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Multiple *ADH* genes modulate risk for drug dependence in both African- and European-Americans

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Abstract

Drug dependence (DD) is commonly co-morbid with alcohol dependence (AD). Many studies have also shown common genetic risk factors for these disorders. We previously reported associations of AD with seven alcohol dehydrogenase (*ADH*) genes. The present study examines the relationship between these genes and DD. We genotyped 16 markers within the *ADH* gene cluster and 38 unlinked ancestry-informative markers in a case-control sample of 718 individuals. All markers were consistent with Hardy-Weinberg equilibrium in controls, but some markers showed Hardy-Weinberg disequilibrium in cases (minimal $P = 0.002$). Genotypes of many markers were associated with DD, both before and after controlling for admixture effects (minimal $P < 1.0 \times 10^{-6}$). Diplotype trend regression analysis showed that *ADH5* and *ADH6* genotypes, and diplotypes at *ADH1A*, *ADH1B*, *ADH1C* and *ADH7* (minimal $P = 0.002$), were associated with DD in European-Americans and/or African-Americans. This first report of an allelic association of these loci with DD provides new insight into the mechanism of genetic risk for DD. These findings, obtained using a series of powerful and reliable analytic methods, may also help to explain the high rate of co-morbidity between AD and DD.

INTRODUCTION

Drug dependence (DD), which refers to cocaine dependence (CD) and/or opioid dependence (OD) in the context of the present study, results in serious medical, legal, social and psychiatric problems and influences many facets of American society, cutting across geographical region, race, ethnicity and socioeconomic status. Cocaine is second only to cannabis as the most commonly used illicit drug in the USA; OD has a lifetime prevalence of 0.4%, and the combined lifetime prevalence of OD and opioid abuse is 0.7%. Risk for DD is influenced by genetic factors, as demonstrated by adoption studies (in the general case of substance dependence) and

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by twin studies [summarized by Gelernter et al. (1,2)]. Elucidating the genetic basis of DD would represent major progress toward understanding the etiology of this disorder.

A genome-wide scan located possible risk regions for CD or CD-related traits at chromosomes 10 [in mixed European-American (EA) and African-American (AA) samples], 3 and 12 (in EAs) and 9 and 18 (in AAs) (1) and located risk regions for OD at chromosomes 17 (in EAs and AAs) and 2 (in AAs) (2). Many population-based case-control association studies have also examined the molecular genetics of DD (3–6). The present study focused on the roles of the alcohol dehydrogenase (*ADH*) genes in risk for DD.

Seven *ADH* genes are located in a cluster within an ~364 kilobase (kb) region at 4q21–25. We recently studied 16 *ADH* markers in relation to alcohol dependence (AD) [MIM 103780] (7). These *ADH* markers span 346 327 bp, covering 95% of the full length of the *ADH* gene cluster, with an average intermarker distance of 21.6 kb, including one *ADH5* [MIM 103710] marker (located in a haplotype block that covers 80% of the full length of *ADH5*), one *ADH6* [MIM 103735] marker (located in a haplotype block that covers the full length of *ADH6*), three *ADH1A* [MIM 103700] markers, four *ADH1B* [MIM 103720] markers, three *ADH1C* [MIM 103730] markers and four *ADH7* [MIM 600086] markers (Table 1). The *ADH* markers were located in several haplotype blocks. Genotype frequency distributions of all markers were in Hardy–Weinberg equilibrium (HWE) in both EA and AA controls, but some of the markers were in Hardy–Weinberg disequilibrium (HWD) in either EA or AA subjects with AD. Genotypes of some *ADH* markers were associated with AD, even after controlling for admixture effects. Diplotype trend regression (DTR) analysis demonstrated that most of the genes studied were risk loci for AD (7). Most of these findings were consistent in an independent sample of pedigrees by investigators in the Collaborative Study on the Genetics of Alcoholism (COGA) (8).

Our initial hypothesis was that, because the *ADH* genes are specifically involved in the metabolism of ethanol, their risk effects would be limited to AD. However, several studies have shown that the susceptibility to AD attributable to gene variation is shared with susceptibility to disorders that are commonly co-morbid with AD. A typical example is DD, one of the most common phenotypes co-morbid with AD (9). DD has many features in common with AD, including symptomatology, neuropsychological impairment, hypothesized pathogenetic mechanisms and response to specific treatments, especially (in the case of CD) disulfiram, an *ALDH2* inhibitor that has been used for more than 50 years for the treatment of AD. Further, DD has been reported to share some susceptibility genes with AD (10–12). For example, we previously observed that variation at the *ADH4* locus [MIM 103740] and *CHRM2* locus [MIM 118493] affected risk for both AD and DD (4–6). In addition, *OPRM1* variation has been reported to affect susceptibility to AD and/or DD (3,13–16). That AD and DD share common genetic risk factors may partially underlie their high rate of co-morbidity.

Thus, in the present study, we investigated the relationships between *ADH* genes and DD on the basis of our initial findings for AD and tested the phenotypic specificity of these genes for risk of AD and DD. To accomplish this, we genotyped the same marker set, including 16 *ADH* markers and 38 ancestry informative markers (AIMs), using the same genotyping methods employed in the initial study (7). We performed all analyses separately within ‘genetic’ EAs (European ancestry proportion > 0.5) and ‘genetic’ AAs (African ancestry proportion > 0.5).

RESULTS

Genotype frequency distributions of all markers (Table 2) were in HWE in controls in both EAs and AAs, but many *ADH* markers were nominally in significant ($P < 0.03$), modest (0.03

$\leq P \leq 0.05$) or suggestive ($0.05 < P < 0.09$) HWD in either EA or AA subjects with DD (Table 3), including *ADH5*[^]SNP1, *ADH1B*[^]Arg/His (SNP16: previously called *ADH2*^{*}1/2), *ADH7*[^]SNP21 and *ADH7*[^]Ala/Gly (SNP22) in EA DD; *ADH1C*[^]Ile/Val (SNP17: previously called *ADH3*^{*}1/2) and *ADH1C*[^]SNP19 in EA DD-only (i.e. in the absence of AD); *ADH1B*[^]Arg/His, *ADH1C*[^]Ile/Val, *ADH1C*[^]Gln/Arg (SNP18) and *ADH1C*[^]SNP19 in AA DD and *ADH6*[^]SNP9, *ADH1A*[^]SNP11, *ADH1B*[^]Arg/Cys (SNP14: previously called *ADH2*^{*}1/3), *ADH7*[^]SNP21 and *ADH7*[^]SNP23 in AA DD-only. Seven *ADH4* markers were also in significant HWD in EA cases, as reported previously (6). These results indicate the existence of associations between these genes and DD.

Using case–control comparison (*P* values shown in Table 6), we found that, in EAs, *ADH5*[^]SNP1 was nominally associated with DD, and *ADH5*[^]SNP1 and *ADH1C*[^]Ile/Val (SNP17) were nominally associated with DD-only, both before and after controlling for admixture effects. Genotypes of seven *ADH4* markers were also significantly associated with DD in EAs, as reported elsewhere (6). In AAs, *ADH1A*[^]SNP11, *ADH1B*[^]-Arg/Cys (SNP14) and *ADH7*[^]SNP21 were associated with DD, and *ADH1A*[^]SNP11, *ADH1B*[^]SNP15 and *ADH7*[^]SNP21 were associated with DD-only, both before and after controlling for admixture effects. *ADH1C*[^]Ile/Val (SNP17), *ADH1C*[^]Gln/Arg (SNP18), *ADH1C*[^]SNP19, and *ADH7*[^]Ala/Gly (SNP22) were associated with DD, and *ADH1B*[^]Arg/Cys (SNP14) was associated with DD-only, before controlling for admixture effects. As shown in Table 2, across different case subgroups, the over-represented heterozygotes (within *ADH1C*) or homozygotes (within other genes) for all markers in significant HWD (i.e. whose observed frequencies were higher than the expected frequencies) were always the same as the risk genotypes in the case–control comparison analysis (i.e. whose frequencies in cases were higher than those in controls).

After correction for multiple comparisons using SNPSpD (an effective Bonferroni-type correction) (17), only *ADH5*[^]SNP1 remained in significant HWD in EA DD ($P = 0.0016$; $\alpha = 0.0033$), and only *ADH7*[^]SNP21 remained significantly associated with AA DD-only ($P = 0.0006$ and $P < 10^{-6}$ before and after controlling for admixture effects, respectively; $\alpha = 0.0017$) (Table 6).

DTR analysis demonstrated that several genes contributed to the risk for DD (Table 4). In EAs and/or AAs, genotypes of *ADH5*[^]SNP1 and *ADH6*[^]SNP9 and some diplotypes at the *ADH1A*, *ADH1B*, *ADH1C* and *ADH7* genes were associated with DD. Some of these risk diplotypes exerted consistent effects on phenotypes across EAs and AAs: diplotype ACGG/TCGG at *ADH7* increased risk for disease in both populations ($\beta > 0$). Some of the risk genotypes or diplotypes exerted opposite effects on phenotypes in EAs and in AAs: all of the diplotypes at *ADH1A* increased risk for DD in EAs ($\beta > 0$), but protected against DD in AAs ($\beta < 0$) [many other rare *ADH1A* diplotypes ($f < 5\%$) were not entered into the regression model (Table 4), and the effects of *ADH1A* diplotypes were referenced to all the covariates and the other five *ADH* genes]; diplotype AGT/GAC at *ADH1C* protected against DD in EAs ($\beta < 0$), but increased risk for DD in AAs ($\beta > 0$). Some of these risk diplotypes exerted effects on phenotypes only in EAs: diplotype CCTG/CCTG at *ADH1B* and diplotype TCGA/TCGA at *ADH7* increased risk for DD ($\beta > 0$); diplotypes AGT/AGT and GAC/GAC at *ADH1C* protected against DD ($\beta < 0$). Some of these risk diplotypes exerted effects on phenotype only in AAs: genotype T/T of *ADH6*[^]SNP9 and diplotypes TCCG/TCCG and TTCG/TCCG at *ADH1B* protected against DD ($\beta < 0$); diplotype TCGG/TCGG at *ADH7* increased risk for DD ($\beta > 0$). All of the above risk genotypes and diplotypes exerted consistent effects both for DD and DD-only (i.e. in the absence of AD). Table 4 lists only those variables that remained in the last step of the DTR equations.

There are several peak *J*-values among the *ADH* markers in both EAs and AAs, which may indicate the proximity of risk alleles for both DD and DD-only (Table 5; Fig. 1). For DD, the

highest J peak in the *ADH* gene cluster is at a functional variant, *ADH1B*^{Arg/His} (SNP16) ($|J| = 1.185$ in EAs, 1.000 in AAs); for DD-only, the highest J peak is at *ADH7*^{SNP21} (7.8 kb from SNP22) ($|J| = 1.000$ in EAs, 3.118 in AAs). Other J peaks and the exact J -values can be seen in Figure 1 and Table 5, respectively. Figure 1 does not include J peaks for AD, for which the exact J -values were shown in Table 5; that figure was reported previously (7).

DISCUSSION

ADH genes have been shown to be important risk factors for AD in EAs and AAs in our initial study (7). In this study, we present the first comprehensive evidence that these genes (specifically *ADH5*, *ADH6*, *ADH1A*, *ADH1B*, *ADH1C* and *ADH7*) also moderate risk for DD.

In our sample, genotypes of all markers were in HWE in controls, but some were in HWD in cases, indicating the existence of associations between genes and disease (6,7). The genotypewise case–control association analysis also showed association of these genes with DD, both before and after controlling for admixture effects. However, we noted that many associations from these conventional analyses became non-significant after correction for multiple testing, indicating that these analyses often led to information loss. To preserve this information, we applied DTR. The HWD test, case–control comparison and structured association (SA) analyses cannot correct for interaction effects between markers and between genes. These issues can also be well addressed by DTR (7).

DTR is a powerful method, and in using it, we detected associations that were not seen using the aforementioned conventional association methods. Several features, more details of which were discussed in Luo *et al.* (7), make DTR more powerful than other conventional association methods in many circumstances. First, it was possible to combine cases and controls, and EAs and AAs, in a single DTR model, thereby increasing the sample size and statistical power (while simultaneously controlling for potential stratification effects from phenotype and population variance). Second, different variables, including different genotypes and diplotypes from different genes, were entered into a single DTR model to avoid the multiple tests that inflate Type 1 error. Third, because DTR does not assume the presence of HWE, analysis was possible using this approach in the presence of deviation from HWE [e.g. for diplotype probabilities predicted by the program PHASE (18,19)]. Under HWD, alleles at a locus or multilocus haplotypes at a gene are not independent of each other, which may invalidate allelewise and haplotype-wise analyses (6,7,20), and thus both analyses were not presented in the present study; however, because genotypewise and diplotypewise analyses may be valid and informative methods, we used them in the present study. Fourth, in the present study, the maximum proportion of individuals with unambiguous diplotypes (i.e. $P = 1$) in a single gene was only 37%. These diplotype data with uncertain phase can be analyzed by DTR. Fifth, age and sex were included in the DTR model to control for potential confounding effects. In the present study, the average age of the controls was 9 years less than for the cases, so a certain proportion of control subjects might still develop substance dependence in their lifetimes. Using such subjects as a control group for a study of substance dependence could reduce power but not produce false-positive results. The positive findings in the present study should be reliable—any bias from this age difference would tend to decrease the significance of the results but not lead to spurious findings. Potential confounding effects by age were controlled in the DTR analysis. Ancestry proportions were also included in the DTR model to control for population stratification and admixture effects. AD was not included in the DTR model as a covariate because of the multicollinearity issue resulting from the strong associations between AD and the *ADH* diplotypes (7). Sixth, DTR takes into account gene–gene interactions, which is a more powerful approach than single-gene analysis (21). Diplotypes incorporate the LD information from different markers, and the interactions between diplotypes (diplotype–diplotype interactions from different genes are more representative of gene–gene interactions

than marker–marker interactions) were considered in the DTR model. Finally, DTR is able to account for LD effects and, additionally, *cis*-acting functional effects. On the basis of these considerations, findings obtained through application of DTR have a high likelihood of being valid.

DTR showed that in EAs and/or AAs, genotypes of *ADH5*[^]SNP1 and *ADH6*[^]SNP9 and diplotypes at the *ADH1A*, *ADH1B*, *ADH1C*, and *ADH7* genes were associated with phenotype (the risk genotypes and diplotypes exerted effects in the same direction for both DD and DD-only). Further, most associations evidenced by DTR analysis showed a higher degree of statistical significance than those based on conventional association methods. In summary, our findings by DTR analysis include the following: (i) Some associations were observed in both populations. This is expected for functional variants and could also occur when the same functional variants are important in both populations, and the markers studied are in similar LD with such variants in both populations. (ii) Some associations were population-specific or even showed opposite effects in the two populations. This is understandable because the diplotype frequency distributions are population-specific in the genetically distinct EAs and AAs, and even the same diplotypes could have different frequencies in these two populations, e.g. *ADH1A*[^]AGA/TCG is quite rare in AAs ($f=0.057$), but more common in EAs ($f=0.181$). The common diplotypes in one population and the rare diplotypes in another population might be in LD with the opposite allele phases of either the same or possibly different unknown risk-influencing variants and could thus exert opposite effects. This is analogous to the situation in which the major allele and the minor allele of the same marker have opposite effects. Additionally, as explored by Luo *et al.* (7), gene–gene interaction effects can be population-specific; of course, the admixture effects could be population-specific also (e.g. AAs show more admixture effects than EAs). These gene–gene interaction effects and admixture effects could affect the direction of association of a certain diplotype in two distinct populations, which could explain why the *ADH1C*[^]AGT/GAC is common in both EAs ($f=0.353$) and AAs ($f=0.275$) but has opposite effects in these two populations. (iii) Of particular note here, *ADH6*[^]SNP9 genotype was suggestively associated with DD-only and only in AAs ($P=0.088$). We cannot exclude the possibility that the failure to reach statistical significance is due to insufficient statistical power.

Results from DTR analysis suggest that the *ADH* gene cluster may harbor risk loci for DD. Risk alleles can be fine-mapped using the *J*-value. If a marker is close to a risk allele for AD, but not close to a DD risk allele (i.e. if the allele affects risk for AD, but not DD), the *J*-value of this marker should be highest for AD, intermediate for DD (which includes co-morbid AD) and lowest in DD-only (Table 5; Fig. 1). For example, the *J*-value at *ADH1B*[^]Arg/His (SNP16: previously called *ADH2*^{*}1/2) is extremely high for AD (11.667 in EAs; 1.000 in AAs), intermediate in DD (1.185 in EAs; 1.000 in AAs) and very low in DD-only (0.008 in EAs; 0.001 in AAs), suggesting that the marker is close to a risk allele for AD, but not for DD. We expected to identify markers that, if they affected risk for DD, did so through co-morbid AD, rather than through a direct effect on DD. Conversely, if a marker is close to a risk allele for DD, but not close to one for AD, the *J*-value of this marker would be high in DD-only, intermediate in DD (which is co-morbid with AD) and low in AD. Although not anticipated, we observed several associations of this kind as well, including those involving SNPs 1, 11, 20 and 22 in EAs and SNPs 9, 14, 17 and 21 in AAs. If a marker is close to risk alleles both for AD and DD (i.e. the allele affects risk for both phenotypes), the *J*-value of this marker should be high in all groups: AD, DD and DD-only. We observed this situation for SNP13 in EAs and SNPs 10 and 22 in AAs. This finding suggests that some risk alleles for AD and DD may be located in similar positions in the *ADH* gene cluster; others may be located in different positions. Some risk alleles for DD are close to some well-known functional variants, such as *ADH1B*[^]Arg/Cys (SNP14: previously called *ADH2*^{*}1/3), *ADH1C*[^]Ile/Val (SNP17: previously called *ADH3*^{*}1/2) and *ADH7*[^]Ala/Gly (SNP22).

All of the earlier-mentioned conventional association analyses, the DTR association analysis and the fine-mapping analysis suggest that risk for DD is affected by multiple *ADH* genes, consistent with their multigenic etiology, i.e. minor effects of different genes with additive effects on risk for DD. There are several possible explanations for the associations between *ADH* genes and DD: (i) the associations are completely driven by the actual associations between these genes and AD, because many patients with DD have co-morbid AD. Alcohol helps to enhance the effects of many drugs. As a specific example, cocaine and alcohol are metabolized to cocaethylene (22), which has biological properties similar to cocaine but is longer acting. Many cocaine abusers therefore prefer to use cocaine together with alcohol, which contributes to the high rate of co-morbidity of AD and CD. Thus, variants affecting risk for AD may indirectly affect risk for DD by modifying alcohol's enhancing effect on other drugs. However, we also observed many associations between these genes and DD-only (i.e. without co-morbid AD), which argues against indirect effects as a general explanation for these observations. (ii) The susceptibility to DD attributable to these genes is shared with AD susceptibility (i.e. the genes contribute independently to risk for the two phenotypes); for example, these alcohol-metabolizing genes may directly modulate risk for DD via specific pathways independent of alcohol metabolism. These pathways may overlap with the mechanism by which disulfiram exerts its treatment effect on CD. (iii) The findings could also be false positives; however, the statistical significance levels we observed argue in favor of other explanations.

A careful look at the range of physiological function of the alcohol-metabolizing enzymes also provides support for the existence of direct genetic effects on DD risk, without invoking mediation by effects on alcohol use. In addition to catalyzing the oxidation of ethanol, ADH and aldehyde dehydrogenase (ALDH) enzymes may be involved in the metabolic pathways of several neurotransmitters, including serotonin, norepinephrine and dopamine (23). For example, (i) 5-hydroxytryptamine (5-HT) is metabolized by monoamine oxidase to 5-hydroxyindole-3-acetaldehyde (5HIAL), which is either oxidized to the major metabolite 5-hydroxyindole-3-acetic acid (5HIAA) by ALDH or reduced to the minor metabolite 5-hydroxytryptophol (5HTOL) by alcohol dehydrogenase (ADH) (24). Hypo-potented γ ADH (mainly) and β ADH (partially) inhibit the turnover of 5-HIAL to 5-HTOL (25). Elevated ALDH2 activity potentiates the oxidization of 5-HIAL to 5-HIAA and decreases the production of 5-HTOL reduced from 5-HIAL (26). Low levels of 5-HTOL may predispose to self-administration of cocaine, a potential exogenous competitor for 5-HTOL, thereby compensating for the reduced effects of 5-HTOL, which could lead to CD. Inhibiting ALDH2 activity with disulfiram (Antabuse) or cyanamide (calcium carbimide, Dipsan) inhibits the oxidization of 5-HIAL to 5-HIAA and increases the production of 5-HTOL from 5-HIAL (26), which could reduce cocaine self-administration and might be the mechanism by which disulfiram exerts therapeutic effects in CD. (ii) π ADH can catalyze the reduction of norepinephrine aldehydes. Increased π ADH activity could lead to a very high turnover of norepinephrine aldehydes (27). Cocaine, which functions as a norepinephrine re-uptake inhibitor, can activate the nor-adrenergic system (28). Plasma epinephrine and norepinephrine concentrations were significantly increased in response to cocaine injection (28). Intravenous opioids also stimulate norepinephrine and acetylcholine release in cerebrospinal fluid (29). Therefore, self-administration of cocaine or opioids could elevate norepinephrine aldehydes, which could reinforce these behaviors, thereby contributing to the development of DD.

These neurotransmission signaling systems may also modulate the reinforcing or rewarding effects of abusable drugs through other neurotransmitter systems, providing a key psychomotor mechanism of the development of DD. For example, (i) the psychomotor stimulant effects of cocaine are most often thought to be mediated through enhanced dopamine or serotonin neurotransmission in mesolimbic areas of the brain (30–32). Blockade of the D3 receptor in the mesolimbic system with SB-277011A, a novel D3-selective antagonist, attenuates cocaine-

enhanced brain stimulation reward, cocaine-induced conditioned place preference and cocaine-induced reinstatement of cocaine-seeking behavior in rats (33). Dopamine signaling in the nucleus accumbens (NAc) is also thought to play an important role in regulating drug-taking and drug-seeking behaviors (34). Dopamine D1 receptor agonists and D2 receptor antagonists attenuate reinstatement of cocaine-seeking in rats through D1-like and D2 dopamine receptors in the NAc (35–37). (ii) Cocaine binding to the dopamine transporter (DAT), serotonin transporter (SERT) and norepinephrine transporter (NET) strongly inhibits the reuptake of these biogenic amines (38). These neurotransmitters play important roles in the pharmacological effects of cocaine, including the pleasurable properties, rewarding and reinforcing effects, and may also produce neurotoxic effects (39,40). Altered activities of ADH enzymes determined by *ADH* gene variation may thus modulate risk for DD via a number of different neurotransmitter signaling pathways.

In summary, we have presented the first comprehensive evidence for association of *ADH* variants with DD, irrespective of co-morbidity with AD. Associations of some of the alcohol-metabolizing enzymes with AD are well known and in fact are some of the oldest and most consistent finds in psychiatric genetics. Associations with DD [except for *ADH4*, which we reported previously (5,6)] have not been reported. Such associations might be expected on the basis of the high rate of co-morbidity of AD and DD or through the modulating effects of polymorphic variation in the genes encoding these enzymes on the effects of alcohol, even in the absence of AD. However, our results, which in some cases show stronger association with DD than AD, are not fully explained by recourse to the effects on alcohol metabolism. These associations may be better explained by physiological effects of alcohol-metabolizing enzymes on neurotransmission or indirect effects through personality (41). These results provide a novel window into the genetic risk mechanisms for DD and new support for the role of the polymorphic variation in genes encoding alcohol-metabolizing enzymes for substance dependence risk. As for any such novel results, these findings require independent replication to support their validity.

MATERIALS AND METHODS

Subjects

A total of 718 unrelated subjects, recruited at the University of Connecticut Health Center or the VA Connecticut Healthcare System—West Haven Campus, were included in this study. All subjects gave informed consent before participating in the study, which was approved by the Institutional Review Board at the respective institutions. The sample consisted of 365 healthy controls (317 EAs and 48 AAs; 219 females and 146 males) and 353 cases [including those with CD ($n = 314$) and/ or OD ($n = 150$)] (208 EAs and 145 AAs; 111 females and 242 males). Among the cases, 122 subjects (79 males and 43 females) had a diagnosis of DD in the absence of AD (i.e. DD-only). All cases met lifetime DSM-III-R criteria (42) for CD or OD, or both of these disorders; some patients ($n = 231$) also met criteria for AD. Alcohol abuse was excluded in all cases, but not all types of alcohol problems (e.g. social drinking) were excluded from this sample (an effect on alcohol problems that do not reach the DSM-III-R thresholds for abuse or dependence would be unlikely to be important as a mechanism in determining DD risk). The healthy controls were recruited from the general population through advertisement, with many recruited from local college campuses. They were screened using the Structured Clinical Interview for DSM-III-R (SCID) (43), the Computerized Diagnostic Interview Schedule for DSM-III-R (C-DIS-R) (44), the Schedule for Affective Disorders and Schizophrenia (SADS) (45) or an unstructured interview to exclude major Axis I mental disorders, including substance use disorders, psychotic disorders (including schizophrenia or schizophrenia-like disorders), mood disorders and major anxiety disorders. Males constituted 68.0% of the cases and 40.0% of the controls. The average ages were 28.1 ± 9.1 years for

controls and 37.1 ± 8.1 years for cases. The cases with co-morbid AD and the control subjects were also included in our initial studies of AD (6,7).

Genotyping

Most *ADH* SNPs were genotyped with a fluorogenic 5' nuclease assay method, i.e. the TaqMan technique; some *ADH* SNPs and the Duffy antigen gene (*FY*) marker (rs2814778) were genotyped with the reaction–restriction fragment length polymorphism (PCR–RFLP) technique; 37 STR markers were genotyped by a fluorescence capillary electrophoresis technique (5,7).

Statistical analysis

HWE test and genotype frequency analysis—HWE for the genotype frequency distribution of each marker was tested within populations and separately in cases and controls, using an exact test ($df = 1$) implemented in the program PowerMarker, with *P*-values shown in Table 3. The HWE test has been used as a valid method to detect disease–gene association in recent years (4,6,7,46–52). Genotype–phenotype associations were analyzed by comparing the genotype frequency distributions between cases and controls, with exact tests ($df = 2$) using the program PowerMarker, and the *P*-values are listed in Table 6.

Population structure analysis and structured association analysis—Both EAs and AAs were assumed to be potentially admixed populations. To measure the extent of admixture (i.e. African ancestry proportions in EAs and European ancestry proportions in AAs), 38 AIMs unlinked to one another and to the *ADH* genes were analyzed to infer ancestry proportions in each subject through the application of the program STRUCTURE (53). Parameter settings used to run STRUCTURE are presented elsewhere (5). Admixture within these two populations could confuse HWD tests or cause spurious associations. By conditioning the association analysis on the ancestry proportions of each subject, admixture effects can be controlled for statistically through an SA analysis using the program STRAT (54) [STRAT parameters were described previously (5)]. It should be noted that, in the present study, owing to the presence of HWD among the *ADH* markers, the association analysis was limited to the genotypewise level, not the allelwise level.

Correction for multiple tests—If the same data are tested multiple times from different perspectives, the tests (including the HWE tests, genotype frequency analyses and SA analyses) need correction. Each phenotype subgroup in the present study was tested for 20 SNPs. These 20 SNPs were found to be equivalent to 15 independent SNPs (calculated by SNPSpD). Thus, the α for HWE test was set at 0.0033 ($=0.05/15$) and the α for genotype frequency analyses (before and after SA analysis) was set at 0.0017 [$=0.05/(15 \times 2)$, where 2 is the test number for 'with and without SA analysis']. However, EA and AA are two separate populations (and not the same set of data) and could (in fact, it has been shown that they often do) have different risk alleles; cases and controls are also two separate populations (not the same set of data); analysis within EA and AA or within cases and controls therefore does not need correction for two tests. The analysis in cases was tested for DD and DD-only separately, but DD-only is not an independent and different phenotype, but a subset of DD, that is, DD and DD-only definitions do not describe the cases from two completely different perspectives; analyses within DD and DD-only therefore were not corrected conservatively for two tests. However, this needs to be kept in mind when interpreting the results.

DTR analysis—DTR (7), a stepwise logistic regression analysis, was used to test associations between genes and diseases. In the DTR model, diagnosis served as the dependent variable, and the covariates included ancestry proportions, age, sex, genotype probabilities at *ADH5* and *ADH6* (diplotype data were not available in these two genes because only one marker was

genotyped in each of them), diplotype probabilities at other genes and interactions among genotypes or diplotypes. More detailed justification for using DTR was introduced previously (7).

Fine-mapping the risk alleles—HWD of a marker in cases sometimes indicates a valid genetic association (4,6,7). Thus, HWD measures can be used for fine-mapping a risk locus. J , an HWD statistic, is the preferred disequilibrium measure for fine-mapping (52), because it is a direct decreasing function of the recombination fraction between the disease and the marker loci. J -value is used for fine-mapping the risk variant among a set of markers within the same locus on the basis of the relative J -values between those markers. J can be derived from the genotype frequency data (6,52). If there are several peak J values in the *ADH* gene cluster, this might suggest that there are several risk alleles for disease within that cluster (Fig. 1). On the basis of these considerations, the J statistic is the one that is best suited for fine-mapping in the present setting, as discussed previously (7).

However, there is no way to test the statistical significance of a J -value directly, to our knowledge. This is a disadvantage of using J as a fine-mapping method. To address this issue, in the present study, we combined DTR and the J statistic for fine-mapping by using DTR to screen potential susceptibility genes (i.e. test the statistical significance for candidate variants) and then using the J -value to fine-map the risk alleles within those genes.

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Web Resources

The URLs for data presented herein are as follows: dbSNP, <http://www.ncbi.nlm.nih.gov/SNP/>; Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for DD, AD, ADH1A, ADH1B, ADH1C, ADH4, ADH5, ADH6, ADH7 and ALDH2); PowerMarker, <http://www.powermarker.net/> (for genetic data analysis software).

References

1. Gelernter J, Panhuysen C, Weiss R, Brady K, Hesselbrock V, Rounsaville B, Poling J, Wilcox M, Farrer L, Kranzler HR. Genomewide linkage scan for cocaine dependence and related traits: significant linkages for a cocaine-related trait and cocaine-induced paranoia. *Am J Med Genet (Neuropsychiatr Genet)* 2005;136:45–52.
2. Gelernter J, Panhuysen C, Wilcox M, Hesselbrock V, Rounsaville B, Poling J, Weiss R, Sonne S, Zhao H, Farrer L, Kranzler HR. Genomewide linkage scan for opioid dependence and related traits. *Am J Hum Genet* 2006;78:759–769. [PubMed: 16642432]
3. Luo X, Kranzler HR, Zhao H, Gelernter J. Haplotypes at the OPRM1 locus are associated with susceptibility to substance dependence in European-Americans. *Am J Med Genet (Neuropsychiatric Genet)* 2003;120B:97–108.
4. Luo X, Kranzler HR, Zuo L, Wang S, Blumberg HP, 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;14:2421–2434. [PubMed: 16000316]

5. Luo X, Kranzler HR, Zuo L, Yang BZ, Lappalainen J, Gelernter J. ADH4 gene variation is associated with alcohol and drug dependence in European Americans: results from family-controlled and population-structured association studies. *Pharmacogenet Genomic* 2005;15:755–768.
6. Luo X, Kranzler HR, Zuo L, Lappalainen J, Yang BZ, Gelernter J. ADH4 gene variation is associated with alcohol and drug dependence in European Americans: results from HWD tests and case–control association studies. *Neuropsychopharmacology* 2006;31:1085–1095. [PubMed: 16237392]
7. Luo X, Kranzler HR, Zuo L, Wang S, Schork NJ, Gelernter J. Diplotype trend regression (DTR) analysis of the ADH gene cluster and ALDH2 gene: multiple significant associations for alcohol dependence. *Am J Hum Genet* 2006;78:973–987. [PubMed: 16685648]
8. Edenberg HJ, Xuei X, Chen HJ, Tian H, Wetherill LF, Dick DM, Almasy L, Bierut L, Bucholz KK, Goate A, et al. Association of alcohol dehydrogenase genes with alcohol dependence: a comprehensive analysis. *Hum Mol Genet* 2006;15:1539–1549. [PubMed: 16571603]
9. Gossop, M. Multiple substance use and multiple dependencies. In: Kranzler, HR.; Tinsley, JA., editors. *Dual Diagnosis and Psychiatric Treatment*. Marcel Dekker; New York: 2004. p. 129–156.
10. Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J, True W, Lin N, Toomey R, Eaves L. Co-occurrence of abuse of different drugs in men: the role of drug-specific and shared vulnerabilities. *Arch Gen Psychiatry* 1998;55:967–972. [PubMed: 9819064]
11. Kendler KS, Jacobson KC, Prescott CA, Neale MC. Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. *Am J Psychiatry* 2003;160:687–695. [PubMed: 12668357]
12. Kreek MJ, Nielsen DA, LaForge KS. Genes associated with addiction: alcoholism, opiate, and cocaine addiction. *Neuromolecular Med* 2004;5:85–108. [PubMed: 15001815]
13. Hoehe MR, Kopke K, Wendel B, Rohde K, Flachmeier C, Kidd KK, Berrettini WH, Church GM. Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. *Hum Mol Genet* 2000;19:2895–2908. [PubMed: 11092766]
14. Szeto CY, Tang NL, Lee DT, Stadlin A. Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. *Neuroreport* 2001;6:1103–1106. [PubMed: 11338173]
15. Schinka JA, Town T, Abdullah L, Crawford FC, Ordorica PI, Francis E, Hughes P, Graves AB, Mortimer JA, Mullan M. A functional polymorphism within the mu-opioid receptor gene and risk for abuse of alcohol and other substances. *Mol Psychiatry* 2002;7:224–228. [PubMed: 11840318]
16. Zhang H, Luo X, Kranzler HR, Lappalainen J, Yang BZ, Krupitsky E, Zvartau E, Gelernter J. Association between two mu-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Hum Mol Genet* 2006;15:807–819. [PubMed: 16476706]
17. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 2004;74:765–769. [PubMed: 14997420]
18. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;68:978–989. [PubMed: 11254454]
19. Stephens M, Donnelly P. A comparison of bayesian methods for haplotypes reconstruction from population genotype data. *Am J Hum Genet* 2003;73:1162–1169. [PubMed: 14574645]
20. Sasieni PD. From genotypes to genes: doubling the sample size. *Biometrics* 1997;53:1253–1261. [PubMed: 9423247]
21. Marchini J, Donnelly P, Cardon LR. Genome-wide strategies for detecting multiple loci that influence complex diseases. *Nat Genet* 2005;37:413–417. [PubMed: 15793588]
22. Harris DS, Everhart ET, Mendelson J, Jones RT. The pharmacology of cocaethylene in humans following cocaine and ethanol administration. *Drug Alcohol Depend* 2003;72:169–182. [PubMed: 14636972]
23. Höög JO, Hedberg JJ, Strömberg P, Svensson S. Mammalian alcohol dehydrogenase—functional and structural implications. *J Biomed Sci* 2001;8:71–76. [PubMed: 11173978]
24. Consalvi V, Mardh G, Vallee BL. Human alcohol dehydrogenases and serotonin metabolism. *Biochem Biophys Res Commun* 1986;139:1009–1016. [PubMed: 2429655]
25. Svensson S, Some M, Lundsjö A, Helander A, Cronholm T, Höög JO. Activities of human alcohol dehydrogenases in the metabolic pathways of ethanol and serotonin. *Eur J Biochem* 1999;262:324–329. [PubMed: 10336614]

26. Beck O, Helander A, Carlsson S, Borg S. Changes in serotonin metabolism during treatment with the aldehyde dehydrogenase inhibitors disulfiram and cyanamide. *Pharmacol Toxicol* 1995;77:323–326. [PubMed: 8778744]
27. Mardh G, Dingley AL, Auld DS, Vallee BL. Human class II (pi) alcohol dehydrogenase has a redox-specific function in norepinephrine metabolism. *Proc Natl Acad Sci* 1986;83:8908–8912. [PubMed: 3466164]
28. Sofuoglu M, Nelson D, Babb DA, Hatsukami DK. Intravenous cocaine increases plasma epinephrine and norepinephrine in humans. *Pharmacol Biochem Behav* 2001;68:455–459. [PubMed: 11325399]
29. Bouaziz H, Tong C, Yoon Y, Hood DD, Eisenach JC. Intravenous opioids stimulate morepinephrine and acetylcholine release in spinal cord dorsal horn. Systematic studies in sheep and an observation in a human. *Anesthesiol* 1996;84:143–154.
30. Kelly PH, Iversen SD. Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. *Eur J Pharmacol* 1976;40:45–56. [PubMed: 1033072]
31. Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. *Psychol, Rev* 1987;94:469–492. [PubMed: 3317472]
32. Hanania T, McCreary AC, Salaz DO, Lyons AM, Zahniser NR. Differential regulation of cocaine-induced locomotor activity in inbred long-sleep and short-sleep mice by dopamine and serotonin systems. *Eur J Pharmacol* 2004;502:221–231. [PubMed: 15476748]
33. Vorel SR, Ashby CR Jr, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, Gardner EL. Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci* 2002;22:9595–9603. [PubMed: 12417684]
34. Bachtell RK, Whisler K, Karanian D, Self DW. Effects of intranucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat. *Psychopharmacology* 2005;183:41–53. [PubMed: 16163523]
35. Milivojevic N, Krisch I, Sket D, Živin M. The dopamine D1 receptor agonist and D2 receptor antagonist LEK-8829 attenuates reinstatement of cocaine-seeking in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 2004;369:576–582.
36. Xi ZX, Newman AH, Gilbert JG, Pak AC, Peng XQ, Ashby CR Jr, Gitajn L, Gardner EL. The Novel Dopamine D(3) Receptor Antagonist NGB 2904 Inhibits Cocaine's Rewarding Effects and Cocaine-Induced Reinstatement of Drug-Seeking Behavior in Rats. *Neuropsychopharmacology* 2006;31:1393–1405. [PubMed: 16205781]
37. Bari AA, Pierce RC. D1-like and D2 dopamine receptor antagonists administered into the shell subregion of the rat nucleus accumbens decrease cocaine, but not food, reinforcement. *Neuroscience* 2005;135:959–968. [PubMed: 16111825]
38. Carroll FI, Kotiau P, Dehghani A, Gray JL, Kuzemko MA, Parham KA, Abraham P, Lewin AH, Boja JW, Kuhar MJ. Cocaine and 3 beta-(4'-substituted phenyl) tropane-2 beta-carboxylic acid ester and amide analogues. New high-affinity and selective compounds for the dopamine transporter. *J Med Chem* 1995;38:379–388. [PubMed: 7830281]
39. Belej T, Manji D, Sioutis S, Barros HM, Nobrega JN. Changes in serotonin and norepinephrine uptake sites after chronic cocaine: pre- vs. post-withdrawal effects *Brain Res* 1996;736:287–296.
40. Sora I, Hall FS, Andrews AM, Itokawa M, Li XF, Wei HB, Wichems C, Lesch KP, Murphy DL, Uhl GR. Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc Natl Acad Sci USA* 2001;98:5300–5305. [PubMed: 11320258]
41. Luo X, Kranzler HR, Zuo L, Wang S, Gelernter J. Personality traits of agreeableness and extraversion are associated with *ADH4* variation. *Biol Psychiatry*. 10.1016/j.biopsych.2006.05.017(in press)
42. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 3. American Psychiatric Press; Washington, D.C.: 1987.
43. Spitzer RL, Williams JBW, Gibbon M, First MB. The Structured Clinical interview for DSM-III-R: I. History, rationale and description. *Arch Gen Psychiatry* 1992;49:624–629. [PubMed: 1637252]
44. Blouin AG, Perez EL, Blouin JH. Computerized administration of the diagnostic interview schedule. *Psych Res* 1988;23:335–344.

45. Endicott J, Spitzer RL. A diagnostic interview: the schedule for affective disorders and schizophrenia. *Arch Gen Psychiatry* 1978;35:837–844. [PubMed: 678037]
46. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R Jr, Ellis MC, Fullan A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399–408. [PubMed: 8696333]
47. Nielsen DM, Ehm MG, Weir BS. Detecting marker–disease association by testing for Hardy–Weinberg disequilibrium at a marker locus. *Am J Hum Genet* 1999;63:1531–1540. [PubMed: 9867708]
48. Hoh J, Wille A, Ott J. Trimming, weighting, and grouping SNPs in human case–control association studies. *Genome Res* 2001;11:2115–2119. [PubMed: 11731502]
49. Lee WC. Searching for disease-susceptibility loci by testing for Hardy–Weinberg disequilibrium in a gene bank of affected individuals. *Am J Epidemiol* 2003;158:397–400. [PubMed: 12936892]
50. Hao K, Xu X, Laird N, Wang X, Xu X. Power estimation of multiple SNP association test of case–control study and application. *Genet Epidemiol* 2004;26:22–30. [PubMed: 14691954]
51. Wittke-Thompson JK, Pluzhnikov A, Cox NJ. Rational inferences about departures from Hardy–Weinberg equilibrium. *Am J Hum Genet* 2005;76:967–986. [PubMed: 15834813]
52. Jiang R, Dong J, Wang D, Sun FZ. Fine-scale mapping using Hardy–Weinberg disequilibrium. *Ann Hum Genet* 2001;65:207–219. [PubMed: 11427179]
53. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;155:945–959. [PubMed: 10835412]
54. Pritchard JK, Stephens M, Rosenberg NA, Donnelly P. Association mapping in structured populations. *Am J Hum Genet* 2000;67:170–181. [PubMed: 10827107]

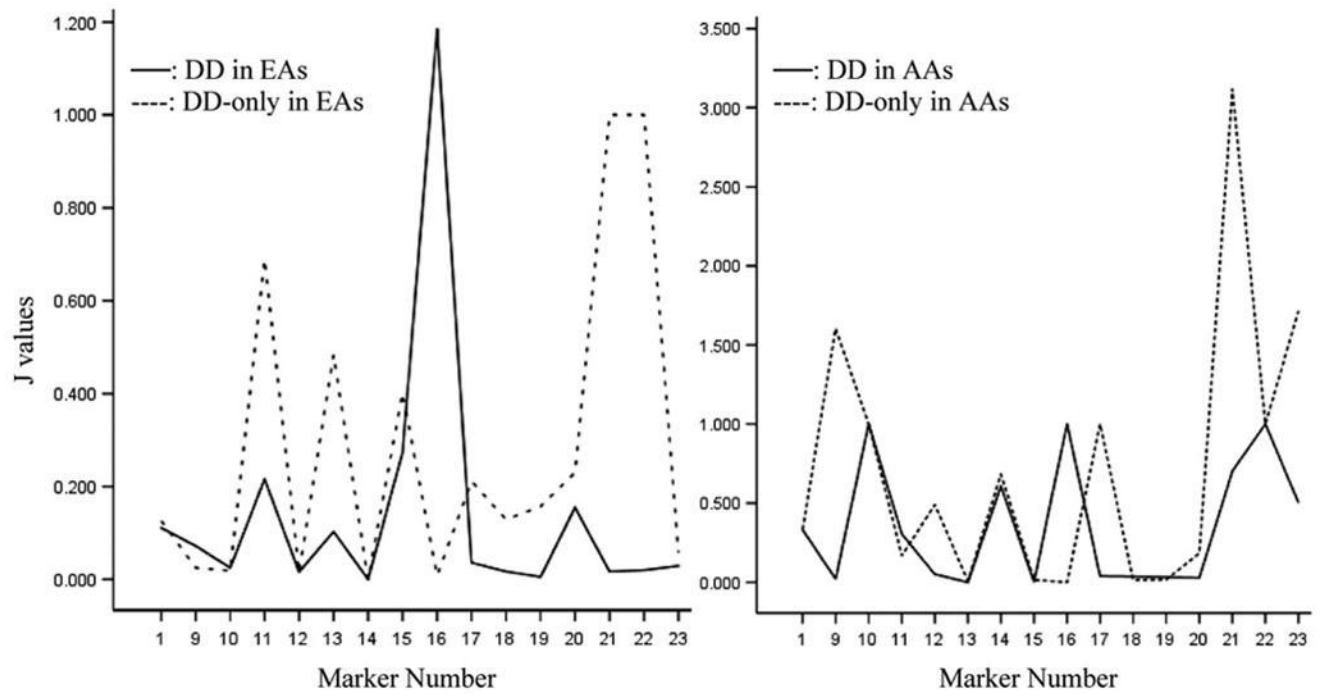


Figure 1.

Fine-mapping the risk alleles at the *ADH* gene cluster in EAs and AAs according to the *J*-values. *X*-axis represents the marker names which correspond to the order as presented in Table 1; *Y*-axis represents the *J*-values. Note that SNPs 2 to 8 are not included in this figure; data regarding these markers (in *ADH*₄) were reported previously (6).

Table 1The information of *ADH* markers examined in the study

Marker	rs no. or hcv no.	Alias	Substitution	Amino acid
ADH5 ^Δ SNP1	rs1154400		C/T	
ADH4 ^Δ SNP2	hcv2033010		T/C	
ADH4 ^Δ SNP3	rs1042364		G/A	Gly/Arg
ADH4 ^Δ SNP4	rs1126671		G/A	Val/Ile
ADH4 ^Δ SNP5	rs1126670		T/G	Pro/Pro
ADH4 ^Δ SNP6	rs7694646		A/T	
ADH4 ^Δ SNP7	rs1800759	A-75C	A/C	
ADH4 ^Δ SNP8	rs1984362		C/T	
ADH6 ^Δ SNP9	hcv320091		A/T	
ADH1A ^Δ SNP10	rs6837311		A/T	
ADH1A ^Δ SNP11	rs975833		C/G	
ADH1A ^Δ SNP12	rs1229966		A/G	
ADH1B ^Δ SNP13	rs1042026		C/T	
ADH1B ^Δ SNP14	rs2066702	ADH2*1/3	C/T	Arg/Cys
ADH1B ^Δ SNP15	rs2066701	C96T	C/T	
ADH1B ^Δ SNP16	rs1229984	ADH2*1/2	G/A	Arg/His
ADH1C ^Δ SNP17	rs698	ADH3*1/2	A/G	Ile/Val
ADH1C ^Δ SNP18	rs1693482		A/G	Gln/Arg
ADH1C ^Δ SNP19	rs1693427		C/T	
ADH7 ^Δ SNP20	rs284786		A/T	
ADH7 ^Δ SNP21	rs971074		C/T	Arg/Arg
ADH7 ^Δ SNP22	rs1573496		C/G	Ala/Gly
ADH7 ^Δ SNP23	rs1154470		A/G	

Chromosome positions, map locations and genotyping methods for these markers were introduced in Luo *et al.* (5,7). rs numbers were obtained from the NCBI SNP database.

Table 2

Genotype and allele frequencies in EAs and AAs

Marker	Genotype and allele	EAs		AAs		Controls		DD-only		Controls			
		f	n	f	n	f	n	f	n	f	n		
ADH5`SNP1	T/T	0.483	97	0.364	136	0.459	71	0.504	29	0.558	20	0.417	20
ADH5`SNP1	T/C	0.353	71	0.409	135	0.456	63	0.447	21	0.404	24	0.500	24
ADH5`SNP1	C/C	0.164	33	0.227	25	0.084	7	0.050	2	0.038	4	0.083	4
ADH5`SNP1	T	0.659	265	0.568	407	0.688	205	0.727	79	0.760	64	0.667	64
ADH5`SNP1	C	0.341	137	0.432	185	0.313	77	0.273	25	0.240	32	0.333	32
ADH6`SNP9	T/T	0.319	65	0.214	83	0.278	108	0.755	44	0.800	35	0.729	35
ADH6`SNP9	A/T	0.466	95	0.486	140	0.468	30	0.210	9	0.164	13	0.271	13
ADH6`SNP9	A/A	0.216	44	0.300	76	0.254	5	0.035	2	0.036	0	0.000	0
ADH6`SNP9	T	0.551	225	0.457	306	0.512	246	0.860	97	0.882	83	0.865	83
ADH6`SNP9	A	0.449	183	0.543	292	0.488	40	0.140	13	0.118	13	0.135	13
ADH1A`SNP10	T/T	0.408	76	0.290	106	0.353	121	0.840	51	0.895	37	0.787	37
ADH1A`SNP10	A/T	0.456	94	0.507	142	0.473	23	0.160	6	0.105	10	0.213	10
ADH1A`SNP10	A/A	0.136	28	0.203	52	0.173	0	0.000	0	0.000	0	0.000	0
ADH1A`SNP10	T	0.636	262	0.543	354	0.590	265	0.920	108	0.947	84	0.894	84
ADH1A`SNP10	A	0.364	150	0.457	246	0.410	23	0.080	6	0.053	10	0.106	10
ADH1A`SNP11	G/G	0.591	120	0.586	169	0.569	76	0.528	23	0.404	21	0.438	21
ADH1A`SNP11	C/G	0.369	75	0.400	108	0.364	52	0.361	21	0.368	26	0.542	26
ADH1A`SNP11	C/C	0.039	8	0.014	20	0.067	16	0.111	13	0.228	1	0.021	1
ADH1A`SNP11	G	0.776	315	0.786	446	0.751	204	0.708	67	0.588	68	0.708	68
ADH1A`SNP11	C	0.224	91	0.214	148	0.249	84	0.292	47	0.412	28	0.292	28
ADH1A`SNP12	A/A	0.394	80	0.493	123	0.423	19	0.133	7	0.121	3	0.063	3
ADH1A`SNP12	A/G	0.478	97	0.420	127	0.436	57	0.399	19	0.328	24	0.500	24
ADH1A`SNP12	G/G	0.128	26	0.087	41	0.141	67	0.469	32	0.552	21	0.438	21
ADH1A`SNP12	A	0.633	257	0.703	373	0.641	95	0.332	33	0.284	30	0.313	30
ADH1A`SNP12	G	0.367	149	0.297	209	0.359	191	0.668	83	0.716	66	0.688	66
ADH1B`SNP13	T/T	0.512	103	0.545	154	0.520	121	0.840	48	0.828	43	0.915	43
ADH1B`SNP13	T/C	0.418	84	0.424	115	0.389	22	0.153	10	0.172	4	0.085	4
ADH1B`SNP13	C/C	0.070	14	0.030	27	0.091	1	0.007	0	0.000	0	0.000	0
ADH1B`SNP13	T	0.721	290	0.758	423	0.715	264	0.917	106	0.914	90	0.957	90
ADH1B`SNP13	C	0.279	112	0.242	169	0.285	24	0.083	10	0.086	4	0.043	4
ADH1B`SNP14	C/C	1.000	198	1.000	280	1.000	96	0.696	33	0.600	24	0.511	24
ADH1B`SNP14	T/C	0.000	0	0.000	0	0.000	35	0.254	16	0.291	22	0.468	22
ADH1B`SNP14	T/T	0.000	0	0.000	0	0.000	7	0.051	6	0.109	1	0.021	1
ADH1B`SNP14	C	0.000	0	0.000	0	0.000	0	0.822	82	0.745	70	0.745	70
ADH1B`SNP14	T	0.000	0	0.000	0	0.000	49	0.178	28	0.255	24	0.255	24
ADH1B`SNP15	C/C	0.513	97	0.571	116	0.487	112	0.818	43	0.782	43	0.915	43
ADH1B`SNP15	T/C	0.434	82	0.397	101	0.424	24	0.175	12	0.218	4	0.085	4
ADH1B`SNP15	T/T	0.053	10	0.032	21	0.088	1	0.007	0	0.000	0	0.000	0
ADH1B`SNP15	C	0.730	276	0.770	333	0.700	248	0.905	98	0.891	90	0.957	90
ADH1B`SNP15	T	0.270	102	0.230	143	0.300	26	0.095	12	0.109	4	0.043	4
ADH1B`SNP16	G/G	0.904	160	0.836	215	0.888	117	0.967	43	0.935	41	0.953	41
ADH1B`SNP16	A/G	0.090	16	0.164	27	0.112	4	0.033	3	0.065	2	0.047	2
ADH1B`SNP16	A/A	0.006	1	0.000	0	0.000	0	0.000	0	0.000	0	0.000	0
ADH1B`SNP16	G	0.949	336	0.918	457	0.944	238	0.983	89	0.967	84	0.977	84
ADH1B`SNP16	A	0.051	18	0.082	27	0.056	4	0.017	3	0.033	2	0.023	2
ADH1C`SNP17	A/A	0.348	64	0.254	105	0.376	85	0.669	38	0.792	35	0.761	35
ADH1C`SNP17	A/G	0.505	93	0.627	126	0.452	42	0.331	10	0.208	10	0.217	10
ADH1C`SNP17	G/G	0.147	27	0.119	48	0.172	0	0.000	0	0.000	1	0.022	1
ADH1C`SNP17	A	0.601	221	0.568	336	0.602	212	0.835	86	0.896	80	0.870	80
ADH1C`SNP17	G	0.399	147	0.432	222	0.398	42	0.165	10	0.104	12	0.130	12
ADH1C`SNP18	G/G	0.371	75	0.309	113	0.382	99	0.683	45	0.789	38	0.792	38
ADH1C`SNP18	A/G	0.480	97	0.574	130	0.439	46	0.317	12	0.211	9	0.188	9

Marker	Genotype and allele	EAS		AAS		Controls		DD-only		Controls	
		DD	n	DD	n	n	f	n	f	n	f
ADH1C*SNP18	A/A	30	0.149	8	0.118	53	0.179	0	0.000	1	0.021
ADH1C*SNP18	G	247	0.611	81	0.596	356	0.601	102	0.841	85	0.885
ADH1C*SNP18	A	157	0.389	55	0.404	236	0.399	12	0.159	11	0.115
ADH1C*SNP19	T/T	68	0.360	17	0.288	111	0.370	43	0.699	38	0.792
ADH1C*SNP19	T/C	91	0.481	35	0.593	132	0.440	13	0.301	9	0.188
ADH1C*SNP19	C/C	30	0.159	7	0.119	57	0.190	0	0.000	1	0.021
ADH1C*SNP19	T	227	0.601	69	0.585	354	0.590	99	0.849	85	0.885
ADH1C*SNP19	T/T	151	0.399	49	0.415	246	0.410	13	0.151	11	0.115
ADH1C*SNP19	A/T	105	0.522	39	0.582	147	0.497	16	0.276	9	0.188
ADH7*SNP20	A/T	77	0.383	23	0.343	116	0.392	23	0.486	22	0.458
ADH7*SNP20	A/A	19	0.095	5	0.075	33	0.111	19	0.328	17	0.354
ADH7*SNP20	T	287	0.714	101	0.754	410	0.693	153	0.524	40	0.417
ADH7*SNP20	A	115	0.286	33	0.246	182	0.307	61	0.476	56	0.583
ADH7*SNP21	C/C	158	0.775	53	0.779	225	0.771	48	0.712	24	0.511
ADH7*SNP21	T/C	40	0.196	15	0.221	62	0.212	7	0.240	20	0.426
ADH7*SNP21	T/T	6	0.029	0	0.000	5	0.017	7	0.048	3	0.064
ADH7*SNP21	C	356	0.873	121	0.890	512	0.877	243	0.832	68	0.723
ADH7*SNP21	T	52	0.127	15	0.110	72	0.123	49	0.168	26	0.277
ADH7*SNP22	G/G	163	0.795	54	0.783	242	0.796	142	0.979	44	0.936
ADH7*SNP22	C/G	36	0.176	15	0.217	56	0.184	3	0.021	3	0.064
ADH7*SNP22	C/C	6	0.029	0	0.000	6	0.020	0	0.000	0	0.000
ADH7*SNP22	G	362	0.883	123	0.891	540	0.888	287	0.990	91	0.968
ADH7*SNP22	C	48	0.117	15	0.109	68	0.112	3	0.010	3	0.032
ADH7*SNP23	G/G	83	0.439	28	0.452	133	0.452	103	0.763	36	0.750
ADH7*SNP23	A/G	81	0.429	25	0.403	118	0.404	31	0.230	11	0.229
ADH7*SNP23	A/A	25	0.132	9	0.145	41	0.140	1	0.007	1	0.021
ADH7*SNP23	G	247	0.653	81	0.653	384	0.658	237	0.878	83	0.865
ADH7*SNP23	A	131	0.347	43	0.347	200	0.342	33	0.122	13	0.135

DD, drug dependence; DD-only, DD in observe of alcohol dependence; n, individual number (for genotype) or chromosome number (for allele); f, frequency. The expected genotype frequencies (p^2 , $2pq$ and q^2) can be derived from the observed allele frequencies (p and q).

Table 3*P*-values for HWE tests in EAs and AAs

Marker	EAs DD	DD-only	AAs DD	DD-only
ADH5 ^Δ SNP1	0.0016	—	—	—
ADH6 ^Δ SNP9	—	—	0.068	0.018
ADH1A ^Δ SNP11	—	—	—	0.061
ADH1B ^Δ SNP14	N/A	N/A	0.080	0.080
ADH1B ^Δ SNP16	0.060	—	0.025	—
ADH1C ^Δ SNP17	—	0.038	0.006	—
ADH1C ^Δ SNP18	—	—	0.011	—
ADH1C ^Δ SNP19	—	0.056	0.015	—
ADH7 ^Δ SNP21	0.047	—	—	0.015
ADH7 ^Δ SNP22	0.037	—	—	—
ADH7 ^Δ SNP23	—	—	—	0.031

N/A, not applicable due to non-polymorphic or rare frequency; absence of a *P*-value denotes $P > 0.10$; markers with $P > 0.10$ in all phenotype groups (including controls) are not listed.

Table 4

DTR analysis in EAs and AAs

EAs Variables	<i>f</i>	<i>P</i> (β) DD	DD-only	AAs Variables	<i>f</i>	<i>P</i> (β) DD	DD-only
European ancestry		0.0317(-)					
Male		2.8E-05 (+)	0.0001(+)	Male		0.0110 (+)	
Age		2.8E-17 (+)	3.9E-08 (+)	Age		0.0113 (+)	
ADH5: C/C		0.0035(+)		ADH6: T/T			0.0880 (-)
ADH1A: AGA/TGA	0.203	0.0167(+)	0.0019(+)	ADH1A: AGA/TCG	0.057	0.0354 (-)	
AGA/TCG	0.181	0.0167(+)	0.0019(+)	ADH1B: TCCG/TCG	0.425	0.0112 (-)	0.0209 (-)
AGA/AGA	0.164	0.0168(+)	0.0019(+)	TTCG/TCCG	0.270	0.0018 (-)	0.0036 (-)
TCG/TGA	0.109	0.0167(+)	0.0019(+)	TCCG/CCTG	0.113	0.0698 (-)	
AGA/TGG	0.088	0.0168(+)	0.0019(+)	ADH1C: AGT/GAC	0.275	0.0252 (+)	
TGA/TGA	0.060	0.0166(+)	0.0019(+)	ADH7: ACGG/TCGG	0.216	0.0122 (+)	0.0114 (+)
TCG/TGG	0.060	0.0168(+)	0.0019(+)	ACGG/ACGG	0.124		0.0035 (+)
TGA/TGG	0.057	0.0166(+)	0.0019(+)	TCGG/TCGG	0.108	0.0445 (+)	0.0035 (+)
ADH1B: CCTG/CCTG	0.075	0.0189(+)					
ADH1C: AGT/AGT	0.445		0.0033(-)				
AGT/GAC	0.353	0.0357(-)	0.0028(-)				
GAC/GAC	0.175		0.0031(-)				
ADH7: ACGG/TCGG	0.094		0.0841(+)				
TCGA/TCGA	0.039		0.0642(+)				

P, *P*-values; β , regression coefficient; only the signs, but not the values, of β are shown in this table (+ values of β reflect increased risk of the disorder when the diplotype is present; - values reflect a protective effect of the diplotype). The diplotype frequencies (*f*) in cases + controls within EAs and AAs are listed.

Table 5*J* values for each marker in two populations

SNPs	EAs AD	DD	DD-only	AAs AD	DD	DD-only
1	0.051	0.111	0.126	0.439	0.334	0.334
9	0.014	0.072	0.025	0.010	0.021	1.604
10	0.018	0.025	0.019	1.000	1.000	1.000
11	0.047	0.216	0.689	0.182	0.306	0.168
12	0.030	0.016	0.015	0.009	0.051	0.491
13	0.226	0.103	0.484	0.004	0.000	0.009
14	0.000	0.000	0.000	0.112	0.609	0.684
15	0.041	0.273	0.401	0.004	0.002	0.015
16	11.667	1.185	0.008	1.000	1.000	0.001
17	0.061	0.036	0.211	0.053	0.039	1.000
18	0.072	0.017	0.129	0.048	0.036	0.014
19	0.070	0.005	0.157	0.038	0.032	0.017
20	0.055	0.155	0.230	0.149	0.028	0.185
21	0.004	0.017	1.000	0.135	0.703	3.118
22	0.006	0.020	1.000	1.000	1.000	1.000
23	0.007	0.029	0.058	0.030	0.504	1.716

SNP numbers correspond to Table 1.

Table 6
P-values for case–control comparisons on genotype frequency distributions

Marker	Before control for admixture				After control for admixture			
	EAs DD	DD- only	AAs DD	DD-only	EAs DD	DD- only	AAs DD	DD-only
ADH5 ^A SNP1	0.008	0.006	—	—	0.012	0.004	—	—
ADH1A ^A SNP11	—	—	0.031	0.004	—	—	0.021	0.001
ADH1B ^A SNP14	N/A	N/A	0.020	0.072	N/A	N/A	0.026	—
ADH1B ^A SNP15	—	—	—	0.059	—	—	0.098	0.061
ADH1C ^A SNP17	—	0.053	0.064	—	—	0.047	—	—
ADH1C ^A SNP18	—	—	0.046	—	—	—	—	—
ADH1C ^A SNP19	—	0.091	0.063	—	—	0.094	—	—
ADH7 ^A SNP21	—	—	0.029	0.0006	—	—	0.016	<1.E-6
ADH7 ^A SNP22	—	—	0.034	—	—	—	—	—

N/A and no *P*-value same as Table 3; markers with *P* > 0.10 in all phenotype groups are not listed. Before control for admixture, conventional case–control comparison before controlling for admixture effects; After control for admixture, case–control comparison after controlling for admixture effects (SA analysis). *P*-values in AD group were presented previously (7).