Association of Alcohol Craving With α-Synuclein (*SNCA*)

Tatiana Foroud, Leah Flury Wetherill, Tiebing Liang, Danielle M. Dick, Victor Hesselbrock, John Kramer, John Nurnberger, Marc Schuckit, Lucinda Carr, Bernice Porjesz, Xiaoling Xuei, and Howard J. Edenberg

Background: Studies have found that genomic variation in the gene *SNCA*, which encodes the protein α-synuclein, may contribute to the variation in alcohol consumption in an inbred rat model of alcohol preference. Studies in humans have provided support for an association between *SNCA* and craving for alcohol.

Methods: To examine the role of this gene in alcohol dependence and related phenotypes, 30 single nucleotide polymorphisms (SNPs) were genotyped across the *SNCA* gene in a sample of 219 multiplex alcoholic families of European American descent. Two phenotypes, alcohol dependence and alcohol craving, were analyzed using the pedigree disequilibrium test.

Results: There was no evidence of association between any of the *SNCA* SNPs and alcohol dependence ($p \geq 0.13$). In contrast, 8 SNPs provided evidence of association ($p < 0.05$) with the phenotype of alcohol craving. Haplotype analysis further supported evidence of an association with alcohol craving; a haplotype encompassing SNPs in intron 4 through the region downstream of the gene was overtransmitted to cravers and a second haplotype was overtransmitted to noncravers.

Conclusions: These results suggest that variation in *SNCA* contributes to alcohol craving, a common, although not uniform, feature of alcohol dependence.

Key Words: Alcoholism, Craving, Genetic Association, Alpha Synuclein, SNP, Family Study.

ALCOHOLISM IS A common disorder with significant morbidity and mortality. Family, adoption, and twin studies all provide evidence that genetic factors have an important role in addiction (Cadoret et al., 1980; Cloninger et al., 1981; Goodwin, 1979; Heath et al., 1997; Kendler et al., 1994; Pickens et al., 1991), with heritability estimates of approximately 50% for alcoholism susceptibility (Heath et al., 1997; McGue, 1999). Alcohol dependence is a heterogeneous clinical diagnosis with individuals meeting criteria for DSM-IV alcohol dependence by endorsing symptoms in 3 or more of 7 criteria in a 12 month period. Thus, different symptoms will contribute to the diagnosis of alcohol dependence in subjects; this suggests that different subsets of genes are likely to act either independently or in concert to affect the risk for alcohol dependence. Therefore, complementary analyses that focus on more homogeneous phenotypic definitions may have greater power to detect association.

In recent years, substantial progress has been made elucidating the genes underlying the risk for alcohol dependence. Several studies have now replicated the initial reports by the Collaborative Study on the Genetics of Alcoholism (COGA) of a role for variation in *GABRA2* (Covault et al., 2004; Edenberg et al., 2004; Fehr et al., 2006; Lappalainen et al., 2005), *ADH4* (Edenberg et al., 2006; Guindalini et al., 2005; Luo et al., 2005b), and *CHRM2* (Luo et al., 2005a; Wang et al., 2004) in disease susceptibility. Additional genes have been implicated in disease risk, including *GABRG3* (Dick et al., 2004) and a bitter taste receptor, *TAS2R16* (Hinrichs et al., 2006). For most of these genes, multiple noncoding variations are associated with disease risk. Despite these successes in dissecting the genetic susceptibility to alcoholism, it is clear that additional genes must also contribute.

Genetic analyses in rodents have sought to identify genes contributing to various aspects of alcohol consumption. The alcohol-preferring (P) and -nonpreferring (NP) rat lines were developed through bi-directional selective breeding as an animal model of alcohol preference (Li et al., 1991). The P rats consume alcohol for its pharmacologic effect as shown by their intragastric and intracerebral administration of alcohol (Li et al., 1993). Genome wide linkage analysis in the inbred alcohol pre-

From the Indiana University School of Medicine, Indianapolis, Indiana (TF, LFW, TL, JN, LC, XX, HJE); Washington University School of Medicine, St. Louis, Missouri (DMD); University of Connecticut School of Medicine, Farmington, Connecticut (VH); University of Iowa Carver College of Medicine, Iowa City, Iowa (JK); University of California, San Diego, California (MS); SUNY Health Sciences Center, Brooklyn, New York (BP).

Received for publication May 4, 2006; accepted December 8, 2006.

This national collaborative study is supported by the NIH Grants U10AA008401 and R01AA010707 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA); and Technology Fund and the Indiana Genomics Initiative (INGEN).

Reprint requests: Tatiana Foroud, PhD, Indiana University School of Medicine, Department of Medical and Molecular Genetics (IB 130), 975 West Walnut Street, Indianapolis, IN 46202-5251; Fax: (317) 278-1100; E-mail: tforoud@iupui.edu

DOI: 10.1111/j.1530-0277.2007.00337.x

ferring (iP) and alcohol nonpreferring (iNP) rats initially detected linkage to rat chromosome 4 with a lod score of 9.2 (Carr et al., 1998). Subsequent analyses implicated the gene encoding rat α-synuclein (*Snca*) as contributing to the variation in alcohol preference (Liang et al., 2003). α-Synuclein mRNA and protein levels in the hippocampus were higher in iP rats than in iNP rats, and sequence analysis identified 2 single nucleotide polymorphisms (SNPs) in the 3′-untranslated region (UTR) of the cDNA. In a previous genome wide analysis, COGA detected linkage of the alcoholism phenotype (defined by DSM-IV) to a broad region on chromosome 4q (Williams et al., 1999) that includes *SNCA*.

Recently, studies in macaque monkeys have further supported an association of α-synuclein mRNA levels with alcohol consumption. Walker and Grant (2006) have shown that monkeys that chronically self-administered ethanol for 18 months had 3-fold higher peripheral blood levels of α-synuclein mRNA than did alcohol naïve control monkeys.

α -Synuclein has an important role in the regulation of dopamine function. Data suggest that α-synuclein regulates dopamine synthesis by interacting with tyrosine hydroxylase, inhibiting its activity and leading to a reduction in dopamine synthesis (Perez et al., 2002). Overexpression of human α-synuclein has been shown to cause the death of dopaminergic neurons in human primary cultures (Zhou et al., 2002), and degeneration of dopaminergic terminals and α-synuclein-immunoreactive inclusions in neurons in the hippocampus, neocortex, and substantia nigra in transgenic mice (Masliah et al., 2000).

Studies in humans have provided additional evidence for the role of α-synuclein in the development of alcohol dependence. Bonsch et al. (2004) found higher levels of α-synuclein mRNA expression in patients with alcohol dependence than in control subjects. They also obtained a significant positive correlation between craving, as measured by the Obsessive Compulsive Drinking Scale (OCDS; Anton et al., 1995) total score and the obsessive subscale, and α-synuclein mRNA expression (Bonsch et al., 2004). The relationship between craving and α-synuclein was strengthened when Bonsch et al. (2005a) again found, in a replication sample of alcohol dependent and control subjects, significant positive correlations between the OCDS total score and the OCDS obsessive and compulsive subscale scores and α-synuclein protein levels. In their samples, alcohol dependent subjects had longer alleles at a polymorphic complex repeat site 9 kb upstream of *SNCA* (Bonsch et al., 2005b). Furthermore, those subjects with longer alleles of *NACP*-REP1 also had higher α-synuclein mRNA expression levels. Bonsch et al. (2005c) reported hypermethylation in the promoter region of alcoholic individuals compared with controls. However, sequence alignment (NM_000345.2; Touchman et al., 2001) indicates that the region examined by Bonsch is actually not in the promoter, but is in exon 1 and intron 1.

Craving is an important and relatively common aspect of alcohol dependence, and while it is not a formal criterion in DSM-IV (American Psychiatric Association, 1994), it is in *ICD-10* (World Health Organization, 1993). Research on this construct has generally focused on obsessive thinking about a substance and/or its compulsive use, possibly related to orbitofrontal functioning (Volkow and Fowler, 2000). Few studies have examined its genetic underpinnings. Ehlers and Wilhelmsen (2005) estimated the heritability of the obsessive component of craving, defined by an affirmative response when individuals were asked "In situations where you couldn't drink, did you ever have such a strong desire for it that you couldn't think of anything else?" to be 65%.

Based on the previous linkage results in the COGA sample and the strong lines of evidence suggesting a role for *SNCA* in human alcoholism and animal alcohol consumption we sought to rigorously test the hypothesis that genomic variation in *SNCA* is associated with alcoholism susceptibility and alcohol craving. We employed a family-based association design testing SNPs throughout the *SNCA* gene.

MATERIALS AND METHODS

Sample

Multiplex alcohol dependent families were ascertained through alcohol treatment programs as part of the ongoing COGA study (Begleiter et al., 1995; Foroud et al., 2000; Nurnberger et al., 2004; Reich et al., 1998). Probands and their families were administered a polydiagnostic instrument, the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview (Bucholz et al., 1994; Hesselbrock et al., 1999). The families that participated in the genetic phase of this study included a proband and at least 2 first-degree relatives who met both lifetime DSM-IIIR criteria for alcohol dependence (American Psychiatric Association, 1987) and lifetime Feighner et al. (1972) criteria for definite alcoholism. Details of the ascertainment and assessment have previously been published (Begleiter et al., 1995; Foroud et al., 2000; Reich et al., 1998).

Phenotypes

As linkage to the chromosome 4 region (Williams et al., 1999) was strongest for the phenotype of alcohol dependence as defined by DSM-IV criteria, we used this definition as our alcoholism phenotype. From the SSAGA data, subjects were classified as affected or unaffected according to DSM-IV criteria. Individuals without a completed SSAGA ($n = 123$) were classified as unknown. A craving phenotype was also developed by analyzing responses to the same SSAGA question employed by Ehlers and Wilhelmsen (2005) for their craving phenotype: "In situations where you couldn't drink, did you ever have such a strong desire for it that you couldn't think of anything else?" Individuals who responded affirmatively to this question, regardless of whether or not they met criteria for DSM-IV alcohol dependence, were classified as alcohol cravers. Subjects who responded negatively to this question, regardless of whether or not they met criteria for DSM-IV alcohol dependence, were classified as noncravers. A total of 219 European-American families containing 1,923 genotyped individuals were employed in the analysis (Table 1).

Table 1. Phenotypic Characteristics of the Genotyped Individuals

	Male	Female
Alcohol Dependent[a]	499	254
Cravers[b]	215	97
Noncravers	284	157
Not Alcohol Dependent	349	698
Cravers	9	8
Noncravers	317	526

[a]There are 64 males and 59 females who have genotypic information but who did not complete a SSAGA and therefore are classified as unknown for both the phenotype of alcohol dependence and alcohol craving.

[b]Among the not alcohol dependent individuals are 23 males and 164 females for whom craving status is undefined.

SSAGA, Semi-Structured Assessment for the Genetics of Alcoholism.

SNP Genotyping

SNCA is about 110 kb in size and contains 6 known exons (NM_000345; Touchman et al., 2001), one more (exon 1) than reported from the Human Genome Assembly version 35.1. The gene is transcribed from qter toward the centromere. For convenience, we have shown the gene structure in the transcribed direction throughout this report (Fig. 1A; Table 2).

Single nucleotide polymorphisms were primarily selected through dbSNP (www.ncbi.nlm.nih.gov/SNP/) and were chosen to provide coverage across the *SNCA* gene. Before the completion of HapMap, SNPs were selected based on position within the gene. To determine approximate SNP allele frequencies, particularly for the SNPs initially selected, SNPs were genotyped in a set of 40 unrelated individuals from the Coriell Caucasian samples. Single nucleotide polymorphisms with minor allele frequencies greater than 0.05 and that were in Hardy–Weinberg Equilibrium were preferentially genotyped in the COGA sample.

There were no *SNCA* coding SNPs in the dbSNP database. Three rare coding mutations in SNCA have been reported to cause Parkinson disease; however, they have been reported in only a small number of families worldwide despite extensive screening in hundreds of PD samples (Gasser, 2001). Therefore, it is very unlikely that these rare coding mutations would be informative in our study.

Genotyping was done using a modified single nucleotide extension reaction, with allele detection by mass spectrometry (Sequenom

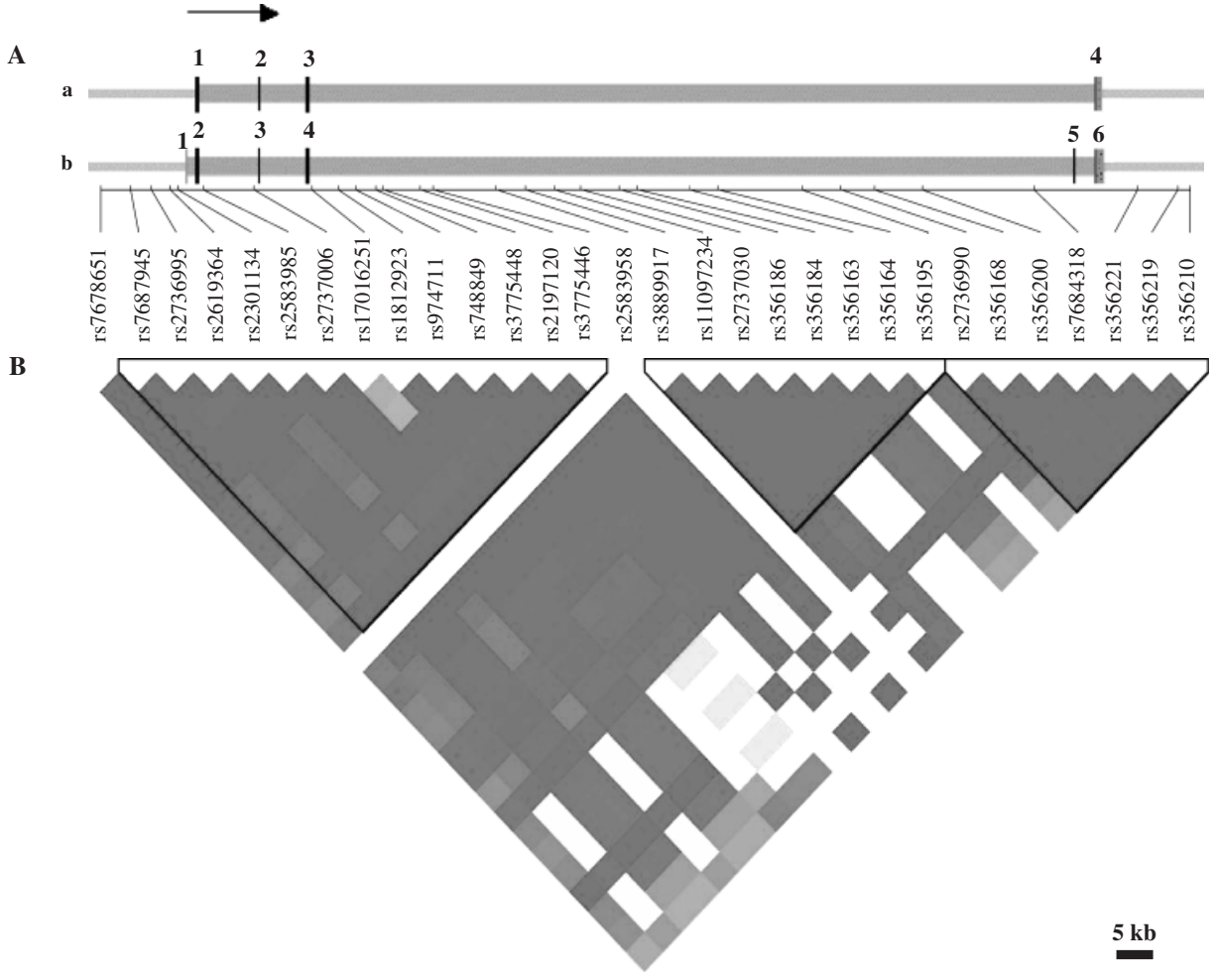

Fig. 1. (A) Genomic structures of *SNCA* based on transcripts NM_007308 (a) and NM_00345 (b) respectively. NM_00345 is known to be commonly transcribed and therefore was used in this study. The direction of transcription and the exons are indicated in arrow and numerical number, respectively. The size of the gene is indicated in scale at the lower right. **(B)** Pairwise linkage disequilibrium (LD). Pairwise LD (D′) estimates among the 30 single nucleotide polymorphisms (SNPs) genotyped in the Collaborative Study on the Genetics of Alcoholism sample. Darkly shaded boxes have strong evidence of LD, defined as a pair of SNPs with the 1-sided upper 95% confidence bound on D′ of 0.98 and the lower bound above 0.7. Lightly shaded boxes have lower LD. The SNPs form 3 blocks across the *SNCA* gene.

Table 2. *SNCA* SNPs and Association Results

	SNP	Position[a]	SNP location[b]	Haplotype block[c]	MAF[d]	Alcohol dependence *p*-value[e]	Alcohol craving *p*-value[e]	3 SNP craving *p*-value[e]
1	rs7678651	91,125,549	Upstream		0.37	0.99	0.65	–
2	rs7687945	91,121,877	Upstream	1	0.48	0.52	0.83	–
3	rs2736995	91,119,375	Upstream	1	0.27	0.44	0.01*	0.02*
4	rs2619364	91,117,065	Upstream	1	0.27	0.44	0.01*	0.08
5	rs2301134	91,116,123	Upstream	1	0.48	0.35	0.69	0.07
6	rs2583985	91,113,117	Intron 2	1	0.27	0.46	0.02*	0.11
7	rs2737006	91,106,864	Intron 2	1	0.27	0.42	0.02*	0.14
8	rs17016251	91,099,817	Intron 4	1	0.45	0.53	0.37	0.15
9	rs1812923	91,096,717	Intron 4	1	0.45	0.95	0.26	0.09
10	rs974711	91,094,505	Intron 4	1	0.45	0.89	0.21	0.76
11	rs748849	91,092,139	Intron 4	1	0.20	0.36	0.39	0.16
12	rs3775448	91,091,283	Intron 4	1	0.08	0.24	0.57	0.65
13	rs2197120	91,086,780	Intron 4	1	0.21	0.78	0.19	1.00
14	rs3775446	91,085,101	Intron 4	1	0.08	0.24	0.57	0.59
15	rs2583958	91,077,543	Intron 4		0.38	0.84	0.95	0.87
16	rs3889917	91,074,030	Intron 4	2	0.07	0.19	0.63	0.71
17	rs11097234	91,070,508	Intron 4	2	0.37	0.88	0.36	0.60
18	rs2737030	91,067,277	Intron 4	2	0.20	0.61	0.35	0.17
19	rs356186	91,062,542	Intron 4	2	0.20	0.46	0.28	0.17
20	rs356184	91,060,411	Intron 4	2	0.28	0.13	0.01*	0.10
21	rs356163	91,054,005	Intron 4	2	0.28	0.34	0.01*	0.22
22	rs356164	91,050,654	Intron 4	2	0.13	0.78	0.06	0.16
23	rs356195	91,040,346	Intron 4	2	0.28	0.16	0.005*	0.13
24	rs2736990	91,035,719	Intron 4	3	0.47	0.62	0.12	0.10
25	rs356168	91,031,609	Intron 4	3	0.48	0.84	0.05*	0.07
26	rs356200	91,025,792	Intron 4	3	0.47	0.71	0.09	0.02*
27	rs7684318	91,012,181	Intron 4	3	0.09	0.49	0.83	0.01*
28	rs356221	90,999,642	Downstream	3	0.47	0.66	0.12	0.01*
29	rs356219	90,994,779	Downstream	3	0.38	0.8	0.39	0.02*
30	rs356210	90,993,371	Downstream	3	0.28	0.52	0.99	0.07

[a]Position in nucleotides from chromosome 4pter, as estimated by BLASTing against the NCBI Human Genome assembly (version 35.1).
[b]Position within or near gene; SNPs are listed in the direction of transcription, which is from qter toward pter.
[c]Haplotype blocks were defined as a set of contiguous SNPs whose average *D′* exceeded a predetermined threshold (Reich et al., 2001; Gabriel et al., 2002).
[d]Minor allele frequency calculated from the COGA dataset.
[e]Pedigree Disequilibrium test average statistic *p*-value.
*Significant ($p \leq 0.05$) association test results.
SNP, single nucleotide polymorphism; MAF, minor allele frequency; COGA, Collaborative Study on the Genetics of Alcoholism.

MassArray system; Sequenom, San Diego, CA). The multiplex assays were designed and run with 2 formats, hME™ or iPLEX™ (Sequenom, San Diego, CA). All SNP genotypes were checked for Mendelian inheritance using the program PEDCHECK (O'Connell and Weeks, 1998). Marker allele frequencies and heterozygosities were computed using the program USERM13 (Boehnke, 1991). Markers were tested for Hardy–Weinberg equilibrium (Barrett et al., 2005).

Statistical Analyses

To ensure that the SNP density was sufficient to evaluate the evidence of association, linkage disequilibrium (LD) was computed using the program HAPLOVIEW (Barrett et al., 2005). The haplotype block structure was also examined with HAPLOVIEW, with blocks defined as a set of contiguous SNPs whose average D′ exceeded the default threshold for strong LD (the 1-sided upper 95% confidence bound on D′ is 0.98, and the lower bound is above 0.7; Gabriel et al., 2002; Reich et al., 2001).

The Pedigree Disequilibrium test (PDT) (Martin et al., 2001) as implemented in the program UNPHASED (Dudbridge, 2003) was used to test for association in the extended, multiplex COGA pedigrees. We report results using the PDTaverage statistic, which weighs each family equally in computing the overall test statistic. Two phenotypes were analyzed: alcohol dependence and alcohol craving.

Multi-SNP haplotypes were constructed to further analyze the evidence of association. Haplotypes were constructed in 2 ways. First, in each block, SNPs were identified which tagged haplotypes with a frequency of 0.05 or greater (Haploview; Barrett et al., 2005). Second, haplotypes were estimated using consecutive, overlapping sets of 3 adjacent SNPs (Edenberg et al., 2004). The haplotypes were used to test for association using the PDT and the global *p*-value is reported for each test of association. The haplotypes were then reviewed in each of the sequential sliding windows to determine whether significant association results were due to the overtransmission of a particular haplotype to affected individuals or to the differential transmission to siblings discordant for the phenotype.

RESULTS

Thirty SNPs were genotyped across the 133 kb region containing *SNCA*, extending 10 kb beyond the gene on both the 5′ and 3′ ends (Fig. 1A). Evaluation of the SNP positions (Fig. 1A) and the pairwise LD in our sample (Fig. 1B) indicated that the SNPs we genotyped adequately covered *SNCA* and its flanking regions. To provide an independent evaluation of the coverage, we used the program Tagger (de Bakker et al., 2005) to calculate LD between the SNPs we genotyped and all 149 SNPs in the

Table 3. Transmission Results

rs2736995	rs17016251	rs1812923	rs2197120	Transmissions to cravers T/NT[a]	Transmissions to discordant siblings C/NC[b]	Haplotype *p*-value
A. Block 1						
C	G	C	G	33/36	96/105	0.77
A	C	A	G	61/59	202/209	0.21
A	C	C	G	14/9	45/37	0.26
A	G	A	G	12/7	43/28	0.60
A	G	C	A	31/40	83/89	0.85

rs3889917	rs11097234	rs356186	rs356163	rs356164	Transmissions to cravers T/NT[a]	Transmissions to discordant siblings C/NC[b]	Haplotype *p*-value
B. Block 2							
T	C	A	C	G	24/24	52/45	0.22
T	C	A	C	C	10/11	44/52	0.35
T	C	G	C	C	14/13	84/68	0.59
T	C	G	A	C	35/36	124/121	0.17
T	G	G	C	C	53/58	189/213	0.01
C	C	G	C	C	14/8	43/37	0.46

rs7684318	rs356221	rs356219	Transmissions to cravers T/NT[a]	Transmissions to discordant siblings C/NC[b]	Haplotype p-value
C. Block 3					
C	A	G	14/10	51/36	0.05
T	A	A	20/13	90/58	0.06
T	A	G	42/55	136/148	0.86
T	T	A	118/116	361/396	0.02

[a]Number of transmissions (T) or nontransmissions (NT) of the haplotype to a craving offspring from a heterozygous parent.
[b]Number of transmissions of the haplotype to a pair of siblings one of whom is a craver (C) and the other is a noncraver (NC).

region that were analyzed by HapMap. Of the 30 SNPs genotyped in our data, 22 SNPs were in the HapMap database and therefore could be evaluated. The average r^2 of these 22 SNPs with all 149 known HapMap SNPs (minor allele frequency ≥ 0.05) in the 133 kb region was 0.72; r^2 was >0.5 for 77% of the SNPs and >0.8% for 64% of the SNPs. Given that we genotyped an additional 8 SNPs not in the HapMap database (and thus not evaluated), our actual coverage was even better.

Table 2 presents the results from family based tests of association (PDT) between each *SNCA* SNP and alcohol dependence. No significant association was found ($p \geq 0.13$).

Analyses were then performed using alcohol craving as a phenotype. Craving was highly correlated with alcohol dependence, and identified a large subset of alcoholic individuals (Table 1). Forty-two percent of the individuals meeting criteria for DSM-IV alcohol dependence also reported alcohol craving. The rate of craving was similar in men and women (43 vs 38%, respectively). In contrast, among the individuals who did not meet criteria for DSM-IV alcohol dependence, only 2% reported alcohol craving.

Association analyses with alcohol craving detected evidence of association ($p \leq 0.05$) with 8 SNPs distributed in the 5′ and 3′ ends of the gene. To further analyze the evidence of association, haplotypes were constructed using 2 approaches. Haplotypes were estimated using tag SNPs selected so as to represent all haplotypes present in that block at a frequency of at least 5% (Table 3). For block 1 (SNP2 through SNP14), haplotypes (composed of 4 tag SNPs) were not associated with alcohol craving ($p = 0.72$). In block 2 (SNP16 through SNP23), 5 tag SNPs were employed and again there was no statistically significant evidence of association ($p = 0.06$). In block 3 (SNP24 through SNP30), haplotypes composed of 3 tag SNPs were significantly associated with craving ($p = 0.02$).

Additional analyses using overlapping sets of 3 adjacent SNPs were performed to assess the consistency of the evidence of association (Table 4). Significant evidence ($p < 0.02$) of association was observed with 4 consecutive haplotypes constructed from SNPs in intron 4 and into the 3′ region of the gene (Table 4). All of these SNPs were part of a single haplotype block (block 3). The same SNP allele included in multiple overlapping haplotypes was significantly associated with the risk of alcohol dependence. As shown in Table 4, an extended haplotype defined as G–G–T–C–A–G (SNP 24–29) was overtransmitted to alcohol cravers while the complementary haplotype defined as A–A–C–T–T–A was overtransmitted to individuals who do not crave alcohol. The frequency of the haplotype overtransmitted to cravers was 10% while the frequency of the complementary haplotype that was overtransmitted to noncravers was 50%. In contrast, only one 3-SNP haplotype in the 5′ end of the gene (rs7678651–rs7687945–rs2736995; $p = 0.02$) provided significant evidence of association, which was due to discordant transmission. One haplotype was overtransmitted ($p = 0.01$) to siblings who were noncravers (C–C–A), while another haplotype (C–C–C) was overtransmitted ($p = 0.02$) to the siblings who were cravers.

DISCUSSION

This paper presents results from the first extensive testing of the association between alcohol dependence and alcohol craving with the α-synuclein gene, *SNCA*. While we did not find evidence of association with alcohol dependence, we did find strong evidence of association

Table 4. Transmission Results

	SNP [23–25]		SNP [24–26]		SNP [25–27]		SNP [26–28]		SNP [27–29]		SNP [28–30]	
Overtransmission	C	NC	C	NC	C	NC	C	NC	C	NC	C	NC
rs356195 [23]		C										
rs2736990 [24]		A	**G**	A								
rs356168 [25]		A	**G**	A	**G**	A						
rs356200 [26]			**T**	C	**T**	C	**T**	C				
rs7684318 [27]					**C**	T	**C**	T	**C**	T		
rs356221 [28]							**A**	T	**A**	T		T
rs356219 [29]									**G**	A		A
rs356210 [30]												C
Haplotype *p*-value[a]		0.01	0.01	0.01	0.02	0.01	0.03	0.01	0.05	0.02		0.04
Global *p*-value[b]	0.07		0.02		0.01		0.01		0.02		0.07	

[a]*p*-Value associated with the differential transmission of that haplotype.
[b]Global *p*-Value for the Pedigree Disequilibrium test using all haplotypes observed with these 3 SNPs.
C, Craver; NC, Noncraver.

with alcohol craving. These results imply that the effect of variations in *SNCA* is not universal among alcoholic individuals. Rather, this effect is found in only a subset of the individuals meeting criteria for alcohol dependence.

A single question in the SSAGA interview concerning alcohol craving allowed us to identify a subset of alcoholicindividuals, 42%, who experienced craving. This estimate is similar to the 30% of alcoholic individuals who reported craving in a Mission Indian sample interviewed with the same instrument (Ehlers and Wilhelmsen, 2005). Although defining craving with this single question seems oversimplified, craving so defined is significantly heritable (Ehlers and Wilhelmsen, 2005) and relatively specific for alcoholic individuals. We found that 42% of the individuals meeting criteria for DSM-IV alcohol dependence also reported alcohol craving, whereas only 2% of the subjects who failed to meet criteria for DSM-IV alcohol dependence reported ever experiencing alcohol craving. Therefore, our analyses of alcohol craving consisted almost entirely (95%) of a subset of the sample of individuals meeting criteria for a lifetime diagnosis of alcohol dependence.

Eight SNPs extending from upstream of the gene through intron 4 gave evidence of association with alcohol craving. The gene fell into 3 haplotype blocks; 4 SNPs in block 1, 3 in block 2 and one in block 3 were significant. However, sliding 3-SNP haplotypes gave the strongest evidence of association in block 3, the region extending from intron 4 to downstream of the gene. It is possible that the increased informativeness of the haplotypes compared with the individual SNP analyses increased the power to detect association. Liang et al. (2003) identified 2 polymorphisms at positions +439 and +679 between the iP and iNP rats in the 3′UTR region. The region associated with craving in the present study encompasses the 3′UTR. Although the obsessive thinking aspect of craving cannot be directly measured in animals, the compulsive use associated with craving is consistent with data from alcohol preferring rats (Liang et al., 2003). Comparison of the physical position of the SNPs associated with the craving phenotype with other vertebrate genomes in the 17-way Vertebrate MultiAlignment (UCSC Genome Browser, v. 141) indicated that none of these SNPs were in evolutionally conserved regions of the gene.

α-Synuclein has been examined for its role in neuropsychiatric disorders, including drug dependence. Mash et al. (2003) reported that α-synuclein levels were 3 fold increased in the midbrain dopamine neurons of chronic cocaine abusers compared with controls. α-Synuclein mRNA levels are also elevated in mice withdrawn from chronic morphine treatment (Ziolkowska et al., 2005). Analyses of SNPs in the noncoding exon 1′ and intron 1 of *SNCA* in a sample of methamphetamine dependent patients and matched controls did not detect significant association (Kobayashi et al., 2004). However, when gender-specific analyses were performed, there was some evidence of association in the female sample. These studies suggest that genomic variation in *SNCA*, which affects the expression of this protein, may also modulate the risk for drug dependence.

Bonsch et al. (2004) have suggested that variation in the 5′ region of *SNCA* contributes to the overexpression of α-synuclein in alcohol cravers. Our study found association of individual upstream and promoter SNPs with alcohol craving. The *NACP*-Rep1 association is a 300 bp repeat located between rs7678651 and rs7687945 about 9 kb upstream from the transcription initiation site (Fig. 1A; Table 2); neither SNP yielded significant evidence of association with alcohol craving when analyzed individually. However, there was some evidence of association when a 3 SNP window comprising the most distal 5′ SNPs (including the 2 flanking the repeat and an associated SNP in the proximal 5′ region, rs2736995) was analyzed ($p = 0.02$). Single nucleotide polynorphisms rs2736995 and rs2619364 are located immediately upstream of the transcription initiation site in the promoter, and both were significantly associated with alcohol craving in this study ($p = 0.01$ for both). Several other SNPs in the promoter region

(rs2619364 and rs2301134) also provided evidence of association with alcohol craving.

Few studies have examined the association of alcohol craving with genomic variation. In addition to the work of Bonsch et al. (2004, 2005a, 2005b, 2005c), described above, a genome screen was completed in the Mission Indians using the phenotype of alcohol craving (Ehlers and Wilhelmsen, 2005). Evidence of linkage to chromosome 5 was reported with a lod score of 4.5. There was no evidence of linkage to the region on chromosome 4 where *SNCA* is located. It should be noted, however, that our study analyzed only European-American families.

The current study has several strengths. First, a large sample of 219 multiplex, well-characterized alcoholic families was analyzed. Second, as SNP allele frequencies and the evolutionary history of a haplotype can differ across racial groups, analyses were limited to an ethnically more homogeneous subset consisting of only the European-American families. Third, family based association analyses were performed, reducing the possible effects of unknown population stratification. Fourth, 30 SNPs were genotyped across the *SNCA* gene, ensuring extensive coverage of the gene.

A limitation of our study is that defining craving as the response to a single, dichotomous question about craving "for a drink" limited the informativeness of the trait and restricted our measurement of craving to obsessive thinking, without including compulsive drinking. However, the fact that approximately 95% of the people reporting craving also met diagnostic criteria for alcohol dependence suggests that the craving for alcohol and the compulsive drinking characteristic of alcoholic individuals are found together in the individuals analyzed.

The COGA study has evaluated candidate genes implicated on the basis of linkage results (Dick et al., 2004; Edenberg et al., 2004, 2006; Hinrichs et al., 2006; Wang et al., 2004), relevant pathways (Xuei et al., 2006), or previous studies in humans or animal models (Dick et al., 2004). The analyses in this manuscript focused on a particular candidate gene, *SNCA*, in a broad region linked to alcohol dependence. *SNCA* was implicated as a candidate gene based on previous studies of alcohol preference and craving in humans, rats and monkeys. Therefore, we were testing 2 specific hypotheses, the role of alpha synuclein in (1) alcohol dependence and (2) alcohol craving. Seven of the 8 SNPs that were found to be associated would still be significant if corrected for 2 independent tests; however, because the phenotypes are correlated, such a correction would be overly conservative. As for all association studies, we believe it is essential that replication of this association be performed in an independent series of samples.

In summary, in a thorough evaluation of the *SNCA* gene, we found evidence of association of SNPs in regions near both ends of the gene with alcohol craving, using a family based test of association in multiplex alcoholic pedigrees. Further analyses in the 3′ end of the gene identified a haplotype overtransmitted to individuals who crave alcohol and a different haplotype, which was preferentially transmitted to noncravers. These results are consistent with a previous report in the iP and iNP rats, which proposed that SNPs in the 3′UTR of *Snca* contribute to alcohol preference.

ACKNOWLEDGMENTS

The COGA, Co-Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes 9 different centers where data collection, analysis, and storage take place. The 9 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); Southwest Foundation (L. Almasy). Zhaoxia Ren serves as the NIAAA Staff Collaborator.Genotyping facilities were provided by the Center for Medical Genomics at Indiana University School of Medicine, supported in part by the Indiana Genomics Initiative (INGEN, supported in part by the Lilly Endowment, Inc.). We thank Jinghua Zhao, Gayathri Rajan and Rachel Thowe for their superb technical support on SNP genotyping.

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.

REFERENCES

American Psychiatric Association (1987) *Diagnostic and Statistical Manual of Mental Disorders*, 3rd ed., revised. American Psychiatric Association Press, Washington, DC.

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

Anton RF, Moak DH, Latham P (1995) The Obsessive Compulsive Drinking Scale: a self-rated instrument for the quantification of thoughts about alcohol and drinking behavior. Alcohol Clin Exp Res 19:92–99.

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265.

Begleiter H, Reich T, Hesselbrock V, Porjesz B, Li T-K, Schuckit MA, Edenberg HJ, Rice AP (1995) The Collaborative Study on the Genetics of Alcoholism. Alcohol Health Res World 19:228–236.

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

Bonsch D, Greifenberg V, Bayerlein K, Biermann T, Reulback U, Hillemacher T, Kornhuber J, Bleich S (2005a) alpha-synuclein protein levels are increased in alcoholic patients and are linked to craving. Alcohol Clin Exp Res 29:763–765.

Bonsch D, Lederer T, Reulback U, Hothorn T, Kornhuber J, Bleich S (2005b) Joint analysis of the NACP-REP1 marker within the alpha synuclein gene concludes association with alcohol dependence. Hum Mol Genet 14:967–971.

Bonsch D, Lenz B, Kornhuber J, Bleich S (2005c) DNA hypermethylation of the alpha synuclein promoter in patients with alcoholism. NeuroReport 17:167–170.

Bonsch D, Reulback U, Bayerlein K, Hillemacher T, Kornhuber J, Bleich S (2004) Elevated alpha synuclein mRNA levels are associated with craving in patients with alcoholism. Biol Psychiatry 56:984–986.

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

Cadoret RJ, Cain CA, Grove WM (1980) Development of alcoholism in adoptees raised apart from alcoholic biologic relatives. Arch Gen Psychiatry 37:561–563.

Carr LG, Foroud T, Bice P, Gobbett T, Ivashina J, Edenberg H, Lumeng L, Li TK (1998) A quantitative trait locus for alcohol consumption in selectively bred rat lines. Alcohol Clin Exp Res 22:884–887.

Cloninger CR, Bohman M, Sigvardsson S (1981) Inheritance of alcohol abuse: cross-fostering analysis of adopted men. Arch Gen Psychiatry 38:861–868.

Covault J, Gelernter J, Hesselbrock V, Nellissery M, Kranzler HR (2004) Allelic and haplotypic association of GABRA2 with alcohol dependence. Am J Med Genet B Neuropsychiatr Genet 129:104–109.

de Bakker PIW, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D (2005) Efficiency and power in genetic association studies. Nature Genet 37:1217–1223.

Dick DM, Edenberg HJ, Xuei X, Goate A, Kuperman S, Schuckit M, Crowe R, Smith TL, Porjesz B, Begleiter H, Foroud T (2004) Association of GABRG3 with alcohol dependence. Alcohol Clin Exp Res 28:4–9.

Dudbridge F (2003) Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 25:115–121.

Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO, Crowe RR, Goate A, Hesselbrock V, Jones K, Kwon J, Li TK, Nurnberger JI Jr., O'Connor SJ, Reich T, Rice J, Schuckit MA, Porjesz B, Foroud T, Begleiter H (2004) Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. Am J Hum Genet 74:705–714.

Edenberg HJ, Xuei X, Chen HJ, Tian H, Wetherill LF, Dick DM, Almasy L, Bierut L, Bucholz KK, Goate A, Hesselbrock V, Kuperman S, Nurnberger J, Porjesz B, Rice J, Schuckit M, Tischfield J, Begleiter H, Foroud T (2006) Association of Alcohol Dehydrogenase Genes with Alcohol Dependence: a comprehensive analysis. Hum Mol Genet 15:1539–1549.

Ehlers CL, Wilhelmsen KC (2005) Genomic scan for alcohol craving in Mission Indians. Psychiatr Genet 15:71–75.

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

Feighner JP, Robins E, Guze SB, Woodruff RA, Winokur G, Munoz R (1972) Diagnostic criteria for use in psychiatric research. Arch Gen Psychiatry 26:57–63.

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

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

Gasser T (2001) Genetics of Parkinson's Disease. J Neurol 248:833–840.

Goodwin DW (1979) The cause of alcoholism and why it runs in families. Br J Addict Alcohol Other Drugs 74:161–164.

Guindalini C, Scivoletto S, Ferreira RG, Breen G, Zilberman M, Peluso MA, Zatz M (2005) Association of genetic variants in alcohol dehydrogenase 4 with alcohol dependence in Brazilian patients. Am J Psychiat 162:1005–1007.

Heath AC, Bucholz KK, Madden PA, Dinwiddie SH, Slutske WS, Bierut LJ, Statham DJ, Dunne MP, Whitfield JB, Martin NG (1997) Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. Psychol Med 27:1381–1396.

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

Hinrichs AL, Wang JC, Bufe B, Kwon JM, Budde J, Allen R, Bertelsen S, Evans W, Dick D, Rice J, Foroud T, Nurnberger J, Tischfield JA, Kuperman S, Crowe R, Hesselbrock V, Schuckit M, Almasy L, Porjesz B, Edenberg HJ, Begleiter H, Meyerhof W, Bierut LJ, Goate AM (2006) Functional variant in a bitter-taste receptor (hTAS2R16) influences risk of alcohol dependence. Am J Hum Genet 78:103–111.

Kendler KS, Neale MC, Heath AC, Kessler RC, Eaves LJ (1994) A twin-family study of alcoholism in women. Am J Psychiatry 151:707–715.

Kobayashi H, Ide S, Hasegawa J, Ujike H, Sekine Y, Ozaki N, Inada T, Harano M, Komiyama T, Yamada M, Iyo M, Shen H-W, Ikeda K, Sora I (2004) Study of association between a-synuclein gene polymorphism and methamphetamine psychosis/dependence. Ann NY Acad 1025:325–334.

Lappalainen J, Krupitsky E, Remizov M, Pchelina S, Taraskina A, Zvartau E, Somberg LK, Covault J, Kranzler HR, Krystal JH, Gelernter J (2005) Association between alcoholism and gamma-amino butyric acid alpha2 receptor subtype in a Russian population. Alcohol Clin Exp Res 29:493–498.

Li TK, Lumeng L, Doolittle DP (1993) Selective breeding for alcohol preference and associated responses. Behav Genet 23:163–170.

Li TK, Lumeng L, Doolittle DP, Carr LG (1991) Molecular associations of alcohol-seeking behavior in rat lines selectively bred for high and low voluntary ethanol drinking. Alcohol Alcohol 1(suppl): 121–124.

Liang T, Spence J, Liu L, Strother WN, Chang HW, Ellison JA, Lumeng L, Li TK, Foroud T, Carr LG (2003) alpha-Synuclein maps to a quantitative trait locus for alcohol preference and is differentially expressed in alcohol-preferring and -nonpreferring rats. Proc Natl Acad Sci U S A 100:4690–4695.

Luo X, Kranzler HR, Zuo L, Wang S, Blumberg HP, Gelernter J (2005a) CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: results from an extended case-control structured association study. Hum Mol Genet 14:2421–2434.

Luo X, Kranzler HR, Zuo L, Yang BZ, Lappalainen J, Gelernter J (2005b) ADH4 gene variation is associated with alcohol and drug dependence: results from family controlled and population-structured association studies. Pharmacogenet Genom 15:755–768.

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

Mash DC, Ouyang Q, Pablo J, Basile M, Izenwasser S, Lieberman A, Perrin RJ (2003) Cocaine abusers have an overexpression of alpha-synuclein in dopamine neurons. J Neurosci 23:2564–2571.

Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L (2000) Dopaminergic loss and inclusion body formation in alpha-synuclein mice: implications for neurodegenerative disorders. Science 287:1265–1269.

McGue M (1999) The behavioral genetics of alcoholism. Curr Directions Psychol Sci 8:109–115.

Nurnberger JI Jr., Wiegand R, Bucholz K, O'Connor S, Meyer ET, Reich T, Rice J, Schuckit M, King L, Petti T, Bierut L, Hinrichs AL, Kuperman S, Hesselbrock V, Porjesz B (2004) A family study of alcohol dependence: coaggregation of multiple disorders in relatives of alcohol-dependent probands. Arch Gen Psychiatry 61:1246–1256.

O'Connell JR, Weeks DE (1998) PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 63:259–266.

Perez RG, Waymire JC, Lin E, Liu JJ, Guo F, Zigmond MJ (2002) A role for alpha-synuclein in the regulation of dopamine biosynthesis. J. Neurosci 22:3090–3099.

Pickens RW, Svikis DS, McGue M, Lykken DT, Heston LL, Clayton PJ (1991) Heterogeneity in the inheritance of alcoholism. A study of male and female twins. Arch Gen Psychiatry 48:19–28.

Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES (2001) Linkage disequilibrium in the human genome. Nature 411:199–204.

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 JIJ, Tischfield JA, Crowe R, Cloninger CR, Wu W, Shears S, Carr K, Crose C, Willig C, Begleiter H (1998) A genome-wide search for genes affecting the risk for alcohol dependence. Am J Med Genet Neuropsychiatr Genet 81:207–215.

Touchman JW, Dehejia A, Chiba-Falek O, Cabin DE, Schwartz JR, Orrison BM, Polymeropoulos MH, Nussbaum RL (2001) Human and mouse alpha-synuclein genes: comparative genomic sequence analysis and identification of a novel gene regulatory element. Genome Res 11:78–86.

Volkow ND, Fowler JS (2000) Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. Cereb Cortex 10: 318–325.

Walker SJ, Grant KA (2006) Peripheral blood alpha-synuclein mRNA levels are elevated in cynomolgus monkeys that chronically self-administer ethanol. Alcohol 38:1–4.

Wang JC, Hinrichs AL, Stock H, Budde J, Allen R, Bertelsen S, Kwon JM, Wu W, Dick DM, Rice J, Jones K, Nurnberger JI Jr., Tischfield J, Porjesz B, Edenberg HJ, Hesselbrock V, Crowe R, Schuckit M, Begleiter H, Reich T, Goate AM, Bierut LJ (2004) 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 13:1903–1911.

Williams JT, Begleiter H, Porjesz B, Edenberg HJ, Foroud T, Reich T, Goate A, Van Eerdewegh P, Almasy L, Blangero J (1999) Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. II. Alcoholism and event-related potentials. Am J Hum Genet 65:1148–1160.

World Health Organization (1993) *International Classification of Disease*, 10th ed. World Health Organization, Geneva.

Xuei X, Dick D, Flury-Wetherill L, Tian HJ, Agrawal A, Bierut L, Goate A, Bucholz K, Schuckit M, Nurnberger J Jr, Tischfield J, Kuperman S, Porjesz B, Begleiter H, Foroud T, Edenberg HJ (2006) Association of the kappa-opioid system with alcohol dependence. Mol Psychiatry 11:1016–1024.

Zhou W, Schaack J, Zawada WM, Freed CR (2002) Overexpression of human alpha-synuclein causes dopamine neuron death in primary human mesencephalic culture. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. Brain Res 926:42–50.

Ziolkowska B, Gieryk A, Bilecki W, Wawrzczak-Bargiela A, Wedzony K, Chocyk A, Danielson PE, Thomas EA, Hilbush BS, Sutcliffe JG, Przewlocki R (2005) Regulation of alpha-synuclein expression in limbic and motor brain regions of morphine-treated mice. J Neurosci 25:4996–5003.