doi:10.1111/j.1360-0443.2007.01871.x

# Alcohol dependence with comorbid drug dependence: genetic and phenotypic associations suggest a more severe form of the disorder with stronger genetic contribution to risk*

**Danielle M. Dick[1], Arpana Agrawal[1], Jen C. Wang[1], Anthony Hinrichs[1], Sarah Bertelsen[1], Kathleen K. Bucholz[1], Marc Schuckit[2], John Kramer[3], John Nurnberger Jr[4], Jay Tischfield[5], Howard J. Edenberg[4], Alison Goate[1] & Laura J. Bierut[1]**

Washington University School of Medicine, St Louis, Missouri,[1] University of California-San Diego, San Diego,[2] University of Iowa, Iowa City, Iowa,[3] Indiana University School of Medicine, Indianapolis, Indiana[4] and Rutgers University, New Brunswick, NJ, USA[5]

## ABSTRACT

**Background** Twin data suggest that alcohol dependence comorbid with illicit drug dependence represents a more heritable form of the disorder. In the Collaborative Study on the Genetics of Alcoholism sample, approximately half the alcohol-dependent individuals also meet diagnostic criteria for illicit drug dependence. In this study, we tested for heterogeneity in the association between the muscarinic acetylcholine M2 receptor gene (*CHRM2*) and alcohol dependence, reported previously in the full sample, among the subgroups of alcohol-dependent individuals with and without comorbid drug dependence. **Methods** Family-based association tests were conducted separately (a) in individuals with alcohol dependence *with* comorbid drug dependence ($n = 477$) and (b) in individuals with alcohol dependence *without* comorbid drug dependence ($n = 433$). These subgroups were subsequently compared on other phenotypic characteristics. **Results** The evidence for association between *CHRM2* and alcohol dependence came entirely from the subgroup of individuals with comorbid drug dependence. There was no evidence of association with *CHRM2* among the alcohol-dependent individuals without drug dependence. Subsequent phenotypic analyses suggest that the subgroup of alcohol-dependent individuals with comorbid drug dependence differ on a number of other phenotypic characteristics, including several measures of the severity of their alcohol problems, personality traits and comorbid psychiatric disorders. **Conclusions** These analyses provide specific genetic evidence suggesting that alcohol dependence with comorbid drug dependence represents a particularly severe form of the disorder, with higher genetic contribution to vulnerability.

**Keywords** Alcohol dependence, *CHRM2*, comorbid drug dependence, genetics, heterogeneity.

*Correspondence to*: Danielle M. Dick, Washington University in St Louis, Department of Psychiatry, Box 8134, 660 South Euclid Avenue, St Louis, MO 63110, USA. E-mail: dickd@psychiatry.wustl.edu
Submitted 1 August 2006; initial review completed 30 November 2006; final version accepted 2 March 2007

## INTRODUCTION

Several large epidemiological surveys conducted in the United States (the Epidemiological Catchment Area Study [1], the National Longitudinal Alcohol Epidemiological Survey [2], the National Comorbidity Survey [3] and the National Epidemiologic Survey on Alcohol and Related Conditions [4]), as well as in other countries (the Australian National Survey of Mental Health and Well Being: NSMH & WB [5]; and the Mental Health Supplement of the Ontario Canada Health Survey: MHS-OHS [6]), demonstrate significant comorbidity between alcohol use

*In memory of Henri Begleiter and Theodore Reich, Principal and Co-Principal Investigators of COGA since its inception; we are indebted to their leadership in the establishment and nurturing of COGA, and acknowledge with great admiration their seminal scientific contributions to the field.

disorders and other psychiatric problems. Illicit drug dependences are among the most frequently co-occurring disorders with alcohol dependence [7–9]. Several studies suggest that individuals with alcohol dependence and comorbid drug dependence differ from individuals with alcohol dependence alone on several dimensions. There are demographic differences: individuals with comorbid alcohol and other drug dependence are more likely to be younger, male, never married and of lower socio-economic status [4]. In addition, there is a suggestion that alcohol dependence with comorbid drug dependence may represent a more severe form of the disorder. Individuals with alcohol dependence who attempt or complete suicide are more likely to have comorbid substance problems [10]. In addition, comorbid individuals have higher use of health services [5] and are more likely to seek treatment [4,11]. Furthermore, alcohol-dependent individuals with comorbid drug problems are more likely to relapse after treatment [12].

There is also an indication that alcohol dependence comorbid with drug dependence may represent a form of the disorder with higher heritability. Electrophysiological abnormalities in alcohol-dependent individuals, which are thought to index genetic vulnerability such as a reduced P3 amplitude compared to controls [13], are most dramatic in alcohol-dependent individuals who also have a diagnosis of illicit drug abuse or dependence, particularly if they also display antisocial personality [14]. In a study of the relationship between parental alcoholism and adolescent psychopathology, adolescents with a parent with comorbid alcohol dependence and drug dependence showed elevated rates of psychological problems in adolescence, whereas adolescents with parents with alcohol dependence only did not show elevated rates of psychopathology [15]. In a small study of monozygotic (MZ) and dizygotic (DZ) twin pairs, DSM-III alcohol dependence in males was found to be heritable only when comorbid with DSM-III drug dependence or mental disorder, but not when the proband had alcohol dependence alone [16]. In an extension of this work, a latent class analysis of alcohol-dependent individuals from the Epidemiological Catchment Area study found that only the severe alcohol dependence subtype, characterized by significantly greater comorbid drug dependence, showed evidence of genetic influence [17].

More recently, individuals have begun to take this comorbidity into account in an attempt to understand specific gene association results more clearly. For example, Kranzler and colleagues examined whether heterogeneity related to association results with the 5-HTTLPR polymorphism in the promoter region of the serotonin transporter gene and alcohol dependence may be explained by a difference in comorbid drug dependence; however, they found no evidence that this was the case in the relation to this polymorphism in their sample [18]. In the Collaborative Study on the Genetics of Alcoholism (COGA) sample, approximately half the alcohol-dependent individuals in the genetic analysis sample meet criteria for comorbid drug dependence. We previously conducted follow-up analyses of the association between a GABA-A receptor gene, *GABRA2*, and alcohol dependence reported in the full sample [19] to test for possible heterogeneity associated with the presence of a comorbid drug dependence diagnosis. We found that the evidence for association came entirely from the subset of alcohol-dependent individuals with comorbid drug dependence; there was no evidence for association among the subgroup of alcohol-dependent individuals without a comorbid drug diagnosis [20].

Here we report parallel analyses for another gene, the muscarinic acetylcholine M2 receptor gene (*CHRM2*), that is associated with alcohol dependence in the COGA sample [21]. *CHRM2* was investigated originally in the COGA project based on its proximity to a linkage peak associated with evoked brain oscillations involved in P3 [22], an electrophysiological endophenotype related to alcohol dependence and drug dependence, as described above [23]. Muscarinic acetylcholine receptors belong to a family of G-protein-coupled receptors that activate a multitude of signaling pathways important for modulating neuronal excitability, synaptic plasticity and feedback of acetylcholine release [24]. They are present in the central nervous system and thought to be involved in many brain functions, including attention, learning, memory and cognition [25]. The association observed between *CHRM2* and alcohol dependence in the COGA sample has been replicated by an independent group [26]. Interestingly, in that replication sample, an association between *CHRM2* and drug dependence was also observed. In this study, we first examined the association between single nucleotide polymorphisms (SNPs) in the *CHRM2* gene among alcohol-dependent individuals with and without comorbid illicit drug dependence. We then conducted further phenotypic analyses comparing these subgroups of alcohol-dependent individuals.

## METHODS

### Sample

COGA is a multi-site project, in which families were collected by 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 in-patient or out-patient alcohol treatment programs by each of 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 [27]. A total of 2282 individuals from 262 multiplex alcoholic families were included in the COGA genetic analysis sample. Of the total sample, 83% of the families reported their race as white, 13% as black and 4% of other descent. The mean age of the sample was 39.5 (standard deviation, SD = 14.8) (range: 17–91) [8,14]. The sample includes slightly more women (54%) than men, although men were more likely to be affected with alcohol dependence (46% versus 20%). The institutional review boards of all participating centers approved the study. Additional details about the study have been published previously [27,28].

### Phenotypes

All phenotypic diagnoses were based on interview data from participants who were administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview [29,30]. Life-time alcohol dependence diagnoses were made using the criteria of the Fourth Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-IV; [31]). 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 diagnoses of other disorders were assessed using the established Third Revised (DSM-IIIR) criteria [29,30]. The rate of alcohol dependence among the genotyped individuals in the sample was 41%. Of the alcohol-dependent individuals in COGA, 52% also met criteria for an illicit drug dependence diagnosis. There were 477 individuals with alcohol dependence *with* comorbid illicit drug dependence and 433 individuals with alcohol dependence *without* comorbid illicit drug dependence. Of the individuals who met criteria for an illicit drug dependence: 71.5% ($n = 341$) met criteria for marijuana dependence; 58.5% ($n = 279$) met criteria for cocaine dependence; 18.5% ($n = 88$) met criteria for opioid dependence; 33.3% ($n = 159$) met criteria for stimulant dependence; and 20.1% ($n = 96$) met criteria for sedative dependence.

### Genotyping

Publicly available databases, dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and HapMap (http://www.hapmap.org), were used to identify SNPs within and flanking the *CHRM2* gene. In addition, a number of novel SNPs were identified by sequencing. We genotyped 27 SNPs within and flanking *CHRM2*. SNPs were selected to cover all exons and flanking intronic sequence as well as putative upstream promoter regions and a region of high sequence conservation in intron 3. The location of genotyped SNPs across the gene is illustrated in [32]. The minor allele frequency was > 0.10 in all cases (average = 0.45). Genotyping was performed using a modified single nucleotide extension reaction, with allele detection by mass spectroscopy (Sequenom MassArray system; Sequenom, San Diego, CA, USA). All genotypic data were checked for Mendelian inheritance of marker alleles with the USERM13 [33] option of the MENDEL linkage computer programs, which was then used to estimate marker allele frequencies. Trio data from Caucasian individuals genotyped in the COGA data set was entered into the program Haploview [34] to examine the linkage disequilibrium structure of the genotyped SNPs (Fig. 1).

### Genetic analyses

Multiplex families of alcoholics were used in tests of association between each of the SNPs and each of the phenotypes studied, using the Pedigree Disequilibrium Test (PDT) [35]. The PDT can analyze data from extended pedigrees, using all available trios in a family (two parents plus child genotyped) as well as discordant siblings. The PDT tests for (1) overtransmission of a particular allele to affected individuals and (2) an increased frequency of the overtransmitted allele in affected siblings compared to their unaffected siblings. This test produces two statistics: the 'PDT-ave', which averages the association statistic over all families, and the 'PDT-sum', which gives greater weight to larger families with more informative trios and discordant siblings [35]. As in previous COGA papers analyzing *CHRM2* [21], the *P*-values presented are uncorrected for multiple testing. This allows for comparison between the association analyses of *CHRM2* reported previously for the full sample, and the results reported here separately for the subgroups of alcohol-dependent individuals with and without drug dependence. Because no consensus exists for the most appropriate way to handle the analysis of correlated phenotypes and correlated SNPs, the strategy generally employed by the COGA group has been to consider as positive a group of SNPs yielding evidence of overtransmission that correspond with the pattern of LD, rather than using strict *P*-value cut-offs for evaluating evidence for association for a particular gene. However, the evaluation of the overall strength of association is less critical in the present paper: multiple positive reports exist for the association between *CHRM2* and alcohol dependence [21,26]; accordingly, the question being addressed in this paper is not the evidence for association *per se*, but the comparison of results across the subgroups to test for genetic heterogeneity in the sample.

Genetic analyses were conducted separately for (a) individuals with alcohol dependence and comorbid drug

 

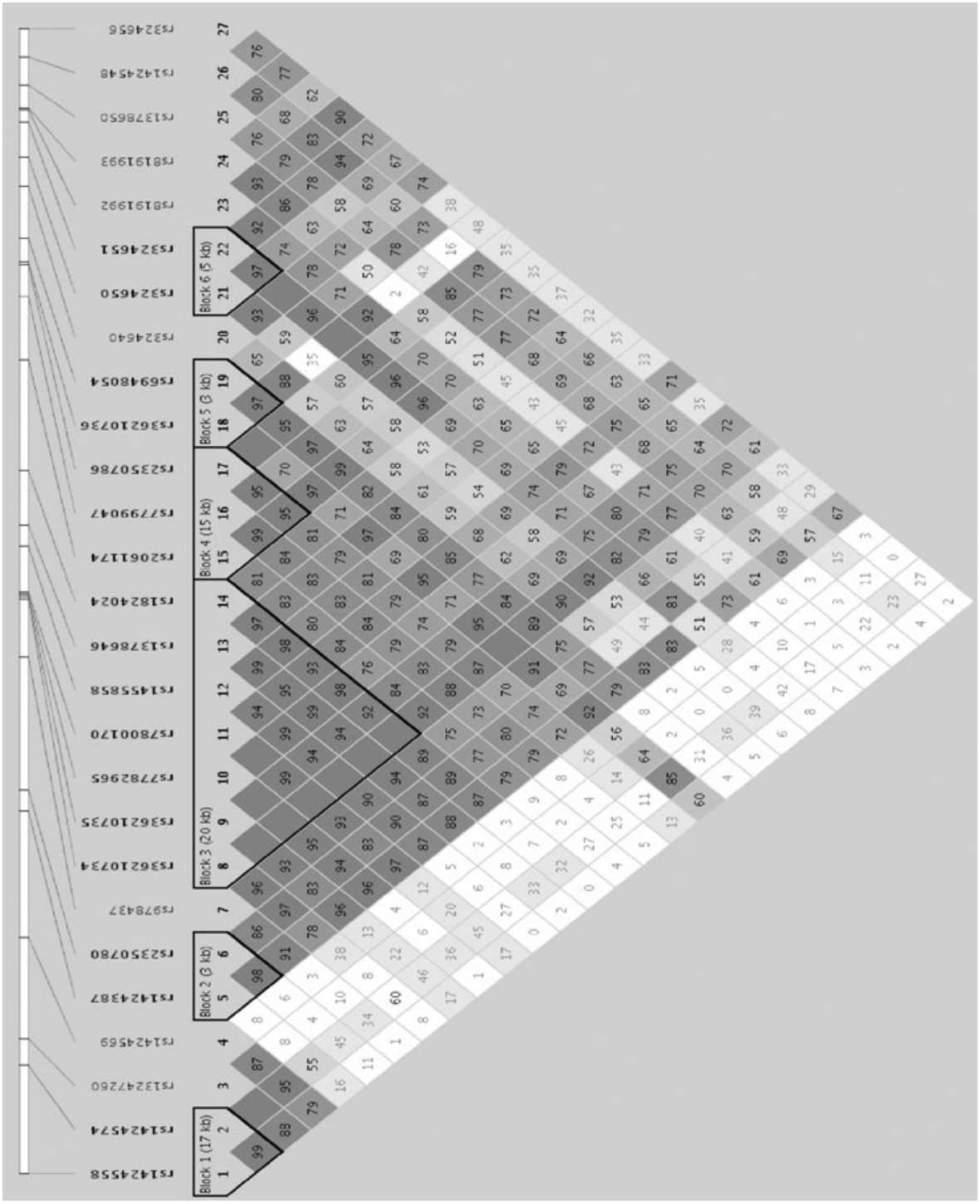


**Figure 1** Haploview plot of linkage disequilibrium structure (D′) across the genotyped single nucleotide polymorphisms (SNPs) using Caucasian individuals from the COGA sample

dependence and (b) individuals with alcohol dependence without a comorbid drug dependence diagnosis. The unaffected group was kept constant across analyses, considering only individuals who reported neither alcohol nor drug dependence as unaffected. For example, consider a family with three siblings, where Sib1 is diagnosed with alcohol and drug dependence, Sib2 is diagnosed with alcohol without drug dependence and Sib3 does not meet criteria for alcohol or drug dependence (i.e. unaffected). For the PDT analyses of alcohol and drug dependence, Sib1 is affected, Sib2 is missing (not used in the analysis) and Sib 3 is unaffected. Therefore, Sib1 is used in

 

computing the overtransmission statistic, and Sib1 and Sib3 form the discordant pair. In contrast, for the PDT analyses of alcohol without drug dependence, Sib1 is missing, Sib2 is affected and Sib 3 is unaffected. Therefore, Sib 2 is used in evaluating overtransmission, and Sib2 and Sib3 form the discordant pair. For an example SNP selected at random, rs1824024, the number of allelic transmissions from heterozygous parents to affected offspring was 473 and the number of discordant sibships was 222 for the alcohol with drug dependence group; for the alcohol without drug dependence group, the number of informative transmissions was 460 and discordant sibships was 145. The exact number of parent–child transmissions and discordant sibships varied slightly across SNPs as a function of the heterozygosity and missing rate of the individual SNPs.

### Phenotypic analyses

A series of additional analyses was conducted comparing the alcohol-dependent individuals with and without drug dependence on a variety of additional phenotypes assessed by COGA: demographic variables, alcohol use patterns, comorbid disorders and personality. Personality was assessed with the Tridimensional Personality Questionnaire [36]. All other variables were obtained in the SSAGA interview. Because the age at time of interview differed significantly between the groups (mean = 42.81, SD = 14.41 among the alcohol-dependence with drug-dependence group; mean = 33.33, SD = 8.00 among the alcohol-dependence without drug-dependence group; $P < 0.0001$), we compared group differences on the additional phenotypes of interest using the GLM procedure in the SAS software program [37] to take into account age at time of interview.

## RESULTS

### Genetic analyses

Table 1 shows the *P*-values from association tests for each of the markers in *CHRM2* with the alcohol-dependent individuals split into two groups: those with comorbid drug dependence and those without drug dependence.

As the table illustrates, the association with alcohol dependence that was observed previously in the full COGA sample [21] comes entirely from the subgroup of individuals with alcohol dependence and comorbid drug dependence. There is no evidence of association with *CHRM2* among the alcohol-dependent individuals who do not have comorbid drug dependence. The failure to observe an association in the group of individuals who are alcohol-dependent without drug dependence is not likely to be due to a lack of power (if the effects are of equal magnitude), because the numbers of alcohol-dependent individuals with and without drug dependence are comparable.

**Table 1** *P*-values from association tests with *CHRM2* SNPs.

| | | *AD with DD* | | *AD without DD* | |
|---|---|---|---|---|---|
| *Marker name* | *Position (bp)* | *Sum* | *Ave.* | *Sum* | *Ave.* |
| rs1424558 | 135988926 | 1.00 | 0.64 | 0.68 | 0.84 |
| rs1424574 | 136006288 | 0.92 | 0.61 | 0.26 | 0.48 |
| rs13247260 | 136010518 | 0.16 | 0.51 | 0.80 | 0.33 |
| rs1424569 | 136026671 | 0.51 | 0.77 | 0.91 | 0.19 |
| rs1424387 | 136046865 | 0.09 | 0.35 | 0.96 | 0.68 |
| rs2350780 | 136050224 | 0.07 | 0.16 | 0.88 | 0.79 |
| rs978437 | 136071433 | **0.001*** | **0.003*** | 0.94 | 0.86 |
| rs36210734 | 136080468 | **0.04*** | **0.04*** | 0.66 | 0.81 |
| rs36210735 | 136080901 | **0.02*** | **0.01*** | 0.97 | 0.98 |
| rs7782965 | 136081388 | **0.01*** | **0.02*** | 0.74 | 0.70 |
| rs7800170 | 136081575 | **0.005*** | **0.01*** | 0.84 | 0.91 |
| rs1455858 | 136088958 | **0.05*** | 0.06 | 0.86 | 0.69 |
| rs1378646 | 136092256 | **0.007*** | **0.006*** | 0.90 | 0.98 |
| rs1824024 | 136100949 | **0.004*** | **0.004*** | 0.94 | 0.89 |
| rs2061174 | 136118655 | **0.05*** | 0.09 | 0.61 | 0.72 |
| rs7799047 | 136128813 | 0.26 | 0.26 | 0.44 | 0.95 |
| rs2350786 | 136133825 | 0.42 | 0.30 | 0.34 | 0.68 |
| rs36210736 | 136134189 | 0.10 | 0.46 | 1.00 | 0.86 |
| rs6948054 | 136138056 | 0.08 | 0.07 | 0.38 | 0.84 |
| rs324640 | 136146251 | 0.07 | 0.07 | 0.91 | 0.96 |
| rs324650 | 136150916 | 0.06 | 0.06 | 0.81 | 0.58 |
| rs324651 | 136156516 | **0.03*** | **0.01*** | 0.75 | 0.75 |
| rs8191992 | 136158563 | 0.06 | **0.05*** | 0.93 | 0.66 |
| rs8191993 | 136158818 | 0.11 | 0.13 | 0.22 | 0.33 |
| rs1378650 | 136162406 | 0.17 | 0.10 | 0.74 | 0.88 |
| rs1424548 | 136167015 | 0.74 | 0.65 | 0.25 | 0.14 |
| rs324656 | 136171367 | 0.47 | 0.78 | 0.52 | 0.39 |

AD = alcohol dependence; DD = drug dependence; SNPs (single nucleotide polymorphisms) in bold type significant at $P \leq 0.05$ shown with an asterisk.

### Phenotypic analyses

These findings led us to conduct analyses comparing the alcohol-dependent individuals with and without comorbid drug dependence on a variety of additional phenotypes to understand more clearly the differences between these groups (Table 2).

Males were slightly over-represented among the individuals with comorbid drug dependence, although this difference did not reach statistical significance. The alcohol-dependent individuals with comorbid drug dependence endorsed a significantly higher number of DSM-IV criteria and were significantly more likely to exhibit high-risk drinking patterns, as indicated by a younger age of onset of alcohol dependence, a younger age of onset of regular drinking and first drunkenness, a higher maximum number of drinks consumed in 24 hours and increased rates of bingeing and with-

**Table 2** Comparison of alcohol-dependent individuals with and without comorbid drug dependence; percentages or means (with standard deviations) presented.

| | *AD with DD (n = 477)* | *AD without DD (n = 432)* | *P-value** |
|---|---|---|---|
| Demographics and drinking variables | | | |
| Gender (% male) | 68.97% | 62.96% | 0.06 |
| Ethnicity (% black/white) | 13.4/82.6% | 11.1/86.6% | 0.48 |
| Number DSM-IV symptoms | 5.63 (1.44) | 4.86 (1.41) | < 0.0001 |
| Age onset AD | 20.76 (5.28) | 26.81 (10.02) | < 0.0001 |
| Treatment for a drinking problem | 66.25% | 47.92% | < 0.0001 |
| Age started regular drinking | 16.13 (3.92) | 18.61 (5.56) | 0.0003 |
| Age first drunk | 14.32 (3.74) | 17.15 (5.10) | < 0.0001 |
| Maximum drinks in 24 hours | 32.88 (21.71) | 24.27 (15.03) | < 0.0001 |
| Bingeing | 65.41% | 46.99% | < 0.0001 |
| DSM-IV withdrawal | 55.46% | 42.92% | < 0.0001 |
| Withdrawal symptoms | 3.28 (3.08) | 2.36 (2.91) | < 0.0001 |
| SRE: first drinking | 4.44 (2.68) | 5.04 (3.99) | 0.1133 |
| Personality | | | |
| Harm avoidance | 14.56 (7.43) | 13.91 (7.45) | 0.2236 |
| Novelty seeking | 18.55 (5.18) | 15.68 (5.26) | < 0.0001 |
| Reward dependence | 12.48 (3.81) | 12.62 (3.79) | 0.17 |
| Comorbidity | | | |
| Antisocial personality disorder | 34.38% | 7.03% | < 0.0001 |
| Conduct disorder | 40.04% | 12.38% | < 0.0001 |
| Anxiety disorders† | 15.55% | 10.33% | 0.0261 |
| Eating disorders‡ | 4.61% | 2.34% | 0.2549 |
| Major depressive disorder | 57.65% | 38.52% | 0.0004 |
| Mania | 4.62% | 1.64% | 0.0392 |
| Habitual smoking | 87.58% | 79.58% | < 0.0001 |

AD = alcohol dependence, DD = drug dependence. †Agoraphobia, obsessive-compulsive disorder, social phobia, panic disorder; ‡anorexia nervosa, bulimia. **P*-values associated with all comparisons except for gender and ethnicity are corrected for age at time of interview.

drawal. They were also more likely to report treatment for a drinking problem. Interestingly, the group of individuals with alcohol dependence without drug dependence reported higher initial sensitivity to alcohol on their initial drinking occasions.

In the personality comparisons, individuals with alcohol dependence comorbid with drug dependence had higher novelty seeking scores. There were no differences in scores on the harm avoidance or reward dependence scales.

Finally, alcohol-dependent individuals with comorbid drug dependence had significantly elevated rates of comorbid psychiatric disorders, across virtually all categories of psychiatric disorders assessed in the SSAGA. Due to the low number of individuals who reported specific anxiety disorders or eating disorders, individual diagnoses were collapsed to compare overall rates of these problems. The most pronounced differences were a nearly fivefold increase in the rate of antisocial personality disorder (ASPD) and a greater than threefold increase in the rate of conduct disorder among the individuals with comorbid alcohol dependence and illicit drug dependence. Major depressive disorder was also significantly more common among individuals with comorbid alcohol dependence and drug dependence. This difference was reduced when only independent episodes of depression (those not related to alcohol use) were considered (27.60% in the alcohol dependence with drug dependence group versus 20.42% in the alcohol dependence without drug dependence group), but the difference remained significant ($P = 0.038$). There was no significant difference in rates of eating disorders between the two groups, although this may have been due to the small number of individuals endorsing these problems. Rates of habitual smoking, as measured by interview report of smoking at least one pack a day for 6 months or longer, were also significantly higher among the alcohol-dependent individuals with comorbid drug dependence.

## DISCUSSION

We found evidence that the association between *CHRM2* and alcohol dependence reported previously [21] and replicated [26] is driven entirely by the subset of the

 

COGA sample with alcohol dependence with comorbid drug dependence. In fact, the association observed in the alcohol-dependent group with comorbid drug dependence yields *P*-values very similar in magnitude to the overall sample [21], despite a nearly 50% reduction in sample size. There was no evidence of association with *CHRM2* in the alcohol-dependent individuals without comorbid drug dependence. These results from *CHRM2* parallel those from *GABRA2* [19,20]. Thus, across two genes associated with alcohol dependence in the COGA sample, and replicated by independent groups, we find evidence that the association with alcohol dependence is driven entirely by the subset of individuals with alcohol dependence with comorbid drug dependence.

More detailed phenotypic comparisons of these two groups of alcohol-dependent individuals suggest that the subgroup with comorbid drug dependence differs significantly on many other characteristics. The individuals who meet diagnostic criteria for both alcohol dependence and illicit drug dependence have a more severe alcohol dependence, as indicated by a higher number of DSM-IV criteria met, an earlier age of regular drinking and of first being drunk and an earlier onset of alcohol dependence. This group has a higher frequency of individuals who binge drink and who have experienced withdrawal. They also have a higher maximum number of drinks within a 24-hour period. We do note that the group reporting alcohol dependence with drug dependence was significantly older at time of interview (43 years of age) than the group reporting alcohol dependence without drug dependence (33 years of age). However, we do not believe this could be entirely responsible for the pattern of results, as (1) the *P*-values associated with differences between the groups were significant, even after taking into account age at time of interview, and (2) both alcohol-dependent groups show no censoring for many of the severity items, such as age of onset of regular drinking, age at first drunkenness and age of onset of alcohol dependence, and these indices also yield a pattern of results suggesting greater severity in the comorbid group.

In an attempt to understand further whether the genetic heterogeneity observed in the sample was a function of comorbid drug dependence, or just a reflection of the greater severity of alcohol dependence in the comorbid group, we conducted a number of exploratory analyses testing for parallel patterns of results when dividing the sample based on other indices of severity. We compared subgroups of the sample based on an earlier onset of alcohol dependence (< 25 years; 66% of alcohol-dependent individuals); an earlier age of onset of regular drinking (< 17 years; 52% of alcohol-dependent individuals); a higher maximum number of drinks in 24 hours [> 29; 38% of the sample (distribution of maximum drinks made a split closer to 50–50 impossible)]; and the presence of withdrawal (reported by 50% of alcohol-dependent individuals). Only the variable age of onset of regular drinking produced a parallel pattern of results to that observed for the drug dependence subgroups, whereby the association was concentrated in one subgroup: e.g. for the most significant SNP, rs978437, PDT-sum *P*-value = 0.0006 among those who reported regular drinking prior to age 17, and $P = 0.79$ among individuals who reported regular drinking starting at age 17 or later. In addition, we compared overtransmission rates across classes of alcohol-dependent individuals meeting an increasing number of DSM-IV criteria to look for a pattern of increasing evidence for genetic association among the alcohol-dependent individuals with higher symptom counts. We found no evidence that this was the case. Accordingly, a younger age of onset of regular drinking also appears to index a particularly severe form of alcohol dependence that is influenced strongly by genetic factors. A striking association between an early onset of drinking and the subsequent development of alcohol dependence has been reported previously [38,39], with a twin study suggesting that this association reflects a genetic propensity toward developing alcohol dependence [40]. Furthermore, age at first drink has also been associated with reduced P3 amplitude, an electrophysiological marker thought to index genetic vulnerability to alcohol dependence, as well as with a broad range of disinhibitory psychopathology, including illicit drug dependence [39]. The rate of illicit drug dependence was also very high (64%) among those reporting early regular drinking in our sample; thus, it is impossible to tease apart these variables completely.

The subgroup with comorbid drug dependence also shows a significant increase in virtually all comorbid psychiatric disorders. Most notable are the substantially elevated rates of ASPD (4.9-fold) and conduct disorder (3.2-fold). In addition, the alcohol-dependent individuals with comorbid drug dependence have higher levels of novelty seeking. Twin studies have demonstrated that a single genetic factor can account for the comorbidity observed across alcohol and drug dependence, conduct disorder, ASPD and disinhibitory personality traits, and that this latent factor is highly heritable (> 80%) [41–43]. Accordingly, it is possible that the subgroup associated with *CHRM2* represents individuals with this highly heritable predisposition toward general disinhibitory psychopathology. The association between *CHRM2* and electrophysiological endophenotypes that are shared across disinhibitory disorders [22] would also seem to support this. Follow-up analyses aimed at further investigating the potential role of *CHRM2* in general externalizing psychopathology are ongoing.

In conclusion, we find evidence that the association between *CHRM2*, *GABRA2* and alcohol dependence

 

observed in the COGA sample is driven entirely by the subset of alcoholic individuals with comorbid drug dependence. These results match neatly with previous evidence from twin, family and adoption studies suggesting that the extent to which genetic factors are important in the development of alcohol dependence can vary as a function of several characteristics [44,45]. In particular, there is evidence that alcohol dependence with comorbid drug dependence represents a more heritable form of the disorder [46]. Interestingly, the study making that observation discussed the possibility that this information could be used to improve our ability to detect specific genetic effects [46]. Here, we find evidence that this is the case.

**Acknowledgements**

The Collaborative Study on the Genetics of Alcoholism (COGA), Co-Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes nine different centers where data collection, analysis and storage take place. The nine sites and Principal Investigators and Co-Investigators are: University of Connecticut (V. Hesselbrock); Indiana University (H. J. Edenberg, J. Nurnberger Jr, P. M. Conneally, T. Foroud); University of Iowa (S. Kuperman, R. Crowe); SUNY Downstate (B. Porjesz); Washington University in St Louis (L. Bierut, A. Goate, J. Rice); University of California at San Diego (M. Schuckit); Howard University (R. Taylor); Rutgers University (J. Tischfield); and South-west Foundation (L. Almasy). Zhaoxia Ren serves as the NIAAA Staff Collaborator. This national collaborative study is supported by NIH grant no. U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA).

**References**

1. Regier D. A., Farmer M. E., Rae D. S., Locke B. Z., Keith S. J., Judd L. L. *et al.* Comorbidity of mental disorders with alcohol and other drug abuse: results from the Epidemiologic Catchment Area (ECA) Study. *JAMA* 1990; **264**: 2511–18.
2. Grant B. F. DSM-III-R and proposed DSM-IV alcohol abuse and dependence in the United States 1988: a nosological comparison. *Alcohol Clin Exp Res* 1992; **16**: 1068–75.
3. De Lima M. S., Lorea C. F., Carpena M. P. Dual diagnosis on 'substance abuse'. *Subst Use Misuse* 2002; **37**: 8–10.
4. Stinson F. S., Grant B. F., Dawson D. A., Ruan W. J., Huang B., Saha T. Comorbidity between DSM-IV alcohol and specific drug use disorders in the United States: results from the National Epidemiological Survey on Alcohol and Related Conditions. *Drug Alcohol Depend* 2005; **80**: 105–16.
5. Burns L., Teesson M. Alcohol use disorders comorbid with anxiety, depression, and drug use disorders. Findings from the Australian National Survey of Mental Health and Well Being. *Drug Alcohol Depend* 2002; **68**: 299–307.
6. Ross H. E. DSM-III-R alcohol abuse and dependence and psychiatric comorbidity in Ontario: results from the Mental Health Supplement to the Ontario Health Survey. *Drug Alcohol Depend* 1995; **39**: 111–28.
7. Kandel D., Yamaguchi K. From beer to crack: developmental patterns of drug involvement. *Am J Public Health* 1993; **83**: 851–5.
8. Degenhardt L., Hall W., Lynskey M. Alcohol, cannabis, and tobacco use among Australians: a comparison of their associations with other drug use and use disorders, affective and anxiety disorders and psychosis. *Addiction* 2001; **96**: 1603–14.
9. Wagner F. A., Anthony J. C. Into the world of illegal drug use: exposure opportunity and other mechanisms linking the use of alcohol, tobacco, marijuana, and cocaine. *Am J Epidemiol* 2002; **155**: 918–25.
10. Sher L. Alcoholism and suicidal behavior: a clincial overview. *Acta Psychiatr Scand* 2006; **113**: 13–22.
11. Kirchner J. E., Booth B. M., Owen R. R., Lancaster A. E., Smith G. R. Predictors of patient entry into alcohol treatment after initial diagnosis. *J Behav Health Serv Res* 2000; **27**: 339–46.
12. Kranzler H. R., Del Boca F. K., Rounsaville B. J. Comorbid psychiatric diagnosis predicts three-year outcomes in alcoholics: a posttreatment natural history study. *J Stud Alcohol* 1996; **57**: 619–26.
13. Porjesz B., Begleiter H., Reich T., Van Eerdewegh P., Edenberg H. J., Foroud T. *et al.* Amplitude of visual P3 event-related potential as a phenotypic marker for a predisposition to alcoholism: preliminary results from the COGA project. *Alcohol Clin Exp Res* 1998; **22**: 1317–23.
14. Malone S. M., Iacono W. G., McGue M. Event-related potentials and comorbidity in alcohol-dependent adult males. *Psychophysiology* 2001; **38**: 367–76.
15. Ohannessian C. M., Hesselbrock V. M., Kramer J., Kuperman S., Bucholz K. K., Schuckit M. A. *et al.* The relationship between parental alcoholism and adolescent psychopathology: a systematic examination of parental comorbid psychopathology. *J Abnorm Child Psychol* 2004; **32**: 519–33.
16. Pickens R. W., Svikis D. S., McGue M., LaBuda M. C. Common genetic mechanisms in alcohol, drug, and mental disorder comorbidity. *Drug Alcohol Depend* 1995; **39**: 129–38.
17. Johnson E. O., Van Den Bree M. B., Pickens R. W. Subtypes of alcohol-dependent men: a typology based on relative genetic and environmental loading. *Alcohol Clin Exp Res* 1996; **20**: 1472–80.
18. Kranzler H. R., Lappalainen J., Nellissery M., Gelernter J. Association study of alcoholism subtypes with a functional promoter polymorphism in the serotonin transporter protein gene. *Alcohol Clin Exp Res* 2002; **26**: 1330–5.
19. Edenberg H. J., Dick D. M., Xuei X., Tian H., Almasy L., Bauer L. O. *et al.* Variations in GABRA2, encoding the α2 subunit of the GABA-A receptor are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet* 2004; **74**: 705–14.
20. Agrawal A., Edenberg H. J., Foroud T., Bierut L., Dunne G., Hinrichs A. L. *et al.* Association of *GABRA2* with drug dependence in the Collaborative Study of the Genetics of Alcoholism sample. *Behav Genet* 2006; **36**: 640–50.
21. Wang J. C., Hinrichs A. L., Stock H., Budde J., Allen R., Bertelsen S. *et al.* Evidence of common and specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. *Hum Mol Genet* 2004; **13**: 1903–11.

 

22. Jones K. A., Porjesz B., Almasy L., Bierut L., Goate A., Wang J. C. *et al.* Linkage and linkage disequilibrium of evoked EEG oscillations with CHRM2 receptor gene polymorphisms: implications for human brain dynamics and cognition. *Int J Psychophysiol* 2004; **53**: 75–90.
23. Porjesz B., Rangaswamy M., Kamarajan C., Jones K., Padmanabhapillai A., Begleiter H. The utility of neurophysiological markers in the study of alcoholism. *Clin Neurophysiol* 2005; **116**: 993–1018.
24. Volpicelli L. A., Levey A. I. Muscarinic acetylcholine receptor subtypes in cerebral cortex and hippocampus. *Prog Brain Res* 2004; **145**: 59–66.
25. Baxter M. G., Chiba A. A. Cognitive functions of the basal forebrain. *Curr Opin Neurobiol* 1999; **9**: 178–83.
26. Luo X., Kranzler H. R., Zuo L., Wang S., Blumberg H. P., Gelernter J. CHRM2 gene predisposes to alcohol dependence, drug dependence, and affective disorders: results from an extended case–control structured association study. *Hum Mol Genet* 2005; **14**: 2421–32.
27. Reich T. A genomic survey of alcohol dependence and related phenotypes: results from the Collaborative Study on the Genetics of Alcoholism (COGA). *Alcohol Clin Exp Res* 1996; **20**: A133–7.
28. Begleiter H., Reich T., Hesselbrock V., Porjesz B., Li T. K., Schuckit M. *et al.* The Collaborative Study on the Genetics of Alcoholism. *Alcohol Health Res World* 1995; **19**: 228–36.
29. Bucholz K. K., Cadoret R., Cloninger C. R., Dinwiddie S. H., Hesselbrock V. M., Nurnberger Jr., J. I. *et al.* A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *J Stud Alcohol* 1994; **55**: 149–58.
30. Hesselbrock M., Easton C., Bucholz K. K., Schuckit M., Hesselbrock V. A validity study of the SSAGA—a comparison with the SCAN. *Addiction* 1999; **94**: 1361–70.
31. American Psychiatric Association (APA). *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn. Washington, DC: APA; 1994.
32. Dick D. M., Aliev F., Kramer J., Wang J. C., Hinrichs A. L., Bertelsen S. *et al.* Association of CHRM2 with IQ: converging evidence for a gene influencing intelligence. *Behav Genet* 2007; **37**: 265–72.
33. Boehnke M. Allele frequency estimation from pedigree data. *Am J Hum Genet* 1991; **48**: 22–5.
34. Barrett J. C., Fry B., Maller J., Daly M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–5.
35. Martin E. R., Monks S. A., Warren L. L., Kaplan N. L. A test for linkage and association in general pedigrees: the Pedigree Disequilibrium Test. *Am J Hum Genet* 2000; **67**: 146–54.
36. Cloninger C. R. *The Tridimensional Personality Questionnaire.* St Louis, MO: Washington University School of Medicine; 1987.
37. SAS Institute, Inc. *SAS System for Windows*, version 8. Cary, NC: SAS Institute, Inc.; 2001.
38. Grant B. F., Dawson D. A. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiological Survey. *J Subst Abuse* 1997; **9**: 103–10.
39. McGue M., Iacono W. G., Legrand L., Malone S., Elkins I. Origins and consequences of age at first drink. I. Associations with substance use disorders, disinhibitory behavior and psycholopathology, and P3 amplitude. *Alcohol Clin Exp Res* 2001; **25**: 1156–65.
40. Prescott C. A., Kendler K. S. Age at first drink and risk for alcoholism: a noncausal association. *Alcohol Clin Exp Res* 1999; **23**: 101–17.
41. Kendler K. S., Prescott C., Myers J., Neale M. C. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Arch Gen Psychiatry* 2003; **60**: 929–37.
42. Krueger R. F. The structure of common mental disorders. *Arch Gen Psychiatry* 1999; **56**: 921–6.
43. Young S. E., Stallings M. C., Corley R. P., Krauter K. S., Hewitt J. K. Genetic and environmental influences on behavioral disinhibition. *Am J Med Genet* 2000; **96**: 684–95.
44. McGue M., Pickens R. W., Svikis D. S. Sex and age effects on the inheritance of alcohol problems: a twin study. *J Abnorm Psychol* 1992; **101**: 3–17.
45. Cloninger C. R., Bohman M., Sigvardsson S. Inheritance of alcohol abuse: cross-fostering analysis of adopted men. *Arch Gen Psychiatry* 1981; **38**: 861–8.
46. Pickens R. W., Svikis D. S., McGue M., Lykken D. T., Heston L. L., Clayton P. J. Heterogeneity in the inheritance of alcoholism. *Arch Gen Psychiatry* 1991; **48**: 19–28.