by NIH Grant U10AA08403 from the NIAAA. Preparation of this manuscript was also supported by AA13358 (D. Dick) and K02-AA00285 (T. Foroud).

Reprint requests: Tatiana Foroud, PhD, Department of Medical and Molecular Genetics, Indiana University School of Medicine, 975 W. Walnut St., IB-130, Indianapolis, IN 46202-5251; Fax: 317-274-3287; E-mail: tforoud@iupui.edu.

DOI: 10.1097/01.ALC.0000108645.54345.98

hybrid mouse A9 cells containing a single human chromosome 15 (Meguro et al., 1997), Edenberg et al. tested for paternal transmission of *GABRA5* and *GABRB3* in the COGA sample and found evidence of association of both genes with alcohol dependence (Song et al., 2003). In a case-control study of Caucasian alcoholics and controls, an association was also reported between *GABRB3* and severe alcoholism, as defined by documented alcohol-induced bodily damage such as cirrhosis; furthermore, there was a significant, progressive decrease in the prevalence of the most frequent allele of *GABRB3* in nonalcoholics, less severe alcoholics, and severe alcoholics (Noble et al., 1998). The β_2 component of the electroencephalogram (EEG), an endophenotype for alcoholism, also has been linked to the chromosome 15 region that contains the $GABA_A$ receptor gene cluster in the sample collected by COGA (Ghosh et al., 2003).

Here we report family-based association analyses using a large sample of multiplex alcoholic families collected as part of the COGA study. Our study has several strengths that build on the existent literature. We used a large family-based association design, which avoids potential problems with population stratification that are introduced by case-control studies. We analyzed both extended families and classic transmission disequilibrium test (TDT) trios (composed of an affected offspring and his or her parents) to examine consistency across analytic methods. We tested multiple single-nucleotide polymorphisms (SNPs) in each gene and looked for consistent trends across SNPs within the gene. Many previous studies have tested only one or two genetic variants in the gene under study; this can lead to a false-negative finding if that particular marker is not in linkage disequilibrium (LD) with the variation or variations that increase risk for disease. Alternatively, a positive association could result if that particular marker is in high LD with variations in a nearby gene; in such a case, although the signal would be real, the actual gene involved would not be the one in which the marker resides. These potentially incorrect conclusions could result because patterns of LD are not a simple function of physical distance across the genome (Abecasis et al., 2001; Gabriel et al., 2002). Therefore, we analyzed LD between SNPs and used this information to help interpret the results of association analyses. By designing the study in this manner and taking these steps to ensure data quality and consistency, we aimed to provide more conclusive evidence regarding the role of the $GABA_A$ receptor genes on chromosomes 15 in affecting the risk for alcohol dependence.

METHODS

Sample

COGA is a multisite project for which families were collected at six centers across the United States: Indiana University, State University of New York Health Science Center, University of Connecticut, University of Iowa, University of California–San Diego, and Washington University, St. Louis. Probands identified through inpatient or outpatient alcohol treatment programs at these six sites were invited to participate if they had a sufficiently large family (usually more than three siblings with parents available) with two or more members in a COGA catchment area (Reich, 1996). The institutional review boards of all participating institutions approved the study. A total of 1227 families of alcohol-dependent probands were recruited for the first stage of the study. Additionally, a sample of control families, obtained through random sources such as driver's license registries and dental clinics, was assessed. These families consisted of two parents and at least three children over the age of 14 years. All individuals were administered the Semi-Structured Assessment for the Genetics of Alcoholism interview (Bucholz et al., 1994; Hesselbrock et al., 1999). For this study, individuals were diagnosed with alcohol dependence by using DSM-IV criteria (American Psychiatric Association, 1994).

Multiplex alcoholic families that were not bilineal and had at least two 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- and third-degree relatives in the families were assessed when they were considered to be informative for the genetic linkage studies. A total of 987 adult individuals from 105 extended families were included in the initial genotyped dataset (Reich et al., 1998). A replication sample was ascertained and genotyped by following identical procedures; it consisted of 1295 individuals from 157 extended families (Foroud et al., 2000). Thus, 2282 individuals from 262 multiplex alcoholic families were available for genetic analyses. An additional 1254 individuals from 227 control families also had blood collected and were included to increase the power of analyses of LD between markers.

DNA Analyses

SNPs were chosen across each candidate gene from public databases; we did not restrict ourselves to coding regions or exons, because allele frequencies for such SNPs are often low. Locations were in most cases determined from the annotations in the National Center for Biotechnology Information human genome assembly. In some cases, position was determined by BLASTing the sequence against the human genome assembly. Allele frequencies are not usually available for public SNPs, so SNPs were genotyped on 2 sets of approximately 40 unrelated individuals from the Coriell Caucasian American and African American samples to determine approximate allele frequencies; we preferentially chose SNPs with high heterozygosities. Genotyping was performed by using a modified single-nucleotide extension reaction, with allele detection by mass spectrometry (Sequenom MassArray system, Sequenom, San Diego, CA). A total of 3536 individuals from the COGA families were genotyped. All genotypic data were checked for Mendelian inheritance of marker alleles with the USERM13 (Boehnke, 1991) option of the MENDEL linkage computer programs; this was then used to estimate marker allele frequencies. Twenty-one SNPs were genotyped in the chromosome 15 $GABA_A$ receptor gene cluster: 6 SNPs in *GABRB3*, 4 SNPs in *GABRA5*, and 11 SNPs in *GABRG3*. The average heterozygosity across the SNPs in chromosome 15 was 0.44. These SNPs, the gene in which they are located, and their chromosomal positions are shown in Table 1.

Statistical Analyses

Multiplex alcoholic families were used in tests of association between each of the SNPs and alcohol dependence. The Pedigree Disequilibrium Test (PDT) (Martin et al., 2000) was used to analyze associations in the extended pedigrees. The PDT uses data from all available trios in a family, as well as discordant sibships. It produces two statistics: the PDT-avg, which averages the association statistic across all families, and the PDT-sum, which gives greater weight to families with more informative trios and discordant sibships (Martin et al., 2001). Because the COGA sample consists of several very large families that might overly influence the PDT-sum statistic, we report the values from the PDT-avg statistic. We also conducted classic TDT trio-based analyses by selecting one trio from each COGA family. We used the program TRANSMIT for these analyses (Clayton, 1999). Finally, because the existent literature on chromosome 15

Table 1. SNPs in the Chromosome 15 $GABA_A$ Receptor Gene Cluster and Their Association With Alcoholism

Marker	Gene	Position	Heterozygosity	PDT-avg *p* Value	TRANSMIT *p* Value	No. trios	Tdtex-maternal	Tdtex-paternal
rs2912582	*GABRB3*	24,210,447	0.03	0.23	—	4	0.11	0.02*
rs2052991	*GABRB3*	24,221,600	0.36	0.51	0.30	120	0.75	0.61
rs1432007	*GABRB3*	24,228,746	0.50	0.95	0.41	142	0.49	0.79
rs1426217	*GABRB3*	24,239,182	0.49	0.13	0.63	146	0.08	0.31
rs1897356	*GABRB3*	24,283,592	0.28	0.02*	0.93	91	0.41	1.00
rs2873027	*GABRB3*	24,285,466	0.48	0.75	0.69	99	0.0002*	1.00
rs140681	*GABRA5*	24,615,673	0.16	0.66	0.61	63	0.29	0.89
rs140682	*GABRA5*	24,615,818	0.49	0.12	0.46	130	0.57	0.50
rs1864793	*GABRA5*	24,617,050	0.49	0.24	0.75	138	0.46	1.00
rs140685	*GABRA5*	24,621,920	0.50	0.19	0.40	135	0.31	0.25
rs2288694	*GABRG3*	24,650,207[a]	0.48	0.25	0.29	135	0.51	0.61
rs1871019	*GABRG3*	25,229,015	0.49	0.28	0.06	136	0.11	0.21
rs3101639	*GABRG3*	25,243,150	0.49	0.11	0.02*	138	0.11	0.21
rs3097493	*GABRG3*	25,243,588	0.49	0.06	0.02*	131	0.14	0.16
rs3101637	*GABRG3*	25,243,756	0.50	0.10	0.04*	134	0.13	0.44
rs3101636	*GABRG3*	25,243,768	0.49	0.13	0.02*	132	0.20	0.26
rs140678	*GABRG3*	25,245,560	0.49	0.13	0.08	135	0.44	0.31
rs140679	*GABRG3*	25,245,731	0.50	0.10	0.01*	143	0.74	0.06
rs2303879	*GABRG3*	25,245,947	0.49	0.11	0.02*	125	0.29	0.22
rs3097490	*GABRG3*	25,246,846	0.49	0.09	0.02*	136	0.13	0.21
rs3097489	*GABRG3*	25,249,098	0.48	0.30	0.10	129	1.00	0.41

Statistics are shown for both the PDT, TRANSMIT, and parent-of-origin effects using TDTEX.

Position is from dbSNP114, May 2003.

[a] The accuracy of this SNP position is uncertain. There is a gap and an unfinished sequence in this region. rs2288694 maps far from the other *GABRG3* SNPs but is annotated as being within 2 kilobases of the transcript; BLASTing shows it within exon 1 of the messenger RNA. The REFSEQ database shows 10 exons for *GABRG3*, but BLASTing the genome (build 33) gives only 9 hits: 1 middle exon is missing. Thus, the exact distance between SNPs is unknown.

* $p \leq 0.05$; *p* values were not corrected for multiple tests.

$GABA_A$ receptor genes suggested that there may be parental transmission effects, we also used the program TDTEX, which performs TDT analyses that allow for the testing of parent-of-origin effects (Statistical Analysis for Genetic Epidemiology, version 4.0, Department of Epidemiology and Biostatistics, Rammelkamp Center for Education and Research, MetroHealth Campus, Case Western University, Cleveland, OH).

Haplotype analyses were conducted, using both the PDT and TRANSMIT, across genes that showed evidence of association at the individual SNP level. We used a sliding-window approach in which groups of three SNPs were sequentially tested across the gene. Thus, the first analysis tests the haplotype composed of SNPs 1, 2, and 3; the second haplotype analysis tests SNPs 2, 3, and 4, and so on. We used haplotypes computed using Simwalk2 (Sobel and Lange, 1996) as input into the PDT. Families with more than one individual with a double recombinant event were omitted from the analyses. More than 90% of all families were retained for all analyses. TRANSMIT computes haplotypes internally by averaging over all possible configurations of parental haplotypes and transmissions consistent with the observed data. Possible haplotype assignments are weighted according to the probability of each assignment by using the expectation-maximization algorithm. Because several rare haplotypes were observed and these can bias the χ^2 statistic yielded by TRANSMIT, only haplotypes observed at a frequency of at least 10% were used in the test statistic.

LD between markers was evaluated by using the program GOLD (Abecasis and Cookson, 2000) with both multiplex alcoholic family data and control family data. This program uses haplotype input from Simwalk2 (Sobel and Lange, 1996) and produces pairwise disequilibrium measures for all markers entered into the analysis. Families that had more than one individual with a double recombination event within a gene were dropped from subsequent analyses. Several measures of LD are produced by GOLD. We show the degree of LD between markers, as measured by Δ^2, for regions for which LD was used to help interpret the association results. The conventional definition for Δ^2 (Devlin and Risch, 1995) is used by GOLD:

$$\Delta^2 = \frac{(p_{11}p_{22} - p_{12}p_{21})^2}{p_1 \cdot p_2 \cdot p_1 \cdot p_2 \cdot}$$

The *p* values were not corrected for multiple testing because the SNPs are not independent. Thus, we conservatively interpreted our results by requiring consistency across analytic methods and requiring consistency between the pattern of association results and the pattern of LD across the region.

RESULTS

The SNPs in *GABRG3* provided consistent evidence of association with alcohol dependence (Table 1). By using the program TRANSMIT, 7 of the 11 SNPs tested in *GABRG3* provided significant evidence of association ($p \leq 0.05$). Three of the four remaining SNPs showed evidence of association at $p \leq 0.10$. Although the results of the PDT did not reach statistical significance, they were generally consistent with the results from TRANSMIT, with 8 of the 11 SNPs yielding *p* values ≤0.13. LD analyses demonstrated that the 1 SNP that showed no evidence of association with either TRANSMIT or the PDT—rs2288694—was not in LD with the other SNPs in *GABRG3* (Table 2); all other SNPs were in reasonably high LD. rs2288694 maps further from the other *GABRG3* SNPs, and there are apparent problems with this region of the genome (see the note to Table 1). Table 3 lists the number of observed versus expected transmissions for the SNPs in *GABRG3*, so the reader can evaluate the degree of deviation from expected values.

The results from the sliding-window haplotype analyses with both the PDT and TRANSMIT yielded further evidence of association (Table 4). The overall χ^2 statistics showed trends toward significance at $p \leq 0.10$ for six of the nine tests performed with the PDT and seven of nine tests

Table 2. LD Between SNPs Within the Chromosome 15 $GABA_A$ Receptor Gene *GABRG3* as Measured by Δ^2

Variable	rs1871019	rs3101639	rs3097493	rs3101637	rs3101636	rs140678	rs140679	rs2303879	rs3097490	rs3097489
rs2288694	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
rs1871019		0.74	0.74	0.81	0.74	0.59	0.74	0.67	0.69	0.73
rs3101639			0.84	0.76	0.76	0.63	0.68	0.69	0.67	0.75
rs3097493				0.75	0.71	0.63	0.69	0.70	0.65	0.74
rs3101637					0.82	0.56	0.78	0.75	0.73	0.83
rs3101636						0.60	0.80	0.79	0.80	0.82
rs140678							0.61	0.62	0.55	0.58
rs140679								0.75	0.74	0.79
rs2303879									0.75	0.76
rs3097490										0.73

SNPs are shown in order across the gene, corresponding to the chromosomal positions listed in Table 1.

Table 3. Number of Observed vs. Expected Transmissions for Each Allele Yielded by TRANSMIT for SNPs Across *GABRG3*

Marker	Allele	Observed	Expected	*p* Value
rs2288694	1	113	120	0.29
	2	157	150	
rs1871019	1	122	134	0.06
	2	150	138	
rs3101639	1	153	137	0.02
	2	123	139	
rs3097493	1	115	131	0.02
	2	147	131	
rs3101637	1	147	133	0.04
	2	121	135	
rs3101636	1	148	133	0.02
	2	116	131	
rs140678	1	123	135	0.08
	2	147	135	
rs140679	1	132	149	0.01
	2	154	137	
rs2303879	1	139	124	0.02
	2	111	126	
rs3097490	1	122	138	0.02
	2	150	134	
rs3097489	1	117	129	0.10
	2	141	130	

p values are also shown to evaluate the degree of transmission deviation for each SNP; these correspond to the values listed in Table 1.

Table 4. *p* Values From Haplotype Analyses With the PDT and TRANSMIT for All SNPs Tested in *GABRG3*

	PDT		TRANSMIT	
Marker	Overall	High risk	Overall	High risk
rs2288694				
rs1871019				
rs3101639	0.132	0.008	0.156	0.019
rs3097493	0.060	0.001	0.047	0.014
rs3101637	0.153	0.005	0.066	0.024
rs3101636	0.040	0.002	0.017	0.053
rs140678	0.105	0.010	0.203	0.073
rs140679	0.128	0.006	0.040	0.012
rs2303879	0.057	0.001	0.059	0.018
rs3097490	0.035	0.000	0.049	0.015
rs3097489	0.020	0.001	0.067	0.026

Haplotype analyses were conducted with a sliding-window approach for groups of adjacent SNPs. Each *p* value represents the test of the haplotype composed of the three SNPs listed before that value. For both the PDT and TRANSMIT, the first column lists the *p* value yielded by the overall χ^2 statistic testing all haplotypes. The second column represents the *p* value yielded for the test of the specific risk haplotype.

with TRANSMIT. Furthermore, the tests for the specific risk haplotypes, composed of the alleles that were over-transmitted in the individual SNP analyses, were significant for nearly all haplotypes tested across both methods of analysis.

There were no consistent patterns of association across any of the other SNPs tested in the two other $GABA_A$ receptor genes on chromosome 15 (Table 1). The results from the parental origin tests performed in S.A.G.E. suggest that there are no consistent parent-of-origin effects (Table 1); we believe that the two SNPs that were significant in parental origin tests are likely false positives, because the other SNPs that they are in LD with show no evidence of parent-of-origin effects.

DISCUSSION

We have tested for association between the $GABA_A$ receptor genes clustered on chromosome 15 and alcohol dependence by using a large sample of multiplex alcoholic families. Our strategy used multiple analytic methods for family-based designs, tested multiple SNPs in each gene, and used patterns of LD among the SNPs to interpret association results and reduce the possibility of false positives. We found consistent evidence that *GABRG3* on chromosome 15 is associated with alcohol dependence. Nearly all SNPs tested showed evidence of association, at least at the trend level, with both classic TDT analyses and the PDT.

Haplotype analyses were highly significant with both the TDT and PDT tests. We are encouraged by the consistency between the PDT and TRANSMIT haplotype analyses because the haplotypes were computed by using different methods in each program (Simwalk2 uses Markov chain Monte Carlo and simulated annealing algorithms versus the expectation-maximization algorithm in TRANSMIT). However, the haplotype analyses were not helpful in further narrowing the region of *GABRG3* that may contain the putative risk variant. Haplotype analyses showed association across the gene, with no particular patterns of greater significance in any one region.

Greater statistical significance was obtained from the TDT, which uses only one independent trio from each family, than with the PDT, which includes information from additional family members. We speculate that this may be due to the inclusion of genotypic data from unaffected individuals in the PDT. In the event of association, it

would be expected that the overtransmitted allele would be more prevalent in affected individuals as compared with unaffected siblings. However, this was not always the case, which had the effect of decreasing the magnitude of the PDT statistic. Analyses with discordant sibling pairs should be interpreted with caution when alcohol dependence is studied, because no allowance is made for the possibility that unaffected siblings may not truly be unaffected, but simply may not have manifested sufficient symptoms to meet diagnostic criteria. The PDT does not take into account an unaffected individual's age or whether he or she has passed through the period of highest risk.

No consistent evidence of association was seen with either of the other $GABA_A$ receptor genes on chromosome 15. The previous studies (Noble et al., 1998; Song et al., 2003) that found evidence of association with *GABRB3* and *GABRA5* both tested microsatellite markers in the genes. Thus, it is possible that those positive association results could have resulted from LD with a nearby $GABA_A$ receptor gene. It is also puzzling that we found evidence of paternal transmission in this gene cluster previously but found no evidence of parent-of-origin effects here. We have no biological explanation for this. However, our more detailed analyses of multiple SNPs in all three genes across the region provide more power to test for such effects than our previous analyses, which relied on single microsatellite markers tested in each gene (Song et al., 2003). Therefore, we were able to more definitively test for paternal transmission in this dataset, and we found no evidence of such effects.

Our results suggest that *GABRG3* is the gene most likely to be responsible for the association observed in our dataset and that it is likely that variants in this gene contribute to the risk for alcoholism. The function of the *GABRG3* receptor gene is not clearly understood. Therefore, it is difficult to speculate why this particular gene, located within a cluster of $GABA_A$ receptor genes, would be associated with alcohol dependence. Acute exposure to ethanol in vitro increases the activity of $GABA_A$ receptor–coupled chloride channels, which are heteropentamers composed of several types of subunits (Buck, 1996). In studies of mice, chronic alcohol consumption caused the messenger RNA content of *Gabrg3* to increase dramatically and similarly in both withdrawal seizure–prone mice and withdrawal seizure–resistant mice; this led the authors to suggest that γ_3 may be involved in the development of tolerance or behavioral sensitization to ethanol, because these traits do not differ between strains (Buck, 1996).

Although the mechanism is unclear, the significant association of alcohol dependence with multiple SNPs and haplotypes in *GABRG3* suggests that variations in this gene are involved in the etiology of alcohol dependence. On the basis of data from the literature on event-related potentials, Begleiter and Porjesz (1999) hypothesized that the predisposition to alcoholism is inherited as a general state of central nervous system disinhibition/hyperexcitability. This state of disinhibition/hyperexcitability is associated with a number of externalizing disorders. They suggested that alcohol dependence is among these disorders because the hyperexcitability is alleviated by the use of alcohol, which provides a normalizing effect. However, the effect is temporary and requires continued use of increasing amounts of alcohol to achieve this state, putting the individual at higher risk of developing alcohol problems and dependence. GABA was proposed to be involved in this pathway because GABAergic interneurons provide feedback inhibition to regulate recurrent excitation. Thus, it is plausible that this state of disinhibition/hyperexcitability could result from an altered response to GABA. Linkage to EEG in this chromosome 15 region (Ghosh et al., 2003), taken together with our finding that alcohol dependence is associated with genetic variation in *GABRG3*, provides support for this theory.

ACKNOWLEDGMENTS

COGA (H. Begleiter, State University of New York Health Science Center at Brooklyn, principal investigator; T. Reich, Washington University, co-principal investigator; and H. Edenberg, Indiana University, co-principal investigator) includes nine different centers where data collection, analysis, and storage take place. The nine sites and principal investigators and coinvestigators are as follows: Howard University (R. Taylor); Indiana University (H. Edenberg, J. Nurnberger Jr, P. M. Conneally, and T. Foroud); Rutgers University (J. Tischfield); Southwest Foundation (L. Almasy); State University of New York Health Sciences Center at Brooklyn (B. Porjesz and H. Begleiter); University of California at San Diego (M. Schuckit); University of Connecticut (V. Hesselbrock); University of Iowa (R. Crowe and S. Kuperman); and Washington University in St. Louis (T. Reich, C. R. Cloninger, J. Rice, and A. Goate). Lisa Neuhold serves as the NIAAA staff collaborator.

REFERENCES

Abecasis GR, Cookson WO (2000) GOLD—graphical overview of linkage disequilibrium. Bioinformatics 16:182–183.

Abecasis GR, Noguchi E, Heinzmann A, Traherne JA, Bhattacharyya S, Leaves NI, Anderson GG, Zhang Y, Lench NJ, Carey A, Cardon LR, Moffatt MF, Cookson WO (2001) Extent and distribution of linkage disequilibrium in three genomic regions. Am J Hum Genet 68:191–197.

American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV*. 4th ed. American Psychiatric Association, Washington, DC.

Begleiter H, Porjesz B (1999) What is inherited in the predisposition toward alcoholism? A proposed model. Alcohol Clin Exp Res 23:1125–1135.

Boehnke M (1991) Allele frequency estimation from pedigree data. Am J Hum Genet 48:22–25.

Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger JI, Reich T, Schmidt I, Schuckit MA (1994) A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. J Stud Alcohol 55:149–158.

Buck KJ (1996) Molecular genetic analysis of the role of GABAergic systems in the behavioral and cellular actions of alcohol. Behav Genet 26:313–323.

Clayton D (1999) A generalization of the transmission/disequilibrium test for uncertain haplotype transmission. Am J Hum Genet 65:1170–1177.

Devlin B, Risch N (1995) A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 29:311–322.

Dick DM, Foroud T (2003) Candidate genes for alcohol dependence: a review of genetic evidence from human studies. Alcohol Clin Exp Res 27:868-879.

Edenberg HJ, Dick DM, Xuei X, Foroud T, COGA collaborators (2002) Genetic analysis of chromosome 4 for alcohol-related phenotypes. Am J Med Genet 114:703.

Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL, Bierut LJ, Conneally PM, Nurnberger JI, Bucholz KK, Li T-K, Hesselbrock V, Crowe R, Schuckit M, Porjesz B, Begleiter H, Reich T (2000) Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. Alcohol Clin Exp Res 24:933–945.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296:2225–2229.

Ghosh S, Begleiter H, Porjesz B, Chorlian DB, Edenberg HJ, Foroud T, Goate A, Reich T (2003) Linkage mapping of beta 2 EEG waves via non-parametric regression. Am J Med Genet 118B:66–71.

Grobin AC, Matthews DB, Devaud LL, Morrow AL (1998) The role of GABA-A receptors in the acute and chronic effects of ethanol. Psychopharmacology 139:2–19.

Hesselbrock M, Easton C, Bucholz KK, Schuckit M, Hesselbrock V (1999) A validity study of the SSAGA—a comparison with the SCAN. Addiction 94:1361–1370.

Martin ER, Bass MP, Kaplan NL (2001) Correcting for a potential bias in the Pedigree Disequilibrium Test. Am J Hum Genet 68:1065–1067.

Martin ER, Monks SA, Warren LL, Kaplan NL (2000) A test for linkage and association in general pedigrees: the Pedigree Disequilibrium Test. Am J Hum Genet 67:146–154.

Meguro M, Mitsuya K, Sui H, Shigenami K, Kugoh H, Nakao M, Oshimura M (1997) Evidence for uniparental, paternal expression of the human $GABA_A$ receptor subunit genes, using microcell-mediated chromosome transfer. Hum Mol Genet 6:2127–2123.

Noble EP, Zhang X, Ritchie T, Lawford BR, Grosser SC, Young RM, Sparkes RS (1998) D2 dopamine receptor and GABA-A receptor B3 subunit genes and alcoholism. Psychiatry Res 81:133–147.

Parsian A, Zhang Z-H (1999) Human chromosomes 11p15 and 4p12 and alcohol dependence: possible association with the GABRB1 gene. Am J Med Genet 88:533–538.

Porjesz B, Almasy L, Edenberg HJ, Wang K, Chorlian DB, Foroud T, Goate A, Rice J, O'Connor SJ, Rohrbaugh J, Kuperman S, Bauer LO, Crowe R, Schuckit M, Hesselbrock V, Conneally PM, Tischfield JA, Li T-K, Reich T, Begleiter H (2002) Linkage disequilibrium between the beta frequency of the human EEG and a $GABA_A$ receptor gene locus. Proc Natl Acad Sci USA 99:3729–3733.

Reich T (1996) A genomic survey of alcohol dependence and related phenotypes: results from the Collaborative Study on the Genetics of Alcoholism (COGA). Alcohol Clin Exp Res 20:133A–137A.

Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, Foroud T, Hesselbrock V, Schuckit MA, Bucholz K, Porjesz B, Li T-K, Conneally PM, Nurnberger JI, Tischfield JA, Crowe RR, Cloninger CR, Wu W, Shears S, Carr K, Crose C, Willig C, Begleiter H (1998) Genome-wide search for genes affecting the risk for alcohol dependence. Am J Med Genet 81:207–215.

Sobel E, Lange K (1996) Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker sharing statistics. Am J Hum Genet 58:1323–1337.

Song J, Koller DL, Foroud T, Rice J, Nurnberger JI Jr, Begleiter H, Porjesz B, Smith TL, Schuckit M, Edenberg HJ (2003) Association of GABA-A receptors and alcohol dependence and the effects of genetic imprinting. Am J Med Genet 117B:39–45.