Published in final edited form as:
J Stud Alcohol Drugs. 2007 September ; 68(5): 625–633.

The Role of the *GABRA2* Polymorphism in Multiplex Alcohol Dependence Families With Minimal Comorbidity: Within-Family Association and Linkage Analyses*

ABIGAIL G. MATTHEWS, PH.D.†, **ERIC K. HOFFMAN, PH.D.**†, **NICHOLAS ZEZZA, B.S.**, **SCOTT STIFFLER, B.S.**, and **SHIRLEY Y. HILL, PH.D.**†
Department of Psychiatry, University of Pittsburgh School of Medicine, 3811 O'Hara Street, Pittsburgh, Pennsylvania 15213

Abstract

Objective—The genes encoding the γ-aminobutyric acid$_A$ (GABA$_A$) receptor have been the focus of several recent studies investigating the genetic etiology of alcohol dependence. Analyses of multiplex families found a particular gene, *GABRA2*, to be highly associated with alcohol dependence, using within-family association tests and other methods. Results were confirmed in three case-control studies. The objective of this study was to investigate the *GABRA2* gene in another collection of multiplex families.

Method—Analyses were based on phenotypic and genotypic data available for 330 individuals from 65 bigenerational pedigrees with a total of 232 alcohol-dependent subjects. A proband pair of same-sex siblings meeting Diagnostic and Statistical Manual of Mental Disorders, Third Edition, criteria for alcohol dependence was required for entry of a family into the study. One member of the proband pair was identified while in treatment for alcohol dependence. Linkage and association of *GABRA2* and alcohol dependence were evaluated using SIBPAL (a nonparametric linkage package) and both the Pedigree Disequilibrium Test and the Family-Based Association Test, respectively.

Results—We find no evidence of a relationship between *GABRA2* and alcohol dependence. Linkage analyses exhibited no linkage using affected/affected, affected/unaffected, and unaffected/unaffected sib pairs (all p's < .13). There was no evidence of a within-family association (all p's > .39).

Conclusions—Comorbidity may explain why our results differ from those in the literature. The presence of primary drug dependence and/or other psychiatric disorders is minimal in our pedigrees, although several of the other previously published multiplex family analyses exhibit a greater degree of comorbidity.

The neurotransmitter γ-aminobutyric acid (GABA) has been studied extensively because it is considered to be the major inhibitory neurotransmitter in the human central nervous system (Barnard et al., 1998). Genetic variation in GABA may be an important source of risk for developing alcohol dependence based on evidence from animal, human, and in vitro models. GABA$_A$ receptors in the human brain are sensitive to ethanol, as shown by altered expression of receptor subunit mRNAs in the cerebral cortex following chronic ethanol

*This research was supported by National Institute on Alcohol Abuse and Alcoholism (NIAAA) grants AA 005909, AA 008082, and AA 05168.

†Correspondence may be sent to Shirley Y. Hill at the above address or via syh50@imap.pitt.edu. Abigail G. Matthews is now a postdoctoral associate at Rockefeller University, New York, NY. Eric K. Hoffman is with the Pittsburgh Institute for Neurodegenerative Diseases, Department of Neurology, University of Pittsburgh, Pittsburgh, PA.

administration (Morrow et al., 1990). Positive emission tomographic studies of the metabolic response (2-deoxyglucose) to a benzodiazepine challenge in persons at risk for alcohol dependence have revealed reduced benzodiazepine inhibition (Volkow et al., 1997).

Animal studies of $GABA_A$ transmission have been shown to influence alcohol preference (Nowak et al., 1998), tolerance (Hood and Buck, 2000), and withdrawal (Keir and Morrow, 1994). Specifically, $GABA_A$ agonists increase ethanol intake whereas antagonists cause a decrease (Boyle et al., 1993; Nowak et al., 1998; Tomkins and Fletcher, 1996), and a decreased sensitivity of the $GABA_A$ receptor in different areas of the murine brain is associated with both ethanol tolerance and dependence (Grobin et al., 1998). The $GABA_A$ receptor is composed of at least 19 known subunits, with the majority containing three types: (1) α, (2) β, and (3) γ (Barnard et al., 1998). Murine studies indicate that the gene encoding the $GABA_A$ γ2 subunit, *GABRG2*, is involved with the severity of ethanol withdrawal and the motor and hypothermic effects of ethanol (Buck and Hood, 1998; Hood and Buck, 2000).

With the substantial evidence showing an association between $GABA_A$ receptors and response to ethanol, a number of studies (three case control and four family) have recently addressed genetic variation in these receptors and risk for alcohol dependence. Edenberg et al. (2004) were among the first to examine $GABA_A$ variation, finding genetic association with alcohol dependence in families with multiple affected members (i.e., multiplex families). This finding was consistent with results from an earlier data set showing a significant 38 cM linkage peak on Chromosome 4p, a region that is within 20 cM of the $GABA_A$ receptor polymorphisms (Reich et al., 1998). In fact, there is a cluster of four $GABA_A$ receptor subunit-encoding genes in this region of Chromosome 4: (1) *GABRG1*, (2) *GABRA2*, (3) *GABRA4*, and (4) *GABRB1*.

Edenberg et al. (2004) used the multiplex families ascertained by the Collaborative Study on the Genetics of Alcoholism (COGA) through probands seeking treatment for alcohol dependence. To be eligible for the genotyping phases of their study, each proband was required to have at least two first-degree relatives with alcohol dependence, as diagnosed using the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R; American Psychiatric Association, 1987), or DSM-IV (American Psychiatric Association, 1994) criteria. This yielded 262 multiplex families containing 2,282 genotyped individuals. A total of 69 single nucleotide polymorphisms (SNPs) were evaluated: 6 in *GABRG1*, 50 in *GABRA2*, 6 in *GABRA4*, 6 in *GABRB1*, and 1 between *GABRA2* and *GABRA4*. All of the evidence of an association was located in *GABRA2*, thus the finer mapping in this gene. Within-family association analyses found 30 SNPs to be significantly associated (unadjusted for number of comparisons) with alcohol dependence, with the most significant SNP being rs279871. Haplotype analyses using a three SNP sliding window revealed several highly significant associations, even after a conservative adjustment for multiple testing using a Bonferroni correction.

Two subsequent analyses of the COGA data have confirmed the rs279871 association (Table 1). First, Dick et al. (2006a) used a regression-based technique to evaluate the effect of the high-risk allele at *GABRA2* on the odds of being alcohol dependent. Generalized linear mixed models were used to account for the correlation of individuals within the same family. This analysis yielded a highly significant increase in odds ($p = .0003$), with an odds ratio of 1.40. The second set of COGA analyses also used a regression-based technique but considered the age at onset of alcohol dependence as the phenotype (Dick et al., 2006b). Having the high-risk genotype for the *GABRA2* gene significantly increased the lifetime risk of alcohol dependence ($p = .004$). These family-based analyses of the multiplex COGA data

provide compelling evidence of an association between alcohol dependence and the *GABRA2* gene that encodes the $GABA_A$ α2 subunit of the $GABA_A$ receptor.

Case-control analyses have sought to replicate the findings of Edenberg et al. (2004) using three independent populations (see Table 1). Covault et al. (2004) found a statistically significant association between seven SNPs within *GABRA2* and alcohol dependence status, which was diagnosed using DSM-III-R and DSM-IV criteria. This study consisted of 446 cases and 334 controls. Two of the SNPs—rs279858 and rs279837—that were significant in Edenberg et al. (2004) were found to be significantly associated with alcohol dependence at the allelic level ($p = .009$ and $p = .008$, respectively). Exclusion of cases with comorbid drug dependence or major depression increased the magnitude of the association for all seven significant SNPs. Examining the same seven SNPs within *GABRA2* studied by Covault et al. (2004) and Lappalainen et al. (2005) found only two SNPs that were significant at the allelic level, rs279869 ($p = .02$) and rs279858 ($p = .03$), using 113 cases and 100 controls. However, none of the SNPs were significant at the genotypic level. Two of the SNPs (rs279858 and rs279837) were used in the original *GABRA2* COGA analysis. A third case-control study sought to replicate the COGA result and the two previously described case-control results. Investigating six SNPs within the *GABRA2* polymorphism, Fehr et al. (2006) studied 257 cases with DSM-IV alcohol dependence and 88 controls. The six selected SNPs were designed to overlap with those studied by Edenberg et al. (2004) and Covault et al. (2004). At the allelic level, four SNPs were significantly associated with alcohol dependence, including rs279871 ($p = .02$) and rs279858 ($p = .02$). Only two SNPs exhibited a genotypic association, both of which were significant in the Edenberg et al. (2004) analysis: rs279845 ($p = .02$) and rs279836 ($p = .04$).

Family-based and case-control studies completed to date provide evidence for a genetic association between the *GABRA2* gene and alcohol dependence. In view of these results, it appeared useful to attempt to replicate these findings using one of the few existing DNA resources of alcohol-dependent multiplex families.

Method

Ascertainment and assessment of probands and relatives

Multiplex families were selected on the basis of the presence of a same-sex sibling pair, each with a diagnosis of alcohol dependence. Identification of the proband pair was accomplished through recruitment of one of the probands while in a treatment facility in the Pittsburgh area. The majority of probands appeared in the inpatient programs at the time of ascertainment. Two ongoing awards from the NIAAA supported the identification of male (Cognitive and Personality Factors in Relatives of Alcoholics Family Study [CPFFS]) and female (Biological Risk Factors in Female Alcoholics Family Study [BRFFS]) alcoholic pairs. Ascertainment criteria in both studies were identical. All participants signed consent forms following a discussion of the goals and requirements of the study.

The result of the ascertainment strategy is that the majority of probands have three or more siblings who have contributed DNA, consented to a clinical interview, and provided family histories. Our rationale for having initiated the study through a double proband sampling scheme was that restricting the study to multiplex families increases the likelihood of finding biological markers (e.g., P300 characteristics) related to the alcohol dependence phenotype by sampling affected, unaffected, and discordant pairs (Hill et al., 1988; Steinhauer et al., 1987). This ascertainment strategy is also highly beneficial for gene finding as the likelihood of finding a severe form of a disorder segregating in families in which multiple cases are found is increased.

All proband pairs were personally interviewed in both family studies. In the CPFFS initiative—in which a male proband pair was used for selection of families—all adult siblings and living parents were interviewed in person, with each first-degree relative also providing family history on every other member of the family, thus allowing for verification of self-reported symptoms. The BRFFS initiative—in which a pair of alcohol-dependent sisters was used to select families—had performed in-person interviews for all family members who provided a DNA sample. (Changes in the institutional review board regulations precluded obtaining family history for specific family members in the BRFFS initiative. Also, fewer family members were interviewed because of regulations requiring the proband to recruit family members.)

The interview format was a semistructured, well-known, psychiatric diagnostic instrument (Diagnostic Interview Schedule [DIS]) developed and tested for reliability and validity in St. Louis, Missouri (Robins et al., 1981). Use of this instrument makes it possible to make diagnoses of alcohol dependence, alcohol dependence by Feighner Criteria (Feighner et al., 1972), and alcohol dependence and abuse by DSM-III (American Psychiatric Association, 1980) and DSM-III-R criteria. Because the majority of individuals were assessed before the release of DSM-IV, no attempt was made to rediagnose the sample to conform to currently prevailing nomenclature. Nevertheless, the use of more than one diagnostic system allows for comparison with other studies using these criteria in major genome searches for alcoholism susceptibility loci (e.g., COGA). A computer algorithm developed by the authors allows for determining if criteria for particular disorders by each nosological system are present. However, to ensure the highest quality data possible, the DIS interview data was supplemented by a second interview, with a second clinician using a more open-ended format designed to cover major areas of psychopathology routinely covered by clinicians in screening diagnostic interviews. Using the DIS information, the second clinician's information, and the family history report of all other participating relatives, a "best-estimate" diagnosis was made using Feighner Criteria and DSM-III.

It is estimated that more than 5,000 families have been screened through an available proband pair to net the 65 cooperative families and 330 genotyped individuals on whom the present report is based. The median sibship size is four, with 80% of the families having three or more siblings genotyped (Table 2). There are 232 alcohol-dependent subjects, with 73% of the men and 47% of the women being affected.

Because the selection criteria required the presence of a same-sex proband pair and excluded families with particular psychiatric histories, only 1 family in 100 could be selected and studied. Families were excluded if the proband or any first-degree relative was considered to be "primary" for drug dependence (drug dependence preceded alcoholism onset by at least 1 year) and if the proband or first-degree relative met the criteria for schizophrenia or a recurrent major depressive disorder. Probands and relatives with mental retardation or physical illness precluding participation were excluded. The minimal comorbidity in the genotyped sample is demonstrated in Table 3. Seven cases in the data set met the criteria for primary drug dependence. This is far lower than would have occurred had the families not been selected for absence of primary drug dependence. Nevertheless, an explanation for these seven cases is needed. Some sibships are large, and additional siblings were included in some pedigrees after the majority of siblings had been screened and tested. Accordingly, some cases were found to meet criteria for primary drug dependence. These cases were included in the present study to improve the informativeness of the pedigrees.

Samples and genotyping

Blood was drawn from the present set of 330 adult family members, with one aliquot used to extract DNA from whole blood and the second aliquot prepared for Epstein-Barr virus

transformation and cryopreservation. Polymerase chain reaction (PCR) conditions were as described in Hill et al. (2004). The following SNPs were genotyped (using radiolabeled methods): rs483160, rs279867, and rs279858. Two SNPs, rs279837 and rs279871, were analyzed using the Biotage PSQ 96MA Pyrosequencer (Biotage AB, Uppsala, Sweden; Table 4). All of these SNPs were studied by Edenberg et al. (2004), with rs279871 reported as the most statistically significant (p = .0004, unadjusted for multiple comparisons).

GABRA2–rs483160—This polymorphism was analyzed by PCR amplification of a 151-base pair (bp) genomic fragment using the forward primer 5′-TGTGTATATTGGA GGGGGAAA-3′ and the reverse primer 5′-TGCAATAA TCCAATGATGCTG-3′. PCR products were digested with *TspRI* (New England Biolabs, Ispwich, MA). The wild-type allele was detected by the presence of an 87-bp DNA digested fragment. The variant allele was identified by the presence of the 151-bp DNA undigested product.

GABRA2–rs279867—This polymorphism was analyzed by PCR amplification of a 150-bp genomic fragment using the forward primer 5′-GCCAATAAGTCAGGAAGTTATGTC-3′ and the reverse primer 5′-AAATCCTTTTGAAATCAT GCTCGT-3′. Reaction conditions included the addition of dimethyl sulfoxide, formamide, and Triton X, each at a final concentration of 1.4% of the total reaction volume. PCR products were digested with *DraI* (New England Biolabs, Ispwich, MA). The wild-type allele was detected by the presence of a 123-bp DNA digested fragment. The variant allele was identified by the presence of the 150-bp DNA undigested product.

GABRA2–rs279858—This polymorphism was analyzed by PCR amplification of a 188-bp genomic fragment using the forward primer 5′-TCGACAAAAATGGAAAGATGAA-3′ and the reverse primer 5′-ACAGCAGAGTCCCATCATCC-3′. PCR products were digested with *MboII* (New England Biolabs, Ispwich, MA). The wild-type allele was detected by the presence of the 188-bp DNA undigested product. The variant allele was identified by the presence of a 127-bp DNA digested fragment.

GABRA2–rs279837—This polymorphism was analyzed by PCR amplification using the biotinylated forward primer 5′-ATGGTCCTGAGCATCCTCTAATC-3′ and the unmodified reverse primer 5′-GCCTTTCTTTACAACCTCATTCC-3′. The Biotage PSQ 96MA Pyrosequencer and accompanying workstation were then used to isolate the biotinylated single strand from double-stranded PCR products. Subsequently, the isolated product was sequenced using the complimentary sequencing primer 5′-TGCCTTCGGATATTTACT-3′. The wild-type allele was detected by the presence of a T nucleotide at the polymorphic site, whereas the variant allele was detected by the presence of a C nucleotide.

GABRA2–rs279871—This polymorphism was analyzed by PCR amplification using the unmodified forward primer 5′-AATCCAAACCCTGAAACACTTCT-3′ and the biotinylated reverse primer 5′-AGAAGGGATCAGAGGTAGAA CAAA-3′. The Biotage PSQ 96MA Pyrosequencer and accompanying workstation were then used to isolate the biotinylated single strand from double-stranded PCR products. Subsequently, the isolated product was sequenced using the complimentary sequencing primer 5′-TGACATGTATGT GATATATT-3′. The wild-type allele was detected by the presence of an A nucleotide at the polymorphic site, whereas the variant allele was detected by the presence of a G nucleotide.

Statistical analysis

Data were first checked for Mendelian inconsistencies using PedCheck (O’Connell and Weeks, 1998). Two inconsistencies were found and recoded as missing. Allele frequencies

in this sample were estimated using the software package FBAT (Laird et al., 2000; Rabinowitz and Laird, 2000) that considers the 130 founders. The E-M algorithm (Dempster et al., 1977) is used to incorporate parents with missing genotype data in the estimation procedure. Deviation from Hardy-Weinberg equilibrium was evaluated using Haploview version 3.31 (Barrett et al., 2005).

Before performing linkage analysis, the linkage disequilibrium (LD) between all five SNPs was evaluated using the Haploview package. This software estimates D′ and r^2 for all pairs of SNPs. D′ and r^2 take on values of 0 through 1, with perfect LD corresponding to D′ and $r^2 = 1$.

Although the focus of our analysis was within-family association, a linkage analysis was planned to provide potential converging evidence in support of either negative or positive evidence of association. To examine linkage, we used the nonparametric linkage package SIBPAL implemented by Statistical Analysis for Genetic Epidemiology (S.A.G.E.; Case Western Reserve University, Cleveland, OH) Version 5.0 (S.A.G.E., 2004). This method compares the mean allele sharing obtained from GENIBD (within S.A.G.E.) with that expected under the null hypothesis. The statistics are computed separately using the following types of sib pairs: concordantly affected, discordant, and concordantly unaffected.

The association between alcohol dependence and *GABRA2* was evaluated using two family-based approaches: the Pedigree Disequilibrium Test (PDT; Martin et al., 2000, 2001, 2003) and the Family-Based Association Test (FBAT; Rabinowitz and Laird, 2000). The former test extends the Transmission Disequilibrium Test (TDT; Spielman et al., 1993) by considering triads (i.e., two parents and one affected child), as well as discordant sib pairs, and adjusts for the fact that multiple triads and sib pairs arise from the same family. Three PDT statistics were calculated: (1) the sum-PDT, (2) avg-PDT, and (3) geno-PDT. Like the TDT, the sum-PDT and avg-PDT are allele-based tests of association. The geno-PDT is an extension of the PDT that considers genotypes instead of alleles. Under dominant or recessive genetic models, the geno-PDT is more powerful than the two allele-based statistics. The FBAT approach is also an extension of the TDT that compares transmission within an entire pedigree with that expected under the null hypothesis of no association. FBAT was implemented assuming an additive model because this approach is most robust to misspecification of the underlying genetic model.

Because of potential population heterogeneity, all of the previously described analyses were repeated using only the 63 Caucasian families (i.e., 2 African American families were excluded).

Results

The frequencies observed in our data set are in the range reported in dbSNP (an online database; National Center for Biotechnology Information, 2006) (see Table 4). Evaluation of LD among the five selected SNPs yielded perfect LD, with D′ and $r^2 = 1$ for four of the SNPs: (1) rs483160, (2) rs279871, (3) rs279867, and (4) rs279858. Accordingly, subsequent linkage analyses were restricted to the rs279871 and rs279837 SNPs. Haploview indicated no deviation from Hardy-Weinberg equilibrium ($p = .09$ and $p = .19$, respectively).

The nonparametric linkage results from SIBPAL are given in Table 5. No evidence was found for a significant deviation in the mean proportion of alleles shared identical-by-descent from that expected under the null hypothesis for the sib pair groups: affected/affected ($n = 201$), affected/unaffected ($n = 173$), and unaffected/unaffected ($n = 45$). Because of the multiplex for the alcohol dependence ascertainment scheme, only a modest number of concordantly unaffected sib pairs were available for analysis.

Results of the within-family association analyses may be seen in Table 6 for the two SNPs providing unique information: (1) rs279871 and (2) rs279837. Association between *GABRA2* and alcohol dependence was tested using both the PDT and the FBAT. None of the results were statistically significant for the five SNPs chosen for analysis. For the PDT analysis of rs279871, 48% of the parental A alleles were transmitted to the affected child, whereas 53% of the parental G alleles were transmitted to the affected child. The frequency of the A allele in the affected member of a discordant sib pair was 53% and 51%, in the unaffected member, respectively. For the rs279837, PDT analysis showed that the parental T allele was transmitted to affected offspring 48% of the time, whereas the parental C allele was transmitted to affected offspring in 52% of cases. Frequencies of the T allele in the affected and unaffected members of the discordant sib pairs were comparable (53% and 50%, respectively). Linkage and association analyses yielded the same results when repeated using only Caucasian families.

Discussion

The literature contains a plethora of evidence from murine studies that the GABA neurotransmitter and $GABA_A$ receptor are involved in the physiological responses to ethanol (e.g., motor incoordination and sedation) as well as ethanol reinforcement, tolerance, and withdrawal severity (Buck and Hood, 1998; Grobin et al., 1998; Hodge et al., 1996; Hood and Buck, 2000; Korpi et al., 1998). These results spurred the COGA researchers to examine the within-family association of four of the genes encoding subunits of the $GABA_A$ receptor. These genes were *GABRG1, GABRA2, GABRA4,* and *GABRB1* corresponding to the $GABA_A\gamma_1$, $GABA_A\alpha2$, $GABA_A\alpha4$, and $GABA_A\beta1$ subunits, respectively. The original analysis of these data (Edenberg et al., 2004) indicated a significant association between 30 SNPs within *GABRA2* and alcohol dependence. This result was replicated in three independent populations using a case-control approach (Covault et al., 2004; Fehr et al., 2006; Lapplainen et al., 2005). The present study was an attempt to replicate the findings of Edenberg et al. (2004) using another data set consisting of multiplex alcohol-dependent families. No evidence of an association or linkage to the alcohol dependence phenotype and *GABRA2* could be found in this sample.

The present failure to replicate the findings of Edenberg et al. (2004) may have occurred for a number of reasons. First, the power to detect within-family association may have been less than that available in those studies specifically using family-based association analyses (Agrawal et al., 2006; Edenberg et al., 2004). The present study may be underpowered because only 63 pedigrees were analyzed. However, post hoc evaluation of power for the FBAT or PDT are infeasible. The complexity of the alcohol dependence phenotype precludes a simple specification of the underlying genetic model that is required for any power evaluation. Also, it is recognized that the COGA data set has a larger number of multiplex pedigrees for analysis than the present data set. However, placing the available power of the present sample in the context of the power of these COGA studies is difficult, because the number of informative families on which these positive COGA results were based was not reported and because power estimates were not available from those analyses.

Although there might have been a lack of power to completely test our hypothesis that these SNPs in the *GABRA2* would be associated with disease status within the multiplex families, the absence of a difference in the transmission probabilities from parents to affected offspring and in the frequencies within the discordant pairs suggests that it is unlikely that our results would have been statistically significant.

It might be argued that performing haplotype analysis on this data set—as Edenberg et al. (2004) did with the COGA data set—might have revealed a significant association. Because

of complete LD between four of the SNPs chosen for analysis, a haplotype analysis on the two remaining SNPs was not performed. At any rate, such an analysis would only have increased the number of degrees of freedom resulting in loss of statistical power.

PDT and FBAT results do not show any evidence for an association using one SNP. This result is consistent with a previous genome-wide analysis in which no linkage findings of note could be seen on Chromosome 4 (Hill et al., 2004). Following up on an earlier suggestive linkage finding between the MNS blood group (4q28-31) and alcohol dependence (Hill et al., 1988), Neiswanger et al. (1995) used the same sample to perform linkage analyses of the 4q21-31 region. No evidence for linkage was found. In fact, all of the lod scores were zero or negative.

Another possible explanation for disparities between our results and those of COGA, as well as subsequent positive associations based on three case-control studies, may be the result of differing methods of sample selection. Using the disorder that occurs first in the participant's life as the primary diagnosis, and those occurring at least 1 year or later as the secondary diagnosis, the study was designed so that families were not included if the proband pair or their first-degree relatives had primary diagnoses other than alcohol dependence. Accordingly, the present data set was selected so that minimal primary comorbidity for drug dependence and other psychiatric disorders was introduced. In contrast, more than half (51%) of the genotyped, alcohol-dependent COGA subjects had a comorbid diagnosis of illicit drug dependence that appeared to be a primary diagnosis. In the present sample, alcohol-dependent relatives of probands were less frequently drug dependent (30%) than in the COGA sample (see Table 3). In addition to error variance that may occur by inclusion of drug dependent cases, other comorbidity may cloud interpretation of findings. Comorbid depression, anxiety, and antisocial personality disorder (ASPD) are common conditions (Helzer et al., 1991) that may influence results. For example, Dick et al. (2006b) have demonstrated a relationship between *GABRA2* and conduct disorder. Because COGA did not exclude any family as a result of comorbid conditions, whereas the present study did limit comorbidity, it may be assumed that depressive and anxiety disorders, as well as ASPD, were less frequent in the present sample.

Taking all of the studies of $GABA_A$ and alcohol dependence together, one might conclude that removing conditions comorbid with alcohol dependence is necessary to find a relationship (Covault et al., 2004). When depression and drug dependence were removed from the Covault et al. (2004) sample, the *GABRA2* association proved to be even stronger. However, the obverse appears to be the case as well. Some studies find that the presence of comorbid conditions is the only situation in which significant results do occur (Agrawal et al., 2006; Drgon et al., 2006; Fehr et al., 2006). Drgon et al. (2006) analyzed data for 415 polysubstance abusers and 239 controls, finding a significant relationship with one of six SNPs in the *GABRA2* gene (rs279871). However, this relationship was considered to be only modest because no particular allele was found to be associated with polysubstance abuse. The importance of substance abuse other than alcohol is apparent in the work of Agrawal et al. (2006). Using the COGA multiplex families, these investigators found no association of any $GABA_A$ SNP with the alcohol dependence phenotype alone (cases with drug dependence removed). Similarly, analysis of alcohol-dependent individuals (n = 65) and unrelated controls (n = 112) from the COGA data set found no significant differences for six selected *GABRA2* SNPs, including the rs279871 SNP (Drgon et al., 2006).

The presence of other psychiatric diagnoses also appears related to whether significant differences between cases and controls are seen. ASPD may be a factor in determining the strength of the gene/dependence relationship. Significant differences between alcohol-dependent cases and controls were seen in individuals with comorbid ASPD, but removal of

the comorbid ASPD cases resulted in no significant difference between the groups (Fehr et al., 2006). These considerations suggest that improving the separation between cases and controls may provide the opportunity for uncovering genetic variation if it exists. Similarly, where no genetic association may be present, removing the comorbidity from cases and controls may uncover true negative findings. Moreover, if several psychiatric disorders have a unique relationship to *GABRA2* variation, studies need to be designed to assess the independent effects of each of these disorders. To date, study of the relationship between disorders other than alcohol dependence and *GABRA2* has been based on comorbid cases.

Another possibility is that a yet-undiscovered endophenotype that is highly correlated with alcohol dependence may, in fact, be what is contributing to previously reported positive findings. These possible endophenotypes might include lower level of response to alcohol reported for another $GABA_A\alpha6$ receptor (Schuckit et al., 1999) or predisposition to anxiety disorders. One endophenotype that has been found to be associated with *GABRA2* is the EEG β phenotype (Edenberg et al., 2004). Additionally, patients with panic disorder have shown a deficit in $GABA_A$ receptors, resulting in enhanced anxiety for threat cues (Crestani et al., 1999). It is noteworthy that through selection criteria used in the present sample, anxiety disorders were exceptionally low. At any rate, the present analyses did not find evidence favoring a relationship between $GABA_A$ and alcohol dependence in this sample of multiplex families.

References

Agrawal A, Edenberg HJ, Foroud T, Bierut LJ, Dunne G, Hinrichs AL, Nurnberger JI, Crowe R, Kuperman S, Schuckit MA, Begleiter H, Porjesz B, Dick DM. Association of GABRA2 with drug dependence in the Collaborative Study of the Genetics of Alcoholism Sample. Behav Genet. 2006; 36:640–650. [PubMed: 16622805]

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-III). Washington, DC: 1980.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders-Revised (DSM-III-R). Washington, DC: 1987.

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

Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, Braestrup C, Bateson AN, Langer SZ. International Union of Pharmacology: XV. Subtypes of γ-aminobutyric $acid_A$ receptors: Classification on the basis of subunit structure and receptor function. Pharmacol Rev. 1998; 50:291–313. [PubMed: 9647870]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

Boyle AE, Segal R, Smith BR, Amit Z. Bidirectional effects of GABAergic agonists and antagonists on maintenance of voluntary ethanol intake in rats. Pharmacol Biochem Behav. 1993; 46:179–182. [PubMed: 8255910]

Buck KJ, Hood HM. Genetic association of a GABA(A) receptor gamma2 subunit variant with severity of acute physiological dependence on alcohol. Mamm Genome. 1998; 9:975–978. [PubMed: 9880662]

Covault J, Gelernter J, Hesselbrock V, Nellissery M, Kranzler HR. Allelic and haplotypic association of GABRA2 with alcohol dependence. Amer J Med Genet Part B - Neuropsychiat Genet. 2004; 129:104–109.

Crestani F, Lorez M, Baer K, Essrich C, Benke D, Laurent JP, Belzung C, Fritschy JM, Luscher B, Mohler H. Decreased $GABA_A$ receptor clustering results in enhanced anxiety and a bias for threat cues. Nature Neurosci. 1999; 2:833–839. [PubMed: 10461223]

Dempster AP, Laird NM, Rubin DB. Maximum likelihood from incomplete data via the EM algorithm. J Royal Stat Soc Series B -Methodol. 1977; 39:1–38.

Dick DM, Agrawal A, Schuckit MA, Bierut L, Hinrichs A, Fox L, Mullaney J, Cloninger CR, Hesselbrock V, Nurnberger JI Jr, Almasy L, Foroud T, Porjesz B, Edenberg H, Begleiter H. Marital status, alcohol dependence, and GABRA2: Evidence for gene-environment correlation and interaction. J Stud Alcohol. 2006a; 67:185–194. [PubMed: 16562401]

Dick DM, Bierut L, Hinrichs A, Fox L, Bucholz KK, Kramer J, Kuperman S, Hesselbrock V, Schuckit M, Almasy L, Tischfield J, Porjesz B, Begleiter H, Nurnberger J Jr, Xuei X, Edenberg HJ, Foroud T. The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. Behav Genet. 2006b; 36:577–590. [PubMed: 16557364]

Drgon T, D'Addario C, Uhl GR. Linkage disequilibrium, haplotype and association studies of a chromosome 4 GABA receptor gene cluster: Candidate gene variants for addictions. Amer J Med Genet Part B - Neuropsychiat Genet. 2006; 141:854–860.

Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO, Crowe RR, Goate A, Hesselbrock V, Jones K, Kwon J, Li T-K, Nurnberger JI Jr, O'Connor SJ, Reich T, Rice J, Schuckit MA, Porjesz B, Foroud T, Begleiter H. Variations in GABRA2, encoding the α2 subunit of the $GABA_A$ receptor, are associated with alcohol dependence and with brain oscillations. Amer J Human Genet. 2004; 74:705–714. [PubMed: 15024690]

Fehr C, Sander T, Tadic A, Lenzen KP, Anghelescu I, Klawe C, Dahmen N, Schmidt LG, Szegedi A. Confirmation of association of the GABRA2 gene with alcohol dependence by subtype-specific analysis. Psychiat Genet. 2006; 16:9–17.

Feighner JP, Robins E, Guze SB, Woodruff RA Jr, Winokur G, Munoz R. Diagnostic criteria for use in psychiatric research. Arch Gen Psychiat. 1972; 26:57–63. [PubMed: 5009428]

Grobin AC, Matthews DB, Devaud LL, Morrow AL. The role of $GABA_A$ receptors in the acute and chronic effects of ethanol. Psychopharmacology. 1998; 139 (1–2):2–19. [PubMed: 9768538]

Helzer, JE.; Burnam, A.; McEvoy, LT. Alcohol abuse and dependence. In: Robins, LN.; Reiger, DA., editors. Psychiatric Disorders in America: The Epidemiologic Catchment Area Study. New York: Free Press; 1991. p. 81-115.

Hill SY, Aston C, Rabin B. Suggestive evidence of genetic linkage between alcoholism and the MNS blood group. Alcsm Clin Exp Res. 1988; 12:811–814.

Hill SY, Shen S, Zezza N, Hoffman EK, Perlin M, Allan W. A genome wide search for alcoholism susceptibility genes. Amer J Med Genet Part B - Neuropsychiat Genet. 2004; 128:102–113.

Hodge CW, Haraguchi M, Chappelle AM, Samson HH. Effects of ventral tegmental microinjections of the $GABA_A$ agonist muscimol on self-administration of ethanol and sucrose. Pharmacol Biochem Behav. 1996; 53:971–977. [PubMed: 8801605]

Hood HM, Buck KJ. Allelic variation in the $GABA_A$ receptor γ2 subunit is associated with genetic susceptibility to ethanol-induced motor incoordination and hypothermia, conditioned taste-aversion and withdrawal on BXD/Ty recombinant inbred mice. Alcsm Clin Exp Res. 2000; 24:1327–1334.

Keir WJ, Morrow AL. Differential expression of $GABA_A$ receptor subunit mRNAs in ethanol-naive withdrawal seizure resistant (WSR) vs. withdrawal seizure prone (WSP) mouse brain. Mol Brain Res. 1994; 25 (3–4):200–208. [PubMed: 7808218]

Korpi ER, Mäkelä R, Uusi-Oukari M. Ethanol: Novel actions on nerve cell physiology explain impaired functions. News Physiol Sci. 1998; 13:164–170. [PubMed: 11390783]

Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. Genet Epidemiol. 2000; 19 (Suppl 1):S36–S42. [PubMed: 11055368]

Lappalainen J, Krupitsky E, Remizov M, Pchelina S, Taraskina A, Zvartau E, Somberg LK, Covault J, Kranzler HR, Krystal JH, Gelernter J. Association between alcoholism and γ-amino butyric acid α2 receptor subtype in a Russian population. Alcsm Clin Exp Res. 2005; 29:493–498.

Martin ER, Bass MP, Gilbert JR, Pericak-Vance MA, Hauser ER. Genotype-based association test for general pedigrees: The genotype-PDT. Genet Epidemiol. 2003; 25:203–213. [PubMed: 14557988]

Martin ER, Bass MP, Kaplan NL. Correcting for a potential bias in the pedigree disequilibrium test. Amer J Human Genet. 2001; 68:1065–1067. [PubMed: 11254459]

Martin ER, Monks SA, Warren LL, Kaplan NL. A test for linkage and association in general pedigrees: The pedigree disequilibrium test. Amer J Human Genet. 2000; 67:146–154. [PubMed: 10825280]

Morrow AL, Monpied P, Lingford-Hughes A, Paul SM. Chronic ethanol and pentobarbital administration in the rat: Effects on $GABA_A$ receptor function and expression in brain. Alcohol. 1990; 7:237–244. [PubMed: 2158791]

National Center for Biotechnology Information. dbSNP (online database). Bethesda, MD: National Library of Medicine, National Institutes of Health; 2006. (available at: ncbi.nlm.nih.gov/projects/SNP)

Neiswanger K, Kaplan B, Hill SY. Exclusion of linkage between alcoholism and the MNS blood group region on chromosome 4q in multiplex families. Amer J Med Genet Part B - Neuropsychiat Genet. 1995; 60:72–79.

Nowak KL, McBride WJ, Lumeng L, Li T-K, Murphy JM. Blocking $GABA_A$ receptors in the anterior ventral tegmental area attenuates ethanol intake of the alcohol-preferring P rat. Psychopharmacology. 1998; 139(1–2):108–116. [PubMed: 9768548]

O'Connell JR, Weeks DE. PedCheck: A program for identification of genotype incompatibilities in linkage analysis. Amer J Human Genet. 1998; 63:259–266. [PubMed: 9634505]

Rabinowitz D, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. Human Hered. 2000; 50:211–223.

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 Jr, Tischfield JA, Crowe RR, Cloninger CR, Wu W, Shears S, Carr K, Crose C, Willig C, Begleiter H. Genome-wide search for genes affecting the risk for alcohol dependence. Amer J Med Genet Part B - Neuropsychiat Genet. 1998; 81:207–215.

Robins LN, Helzer JE, Croughan J, Ratcliff KS. National Institute of Mental Health Diagnostic Interview Schedule: Its history, characteristics, and validity. Arch Gen Psychiat. 1981; 38:381–389. [PubMed: 6260053]

SAGE. Statistical Analysis for Genetic Epidemiology, Version 5.0 Computer Program Package. Cleveland, OH: Department of Epidemiology and Biostatistics, Case Western Reserve University; 2004. (available at: http://darwin.cwru.edu/sage)

Schuckit MA, Mazzanti C, Smith TL, Ahmed U, Radel M, Iwata N, Goldman D. Selective genotyping for the role of $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$, and $GABA\alpha_6$ receptors and the serotonin transporter in the level of response to alcohol: A pilot study. Biol Psychiat. 1999; 45:647–651. [PubMed: 10088053]

Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: The insulin gene region and insulin-dependent diabetes mellitus (IDDM). Amer J Human Genet. 1993; 52:506–516. [PubMed: 8447318]

Steinhauer SR, Hill SY, Zubin J. Event-related potentials in alcoholics and their first-degree relatives. Alcohol. 1987; 4:307–314. [PubMed: 3620100]

Tomkins DM, Fletcher PJ. Evidence that $GABA_A$ but not $GABA_B$ receptor activation in the dorsal raphe nucleus modulates ethanol intake in Wister rats. Behav Pharmacol. 1996; 7:85–93. [PubMed: 11224398]

Volkow ND, Wang G-J, Overall JE, Hitzemann R, Fowler JS, Pappas N, Frecska E, Piscani K. Regional brain metabolic response to lorazepam in alcoholics during early and late alcohol detoxification. Alcsm Clin Exp Res. 1997; 21:1278–1284.

Table 1

Literature review of association between *GABRA2* and alcohol dependence

Study	Study design	*n*	Statistical methods	NCBI SNP reference IDs	Results[a]	Multiple comparisons[b]	Minimal comorbid disorders[c]
Edenberg et al. (2004)	Multiplex families (COGA)	262[d]	avg-PDT at allele and haplotype levels	69 total SNPs; 52 within *GABRA2* including rs279858	√	√	√
Covault et al. (2004)	Case-control	446/334	χ^2 analyses at allele and haplotype levels	rs567926, rs534459, rs529826, rs279869, rs279858, rs279844, rs279837, rs1372472	√	NA	√
Lappalainen et al. (2005)	Case-control	113/100	χ^2 analyses at genotype, allele, and haplotype levels	rs567926, rs534459, rs529826, rs279869, rs279858, rs279837	√/✗	NA	√
Fehr et al. (2006)	Case-control	257/88	χ^2 analyses at genotype, allele, and haplotype levels	rs567926, rs279871, rs279858, rs279845, rs279836, rs137472	√	NA	✗
Dick et al. (2006a)	Multiplex families (COGA)	262[d]	GLMM	rs279871	√	NA	✗
Dick et al. (2006b)	Multiplex families (COGA)	860[e]	Log-rank test	rs279871	√/✗	NA	✗
Agrawal et al. (2006)	Multiplex families (COGA)	262[d]	avg-PDT and PDTPhase	69 total SNPs; 52 within *GABRA2* including rs279858	√	√	✗
Drgon et al. (2006)	Case-control (COGA)	112/65	χ^2 analyses at genotype, allele, and haplotype levels	rs573400, rs505474, rs279871, rs279867, rs1442061, rs3756007	✗	NA	✗

Notes: NCBI = National Center for Biotechnology Information; SNP = single nucleotide polymorphisms; COGA = Collaborative Study on the Genetics of Alcoholism; PDT = Pedigree Disequilibrium Test; NA = not applicable; GLMM = generalized linear mixed models.

[a] √: evidence of an association; ✗: no evidence of an association;

[b] √: adjustment made for multiple comparisons; ✗: no adjustment for multiple comparisons;

[c] √: minimal comorbid psychiatric disorders present including drug dependence; ✗: comorbid psychiatric disorders are not minimal or were not discussed by the author;

[d] COGA studies genotyped 2,282 individuals from these 262 families;

[e] number of genotyped children.

Table 2

Distribution of sibship size

Sibship size	Frequency (%)
2	13 (20%)
3	15 (23%)
4	17 (26%)
5	12 (19%)
≥6	8 (12%)

Table 3

Lifetime prevalence of comorbid disorders percentage of cases in genotyped sample

	Male (*n* = 168)			Female (*n* = 162)		
Disorder	**Alcohol dependence primary (*n* = 118)**	**Alcohol dependence secondary (*n* = 3)**	**No alcohol dependence (*n* = 47)**	**Alcohol dependence primary (*n* = 67)**	**Alcohol dependence secondary (*n* = 13)**	**No alcohol dependence (*n* = 82)**
Major depression	0.0	0.0	2.1	4.5	38.5	16.3[b]
Antisocial personality	21.4	0.0	6.4	22.4	7.7	1.3
Drug dependence	23.9[a]	66.6	0.0	40.3[a]	76.9[d]	2.6[c]
Anxiety disorder	1.7	0.0	0.0	3.0	7.7	3.9
Any comorbidity	36.7	66.6	8.5	56.7	100.0	20.0

[a] Secondary drug dependence; all probands were selected to be primary for alcohol dependence; primary designation was based on the age of onset of each disorder; a disorder had to precede another disorder by at least 1 year to be considered primary;

[b] each of the major depressive disorders found in these relatives was the primary disorder; as noted, none were alcohol dependent and none were drug dependent;

[c] this percentage is based on two individuals who met criteria for primary drug dependence and were relatives of the proband pair (none were probands);

[d] this percentage is based on 10 cases who either met criteria for primary drug dependence (4 cases) or met criteria for secondary drug dependence (6 cases); none were probands.

Table 4

GABRA2 SNPs selected for genotyping

NCBI reference ID	Location, bp	Alleles	Study frequencies	dbSNP frequencies	Edenberg et al. (2004)*p*[g]
rs483160	45981832	A/C	.439/.561	.509/.491[a]	.150
rs279871	46000490	A/G	.561/.439	.600/.400[b]	
				.571/.429[c]	.0004
rs279867	46003060	G/T	.439/.561	.435/.565[d]	
				.490/.551[e]	.240
rs279858	46009350	A/G	.561/.439	.483/.517[a]	
				.542/.458[d]	.009
rs279837	46034080	T/C	.550/.450	Unavailable[f]	
		A/G		.542/.458[d]	
				.660/.340[e]	.035

Notes: SNPs = single nucleotide polymorphisms; NCBI = National Center for Biotechnology Information; bp = base pair; dbSNP = SNP database.

[a]Hapmap-CEU (30 mother/father/child trios from Centre d'Etude du Polymorphism Human [CEPH] collection [Utah residents with Northern and Western European ancestry]);

[b]TSC Caucasian sample of 12 DNAs from Coriell Cell Repositories from CEPH/UTAH library;

[c]TSC panel consisting of 42 Caucasian individuals;

[d]AFD Eur Panel (24 samples from Coriell Cell Repository primarily of European American descent. Primarily CEPH unrelated parents);

[e]CEPH (Genomic DNA samples were obtained for a panel of 92 unrelated individuals chosen from CEPH; the pooled sample was comprised of 93% Utah, 4% French, and 3% Venezuelan samples);

[f]reverse primers were used resulting in T/C product;

[g]single SNP association results; unadjusted *p*.

Table 5

Results of nonparametric linkage analysis

Type	No. of pairs	Mean proportion of alleles IBD	*p*
rs279871			
Concordantly affected	201	.504	.385
Discordant	173	.481	.102
Concordantly unaffected	45	.453	.935
rs279837			
Concordantly affected	201	.504	.385
Discordant	173	.484	.136
Concordantly unaffected	45	.504	.935

Table 6

Results of family-based association tests

Tests	No. of informative families	*p*
rs279871		
PDT		
Sum-PDT	47	.429
Avg-PDT	47	.760
Geno-PDT	47	.728
FBAT	33	.415
rs279837		
PDT		
Sum-PDT	47	.733
Avg-PDT	47	.868
Geno-PDT	47	.761
FBAT	34	.841

Notes: PDT = Pedigree Disequilibrium Test; FBAT = Family-Based Association Test.