Association of *GABRA2* with Drug Dependence in the Collaborative Study of the Genetics of Alcoholism Sample

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Results from twin studies suggest that overlapping genetic factors influence alcohol dependence and illicit drug dependence. Using data from the Collaborative Study on the Genetics of Alcoholism (COGA), we examined the association between 69 SNPs in the GABA$_A$ receptor gene cluster on chromosome 4 and marijuana and illicit drug dependence, individually, and as co-occurring phenotypes with alcohol dependence. Results suggested association between marijuana dependence and illicit drug dependence with SNPs in the *GABRA2* gene. Interestingly, the evidence for association previously observed with alcohol dependence came only from individuals with comorbid illicit drug dependence. There was no association with other genes in the GABA$_A$ cluster on chromosome 4 with illicit drug dependence.

KEY WORDS: Alcoholism; association analyses; COGA; drug dependence; GABRA2; PDT.

INTRODUCTION

Illicit drug use is a source of significant morbidity and mortality. According to the 2003 National Household Survey on Drug Abuse and Health (Substance Abuse and Mental Health Services Administration (SAMHSA), 2005), 19.5 million Americans aged 12 years or older (8.2% of this population) were current illicit drug users, with 75% of these users reporting cannabis use. The same report estimated 22.2 million people required treatment for an alcohol or illicit drug problem in 2003, 91% of whom did not receive any assistance at a specialty facility. These findings underscore the persistent public and mental health concerns caused by illicit drug involvement.

A number of genetic epidemiological studies have investigated latent risk factors that contribute to the variance in illicit drug involvement. Results from family studies have suggested that drug disorders aggregate in families and that relatives of individuals with illicit drug problems are at increased risk for presenting with drug problems themselves (Bierut *et al.*, 1998; Meller *et al.*, 1988; Merikangas *et al.*, 1998; Mirin *et al.*, 1991). Data from identical and fraternal twin pairs have been used to investigate the extent to which population variation in illicit drug use, abuse and dependence is due to genetic *versus* environmental factors. Results from adult (Kendler *et al.*, 1999a, 2000; Kendler and

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Prescott, 1998a; b; Miles *et al.*, 2001; van den Bree *et al.*, 1998; Tsuang *et al.*, 2001) and adolescent (McGue *et al.*, 2000; Maes *et al.*, 1999; Rhee *et al.*, 2003) samples suggest that illicit drug use and abuse/dependence are under significant genetic influence, with heritability estimates for drug abuse/dependence ($h^2 = 0.26$–0.79) being consistently greater than the heritability of drug use ($h^2 = 0.11$–0.74). Partitioning these genetic risk factors into genetic influences shared by drug use and abuse/dependence and genetic risk factors specific to abuse/dependence, researchers have shown that even after accounting for genetic influences shared between drug use and abuse/dependence (approximately 50%), individual differences in illicit drug abuse/dependence are in part due to genetic influences that are unique to abuse/dependence itself (Agrawal *et al.*, 2005b; Kendler *et al.*, 1999b). For example, results from traditional twin models have suggested that marijuana use ($h^2 = 0.17$–0.72) and marijuana dependence ($h^2 = 0.33$–0.78) are both heritable, and that the genetic influences on marijuana dependence are partly shared with marijuana use (64%) and partly specific to marijuana dependence (36% or abuse/dependence-specific $h^2 = 0.17$) (Kendler and Prescott, 1998a; Kendler *et al.*, 1999b). Multivariate studies that have investigated the relationship across classes of illicit drugs have consistently indicated overlap of genetic factors that influence a number of illicit drugs, such as cannabis, cocaine, stimulants, sedatives, hallucinogens and opioids (Karkowski *et al.*, 2000; Kendler *et al.*, 2003a; McGue *et al.*, 1999; Tsuang *et al.*, 1998). One recent study of adult male twins failed to find any influence of drug-specific genetic factors on abuse/dependence in a multivariate study of illicit drug abuse/dependence (Kendler *et al.*, 2003b). Results from a linkage study of families ascertained using heavy smoking probands (Madden *et al.*, 2005, Abstract) also supports common genetic influences on multiple illicit drugs. In a whole genome scan for multiple illicit drug dependence in Australian families, Agrawal and colleagues observed a peak (LOD = 1.57) on 4p in the region of the GABA$_A$ receptor gene cluster (Agrawal *et al.*, 2005a, Abstract), where linkage to alcohol-related phenotypes has previously been reported (Porjesz *et al.*, 2002; Saccone *et al.*, 2000; Williams *et al.*, 1999).

Illicit drug dependence and alcohol dependence frequently co-occur (Degenhardt *et al.*, 2001; Kandel and Yamaguchi, 1993; Wagner and Anthony, 2002). Alcohol dependence is also under a significant degree of genetic influence, with heritability estimates in the range of 50–60% for both men and women (Heath *et al.*, 1997; McGue, 1999). This raises the possibility that a shared genetic predisposition may contribute to the co-occurrence of illicit drug dependence and alcohol dependence. MZ and DZ cross-twin cross-trait correlations, reported by Pickens and colleagues, suggested that the relationship between alcoholism and illicit drug dependence may be due to shared genetic factors. However, higher cross-MZ *versus* DZ concordance for alcoholism and drug dependence was only observed in male twin pairs (Pickens *et al.*, 1995). Jang *et al.* (1995) also have found that common genetic factors were responsible for the genetic overlap across items assessing alcohol and drug misuse. Similarly, Kendler and colleagues have reported that a common genetic factor contributed to the total variance in alcohol dependence, illicit drug abuse/dependence, conduct disorder and antisocial personality disorder (Kendler *et al.*, 2003c). Interestingly, some authors have proposed that alcohol dependence coupled with illicit drug dependence may represent a more heritable form of the disorder (McGue *et al.*, 1992; Pickens *et al.*, 1995).

Given this observed association between alcohol dependence and illicit drug involvement and the possible role of overlapping genetic factors, it is possible that biologically relevant genes previously shown to be associated with alcoholism are also associated with illicit drug dependence. The Collaborative Study on the Genetics of Alcoholism recently reported a significant association between *GABRA2* and alcohol dependence, using family-based association methods (Edenberg *et al.*, 2004) Association ($p \leq 0.05$) was observed with 31 SNPs tested across *GABRA2*, and haplotype analyses were also highly significant. This finding has subsequently been replicated using a case-control sample ascertained through the University of Connecticut Health Center (UCHC) (Covault *et al.*, 2004), in a Russian population from St. Petersburg (Lappalainen *et al.*, 2005), and in a German population (Fehr *et al.*, 2006). Perhaps surprisingly, the UCHC study found that when individuals with comorbid illicit drug dependence (cocaine or opioid) were eliminated from the analyses (along with those with major depressive disorder), the strength of the association increased.

This manuscript further explores the relationship between *GABRA2* and illicit drug dependence in the COGA sample, with the following goals:

(1) to examine whether genes in the GABA$_A$ receptor gene cluster on 4p3-12 were associated with marijuana and other illicit drug dependence.

(2) to examine the extent to which the association between these genes (*GABRA2*, *GABRA4*, *GABRB1* and *GABRG1*) and alcohol dependence varied according to the inclusion/exclusion of individuals with co-occurring drug dependence.

METHODS

Sample

Using methods approved by all relevant institutional review boards, the Collaborative Study on the Genetics of Alcoholism (COGA) collected data on families 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 sibships > 3 with parents available) with two or more members in a COGA catchment area (Reich *et al.*, 1998). A total of 1227 families of alcohol dependent probands were recruited for the first stage of the study, each of whom were administered the polydiagnostic, Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview (Bucholz *et al.*, 1994; Hesselbrock *et al.*, 1999).

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 data set (Reich *et al.*, 1998). A replication sample was ascertained and genotyped following identical procedures; it consisted of 1295 individuals from 157 extended families (Foroud *et al.*, 2000). Thus, a total of 2282 individuals from 262 multiplex alcoholic families are included in genetic analyses. The sample consists of 49% females and 51% males. Approximately 83% of the geno-

typed sample is Caucasian, 13% is African–American and the remaining families are of other (or mixed) racial backgrounds. The mean age of participants was 40.6 years (range 17–91 years).

DNA Analyses

SNP selection and genotyping in this region has previously been described by Edenberg *et al.* (2004). Briefly, SNPs were chosen across each of the four GABA$_A$ receptor genes clustered on chromosome 4, from public databases; they were not restricted to coding regions or exons, and were preferentially selected based on high heterozygosities. Genotyping was done using a modified single nucleotide extension reaction, with allele detection by mass spectrometry (Sequenom MassArray system; Sequenom, San Diego, CA). All genotypic data were checked for Mendelian inheritance of marker alleles with the USERM13 (Boehnke, 1991) option of the MENDEL linkage computer programs, which was then used to estimate marker allele frequencies. A total of 69 SNPs were genotyped in the chromosome 4 GABA$_A$ receptor gene cluster: 52 SNPs in *GABRA2*, 6 SNPs in *GABRG1*, 5 SNPs in *GABRA4* and 6 SNPs in *GABRB1*. The disproportionate number of SNPs in *GABRA2* is the result of more extensive genotyping after significance was observed with the initial set of screening SNPs in this gene. The average heterozygosity was 0.44. LD was generally high within genes, and low between genes, allowing us to distinguish association between each of the four genes in the cluster (Edenberg *et al.*, 2004).

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 association in the extended pedigrees. The PDT utilizes 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 a larger number of informative trios and discordant sibships (Martin et al., 2001). Because the COGA sample consists of several very large families that might influence the PDT-sum statistic, we report the values from the PDT-avg statistic. This is also the statistic reported in the original *GABRA2* publication (Edenberg *et al.*, 2004)

reporting association with alcohol dependence in the COGA sample.

PDTPhase, which is part of the UNPHASED (Dudbridge, 2003) software program for association analyses was used for haplotype association tests. An extension of PDT, PDTPhase allows for convenient testing of haplotype association using an EM algorithm to include haplotypes with uncertain frequencies.

Phenotypes

All phenotypic diagnoses were based on interview data from participants who were administered the SSAGA. Alcohol dependence was diagnosed using the criteria of the Fourth Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-IV) (American Psychiatric Association, 1994). The SSAGA was created at the time that DSM-IV was being developed; accordingly we included criteria to diagnose alcohol dependence (the primary phenotype of study in COGA) according to DSM-IV, while other diagnoses were assessed using the established Third Revised (DSMIIIR) criteria (Bucholz *et al.*, 1994; Hesselbrock *et al.*, 1999) (but see footnote to Table I). We analyzed five phenotypes in our association analyses:

(i) Marijuana dependence was defined as a diagnosis of DSM-IIIR marijuana dependence.
(ii) Any illicit drug dependence was defined as a DSM-IIIR diagnosis of marijuana, cocaine, stimulant, sedative *or* opioid dependence.
(iii) Alcohol or drug dependence was defined such that individuals were classified as affected if they had *either* a diagnosis of alcohol dependence *or* a diagnosis of dependence on any illicit drug.
(iv) Alcohol and drug dependence was defined such that affected individuals had a diagnosis of alcohol dependence *and* a diagnosis of dependence for any illicit drug; individuals who had a diagnosis of alcohol dependence or drug dependence (but not both) were considered unaffected for this phenotype.
(v) Finally, we also analyzed individuals who had a diagnosis of DSM-IV alcohol dependence, but who did not meet criteria for any illicit drug dependence.

Approximately 41% of the genotyped sample presented with a diagnosis of alcohol dependence at some point in their lives, and 29% presented with a lifetime diagnosis of illicit drug dependence. Of those with illicit drug dependence, 75% met criteria for marijuana dependence, 35% met criteria for stimulant dependence, 50% for cocaine dependence, 17% for opiate dependence and 21% for sedative dependence. Overall, 49% of the participants in the genotyped sample had a diagnosis of either alcohol or illicit drug dependence. Of those with illicit drug dependence, 26% were dependent on an illicit drug other than marijuana (74% with a diagnosis of marijuana dependence and other illicit drug dependence). In the genotyped sample, marijuana dependence was prevalent at 20%. Of those participants diagnosed with drug dependence, 75% also had a comorbid diagnosis of alcohol dependence. Likewise, 51.3% of those with alcohol dependence also had a comorbid diagnosis of illicit drug dependence. In those that had both alcohol and illicit drug dependence, 76% reported marijuana dependence, 53.8% cocaine dependence, 37.8% stimulant dependence, 19.5% opiate dependence and 22.8% sedative dependence.

RESULTS

The PDT (Pedigree Disequilibrium Test), a family-based association test was used to examine the association between five drug-related phenotypes (marijuana dependence, any illicit drug dependence, alcoholism and drug dependence, alcoholism or drug dependence and alcoholism without drug dependence) and the *GABRA2* gene. The same test statistic was used to evaluate the association between the aforementioned phenotypes and *GABRA4*, *GABRG1* and *GABRB1* that constitute the GABA$_A$ receptor gene cluster spanning a 16 cM region on the p-arm of chromosome 4.

GABRA2 Association Findings

Table I includes the *p*-values for all SNPs across *GABRA2* for all phenotypes. Although fewer SNPs across *GABRA2* were associated with marijuana dependence and any illicit drug dependence (Table I) than with alcohol dependence, the number of individuals included in these analyses was considerably smaller than for alcohol dependence. Interestingly, 4 SNPs that were among those showing the strongest association with alcohol dependence were also associated with marijuana and any illicit drug dependence. These included rs279871, rs279826, and rs279836, three SNPs that constituted a high-risk haplotype used to select individuals for sequencing as

GABRA2 and Drug Dependence

					p-Value		
Marker	Position (bp)	Alcohol dependence ($N = 909$)*	Marijuana dependence ($N = 440$)	Any illicit drug dependence ($N = 636$)	Alcohol dependence OR Drug dependence ($N = 1068$)	Alcohol dependence WITH Drug dependence ($N = 477$)	Alcohol dependence WITHOUT Drug dependence ($N = 432$)
Rs490434	46034207	0.004	0.17	0.15	0.10	0.003[#]	0.67
Rs576666	46043305	0.09	0.74	0.53	0.40	0.08	0.42
Rs531460	46051198	0.02	0.21	0.18	0.19	0.02	0.49
Rs561779	46079706	0.05	0.33	0.31	0.44	0.03	0.27
Rs495818	46088826	0.02	0.16	0.19	0.27	0.0097	0.25
Rs497068	46091605	0.006	0.05	0.07	0.08	0.005[#]	0.50
Rs572227	46092321	0.04	0.44	0.47	0.48	0.04	0.39
Rs573400	46092994	0.06	0.08	0.11	0.38	0.0099	0.14
Rs541418	46094144	0.03	0.35	0.19	0.23	0.03	0.162
Rs481311	46095310	0.06	0.18	0.21	0.40	0.03	0.30
Rs507788	46098386	0.03	0.18	0.16	0.23	0.02	0.29
Rs532780	46102294	0.08	0.53	0.46	0.42	0.08	0.58
Rs548583	46104272	0.01	0.29	0.31	0.23	0.02	0.59
Rs10938435	46104792	0.11	0.56	0.47	0.40	0.16	0.45
Rs496650	46105313	0.03	0.05	0.02	0.24	0.005	0.16
Rs540363	46115174	0.06	0.15	0.08	0.15	0.04	0.29
Rs526752	46117557	0.05	0.51	0.53	0.54	0.10	0.45
Rs530329	46122047	0.04	0.25	0.26	0.33	0.02	0.29
Rs483160	46128003	0.16	0.47	0.45	0.50	0.12	0.41
Rs279871	46146661	0.0005[#]	0.03	0.008	0.02	0.0003[#]	0.50
Rs279869	46148923	0.09	0.40	0.21	0.63	0.02	0.20
Rs279867	46149231	0.26	0.74	0.92	0.78	0.26	0.77
Rs279866	46150692	0.03	0.32	0.27	0.26	0.06	0.87
Rs1808851	46152375	0.02	0.43	0.49	0.27	0.05	0.86
Rs279863	46153950	0.02	0.27	0.17	0.19	0.02	0.54
Rs279861	46154253	0.04	0.24	0.19	0.37	0.02	0.18
Rs279858	46155521	0.0097	0.004	0.02	0.08	0.0027[#]	0.32
Rs175931	46157251	0.11	0.23	0.28	0.43	0.06	0.34
Rs279843	46166132	0.04	0.12	0.16	0.18	0.02	0.37
Rs279845	46170651	0.01	0.18	0.08	0.06	0.02	0.45
Rs279846	46170814	0.02	0.15	0.09	0.06	0.03	0.44
Rs183961	46171956	0.05	0.24	0.16	0.26	0.02	0.19
Rs1440130	46174181	0.01	0.15	0.09	0.06	0.02	0.41
Rs279826	46175137	0.003	0.06	0.005	0.06	0.0002[#]	0.16
Rs11503016	46175422	0.02	0.29	0.65	0.08	0.016	0.24
Rs279827	46175630	0.007	0.14	0.09	0.05	0.01	0.52
Rs279828	46175738	0.008	0.17	0.07	0.07	0.01	0.53
Rs279834	46179227	0.02	0.06	0.13	0.12	0.02	0.48
Rs279836	46179998	0.0054	0.05	0.04	0.02	0.007	0.73
Rs279837	46180251	0.04	0.10	0.12	0.23	0.02	0.27
Rs279841	46181691	0.05	0.14	0.13	0.30	0.01	0.15
Rs189957	46197607	0.06	0.66	0.30	0.19	0.13	0.79
Rs1442059	46197880	0.04	0.21	0.19	0.29	0.02	0.26
Rs1442061	46212148	0.39	0.30	0.73	0.62	0.38	0.90
Rs1442062	46218004	0.24	0.21	0.54	0.37	0.27	0.75
Rs11503015	46228072	0.62	0.52	0.63	0.64	0.64	0.15
Rs11503014	46231793	0.89	0.99	0.47	0.90	0.47	0.27
Rs3756007	46231992	0.98	0.62	0.68	0.78	0.64	0.49
Rs894269	46234540	0.10	0.72	0.81	0.17	0.40	0.03
Rs1372472	46237126	0.22	0.33	0.72	0.94	0.49	0.15

Table I. Continued

		p-Value					
Marker	Position (bp)	Alcohol dependence (*N* = 909)*	Marijuana dependence (*N* = 440)	Any illicit drug dependence (*N* = 636)	Alcohol dependence OR Drug dependence (*N* = 1068)	Alcohol dependence WITH Drug dependence (*N* = 477)	Alcohol dependence WITHOUT Drug dependence (*N* = 432)
Rs2165607	46241149	0.44	0.71	0.39	0.28	0.38	0.45
Rs1545234	46245341	0.22	0.95	0.71	0.49	0.86	0.02

"*N*" corresponds to number of affected individuals for each phenotype.
*As reported in Edenberg *et al.*, 2004 (base pair positions have changed due to updated build (build124) of dbSNP).
SNPs that were significant as compared to the B-H *p*-value after applying a FDR correction.
A subset of the SNPs in *GABRA2* were re-analyzed for association with comorbid phenotypes that used the DSM-IIIR alcohol dependence and drug dependence diagnoses. Results were largely unchanged (available from the authors upon request).

reported in Edenberg *et al.* (2004). A SNP encoding a silent polymorphism in exon 5 of the *GABRA2* gene (rs279858) also showed association with marijuana dependence, any illicit drug dependence and alcohol dependence with drug dependence.

The most significant *p*-values were observed for alcohol dependence comorbid with drug dependence, with 32 of the 52 SNPs significant at the 0.05 level. The *p*-values observed for the alcohol dependence with drug dependence phenotype were of a similar level of significance, and occasionally more significant, than those reported in the original manuscript by Edenberg *et al.* for alcohol dependence alone, despite the fact that the sample size was reduced nearly by half. In contrast, when we analyzed alcohol dependence without drug dependence, there was no association with any SNPs in *GABRA2*. This is despite the fact that the sample size was very close to the sample size used in analyses of alcohol dependence with comorbid drug dependence, as 52.5% of the alcohol dependent individuals had comorbid drug dependence.

The *p*-values reported in Table I are the uncorrected *p*-values from the PDT analyses. To take into account multiple testing, we performed a false discovery rate (FDR) correction, at a significance level of $p = 0.05$, using the method proposed by Benjamini & Hochberg (Benjamini and Hochberg, 1995). Observed *p*-values for each phenotype were rank-ordered and compared with the BH (Benjamini & Hochberg) threshold *p*-value, which was computed using the formula 0.05*i/52, where $i = 1...52$ (ranging from 0.05 for the highest (0.05*52/52) to the Bonferroni-corrected *p*-value of 0.001 for the lowest (0.05*1/52)). After correcting for multiple testing, the *p*-values for association with marijuana dependence did not reach

significance (where the lowest observed *p*-value of 0.004 for rs279858 was not less than the lowest BH *p*-value of 0.001). Similarly, for any illicit drug dependence, the lowest *p*-value of 0.005 for rs279826 was not significant as it exceeded the BH *p*-value of 0.001. However, trend level significance for these SNPs was observed. In addition, we note that only 1 SNP reached significance for association with alcohol dependence after correcting for multiple testing ($p = 0.0005$ for rs279871), despite the fact that this association has been replicated in three independent samples now. With the comorbid phenotype, alcohol dependence with drug dependence, five SNPs reached significance, exceeding the corrected BH *p*-value.

We selected the three SNPs (rs279871, rs279858, rs279826) that were associated at $p < 0.05$ with all 6 phenotypes for haplotype-association analyses (Table II). For marijuana dependence, the global chi-square statistic was significant at $p < 0.05$, while for any illicit drug dependence, the global *p*-value for the haplotype analysis was 0.005. The haplotype A-A-A (1-1-1) showed overtransmission with marijuana dependence ($p = 0.006$), with illicit drug dependence ($p = 0.0008$), with alcohol or drug ($p = 0.008$) as well as alcohol and drug dependence ($p < 0.0001$). The alternative haplotype G-G-G (2-2-2), which had the second highest frequency, was also associated with alcohol or drug dependence ($p = 0.0003$), marijuana dependence ($p = 0.01$) and any illicit drug dependence ($p = 0.02$) although to a lesser extent.

Association With Other GABA$_A$ Genes

Finally, we examined association of SNPs in the remaining GABA$_A$ genes in the receptor cluster to

GABRA2 and Drug Dependence

Table II. Haplotype Association Analyses with Significant SNPs (rs279871, rs279858 and rs279826) for Drug and Alcohol-related Phenotypes in the COGA Sample: Marijuana Dependence, Any Illicit Drug Dependence, Alcohol or Drug Dependence, Alcohol and Drug Dependence and Alcohol Without Drug Dependence

Haplotype		Alcohol dependence		Marijuana dependence		Any illicit drug dependence		Alcohol dependence OR Drug dependence		Alcohol dependence WITH Drug dependence		Alcohol dependence WITHOUT Drug dependence	
SNP	Frequency	Z-score	p-value	Z-score	p-value	Z-Score	p-value	Z-score	p-value	Z-score	p-value	Z-Score	p-value
A-A-A	0.53	3.69	0.0002	2.74	0.006	3.36	0.0008	2.7	0.008	4.42	< 0.0001	1.12	0.26
A-A-G	0.08	0.41	0.69	0.50	0.62	−1.18	0.24	0.15	0.88	−0.98	0.33	1.39	0.16
A-G-A	0.004	−0.99	0.32	−0.44	0.66	0.53	0.60	−1.09	0.28	0.99	0.32	−1.89	0.06
A-G-G	0.006	−0.97	0.33	0.45	0.65	0.21	0.84	0.28	0.78	1.02	0.31	−1.37	0.17
G-A-A	0.005	−1.14	0.25	−1.37	0.17	−1.53	0.13	−1.26	0.21	−1.58	0.11	−1.22	0.22
G-A-G	0.004	−2.25	0.02	−0.79	0.43	−1.46	0.14	−2.03	0.04	−2.03	0.04	−0.80	0.42
G-G-A	0.006	−0.51	0.61	−0.32	0.74	−0.44	0.65	−0.32	0.75	−0.74	0.46	0.0003	0.99
G-G-G	0.37	−3.00	0.003	−2.53	0.01	−2.39	0.02	−1.81	0.07	−3.63	0.0003	−1.04	0.30
Global test		27.46	0.0003	15.00	0.04	20.49	0.005	15.3	0.03	37.5	< 0.0001	10.37	0.17

R^2 for SNPs is rs279871 with rs279858: 0.69; rs279871 with rs279826: 0.60; rs279858 with rs279826: 0.60.

test whether our results were limited to the *GABRA2* gene, as with the previous alcohol dependence results, or whether other GABA$_A$ receptor genes in the region might be involved in other drug dependence (Table III). There was no consistent evidence for association between *GABRA4*, *GABRG1* or *GABRB1* and our phenotypes.

DISCUSSION

GABRA2 has been demonstrated to be associated with alcohol dependence in the U.S. COGA family study (Edenberg *et al.*, 2004). This association has been replicated using a different study design, a case–control approach, in samples collected in the

Table III. Association of SNPs for *GABRG1 GABRA4* and *GABRB1* with Marijuana Dependence, Any Illicit Drug Dependence, Alcohol or Drug Dependence, Alcohol and Drug Dependence and Alcohol Without Drug Dependence

Marker	Gene	Position (bp)	Alcohol dependence (N = 909)*	Marijuana dependence (N = 440)	Any illicit drug dependence (N = 636)	Alcohol dependence OR Drug dependence (N = 1068)	Alcohol dependence WITH Drug dependence (N = 477)	Alcohol dependence WITHOUT Drug dependence (N = 432)
Rs1497570	*GABRG1*	45887962	0.65	0.70	0.68	0.99	0.53	0.42
Rs1948609	GABRG1	45903700	0.88	0.78	0.96	0.73	0.47	0.38
Rs1391175	GABRG1	45942092	**0.05**	0.59	0.55	**0.05**	0.57	0.14
Rs2221020	GABRG1	45944493	0.07	0.49	0.23	0.30	**0.03**	0.22
Rs1391168	GABRG1	45956087	0.46	0.21	0.51	0.91	0.09	0.08
Rs904154	GABRG1	45967299	0.19	0.65	0.39	0.38	0.18	0.99
Rs2036943	*GABRA4*	46717487	0.85	0.11	0.14	0.54	0.24	0.02
Rs2055943	*GABRA4*	46808207	0.72	0.42	0.60	0.78	0.66	0.31
Rs1512135	GABRA4	46814816	0.63	0.79	0.91	0.39	0.96	0.20
Rs1877400	GABRA4	46818229	0.90	0.35	0.34	0.33	0.84	0.95
Rs2229940	GABRA4	46836294	0.38	0.71	0.19	0.23	0.49	0.74
Rs989808	*GABRB1*	47007709	0.77	0.29	0.59	0.95	0.82	0.85
Rs1372496	GABRB1	47048736	0.98	0.89	0.48	0.85	0.65	0.92
Rs1372497	GABRB1	47063266	0.53	0.84	0.67	0.88	0.62	0.45
Rs6284	GABRB1	47163147	0.98	0.41	0.53	0.69	0.30	0.54
Rs2070922	GABRB1	47246976	0.22	0.52	0.27	0.08	0.44	0.80
Rs6289	GABRB1	47249637	0.63	0.72	0.25	0.69	0.45	0.09

*As reported in Edenberg et al. (2004) (base pair positions have changed due to updated build (build124) of dbSNP).

U.S. (Covault *et al.*, 2004) in Russia (Lappalainen *et al.*, 2005), and in Germany (Fehr *et al.*, 2006). In the current study, we investigated the relationship between *GABRA2* and drug dependence, by extending our analyses in the COGA sample to incorporate illicit drug dependence and comorbid alcohol dependence-drug dependence phenotypes. Our results suggest that *GABRA2* is most strongly associated with co-occurring alcohol and illicit drug dependence. There was weaker association with marijuana dependence alone and with a broader category of any illicit drug dependence (including marijuana). Surprisingly, alcohol dependence without co-occurring drug dependence showed no evidence of association with *GABRA2* in our sample.

One prior study examined the association of *GABRA2* with alcoholism in the presence and absence of comorbid drug dependence (Covault *et al.*, 2004). Contrary to our findings, Covault and colleagues reported that the association of *GABRA2* with alcoholism increased when individuals with comorbid drug dependence were excluded. The divergence in findings may be due to sample characteristics based on the nature of the recruitment. The COGA families, which were already part of a sample enriched for alcoholism, were required to have at least two first-degree relatives with alcohol dependence. Additionally, probands with intravenous drug use more than 30 times, or within 6 months of screening, were excluded, with the rationale that alcohol dependence may be secondary to illicit drug problems in these subjects and that the pathway and underlying risk factors among this group may differ. In contrast, in the study by Covault *et al.*, subjects were recruited as part of ongoing studies of the genetics of substance use disorders or from clinical trials for the treatment of substance use. Thus, the drug dependent individuals excluded from their analyses that led to greater significance may have been similar to the drug dependent individuals screened out of the COGA sample at the initial ascertainment of probands. In addition, the subset of analyses reported in the Covault paper do not directly parallel the analyses reported here, as major depression was also excluded in those analyses.

Other analyses of the COGA dataset also corroborate the involvement of *GABRA2* in illicit drug dependence. Incorporating data from children and adults genotyped as part of the COGA project into lifetime survival analyses, *GABRA2* was associated with the onset of drug dependence both in childhood/adolescence and across the lifespan (Dick

et al., 2006). However, it is particularly interesting that the family-based association analyses reported here suggest that *GABRA2* is most strongly related to alcohol dependence comorbid with illicit drug dependence, and that there is no evidence of association with alcohol dependence without other drug dependence. These results are in accord with the findings from previous work by Pickens and McGue (McGue *et al.*, 1992; Pickens *et al.*, 1995) demonstrating that alcohol dependence comorbid with other drug dependence is highly heritable, whereas alcohol dependence without other drug dependence was more strongly influenced by the environment.

Biologically, it is plausible that the $GABA_A$ receptor (Kaupmann *et al.*, 1997) is involved in illicit drug dependence in addition to alcohol dependence. Although there has been considerably less research on the $GABA_A$ receptors and illicit drug dependence, as compared to alcohol dependence, Buck and Finn (2001) have implicated $GABA_A$ receptors in acute and chronic barbiturate withdrawal in QTL mapping and candidate gene analyses in murine models (Buck and Finn, 2001). Additionally, cannabinoid(1)-receptor mediated inhibition of cortical GABA production has been studied, in vivo, in rat brains (Ferraro *et al.*, 2001a; Ferraro *et al.*, 2001b) leading to speculation that cannabinoids may target cortical GABA to induce symptoms of loss of memory and cognitive disturbance. Therefore, while there is growing evidence for the involvement of $GABA_A$ receptors in mediating drug dependence, much work is needed to elucidate its precise role in comorbid drug and alcohol dependence.

The findings of the current manuscript should be viewed with the following limitations in mind: The COGA sample used for these analyses was ascertained using alcohol dependent probands and their families. Results reported here may not generalize to other samples. In addition, intravenous drug use more than 30 times lifetime, or within 6 months of screening, were used as exclusionary criteria for participation in the study, as previously mentioned. Individuals affected with HIV were also excluded. This was to minimize diagnostic confusion, as individuals with HIV may develop secondary psychiatric symptoms due to their infection. This again may limit generalization to other drug abusing populations. Finally, we did not examine the association between the genes in the $GABA_A$ receptor cluster and illicit drug dependence without comorbid alcohol dependence. This was primarily due to sample size

restrictions as only 25% of the 636 individuals with illicit drug dependence did not have a comorbid diagnosis of alcohol dependence.

CONCLUSIONS

The study of the observed co-occurrence between different classes of illicit drugs and also between alcohol and illicit drug dependence has drawn considerable interest from epidemiologists and behavioral geneticists. We used this literature to guide exploration of the effect of a specific gene, *GABRA2*, on various forms of drug dependence. The Collaborative Study of the Genetics of Alcoholism has previously reported an association between *GABRA2* and alcohol dependence. Here, we have extended these analyses to explore the relationship between *GABRA2* and illicit drug dependence, as well as the comorbidity between alcohol and other drug dependence. We find some evidence of association between *GABRA2* and marijuana and other illicit drug dependence. In addition, we find that the association between *GABRA2* and alcohol dependence is largely limited to individuals with co-occurring drug dependence in the COGA sample.

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