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## Association of alcohol dehydrogenase genes with alcohol-related phenotypes in a Native American community sample

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### Abstract

**Background**—Previous linkage studies, including a study of the Native American population described in the present report, have provided evidence for linkage of alcohol dependence and related traits to chromosome 4q near a cluster of alcohol dehydrogenase (ADH) genes, which encode enzymes of alcohol metabolism.

**Methods**—The present study tested for associations between alcohol dependence and related traits and 22 single nucleotide polymorphisms (SNPs) spanning the seven ADH genes. Participants included 586 adult men and women recruited from eight contiguous Native American reservations. A structured interview was used to assess DSM-III-R alcohol dependence criteria as well as a set of severe alcohol misuse symptoms and alcohol withdrawal symptoms.

**Results**—No evidence for association with the alcohol dependence diagnosis was observed, but a SNP in exon 9 of *ADH1B* (rs2066702; *ADH1B*\*3) and a SNP at the 5' end of *ADH4* (rs3762894) showed significant evidence of association with the presence of withdrawal symptoms ( $p=0.0018$  and  $0.0012$ , respectively). Further, a haplotype analysis of these two SNPs suggested haplotypes containing either of the minor alleles were protective against alcohol withdrawal relative to the ancestral haplotype ( $p=0.000006$ ).

**Conclusions**—These results suggest that variants in the *ADH1B* and *ADH4* genes may be protective against the development of some symptoms associated with alcohol dependence.

### Keywords

alcohol dehydrogenase; alcohol dependence; alcohol withdrawal; genetic association; candidate gene analysis

### Introduction

Numerous family, twin, and adoption studies have suggested that alcohol dependence represents a heritable condition, with approximately 50–60% of the variance in the development of alcohol dependence explained by genetic influences (e.g., Allgulander et al., 1991; Goodwin et al., 1973; Heath et al., 1997; Kendler et al., 1997; Reich et al., 1988). Most of these studies, as well as molecular genetic studies of alcohol dependence, have been

conducted using Caucasian samples, despite similar or higher prevalence rates in some minority groups, including some Native American communities. Notably, there may be specific advantages in conducting genetic studies of complex diseases, such as alcoholism, in well-defined ethnic populations such as Native American tribes (Lander and Schork, 1994). Often such populations are more environmentally and genetically homogeneous, more geographically restricted, and frequently have large extended pedigrees, all of which can allow for greater power to detect genetic linkage and/or association. Further, specific facets of the studied trait may be more prevalent within the ethnic population under study than the general population thus aiding gene identification (Burchard et al., 2003). In an attempt to capitalize on these advantages, the present study sought to investigate the relations between alcohol dependence and genes involved in alcohol metabolism within a Native American sample.

Genetically influenced metabolic factors have been implicated in the etiology of alcoholism in a number of ethnic groups. The major enzyme families involved in alcohol metabolism, alcohol dehydrogenase (ADH), which is responsible for the oxidation of alcohol to acetaldehyde, and aldehyde dehydrogenase (ALDH), which is responsible for the oxidation of acetaldehyde to acetate, exist as multiple isozymes that differ in their kinetic properties. The genes that encode them have been considered candidate genes that are likely to contribute to variation in alcohol metabolism, variability in response to alcohol, and differences in individual vulnerability for developing alcohol dependence and alcohol-related disability (see Bosron et al., 1993; Chen et al., 2009; Crabb, 1995; Edenberg, 2007; Li, 2000). A large proportion of individuals of Far East Asian descent (often in the range of 30%), possess a mutation in the *ALDH2* gene, which produces a largely inactive form of the enzyme resulting in elevated acetaldehyde levels, an alcohol induced flushing reaction, an increased level of response to alcohol, and lower rates of alcohol use and alcoholism (Higuchi et al., 1995; Luczak et al., 2002; Takeshita et al., 1994; Thomasson et al., 1991; Shen et al., 1997; Wall et al., 1992, 1993, 1999).

In addition to *ALDH2*, one of the more replicable findings described in the genetics of alcohol dependence literature has been evidence of an association for alcohol dependence and related behaviors to chromosome 4 near the *ADH* gene cluster. This gene cluster is approximately 364 kilobases (kb) in length, and the genes are all transcribed from the same DNA strand (4qter to 4pter). The order of genes from qter to pter is *ADH7*, *ADH1C*, *ADH1B*, *ADH1A*, *ADH6*, *ADH4*, and *ADH5*, with each *ADH* gene coding for a unique isozyme. The relation between this chromosomal region and alcohol dependence has been reported in a number of linkage studies of diverse ethnic groups (e.g., Corbett et al., 2005; Long et al., 1998; Prescott et al., 2006; Williams et al., 1999) including the Native American sample presented in this report (Ehlers et al., 2004b). Additionally, genome screens for both the “unaffected by alcoholism” (Reich et al., 1998) and “maximum drinks ever consumed in a 24 hour period,” (Saccone et al., 2000) phenotypes were found to yield evidence of linkage to chromosome 4 in the region of the *ADH* gene cluster in the Collaborative Study of the Genetics of Alcoholism (COGA).

Given that the *ADH* cluster consists of seven genes, researchers have sought to identify which of the *ADH* genes might be involved in the etiology of alcohol dependence. Because the class 1 *ADH* isozymes account for the majority of alcohol metabolism in the liver and have been shown to contain nonsynonymous coding SNPs that alter the kinetic properties of *ADH*, the genes encoding these isozymes, *ADH1A*, *ADH1B*, and *ADH1C*, have received the most initial attention with each gene showing evidence of association with alcohol dependence and related phenotypes (Borras et al., 2000; Chen et al., 1996; Chen et al., 1999; Edenberg et al., 2006; Hasin et al. 2002; Ma et al., 2005; Mulligan et al., 2003; Neumark et al., 2004; Nishimura et al., 2009; Spivak et al., 2007; Thomasson et al., 1991; Wall et al.,

2005). For example, the *ADH1B\*2* allele (rs1229984, A allele) located in exon 3 of *ADH1B* results in an arginine to histidine amino acid change that alters the kinetics of the enzyme (Hurley et al., 1990) and has demonstrated a protective relation with alcohol dependence and related phenotypes (e.g., MacGregor et al., 2009; Shen et al., 1997; Thomasson et al., 1991, 1994; Whitfield, 1997). Similarly, the *ADH1B\*3* allele (rs2066702, located in exon 9 of *ADH1B*) results in an arginine to cysteine change (Carr et al., 1989) that has shown a protective association in the development of alcohol dependence in samples of African descent (Edenberg et al., 2006; Ehlers et al., 2001a, 2007; Luo et al., 2006; McCarthy et al., 2010) and a subset of the Native American population described in the present study (Wall et al., 2003). More recently, researchers have begun to study the remaining *ADH* genes to evaluate whether variants in these genes might also be related to alcohol dependence and related phenotypes (e.g., Birley et al., 2009; Hall et al., 2007; Han et al., 2007; Kuo et al., 2008; Luo et al., 2007; Sherva et al., 2009; van Beek et al., 2010), with several of these studies reporting evidence of association with *ADH4* (Edenberg et al., 2006; Guindalini et al., 2005; Kimura et al., 2009; Luo et al., 2005; MacGregor et al., 2009; Preuss et al., 2011) as well as with *ADH1A* and *ADH1B* (Edenberg et al., 2006).

The present report is part of a larger study exploring risk factors for alcoholism in a Native American community (Ehlers and Wilhelmsen, 2005; Ehlers et al., 1998, 1999, 2001a, 2001b, 2004a, 2004b; Garcia-Andrade et al., 1996, 1997; Gilder et al., 2002; 2004; Wall et al., 1996, 2000, 2003). In previous studies, we have demonstrated the utility of examining evidence of linkage and association using the alcohol dependence diagnosis as well as a severe use and a withdrawal phenotype, with the latter phenotypes selected to identify a more severe form of alcohol dependence given the high prevalence rate of alcohol dependence in this sample. Specifically, the severe use phenotype consists of four alcohol use items that indicate an advanced clinical course in this population (Ehlers et al., 2004a) and has previously shown evidence of linkage to the chromosome 4q *ADH* gene cluster. The withdrawal phenotype was selected given studies suggesting that withdrawal symptoms are late developing and indicate a particularly severe form of the disorder (Bucholz et al., 1996; Gilder et al., 2011; Martin et al., 2006; Nelson et al., 1996; Saha et al., 2006). A previous study conducted in a subset of the present Native American sample yielded evidence of association between *ADH1B* and DSM-III-R defined alcohol dependence (Wall et al., 2003). Thus, the present study sought to extend these findings by testing for associations between alcohol dependence as well as severe use and withdrawal phenotypes and SNPs in *ADH* genes in an expanded Native American sample.

## Methods and Materials

The protocol for the study was approved by the Scripps Institutional Internal Review board and Indian Health Council, a tribal review group overseeing health issues for the reservations where recruitments took place. Written informed consent was obtained from each participant after study procedures had been fully explained. Participants were compensated for their time spent in the study.

### Participants

Participants who were of at least one-sixteenth Native American Heritage were targeted for study and recruited from eight geographically contiguous reservations with a total population of about 3,000 individuals. Participants who were mobile and between the ages of 18–82 years were recruited using a combination of a venue-based method for sampling hard-to-reach populations (Kalton and Anderson, 1986; Muhib et al., 2001) and a respondent-driven procedure (Heckathorn, 1997), as reported previously (Ehlers et al., 2004a). Women were intentionally over-sampled to provide adequate numbers for meaningful analyses.

Demographic characteristics of this Native American population have been reported previously (Ehlers et al. 2004a). Individuals with blood samples genotyped for the present study (N=586) had a mean age of 31 (range 18–82) years (SD= 12 years), with 42% of the sample being male. Participants had a mean of 11.6 years of education (SD=1.6), 47% of the sample by self report had at least 50% Native American heritage as indicated from their federal Indian blood quantum. Among participants, 59% (n = 346) had a lifetime diagnosis of alcohol dependence, 70% (n = 410) reported a first degree family history of alcoholism, 42% (n = 246) reported experiencing one or more of the severe use symptoms, and 32% (n = 185) reported experiencing one or more withdrawal symptoms.

Participants completed an interview with the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) which was used to gather demographic information and make a lifetime diagnosis of alcohol dependence according to DSM-III-R criteria (American Psychiatric Association, 1980). The SSAGA is a polydiagnostic psychiatric interview that has undergone both reliability and validity testing (Bucholz et al., 1994; Hesselbrock et al., 1999). It has been successfully used in Native American populations previously (Hesselbrock et al., 2000; Wall et al., 2003). Interviewers were all trained by Collaborative Study of the Genetics Alcoholism (COGA) personnel. All best final diagnoses were made by one research psychiatrist/addiction specialist (DAG) using a best estimate procedure.

The SSAGA groups individual interview response items into nine categories that correspond to the nine DSM-III-R criteria used for making a dependence diagnosis. These items include: alcohol use severity items, legal, family, work and medical problems, tolerance, wanting/unable to quit and withdrawal. Two phenotypes hypothesized to most likely have a heritable basis were defined using these categories. The first phenotype grouped responses only on four alcohol use severity items: (1) drank more than intended/more days in a row or when promised self wouldn't for three or more times, (2) drank when didn't want to three or more times, (3) during drinking or recovering from the effects of drinking had little time for anything else, and (4) given up or greatly reduced important activities to drink. Each subject was scored as having 1 or more alcohol severity symptoms. The second phenotype was DSM-III-R physiological alcohol withdrawal. The diagnosis of withdrawal was made if the participant had a history of any of the following occurring when he or she stopped, cut down or went without drinking after a prolonged period of steady drinking: (1) the shakes (trembling of the hands), unable to sleep, anxiety or depression, sweating, rapid heart rate, nausea or vomiting, feeling physically weak, headache, auditory or visual hallucinations; (2) seizures; and (3) delirium tremens (DT's).

## Genotyping

A blood sample was obtained by venipuncture from each participant and DNA was isolated from leukocytes. Samples were sent to Indiana University for genotyping (see Edenberg et al., 2006). The genotyped sample of 586 participants was collected from 174 families. Sixty-eight of these families consisted of a single participant, and thus were only included in the linkage disequilibrium block analyses described below. The remaining 518 participants came from 104 families that contained between 2 and 16 genotyped individuals (average family size = 5.2, standard deviation = 3.6).

A set of 22 SNPs that have either shown prior evidence of a functional effect on gene expression or evidence of association with alcohol dependence in a previous study (Edenberg et al., 2006) were genotyped (see Table 1 for details regarding each SNP). An additional 47 SNPs were genotyped in a subset of 184 participants thus providing a more complete assessment of the haplotype block structure underlying the ADH gene cluster in this Native American sample. Quality of the genotypes was assessed by examining the call rate, departures from Hardy-Weinberg equilibrium, and number of Mendelian

inconsistencies observed within pedigrees. All SNPs had call rates greater than 95%. Tests of Hardy-Weinberg equilibrium were conducted using the software program Pedstats (Wigginton and Abecasis, 2005). All SNPs had genotypes consistent with Hardy-Weinberg equilibrium with  $p$ -values that were  $>0.01$  with the exception of rs1693482, which yielded a  $p$ -value of 0.0014. Mendelian inconsistencies were identified using Pedstats (Wigginton and Abecasis, 2005). A total of 58 Mendelian inconsistencies were observed among the 21098 called genotypes suggesting an error rate  $<.3\%$ .

### Statistical Analysis

Initial analyses were conducted using Haploview (Barrett et al., 2005) to evaluate the haplotype block structure of the ADH gene cluster in this Native American sample. Blocks were defined using the confidence interval approach described by Gabriel et al. (2002). Given that a proportion of the study sample reported a mixed ethnic background, within-family tests of association were conducted to reduce potential bias due to population stratification. Thus, tests of association were conducted using the PDT (Martin et al., 2001) as implemented in the UNPHASED software package (Dudbridge, 2003). The PDT analyzes all informative trios within an extended pedigree as well as discordant sibships with one affected and one unaffected sibling that possess discrepant genotypes at the marker to be analyzed. The 'averaged' PDT statistic, which gives equal weight to all families included in the analysis, was used to evaluate the evidence for association. Three phenotypes (i.e., DSM-III-R defined Alcohol Dependence, presence of withdrawal symptoms, and presence of 'severe' alcohol dependence symptoms) were tested for association. To correct for multiple testing across these three phenotypes and the 22 SNPs genotyped in the full sample, a critical  $p$ -value of 0.002 was selected to reduce the potential for Type I error while considering the correlations between SNP genotypes (average  $R^2=.44$  for adjacent SNP pairs).

### Results

One hundred and three unrelated individuals were drawn from the larger sample to calculate linkage disequilibrium (LD) statistics and estimate the haplotype block structure of the region in the present sample using Haploview (Barrett et al., 2005). The degree of LD between SNPs and the haplotype block structure are shown in Figure 1. Evidence of LD was observed across the region with an average  $D'$  value across SNP pairs of 0.64. Nonetheless, there was stronger LD observed within than between genes with an average  $D'$  value across SNP pairs within genes of 0.91 and an average  $D'$  value across SNP pairs of adjacent genes of 0.70. Further, each gene appeared to be defined by a unique haplotype block in this population with the exception of *ADH6* and *ADH1A*, which were fully contained within a single block. The only strong evidence of recombination within a gene was observed for *ADH7* and to a lesser extent for *ADH1B*. For example, in *ADH7* the SNPs at the 5' end show evidence of LD with one another, but those at the 3' end do not. These results are largely consistent with those from the European-American sample described by Edenberg et al (2006), which showed evidence for LD across the ADH gene cluster that was stronger within than between genes.

The pedigree disequilibrium test (PDT) was used to test for association between *ADH* gene cluster SNPs and 3 alcohol dependence related phenotypes: (1) DSM-III-R defined alcohol dependence, (2) the presence of alcohol withdrawal symptoms, and (3) the presence of 'severe' alcohol dependence symptoms (see Table 2 for complete results). For the alcohol dependence diagnosis and 'severe' alcohol dependence symptoms, several SNPs were nominally significant ( $p<0.05$ ) but none of the SNPs yielded evidence for association at  $p<0.002$ . For the withdrawal symptoms phenotype, two SNPs showed significant evidence for association at  $p<0.002$ : rs3762894 located in the promoter region of *ADH4* (chi-

square=10.53,  $p=0.0012$ ) and rs2066702 located in exon 9 of *ADH1B* (chi-square=9.77,  $p=0.0018$ ). For both SNPs, transmission of the minor allele (rs3762894 - C allele; rs2066702 - T allele) was associated with reduced risk for withdrawal symptoms. The locations of these SNPs as well as the results for all tests of association are shown in the Manhattan plot displayed in Figure 2.

A set of follow-up analyses were then conducted to test for association between haplotypes constructed for a set of six SNPs, rs1126672, rs3762894, rs2066702, rs1229984, rs698, and rs1693482, that yielded significant evidence for association in the present study or have a suggested functional impact on gene expression. A sliding window of 2 SNPs was used to construct haplotypes for all contiguous SNP pairs, which were then evaluated for evidence of association with the three described alcohol use phenotypes using the PDT. The strongest evidence for association across all phenotypes was observed for the analysis of rs3762894 and rs2066702 (chi-square=24.01,  $p=0.000006$ ; see Table 3a for complete results). Analysis of the individual haplotypes suggested that transmission of haplotypes containing a minor allele at either SNP (C allele of rs3762894 or T allele of rs2066702) was associated with a reduced risk for experiencing withdrawal symptoms (Table 3b).

## Discussion

The present study sought to evaluate the evidence for association between the 7 *ADH* genes located on chromosome 4q and alcohol dependence as well as phenotypes associated with long-term alcohol misuse. Two SNPs, rs2066702 located in exon 9 of *ADH1B* and rs3762894 located at the 5' end of *ADH4*, yielded significant evidence for an association with withdrawal symptoms. For both SNPs, the minor allele (C allele of rs3762894; T allele of rs2066702) showed a protective relation to the development of withdrawal symptoms. This result was further supported by an analysis of haplotypes constructed from these two SNPs that suggested haplotypes containing either of the minor alleles was protective against alcohol withdrawal relative to the ancestral haplotype (i.e., a haplotype consisting of the major allele at both SNPs). Only nominally significant evidence of an association with alcohol dependence or the severe use phenotype was found with the tested polymorphisms. Though previous studies conducted in this population reported evidence of linkage between the region and the severe use phenotype (Ehlers et al., 2004b) and associations between SNPs located in *ADH1B* and *ADH1C* and DSM-III-R defined alcohol dependence diagnoses (Wall et al., 2003), the present study included an expanded sample using a distinct analytic approach, which may have led to difficulties in reproducing the previous findings.

Both of the SNPs associated with withdrawal symptoms have shown previous evidence of association with alcohol dependence and related phenotypes. Specifically, rs2066702, which has been described in the literature as identifying the *ADH1B*\*3 allele, has been associated with alcohol dependence in samples of African descent (Edenberg et al., 2006; Ehlers et al., 2001a, 2007; Luo et al., 2006) and an earlier study of this Native American population that used a subset of the participants described in the present study (Wall et al., 2003). Similarly, rs3762894 has been associated with alcohol dependence in several recent studies of *ADH4* polymorphisms (Edenberg et al., 2006; MacGregor et al., 2009). Both SNPs have also been shown to affect the kinetic properties of ADH with the minor alleles of each SNP producing more active ADH isozymes than the major alleles (Birley et al., 2009; Edenberg, 2007; Thomasson et al., 1995). It has been suggested that these more active isozymes lead to a more rapid buildup of acetaldehyde, thus leading to a stronger response to alcohol and possibly more severe withdrawal symptoms. Nonetheless, there have been negative results reported for these SNPs (e.g., Kuo et al., 2008), thus posing the question of why the associations have been observed in some studies but not others.

Genetically complex disorders like alcohol dependence are likely influenced by a number of genes each exerting only a small effect on the broad clinical phenotype (Lander and Schork, 1994). These small effect sizes can complicate the search for susceptibility loci given that normal sampling variability will produce both positive and negative results when studies are insufficiently powered due to small sample sizes as is frequently the case. Nonetheless, such genes might be detected if they have a larger effect on a more narrowly defined phenotype. For example, withdrawal symptoms, the presence of which can indicate alcohol dependence with a "physiological component" as defined by DSM-IV (American Psychiatric Association 1994), appear to have particular clinical relevance and may identify an important subpopulation of alcohol dependent individuals with a more severe clinical course (Langenbucher et al., 2000; Schuckit et al., 1998). It is possible that genes involved in alcohol metabolism may play an important role in the etiology of the disorder within this subpopulation relative to individuals diagnosed with alcohol dependence without a "physiological component." The results of the present study as well as those of a previous linkage scan for alcohol dependence and related phenotypes (Ehlers et al., 2004b) are consistent with these conclusions in demonstrating that genetic linkage and association can be detected for withdrawal symptoms even when such associations are not observed for the broader alcohol dependence diagnosis.

The results of the present study also provide evidence that the observed associations between the *ADH4* and *ADH1B* SNPs and alcohol dependence phenotypes previously reported in African, Asian, and Caucasian populations can be extended to Native American populations. This is particularly relevant for Native American populations given that the increased prevalence of alcohol dependence among Native Americans relative to Caucasians has unfortunately resulted in many negative stereotypes of Native Americans. Among these is the common belief that Native Americans may be more genetically susceptible to developing alcohol dependence due to unique differences in the metabolism of alcohol. The results of the present study suggest that in relation to the *ADH* genes located on chromosome 4q, the evidence for association is consistent with that observed in other ethnic groups, thus casting doubt on such theories.

The results of the present study should be interpreted in the context of several limitations. First, the findings may not generalize to other Native Americans or represent all Native Americans in this population. Second, the study gathered clinical data using retrospective methods; therefore, more information is needed using longitudinal techniques. Third, the association between *ADH* variants and alcohol-related phenotypes in this Native American population may not extend to other large population samples due to differences in genetic and environmental variables. Despite these limitations, this report represents an important step in an ongoing investigation to determine risk and protective factors associated with the development of substance use disorders in this high risk and understudied ethnic group.

In summary, the present study examined evidence for association between the cluster of *ADH* genes located on chromosome 4q and alcohol dependence and related phenotypes. Only nominal evidence for association between SNPs in these gene and alcohol dependence was observed, but the presence of withdrawal symptoms was significantly related to a SNP at the 5' end of *ADH4* and a coding SNP in exon 9 of *ADH1B* with a known functional impact on ADH activity. Evidence of association between these two genes and alcohol misuse phenotypes has been reported in previous studies of ethnic groups including Caucasians, individuals of African descent, and Native Americans.

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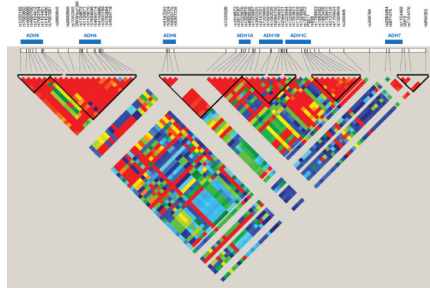
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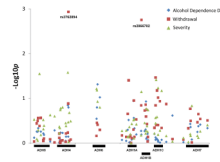
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**Figure 1.** Linkage disequilibrium (LD) between SNPs genotyped across the chromosome 4q ADH gene cluster. Gene locations are indicated by the blue bars. Absent or low levels of LD are indicated by the blue and green squares, respectively, whereas increasing levels of LD are indicated by the yellow, orange, and red squares in ascending order of LD.



**Figure 2.** Manhattan plot of the association signals for the SNPs typed across the ADH gene cluster and the three analyzed phenotypes. The X-axis displays the location of the SNP with each gene indicated by a labeled black bar, and the y-axis displays the  $-\log(p)$  value for each SNP.

Table 1

Locations and minor allele frequencies of genotyped SNPs.

SNP	Chromosome Position	Gene	SNP Type/Location	Alleles <sup>1</sup>	Minor Allele Frequencies			
					Indians	Caucasians <sup>2</sup>	African-Americans <sup>2</sup>	African-Americans <sup>2</sup>
rs1230155	99989258	ADH5	intergenic	A/G	0.200	0.345	0.318	0.318
rs7683802	99995137	ADH5	intron 7	T/G	0.063	0.102	0.107	0.107
rs4699699	99997178	ADH5	intron 6	C/G	0.06	0.108	0.071	0.071
rs4699700	99998334	ADH5	intron 4	A/G	0.074	0.148	0.261	0.261
rs1154412	100001246	ADH5	intron 4	C/T	0.135	0.19	0.031	0.031
rs7683704	100004225	ADH5	intron 2	C/T	0.07	0.108	0.268	0.268
rs1154401	100009737	ADH5	intron 1	C/G	0.213	0.355	0.364	0.364
rs1154400	100010009	ADH5	5' end	T/C	0.145	0.328	0.341	0.341
rs7667261	100011299	ADH5	5' end	C/G	0.015	0.044	0.212	0.212
rs2602846*	100025150	ADH5/ADH4	intergenic	A/T	0.133	0.299	0.123	0.123
rs2602866	100034996	ADH5/ADH4	intergenic	G/A	0.13	0.295	0.135	0.135
rs1042365*	100045499	ADH4	exon 9	C/A	0.136	0.294	0.138	0.138
rs1042364*	100045573	ADH4	exon 9	G/A	0.096	0.294	0.138	0.138
rs29001229 (DWSHpy1881)*	100046822	ADH4	IVS8	T/C	0.136	0.295	0.135	0.135
rs1126672*	100047811	ADH4	exon 8 coding-synon	C/T	0.133	0.305	0.165	0.165
rs1126671	100048413	ADH4	exon 7	G/A	0.208	0.319	0.223	0.223
rs1126670	100052732	ADH4	exon 6	T/G	0.21	0.315	0.217	0.217
rs7694646*	100059731	ADH4	intron 4	T/A	0.152	0.294	0.135	0.135
rs4699714*	100060537	ADH4	intron 4	A/G	0.135	0.304	0.119	0.119
rs4148886	100064648	ADH4	intron 1	A/G	0.464	0.242	0.49	0.49
rs1800759*	100065508	ADH4	5'UTR	A/C <sup>3</sup>	0.428	0.581	0.233	0.233
rs3762894*	100066083	ADH4	5'UTR	T/C	0.108	0.166	0.225	0.225
rs4699718*	100067790	ADH4/ADH6	intergenic	G/A	0.136	0.298	0.122	0.122
rs1984362*	100070972	ADH4/ADH6	intergenic	C/T	0.094	0.294	0.094	0.094



SNP	Chromosome Position	Gene	SNP Type/Location	Minor Allele Frequencies			
				Alleles/ <sup>3</sup>	Indians	Caucasians <sup>2</sup>	African-Americans <sup>2</sup>
rs4147545	100128752	ADH6	intron 6	G/A <sup>3</sup>	0.438	0.658	0.453
rs3857224	100129684	ADH6	intron 6	T/C <sup>3</sup>	0.469	0.667	0.453
rs6833176	100131162	ADH6	intron 5	C/G	0.397	0.461	0.186
rs9307238	100136181	ADH6	intron 2	G/A	0.409	0.488	0.359
rs1230026	100185618	ADH6/ADH1A	intergenic	A/C	0.235	0.223	0.074
rs1618572	100195120	ADH6/ADH1A	intergenic	C/G	0.241	0.224	0.056
rs2866151*	100198511	ADH1A	intron 8	A/T	0.425	0.456	0.233
rs3819197	100200508	ADH1A	intron 8	C/T	0.819	0.749	0.725
rs1229976	100202077	ADH1A	intron 6	T/C	0.242	0.224	0.073
rs1229970	100204379	ADH1A	intron 5	G/T	0.227	0.221	0.056
rs6828526	100205886	ADH1A	exon 4	G/A	0.000	0.001	0.009
rs1229967	100207577	ADH1A	intron 3	G/C	0.241	0.223	0.055
rs3805325	100211398	ADH1A	intron 1	T/C	0.282	0.078	0.128
rs4147531*	100212196	ADH1A	5'UTR	C/T	0.429	0.457	0.233
rs1229966	100213432	ADH1A	5' end	T/C <sup>4</sup>	0.328	0.346	0.605
rs1826909*	100217742	ADH1A/1B	intergenic	C/T	0.146	0.373	0.171
rs1042026	100228465	ADH1B	3' UTR	A/G	0.171	0.306	0.132
rs2066702*	100229016	ADH1B	exon 9 coding-nonsyn	C/T	0.041	0.004	0.165
rs1789883	100236374	ADH1B	intron 5	G/A	0.118	0.027	0.015
rs2066701	100238412	ADH1B	intron 3	C/T	0.173	0.304	0.122
rs1229984*	100239318	ADH1B	coding-nonsyn	G/A	0.028	0.034	0.019
rs1229983	100240001	ADH1B	exon 2	T/C	0.110	0.024	0.044
rs1353621	100241574	ADH1B	intron 1	A/G	0.142	0.370	0.123
rs1159918*	100243008	ADH1B	3'UTR	T/G <sup>3</sup>	0.292	0.652	0.355
rs1229982*	100243931	ADH1B	3'UTR	G/T	0.273	0.22	0.443
rs1614972	100258154	ADH1C	intron 8	C/T	0.147	0.330	0.493
rs35719513 (P351T)	100260782	ADH1C	exon 8	C/A	0.079	0.005	0

SNP	Chromosome Position	Gene	SNP Type/Location	Alleles <sup>1</sup>	Minor Allele Frequencies			
					Indians	Caucasians <sup>2</sup>	African-Americans <sup>2</sup>	African-Americans <sup>2</sup>
rs698*	100260788	ADH1C	exon 8 coding-nonsyn	A/G	0.403	0.389	0.185	0.185
rs1789903	100262040	ADH1C	intron 6	C/G	0.410	0.389	0.169	0.169
rs1693482*	100263964	ADH1C	exon 6 coding-nonsyn	C/T	0.391	0.383	0.178	0.178
rs1693426	100266329	ADH1C	intron 4	A/G	0.409	0.387	0.171	0.171
rs1789915	100266370	ADH1C	exon 4	A/G	0.197	0.261	0.144	0.144
rs3133158	100270611	ADH1C	intron 1	C/G	0.204	0.285	0.160	0.160
rs1789924	100274285	ADH1C	5' end	C/T	0.414	0.389	0.179	0.179
rs1229849	100284684	ADH1C/ADH7	intergenic	T/A	0.197	0.280	0.147	0.147
rs283406	100298470	ADH1C/ADH7	intergenic	C/T	0.033	0.079	0.082	0.082
rs284794	100323028	ADH1C/ADH7	intergenic	A/T	0.055	0.109	0.157	0.157
rs284786*	100333976	ADH7	3'UTR	A/T	0.294	0.272	0.459	0.459
rs284779*	100338260	ADH7	intron 7	C/G	0.392	0.458	0.170	0.170
rs2584464	100339048	ADH7	intron 7	A/G <sup>5</sup>	0.416	0.516	0.678	0.678
rs971074	100341860	ADH7	exon 6	G/A	0.073	0.104	0.177	0.177
rs1154468	100354256	ADH7	intron 1	A/T	0.488	0.328	0.161	0.161
SBP2	100356465	ADH7	5' end	A/G	0.102	0.130	0.051	0.051
rs1154476	100360726	ADH7	intergenic	G/A	0.497	0.384	0.138	0.138
rs894363	100376845	ADH7	intergenic	C/T	0.497	0.379	0.165	0.165

Notes:

\* Indicates SNPs that were genotyped in the full sample,

<sup>1</sup> Alleles indicated are from the + strand and are ordered major/minor based on prevalence estimates from the present sample,

<sup>2</sup> Allele frequencies cited from Edenberg et al. (2006),

<sup>3</sup> The designated 'minor' allele in the Native American sample was observed at a frequency >0.50 among the European-American sample,

<sup>4</sup> The designated 'minor' allele in the Native American sample was observed at a frequency >0.50 among the African-American sample,

<sup>5</sup> The designated 'minor' allele in the Native American sample was observed at a frequency >0.50 among the European-American and African-American samples

Table 2

Pedigree Disequilibrium Tests of association for individual SNPs and three alcohol dependence phenotypes.

SNP	Gene	<i>p</i> -values		
		Alcohol Dependence Diagnosis	Experienced Withdrawal Symptoms	Experienced Severe Symptoms
rs1230155	ADH5	0.7561	0.9254	0.2974
rs7683802	ADH5	0.5527	0.3642	0.8073
rs4699699	ADH5	0.5560	0.3206	0.8734
rs4699700	ADH5	0.4647	0.2767	0.5125
rs1154412	ADH5	0.4147	0.4171	<b>0.0285</b>
rs7683704	ADH5	0.5539	0.2733	0.6551
rs1154401	ADH5	0.8639	0.8597	0.3020
rs1154400	ADH5	0.6093	0.8938	0.4862
rs7667261	ADH5	0.6547	0.7970	0.4071
rs2602846*	ADH5/ADH4	0.9198	0.8234	0.2365
rs2602866	ADH5/ADH4	0.5191	0.9668	0.2510
rs1042365*	ADH4	0.9770	0.7640	0.5993
rs1042364*	ADH4	0.9975	0.6163	0.4294
rs29001229 (DWSHpy188I)*	ADH4	0.8111	0.9402	0.4591
rs1126672*	ADH4	0.9336	0.9973	0.1815
rs1126671	ADH4	0.5827	0.9056	0.1670
rs1126670	ADH4	0.5911	0.8728	0.1592
rs7694646*	ADH4	0.8928	0.9365	0.3564
rs4699714*	ADH4	0.7175	0.6134	0.4137
rs4148886	ADH4	0.1427	0.5866	<b>0.0266</b>
rs1800759*	ADH4	0.7167	0.1312	0.3405
rs3762894*	ADH4	0.1596	<b>0.0012</b>	0.9496
rs4699718*	ADH4/ADH6	0.9005	0.8942	0.2098
rs1984362*	ADH4/ADH6	0.8366	0.8358	0.3232
rs4147545	ADH6	0.2857	0.6914	0.0615
rs3857224	ADH6	0.1595	0.3529	0.0663
rs6833176	ADH6	<b>0.0487</b>	0.3893	0.1185
rs9307238	ADH6	0.0950	0.5078	0.1592
rs1230026	ADH6/ADH1A	0.8477	0.6762	0.5316
rs1618572	ADH6/ADH1A	0.8908	0.7292	0.5229
rs2866151*	ADH1A	0.5055	0.2234	0.7288
rs3819197	ADH1A	0.3042	<b>0.0396</b>	0.7143
rs1229976	ADH1A	0.9635	0.7384	0.4354
rs1229970	ADH1A	0.9563	0.6809	0.6310

SNP	Gene	<i>p</i> -values		
		Alcohol Dependence Diagnosis	Experienced Withdrawal Symptoms	Experienced Severe Symptoms
rs6828526	ADH1A	1.0000	1.0000	1.0000
rs1229967	ADH1A	0.8321	0.7754	0.3722
rs3805325	ADH1A	0.1138	0.5002	0.3048
rs4147531*	ADH1A	0.2741	0.1124	0.7801
rs1229966	ADH1A	0.4680	0.7293	0.2949
rs1826909*	ADH1A/1B	0.6383	0.2966	0.3797
rs1042026	ADH1B	0.5340	0.1445	0.6680
rs2066702*	ADH1B	0.6446	<b>0.0018</b>	0.0717
rs1789883	ADH1B	0.5377	0.1878	0.3544
rs2066701	ADH1B	0.4972	0.1383	0.6085
rs1229984*	ADH1B	0.1177	0.2561	0.5283
rs1229983	ADH1B	0.8007	0.1807	0.1709
rs1353621	ADH1B	0.7413	0.3749	0.4154
rs1159918*	ADH1B	0.6215	0.9254	0.5548
rs1229982*	ADH1B	0.8587	0.7335	0.1824
rs1614972	ADH1C	0.6216	0.4558	0.6042
rs35719513 (P351T)	ADH1C	0.7438	0.1446	0.3611
rs698*	ADH1C	0.8423	<b>0.0344</b>	0.2718
rs1789903	ADH1C	0.8085	0.3926	<b>0.0389</b>
rs1693482*	ADH1C	0.8101	0.0668	0.2606
rs1693426	ADH1C	0.8665	0.3960	0.0590
rs1789915	ADH1C	0.9266	0.0964	0.6475
rs3133158	ADH1C	0.9713	0.1166	0.5221
rs1789924	ADH1C	0.8435	0.4608	0.0679
rs1229849	ADH1C/ADH7	0.9401	0.1347	0.6898
rs283406	ADH1C/ADH7	0.2419	0.8143	0.9149
rs284794	ADH1C/ADH7	0.9504	0.8343	0.7660
rs284786*	ADH7	0.9911	0.4685	0.9435
rs284779*	ADH7	0.3723	0.1719	0.6271
rs2584464	ADH7	0.4379	0.3188	0.6852
rs971074	ADH7	0.8276	0.9676	0.1473
rs1154468	ADH7	0.5625	0.3428	0.6853
SBP2	ADH7	0.4194	0.2265	0.7587
rs1154476	ADH7	0.3853	0.2905	0.5450
rs894363	ADH7	0.4638	0.3110	0.3960

Notes:

\* Indicates SNPs that were genotyped in the full sample, italicized text indicates *p*-value<0.05, bold text indicates *p*-value<0.002.

**Table 3**

Haplotype analysis of putatively functional SNPs using a sliding window of 2 SNPs.

**a.) Summary of results for all sets of SNPs.**

SNPs	<i>p</i> -values		
	Alcohol Dependence Diagnosis	Experienced Withdrawal Symptoms	Experienced Severe Symptoms
rs1126672,rs3762894	0.6408	0.01528	0.7022
rs3762894,rs2066702	0.2499	0.000006126	0.3977
rs2066702,rs1229984	0.2573	.007018	0.2022
rs1229984,rs698	0.8451	0.02986	0.2704
rs698,rs1693482	0.04523	0.004394	0.009667

**b.) Association results for withdrawal symptoms and haplotypes constructed from rs3762894 and rs2066702.**

Haplotype	Z	<i>p</i> -value	Not Transmitted		Frequency
			Transmitted	Not Transmitted	
C/C	-3.274	0.001061	12.63	20.23	0.103
T/C	4.085	0.00004403	192.4	180.2	0.8762
T/T	-2.933	0.003359	1	5.598	0.02081

Global test: Chi-square = 24.01, *p* = 6.126e-006.