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Alcohol metabolizing genes and alcohol phenotypes in an Israeli household sample

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Abstract

Background—*ADH1B* and *ADH1C* variants have been robustly associated with alcohol phenotypes in East Asian populations but less so in non-Asian populations where prevalence of the most protective *ADH1B* allele is low (generally <5%). Further, the joint effects of *ADH1B* and *ADH1C* on alcohol phenotypes have been unclear. Therefore, we tested the independent and joint effects of *ADH1B* and *ADH1C* on alcohol phenotypes in an Israeli sample, with higher prevalence of the most protective *ADH1B* allele than other non-Asian populations.

Methods—A structured interview assessed lifetime drinking and alcohol use disorders (AUDs) in adult Israeli household residents. Four single nucleotide polymorphisms (SNPs) were genotyped: *ADH1B* (rs1229984, rs1229982, rs1159918) and *ADH1C* (rs698). Regression analysis examined the association between alcohol phenotypes and each SNP (absence vs. presence of the protective allele) as well as rs698/rs1229984 diplotypes (also indicating absence or presence of protective alleles) in lifetime drinkers (N=1,129).

Results—Lack of the *ADH1B* rs1229984 protective allele was significantly associated with consumption- and AUD-related phenotypes (OR=1.77 for AUD; OR=1.83 for risk drinking), while lack of the *ADH1C* rs698 protective allele was significantly associated with AUD-related phenotypes (OR=2.32 for AUD). Diplotype analysis indicated that jointly, *ADH1B* and *ADH1C* significantly influenced AUD-related phenotypes. For example, among those without protective alleles for *ADH1B* or *ADH1C*, OR for AUD was 1.87 as compared to those without the protective

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allele for *ADH1B* only and 3.16 as compared to those with protective alleles at both *ADH1B* and *ADH1C*.

Conclusions—This study adds support for the relationship of *ADH1B* and *ADH1C* to alcohol phenotypes in non-Asians. Further, these findings help clarify the mixed results from previous studies by showing that *ADH1B* and *ADH1C* jointly effect AUDs, but not consumption. Studies of the association of alcohol phenotypes and either *ADH1B* or *ADH1C* alone may employ an oversimplified model, masking relevant information.

5 key words/phrases

ADH1B; *ADH1C*; Alcohol use disorders; Alcohol consumption; Israel

Introduction

Heavy alcohol consumption and alcohol use disorders (AUDs) significantly impact public health by increasing physical and mental health problems and related costs (Rehm et al., 2009). Many factors influence the risk for these drinking phenotypes, including genetic variation (Kendler et al., 2007). Among the most widely studied risk genes for AUDs are alcohol dehydrogenase genes, which control the oxidation of alcohol (ethanol) to acetaldehyde in alcohol metabolism.

The association between alcohol dehydrogenase 1B (*ADH1B*) and alcohol phenotypes (e.g., alcohol dependence [AD] and abuse) has been studied extensively (Li et al., 2011). The most consistent results come from East Asian populations, with fewer positive findings in Europeans (Li et al., 2011; Toth et al., 2011; Bierut et al., 2012). One explanation for differences by population is varying prevalence of the most protective *ADH1B* allele (in single nucleotide polymorphism [SNP] rs1229984, referred to as *ADH1B*2*), which is common in East Asians (>60%; Eng et al., 2007; Li et al., 2007), but rare in those with Western and Central European origin (<5%; Hurley and Edenberg, 2012). Most Jewish populations are generally of European origin, but have higher prevalence of *ADH1B*2* than other European populations (20-21% (Neumark et al., 1998; Hasin et al., 2002b)). Therefore, the Israeli Jewish population represents a potentially better powered non-Asian population in which to further study this association. Furthermore, rs1229984 is the most widely studied *ADH1B* SNP in European populations, but two additional functional SNPs (rs1159918, rs1229982), found in the proximal promoter region, were associated with alcoholism in some studies (Edenberg, 2007; Pochareddy & Edenberg, 2011). These associations have not been confirmed in other populations and association between these SNPs and additional alcohol phenotypes (consumption, DSM-IV AUD) has not been examined; further investigation is warranted.

Alcohol dehydrogenase 1C (*ADH1C*) is another widely studied gene related to alcohol metabolism. Meta-analysis indicated an association between the protective *ADH1C* allele (in SNP rs698, referred to as *ADH1C*2*) and lower consumption and/or AUDs (Li et al., 2012). As with *ADH1B*, this association is found more consistently in East Asians (Li et al., 2012) than non-Asians (Zintzaras et al., 2006; Tolstrup et al., 2008; Toth et al., 2011; Olfson & Bierut, 2012). Earlier work on rs698 in Jewish Israeli samples found no association with consumption (Neumark et al., 1998), or alcohol elimination rates (Neumark et al., 2004), perhaps due to low power to detect associations in small samples. In addition, these studies did not examine AUDs. Therefore, investigation of rs698 and multiple alcohol phenotypes in non-Asian populations is warranted.

Because *ADH1B* and *ADH1C* are adjacent on chromosome 4, two main possibilities exist for their effects on alcohol phenotypes: (1) each influences the risk for alcohol phenotypes

or (2) the associations found with one gene are due to "real" associations with the other. There is considerable linkage disequilibrium (LD) across the set of ADH genes in individuals of European decent (Luo et al., 2006; Edenberg, 2007). Further, different combinations of *ADH1B* and *ADH1C* (based on different genotypes) lead to interindividual differences in ethanol oxidation (Hurley et al., 1990). Therefore, both risk variants should be analyzed jointly to further understand how these SNPs influence alcohol phenotypes.

Understanding the joint effects of these variants on alcohol use behavior is important. Numerous studies have examined interaction effects between ADH genes in East Asians (Wu et al., 2012), but only three studies have explicitly examined the joint effect of *ADH1B*-rs1229984 and *ADH1C*-rs698 on alcohol outcomes in non-Asian populations (Neumark et al., 2004; Tolstrup et al., 2008; Toth et al., 2011). While all three studies showed an effect of *ADH1B* on alcohol-related phenotypes, only one study found an effect of *ADH1C* on alcohol-related phenotypes (alcohol problems) as well as a joint effect of *ADH1B* and *ADH1C* (Toth et al., 2011). These studies varied in many respects, including ancestry and genotype frequencies, phenotypes, and methods of testing interaction between *ADH1B* and *ADH1C* SNPs, leaving the cumulative interpretation unclear. Therefore, investigating the joint effects of *ADH1B* and *ADH1C* on alcohol phenotypes (DSM-IV AUDs, consumption) in large samples with sufficient power remains necessary.

Given the gaps in understanding the independent and joint effects of *ADH1B* and *ADH1C* on alcohol consumption and AUDs, specifically in non-Asian populations, this study addressed the following: (1) association of *ADH1B*-rs1229984 with multiple alcohol phenotypes (both consumption and AUDs); (2) association of alcohol phenotypes and regulatory *ADH1B* SNPs (rs1229982, rs1159918); (3) association of *ADH1C*-rs698 and alcohol phenotypes; and (4) joint effects of *ADH1B*-rs1229984 and *ADH1C*-rs698, examined through diplotype analysis. For analysis, each individual SNP was coded as a risk factor, indicating absence of the protective allele (1-3); for diplotype analysis, three risk categories were hypothesized based on absence of protective alleles for *ADH1B*-rs1229984 and *ADH1C*-rs698. We used data from a large general population sample of Israeli Jews (Shmulewitz et al., 2010; Shmulewitz et al., 2012). This sample consists of Israelis from a variety of backgrounds with different drinking cultures (Shmulewitz et al., 2012) and some genetic heterogeneity, reflecting contributions from Northern and Southern European ancestral populations, but little Asian or African contributions (Listman et al., 2010).

Materials and Methods

Study procedures and sample

Data were collected in 2007-2009 from 1,349 adult household residents (Shmulewitz et al., 2010; Shmulewitz et al., 2012). This study was designed to investigate environmental and genetic influences on alcohol-related traits; drinking among Israeli women has low prevalence, so males were oversampled (Hasin et al., 1998; Spivak et al., 2007; Shmulewitz et al., 2010). Interviewers received structured training and administered face-to-face computer-assisted interviews after obtaining written informed consent, as approved by relevant American and Israeli IRBs (Shmulewitz et al., 2010). The overall response rate was 68.9%. Quality control included field observation, reviews of recorded interviews, and telephone verification of responses. The association analysis included 1,129 ever-drinkers (drank any alcohol, lifetime) who were genotyped for the *ADH1B* and *ADH1C* SNPs. Of these, 25.2% (N=284) were 21-29 years old, 33.7% (N=380) were 30-44, and 41.2% (465) were 45+; 78.3% (N=884) were male; and 23.9% (N=270) were immigrants from the Former Soviet Union (FSU).

Measures

Lifetime alcohol use disorders (AUD)

The Alcohol Use Disorders and Associated Disabilities Interview Schedule (AUDADIS; (Grant et al., 1995; Grant et al., 2003) was used to diagnose lifetime alcohol dependence (AD) following the DSM-IV guidelines, i.e., three or more dependence criteria within a 12-month period (American Psychiatric Association, 2000). Since DSM-5 will combine AD and alcohol abuse (AA) (American Psychiatric Association, 2010), we also included "any AUD", based on presence of AD or AA (one or more abuse criteria in the absence of lifetime AD). As reviewed previously (Hasin et al., 2006), reliability and validity of AUDADIS-IV alcohol diagnoses in international general population samples ranges from good to excellent. We also included count phenotypes: AD severity (number of AD criteria endorsed, range 0-7) and AUD severity (number of AD and AA criteria endorsed, range 0-11). Test-retest reliability of AUDADIS count variables is good to excellent in general and clinical samples (intraclass correlation coefficients [ICC]=0.63-0.86;(Grant et al., 1995; Hasin et al., 1997a). Combining AD and AA criteria was supported this sample, showing that the alcohol criteria were unidimensional (Shmulewitz et al., 2010).

Alcohol consumption

Using the AUDADIS alcohol consumption measures, we created a variable indicating maximum number of drinks in a 24 hour period (Maxdrinks;(Saccone et al., 2000; Shmulewitz et al., 2012). AUDADIS consumption items have good psychometric properties and good to excellent inter-rater reliability (ICC=0.59-0.88 for quantity consumed) (Hasin et al., 1997a; Grant et al., 2003). Weekly at-risk drinking (risk drinking) was defined as ≥5 drinks in a day for males or ≥4 in a day for females at least once a week during period of heaviest drinking, and assessed with questions querying the frequency of drinking specific quantities, as described previously (Keyes et al., 2009). Reliability of AUDADIS lifetime drinking frequency was very good to excellent (ICCs 0.72-0.76), and fair-to-good for ≥5 drinks (ICCs 0.47-0.69) (Hasin et al., 1997b; Grant et al., 2003).

Genotyping

DNA was extracted from blood or saliva using standard DNA isolation products (Roche Diagnostics, Germany; QIAGEN, USA; DNA Genotek, USA). The four SNP assays (*ADH1B*: rs1229984, rs1229982, rs1159918; and *ADH1C*: rs698) were designed for the Sequenom MassArray system (Sequenom, USA). Genotyping was done using a modified single nucleotide extension reaction (iPLEX assays) with allele detection by mass spectrometry (Sequenom MassArray System). For quality control, assays were tested on two sets of 40 unrelated individuals from the Coriell European and African-American samples (Coriell Institute for Medical Research, USA), and Hardy-Weinberg equilibrium (HWE) was confirmed in both sets. A panel of 32 ancestry informative short tandem repeat markers (AIMs) to assess population substructure (and shown to be adequate to this purpose; (Listman et al., 2010) was genotyped on a subset of the sample (N=1,096) as well as other related samples (Listman et al., 2010). Briefly, STRUCTURE 2.2 (Pritchard et al., 2000) was used to identify four parental populations, with two main subpopulations reflecting the Northern-to-Southern European cline (average proportions of contribution to present sample: 47.3% Northern Europe, 48.3% Southern Europe) with only minor contributions from "African" (1.7%) and "Asian" (2.7%) ancestral populations (Listman et al., 2010).

Analysis

Genetic markers

Using SAS 9.2 (www.sas.com), the χ^2 goodness-of-fit test tested for deviations from HWE for each SNP, and correlation and Lewontin's D′ (Lewontin, 1964) was calculated to measure linkage disequilibrium (LD) between the SNPs, in the entire set of genotyped samples, i.e, not limited to lifetime drinkers, since these are population based estimates (Tables 1, 2).

Genetic “risk model”: genotypes

For each of the four SNPs, prior to formal analysis, we determined the genetic risk model, i.e. if the effect was more likely due to the presence of a specific allele (e.g., A vs. G for rs1229984) or a specific genotype (e.g., AA, AG, or GG), by evaluating the prevalence of binary phenotypes (AD, any AUD, and risk drinking) and means of count phenotypes (AD severity, AUD severity, and maxdrinks) in each genotype group. Preliminary results showed similar prevalences and means for the alcohol phenotypes based on the presence or absence of a specific allele. For example, for rs12299884, both genotype groups with protective allele A (AA and AG) had similar phenotype values that were lower than group GG (absence of allele A), which was termed the “high risk” group. Consequently, further analysis compared the “high risk” group to the “low risk” group (AA or AG for rs1229984). Similarly, for the other three SNPs, high risk groups were as follows: rs1229982, GG (TT/GT low risk); rs1159918, TT (GG/GT low risk); rs698, GG (AA/AG low risk).

Regression analysis

Regression procedures were used to investigate the association of the each of the four allelic risk factors with each alcohol phenotype. Analyses were adjusted for sex, age, and FSU status, as drinking behavior differs by these subgroups in Israel (Hasin et al., 2002a; Hasin et al., 2002b; Spivak et al., 2007; Shmulewitz et al., 2012). Logistic regression was used for binary phenotypes and Poisson regression (with overdispersion) was used for the count phenotypes, as they showed skewed distributions. The count phenotypes were also modeled using normal, Poisson (without overdispersion), negative binomial, and zero-inflated distributions; data best fit the overdispersed Poisson distribution based on the largest likelihood and smallest goodness-of-it indices (Akaike's Information Criterion and the Bayesian Information Criterion). For binary phenotypes, results are reported as odds ratios (ORs), indicating the increase in the odds of the phenotype given the absence of the protective allele. For count phenotypes, results are reported as risk ratios (RRs), indicating the relative increase in the mean phenotype value given the absence of the protective allele (Hasin et al., 2002a). To exclude association due to population substructure, significant associations were re-analyzed adjusting for population structure among the subset of lifetime drinkers for whom AIMS were available (N=1,009), by including the probability of subpopulation membership as continuous control variables in the regression analysis. Last, to determine that positive results were not merely due to correlation with another associated SNP, significant results were reanalyzed, controlling for each of the other SNPs.

Diplotype analysis

As variants in both *ADH1C*-rs698 and *ADH1B*-rs1229984 showed similar relationships with alcohol phenotypes, we constructed haplotypes with these two SNPs (SAS 9.2, HAPLOTYPE procedure, www.sas.com). Three haplotypes with frequencies >1% where found (rs698-rs1229984): A-A, A-G, and G-G. After removing 11 individuals with the rare haplotype (G-A), haplotypes were assigned with 100% certainty to all remaining individuals (N=1,118). Because haplotypes were assigned instead of estimated, deviations from HWE

due to association (such as HW disequilibrium among AUD cases for rs1229984) should not lead to inaccurate haplotype assignment. To ensure this, we assigned haplotypes separately in cases and in controls, and found no differences. Haplotypes were combined into six diplotypes: A-A, A-A; A-A, A-G; A-A, G-G; A-G, A-G; A-G, G-G; G-G, G-G. These diplotypes were categorized to match the allelic risk model described above according to the level of risk they were hypothesized to confer. The diplotype hypothesized to confer high risk included the absence of the protective allele for both rs698 and rs1229984: G-G, G-G. Diplotypes hypothesized to confer intermediate risk included the absence of the protective allele for rs1229984 only: A-G, A-G (rs698=AA, rs1229984=GG) and A-G, G-G (rs698=AG, rs1229984=GG). Of those without the protective allele for rs698, 95.7% also lacked the protective allele for rs1229984; thus, the diplotype categories can be viewed as dividing up the rs1229984 group without the protective allele into those with the protective allele for rs698 (intermediate risk group) and those without the protective allele for rs698 (high risk group). (Note that there is no category for absence of protective allele for rs698 with presence of protective allele for rs1229984 due to the haplotype structure. Accordingly, we did not formally test interaction between *ADH1B*-rs1229984 and *ADH1C*-rs698.) Diplotypes hypothesized to confer low risk had protective alleles for both rs698 and rs1229984. To investigate the association of the diplotype variable with the alcohol phenotypes, adjusted logistic or Poisson regression analyses were carried out, and ORs/RRs were calculated for all comparisons, to determine if both *ADH1B* and *ADH1C* played a role in increasing risk.

Results

Allele and genotype prevalences for the SNPs for the entire sample and the subset of ever-drinkers (N=1,129) are shown in Table 1. All SNP distributions were consistent with Hardy-Weinberg equilibrium expectations (Table 1). *ADH1B*-rs1229984 and *ADH1B*-rs1229982 were in strong LD (r^2=0.67, D′=0.75), *ADH1B*-rs1229984 and *ADH1C*-rs698 showed moderate LD (r^2=0.32, D′=0.44), and other marker combinations showed weaker LD (Table 2). Prevalence for the rs698-rs1229984 diplotype variable was as follows: low risk, 47.0%; intermediate risk, 47.1%; high risk, 6.0%.

Among the ever-drinkers, 12.5% met criteria for AD, 25.2% met criteria for AUD, and 14.7% endorsed risk drinking. Alcohol phenotypes were highly skewed (Figure S1); average number of AD criteria was 1.18 (s.d.=1.50), number of AUD criteria was 1.48 (s.d.=1.96), and maxdrinks was 4.96 (s.d.=6.01).

Association of individual SNPs and alcohol-related traits

ADH1B-rs1229984 was significantly associated with all alcohol phenotypes: the high risk group (absence of the protective allele) had higher prevalence of AD, any AUD, and risk drinking, and higher mean values of AD and AUD severity and maxdrinks (Table 3). For example, the high risk group had 2.28 times greater odds of AD, and 1.83 times greater odds of risk drinking (Table 3). All results remained significant ($p<0.02$) after adjusting for population structure (Table 3) and when controlling for the other SNPs (for rs1229982, $p<0.007$; for rs1159918, $p<0.001$; for rs698, $p<0.002$). The other *ADH1B* SNPs gave less consistent results. *ADH1B*-rs1229982 was significantly associated with a subset of phenotypes, including AD, AD and AUD severity, with weaker associations than those for rs1229984 (Table S1). Furthermore, *ADH1B*-rs1229982 was no longer associated with these phenotypes when controlling for *ADH1B*-rs1229984. Although the *ADH1B*-rs1159918 high risk group showed higher prevalence for any AUD, this was not significant after adjusting for demographics in regression analysis (Table S2).

ADH1C-rs698 was significantly associated with alcohol disorder phenotypes; the high risk group had a higher prevalence of AD, any AUD, and higher mean values of AD and AUD severity (Table 4). For example, the high risk group had 2.67 times greater odds of AD and 1.47 times higher mean AD severity than the low risk group (Table 4). These results remained significant ($p \leq 0.02$) after adjusting for population structure (Table 4) and when controlling for the other SNPs (for rs1229984, $p < 0.05$; for rs1229982, $p < 0.01$; for rs1159918, $p < 0.003$). While risk was elevated for risk drinking (OR=1.5) and maxdrinks (RR=1.2), there was insufficient evidence for significance of these relationships. Furthermore, after controlling for *ADH1B*-rs1229984, there was no evidence for association of *ADH1C*-rs698 with consumption phenotypes (risk drinking, OR=1.17, 95% CI=0.60-2.25; maxdrinks, RR=1.03, 95% CI=0.81-1.32).

Association of ADH1C-rs698/ADH1B-rs1229984 diplotypes and alcohol-related traits

Mean values were significantly different from each other for AD, any AUD, and AD and AUD severity, with increased ORs or RRs when comparing diplotype groups (Table 5), suggesting that *ADH1B* and *ADH1C* both affect these phenotypes. For example, the intermediate risk group (high risk for *ADH1B* but not *ADH1C*) had 2.06 times increased odds of AD compared to the low risk group (low risk for both *ADH1B* and *ADH1C*), while the high risk group (high risk for both *ADH1B* and *ADH1C*) had 4.31 times increased odds compared to the low risk group and 2.09 times increased odds compared to the intermediate group (Table 5). For risk drinking and maxdrinks, the intermediate-risk and high-risk diplotype groups were not significantly different, suggesting that in this sample, only *ADH1B* plays a role.

Discussion

In this study, we found that variants in two widely studied ADH genes, *ADH1B* and *ADH1C*, were associated with multiple alcohol phenotypes in an Israeli household sample, replicating and extending previous studies. Specifically, we found association between *ADH1B*-rs1229984 and AD, any AUD, risk drinking, AD and AUD severity, and maxdrinks. While we found association between *ADH1C*-rs698 and AD, AUD, and AD and AUD severity, there was no evidence of association with either risk drinking or maxdrinks. When considered together, joint diplotypes of *ADH1B* and *ADH1C* variants were found to influence AD, AUD, and AD and AUD severity.

ADH1B

Results for associations of *ADH1B* with AUDs and alcohol consumption are consistent with recent studies of European-ancestry (but not predominantly Jewish) populations (Toth et al., 2011; Bierut et al., 2012) and previous Israeli studies (Neumark et al., 1998; Hasin et al., 2002a; Hasin et al., 2002b; Neumark et al., 2004). We have extended these results by examining binary and count versions of consumption and AD phenotypes, as well as a proxy for the DSM-5 version of AUD that combines AD and AA, in the largest population-based sample of Israeli Jews studied to date. We also tested rs1229982 and rs1159918, functional polymorphisms in the *ADH1B* promoter region that were previously associated with alcohol-related phenotypes (Pochareddy & Edenberg, 2011) to ensure that rs1229984 associations were not due to correlation with these other SNPs. In this sample, the promoter polymorphisms were either weakly associated with alcohol-related traits (rs1229982) or not associated (rs1159918). The similar allele frequencies observed between the *ADH1B* SNPs suggests that these results were not merely due to low power. Furthermore, the weak associations with rs1229982 were not significant after controlling for rs1229984, suggesting that the observed rs1229982 associations were due to correlation between rs1229982 and

rs1229984 (r^2=0.67). Thus, rs1229984, which has a known functional role in affecting the kinetic properties of the encoded enzyme, is more likely to be the relevant polymorphism.

ADH1C

Association between *ADH1C* and alcohol phenotypes has been robust in East Asian populations (Thomasson et al., 1991; Luo et al., 2006; Li et al., 2012), inconsistent in European populations (Zintzaras et al., 2006; Tolstrup et al., 2008; Toth et al., 2011), and null in Jewish populations (Neumark et al., 1998; Neumark et al., 2004). Results presented here provide support for the association between *ADH1C*-rs698 and AUDs. However, we found no evidence of significant association between *ADH1C*-rs698 and measures of consumption within this sample. These results agree with both the Neumark et al. (Neumark et al., 1998; Neumark et al., 2004) studies showing no association with phenotypes related to alcohol consumption, and with the studies that found association with AUDs (Zintzaras et al., 2006; Tolstrup et al., 2008), in that *ADH1C*-rs698 is associated with problematic AUDs, but not with measures of more normative heavy alcohol consumption or rates of elimination. As with *ADH1B*, we have extended these previous studies by examining multiple phenotypes involving consumption and AUDs, within a large population based sample of Israeli Jews.

ADH1B and ADH1C

We found that while *ADH1B*-rs1229984 and *ADH1C*-rs698 were correlated (r^2=0.32; D′=0.44), they did not represent a single signal; each variant contributed to risk independently. Due to the unique haplotype structure in this population, we were able to directly examine the joint effects of *ADH1B*-rs1229984 and *ADH1C*-rs698 on alcohol phenotypes by identifying *ADH1B*-rs1229984 and *ADH1C*-rs698 diplotypes with certainty, categorizing them into three risk groups, and testing the specific combinations of risk variants for alcohol phenotypes. For both the binary and count measures of AD and AUD, significant differences were observed across each of the three diplotype risk groups, indicating that both SNPs play a role in these outcomes. These results agree with the single SNP analyses, which indicated that both *ADH1B*-rs1229984 and *ADH1C*-rs698 were associated with AUDs, while *ADH1C*-rs698 was not significantly associated with consumption. While the diplotypes explained roughly the same amount of the variance in these traits (e.g. ~19% of the variance in AD) as either *ADH1B*-rs1229984 (~18% of the variance in AD) or *ADH1C*-rs698 (~17% of the variance in AD) individually, considering these SNPs together provides a model that more accurately describes the polygenic architecture of these traits. Thus, the higher effect sizes (ORs or RRs) in the diplotype analysis (for the high vs. low risk comparison) than in the single marker analyses was most likely due to better characterization of the gene effect due to partitioning the *ADH1B*-rs1229984 high risk group into two groups based on the presence or absence of the *ADH1C*-rs698 high risk group.

While prior studies have examined gene-gene interactions between ADH genes (Wu et al., 2012), only three studies explicitly examined the joint effect of these specific ADH SNPs for alcohol outcomes (Neumark et al., 2004; Tolstrup et al., 2008; Toth et al., 2011). Results from these studies were mixed; the Neumark study found no evidence of interaction between these two SNPs, while the more recent studies indicated that the joint effects of these ADH risk alleles (using haplotypes/diplotypes) increased risk for excessive consumption and AUDs (Tolstrup et al., 2008; Toth et al., 2011). Results from our study can potentially clarify these discrepancies in that we found the joint effect (of both *ADH1B* and *ADH1C*) is related to AUDs, but not significantly to measures of consumption in this large population of Israeli Jews. While a genetic variant could plausibly be consistently associated with all related phenotypes (consumption and AUDs), that is not necessarily the case if different

measures tap into different aspects of a related phenotype which may have unique etiological risk factors (Meyers et al., 2012). Further studies are needed to examine the association of AUDs and consumption with *ADH1C*.

Although many gene mapping studies are carried out in Ashkenazi Jewish (AJ) samples (i.e., all four grandparents born in Europe; (Guha et al., 2012), due to the unique genetic structure of that population (Guha et al., 2012), we decided to also include non-Ashkenazi Jews (NAJ; mainly of Middle Eastern origin), to determine if the same genetic effects were found in both potential subpopulations (AJ/NAJ). The AJ/NAJ distinction also connotes cultural differences, possibly encompassing differences in drinking behavior. Although we asked respondents to self-identify as “Ashkenaz” or “non-Ashkenaz”, close to 20% of the sample chose “both” or “other”, reflecting the blurred distinctions between these categories, both in terms of cultural practices and genetic origins (i.e. individuals with grandparents from both groups), in modern-day Israel. Therefore the traditional AJ/NAJ distinction was not useful in this sample. Rather, we used AIMs, which produced a continuum of genetic ancestry measuring the Northern/Southern European cline, with higher proportion of “Northern” ancestry indicating more Ashkenazi origin, and higher proportion “Southern” ancestry indicating more non-Ashkenazi origin (Listman et al., 2010). We analyzed the whole sample (instead of AJ/NAJ separately), including ancestry proportions in regression analyses to ensure that even if there were cultural differences in drinking related to ancestral origins, the association results were not due to genetic admixture. Similar results with and without the ancestry proportions suggest that the ADH gene effects were similar across subpopulations, and support utility of gene association studies in mixed Jewish populations.

Study limitations are noted. First, we do not claim to have identified all the causative genetic variants; we may be detecting significant signals where there are contributions from LD with an additional untested, “true” variant (or variants), not only in *ADH1B* but across the ADH gene cluster. However, enzymatic studies show that the *ADH1B**2 and *ADH1C**2 variants are functional, affecting enzyme kinetics (Bosron et al., 1983; Bosron & Li, 1986). Next, the unique haplotype structure in this population was useful in enabling us to identify diplotypes with certainty and to categorize them into three risk groups. However, to more fully understand the joint or related interaction effects, samples with different LD patterns and greater prevalence of the rare haplotypes/diplotypes (e.g., lack of *ADH1C* protective allele with the presence of the *ADH1B* protective allele) should be studied. In addition, this study examined two genes in relation to six related alcohol outcomes in an effort to systematically assess the association. Prior evidence for genetic association, along with the high degree of correlation between the alcohol phenotypes (r^2 range from 0.35-0.96) and SNPs (r^2 from 0.01-0.75), mitigates the concern that results are merely a consequence of multiple testing. Nevertheless, when we applied the Benjamini-Hochberg procedure to control the false discovery rate at 0.05 (Benjamini & Hochberg, 1995), all results remained significant except that *ADH1B*-rs1229982 was not associated with AUD severity. Last, in Israel, consuming several drinks on one occasion is common in religious practice (e.g., Purim and the Passover Seder), and might occur for cultural/environmental reasons even among individuals who would otherwise drink much less. As a sensitivity analysis, we conducted *post-hoc* analyses of another measure, “usual drinks,” which indicates how many drinks a respondent usually had in a single day. Association results were similar in that *ADH1B* was significantly related to usual drinks, and *ADH1C*, when analyzed jointly with *ADH1B*, was not related to usual drinks.

In conclusion, this study adds support for the role of *ADH1B* and *ADH1C* in alcohol phenotypes in a large Israeli household population. Further, the results suggest that considering these SNPs together provides a model that more accurately depicts the polygenic architecture of AUD traits. Studies of the association of alcohol phenotypes and

either *ADH1B* or *ADH1C* alone are employing an oversimplified model, which may mask relevant information regarding the genetic influence of these ADH variants on behavioral alcohol phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th. American Psychiatric Association; Washington, DC: 2000. text revision

American Psychiatric Association. [Accessed September 19, 2011] DSM-5 Development, Proposed revision: Substance use disorder. 2010. Available at: http://www.dsm5.org/ProposedRevisions/Pages/proposedrevision.aspx?rid=431

Benjamini Y, Hochberg Y. Controlling false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B. 1995; 57:289–300.

Bierut LJ, Goate AM, Breslau N, Johnson EO, Bertelsen S, Fox L, Agrawal A, Bucholz KK, Grucza R, Hesselbrock V, Kramer J, Kuperman S, Nurnberger J, Porjesz B, Saccone NL, Schuckit M, Tischfield J, Wang JC, Foroud T, Rice JP, Edenberg HJ. ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. Mol Psychiatry. 2012; 17:445–450. [PubMed: 21968928]

Bosron WF, Crabb DW, Li TK. Relationship between kinetics of liver alcohol dehydrogenase and alcohol metabolism. Pharmacol Biochem Behav. 1983; 18(1):223–227. [PubMed: 6356161]

Bosron WF, Li TK. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. Hepatology. 1986; 6:502–510. [PubMed: 3519419]

Edenberg HJ. The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health. 2007; 30:5–13. [PubMed: 17718394]

Grant BF, Dawson DA, Stinson FS, Chou PS, Kay W, Pickering R. The Alcohol Use Disorder and Associated Disabilities Interview Schedule-IV (AUDADIS-IV): reliability of alcohol consumption, tobacco use, family history of depression and psychiatric diagnostic modules in a general population sample. Drug Alcohol Depend. 2003; 71:7–16. [PubMed: 12821201]

Grant BF, Harford TC, Dawson DA, Chou PS, Pickering RP. The Alcohol Use Disorder and Associated Disabilities Interview schedule (AUDADIS): reliability of alcohol and drug modules in a general population sample. Drug Alcohol Depend. 1995; 39:37–44. [PubMed: 7587973]

Guha S, Rosenfeld JA, Malhotra AK, Lee AT, Gregersen PK, Kane JM, Pe'er I, Darvasi A, Lencz T. Implications for health and disease in the genetic signature of the Ashkenazi Jewish population. Genome Biol. 2012; 13:R2. [PubMed: 22277159]

Hasin D, Aharonovich E, Liu X, Mamman Z, Matseoane K, Carr Lg, Li TK. Alcohol dependence symptoms and alcohol dehydrogenase 2 polymorphism: Israeli Ashkenazis, Sephardics, and recent Russian immigrants. Alcohol Clin Exp Res. 2002a; 26:1315–1321. [PubMed: 12351924]

Hasin D, Aharonovich E, Liu X, Mamman Z, Matseoane K, Carr L, Li TK. Alcohol and ADH2 in Israel: Ashkenazis, Sephardics, and recent Russian immigrants. Am J Psychiatry. 2002b; 159:1432–1434. [PubMed: 12153842]

Hasin D, Carpenter KM, Mccloud S, Smith M, Grant BF. The alcohol use disorder and associated disabilities interview schedule (AUDADIS): reliability of alcohol and drug modules in a clinical sample. Drug Alcohol Depend. 1997a; 44:133–141. [PubMed: 9088785]

Hasin D, Hatzenbuehler ML, Keyes K, Ogburn E. Substance use disorders: Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) and International Classification of Diseases. Addiction (tenth). 2006; 101(1):59–75. (ICD-10). [PubMed: 16930162]

Hasin D, Rahav G, Meydan J, Neumark Y. The drinking of earlier and more recent Russian immigrants to Israel: comparison to other Israelis. J Subst Abuse. 1998; 10:341–353. [PubMed: 10897288]

Hasin DS, Van Rossem R, Mccloud S, Endicott J. Differentiating DSM-IV alcohol dependence and abuse by course: community heavy drinkers. J Subst Abuse. 1997b; 9:127–135. [PubMed: 9494944]

Hurley TD, Edenberg HJ, Bosron WF. Expression and kinetic characterization of variants of human beta 1 beta 1 alcohol dehydrogenase containing substitutions at amino acid 47. J Biol Chem. 1990; 265:16366–16372. [PubMed: 2398055]

Kendler KS, Myers J, Prescott CA. Specificity of genetic and environmental risk factors for symptoms of cannabis, cocaine, alcohol, caffeine, and nicotine dependence. Arch Gen Psychiatry. 2007; 64:1313–1320. [PubMed: 17984400]

Keyes KM, Geier T, Grant BF, Hasin DS. Influence of a drinking quantity and frequency measure on the prevalence and demographic correlates of DSM-IV alcohol dependence. Alcohol Clin Exp Res. 2009; 33:761–771. [PubMed: 19298332]

Lewontin RC. The Interaction of Selection and Linkage. Ii. Optimum Models. Genetics. 1964; 50:757–782. [PubMed: 14221879]

Li D, Zhao H, Gelernter J. Strong association of the alcohol dehydrogenase 1B gene (ADH1B) with alcohol dependence and alcohol-induced medical diseases. Biol Psychiatry. 2011; 70:504–512. [PubMed: 21497796]

Li D, Zhao H, Gelernter J. Further clarification of the contribution of the ADH1C gene to vulnerability of alcoholism and selected liver diseases. Hum Genet. 2012; 131:1361–1374. [PubMed: 22476623]

Li H, Mukherjee N, Soundararajan U, Tarnok Z, Barta C, Khaliq S, Mohyuddin A, Kajuna SL, Mehdi SQ, Kidd JR, Kidd KK. Geographically separate increases in the frequency of the derived ADH1B*47His allele in eastern and western Asia. Am J Hum Genet. 2007; 81:842–846. [PubMed: 17847010]

Listman JB, Hasin D, Kranzler HR, Malison RT, Mutirangura A, Sughondhabirom A, Aharonovich E, Spivak B, Gelernter J. Identification of population substructure among Jews using STR markers and dependence on reference populations included. BMC Genet. 2010; 11:48. [PubMed: 20546593]

Luo X, Kranzler HR, Zuo L, Wang S, Schork NJ, Gelernter J. Diplotype trend regression analysis of the ADH gene cluster and the ALDH2 gene: multiple significant associations with alcohol dependence. Am J Hum Genet. 2006; 78:973–987. [PubMed: 16685648]

Meyers JL, Nyman E, Loukola A, Rose RJ, Kaprio J, Dick DM. The association between DRD2/ANKK1 and genetically informed measures of alcohol use and problems. Addict Biol. 201210.1111/j.1369-1600.2012.00490.x

Neumark YD, Friedlander Y, Durst R, Leitersdorf E, Jaffe D, Ramchandani VA, O'connor S, Carr LG, Li TK. Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. Alcohol Clin Exp Res. 2004; 28:10–14. [PubMed: 14745297]

Neumark YD, Friedlander Y, Thomasson HR, Li TK. Association of the ADH2*2 allele with reduced ethanol consumption in Jewish men in Israel: a pilot study. J Stud Alcohol. 1998; 59:133–139. [PubMed: 9500299]

Olfson E, Bierut LJ. Convergence of genome-wide association and candidate gene studies for alcoholism. Alcohol Clin Exp Res. 2012; 36:2086–2094. [PubMed: 22978509]

Pochareddy S, Edenberg HJ. Variation in the ADH1B proximal promoter affects expression. Chem Biol Interact. 2011; 191:38–41. [PubMed: 21168396]

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155:945–959. [PubMed: 10835412]

Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J. Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. Lancet. 2009; 373:2223–2233. [PubMed: 19560604]

Saccone NL, Kwon JM, Corbett J, Goate A, Rochberg N, Edenberg HJ, Foroud T, Li TK, Begleiter H, Reich T, Rice JP. A genome screen of maximum number of drinks as an alcoholism phenotype. Am J Med Genet. 2000; 96:632–637. [PubMed: 11054770]

Shmulewitz D, Keyes K, Beseler C, Aharonovich E, Aivadyan C, Spivak B, Hasin D. The dimensionality of alcohol use disorders: results from Israel. Drug Alcohol Depend. 2010; 111:146–154. [PubMed: 20537809]

Shmulewitz D, Wall MM, Keyes KM, Aharonovich E, Aivadyan C, Greenstein E, Spivak B, Weizman A, Frisch A, Hasin D. Alcohol use disorders and perceived drinking norms: ethnic differences in Israeli adults. J Stud Alcohol Drugs. 2012; 73:981–990. [PubMed: 23036217]

Spivak B, Frisch A, Maman Z, Aharonovich E, Alderson D, Carr LG, Weizman A, Hasin D. Effect of ADH1B genotype on alcohol consumption in young Israeli Jews. Alcohol Clin Exp Res. 2007; 31:1297–1301. [PubMed: 17559546]

Thomasson HR, Edenberg HJ, Crabb DW, Mai XL, Jerome RE, Li TK, Wang SP, Lin YT, Lu RB, Yin SJ. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. Am J Hum Genet. 1991; 48:677–681. [PubMed: 2014795]

Tolstrup JS, Nordestgaard BG, Rasmussen S, Tybjaerg-Hansen A, Gronbaek M. Alcoholism and alcohol drinking habits predicted from alcohol dehydrogenase genes. Pharmacogenomics J. 2008; 8:220–227. [PubMed: 17923853]

Toth R, Fiatal S, Petrovski B, Mckee M, Adany R. Combined effect of ADH1B RS1229984, RS2066702 and ADH1C RS1693482/ RS698 alleles on alcoholism and chronic liver diseases. Dis Markers. 2011; 31:267–277. [PubMed: 22048268]

Wu C, Kraft P, Zhai K, Chang J, Wang Z, Li Y, Hu Z, He Z, Jia W, Abnet CC, Liang L, Hu N, Miao X, Zhou Y, Liu Z, Zhan Q, Liu Y, Qiao Y, Zhou Y, Jin G, Guo C, Lu C, Yang H, Fu J, Yu D, Freedman ND, Ding T, Tan W, Goldstein AM, Wu T, Shen H, Ke Y, Zeng Y, Chanock SJ, Taylor PR, Lin D. Genome-wide association analyses of esophageal squamous cell carcinoma in Chinese identify multiple susceptibility loci and gene-environment interactions. Nat Genet. 2012; 44:1090–1097. [PubMed: 22960999]

Zintzaras E, Stefanidis I, Santos M, Vidal F. Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? Hepatology. 2006; 43:352–361. [PubMed: 16440362]

Table 1

ADH1B and *ADH1C* genotypes

SNP	Chromosomal Location[a]	N[b]	HWE		Allele	prevalence (%)		Genotype	prevalence (%)		Risk status (prevalence [%] in drinkers)
			χ^2	p-value		Whole set	Drinkers (N=1,129)		Whole set	Drinkers (N=1,129)	
					ADH1B						
rs1229984	100239319	1,201	2.04	0.141	A	28.3	28.2	AA	8.8	9.0	Low risk [47.5%]
					G	71.7	71.8	AG	38.9	38.5	
								GG	52.3	52.5	High risk [52.5%]
rs1229982	100343932	1,201	3.48	0.089	T	37.4	36.8	TT	15.2	14.4	Low risk [59.2%]
					G	62.6	63.2	TG	44.3	44.7	
								GG	40.5	40.8	High risk [40.8%]
rs1159918	100243009	1,210	0.18	0.683	T	30.5	30.1	TT	9.6	9.0	High risk [9.0%]
					G	69.5	69.9	GT	41.9	42.2	Low risk [91.0%]
								GG	48.5	48.8	
					ADH1C						
rs698	100260789	1,211	0.00	1.000	G	24.9	24.8	GG	6.2	6.2	High risk [6.2%]
					A	75.1	75.2	AG	37.5	37.3	Low risk [93.8%]
								AA	56.3	56.5	

HWE = Hardy-Weinberg Equilibrium

[a] All SNPs located on chromosome 4; positions based upon GRCh37.p5.

[b] N = number successfully genotyped.

Table 2

Linkage disequilibrium (LD) between *ADH1B* and *ADH1C* SNPs (n=1,201): r^2 shown in the upper half, D' in the lower half

Gene	***ADH1B***			***ADH1C***
SNP	***rs1229984***	***rs1229982***	***rs1159918***	***rs698***
rs1229984	--	0.666	0.059	0.323
rs1229982	0.754	--	0.159	0.234
rs1159918	0.071	0.177	--	0.005
rs698	0.445	0.287	0.005	--

Table 3

Relationship between alcohol-related phenotypes and *ADH1B*-rs1229984 among ever-drinkers (N=1,129)

	Binary Alcohol Phenotype		
	Alcohol dependence (AD)	**Any Alcohol use disorder (AUD)**	**Consumption: Risk drinking[a]**
		Prevalence (%)	
Presence of protective allele (AA/AG; N=536)	08.4	20.2	10.8
Absence of protective allele (GG; N=593)	16.2	29.7	18.2
		Regression analysis[b]	
Odds ratio (OR; 95% CI)	2.28 (1.54-3.38)[h]	1.77 (1.32-2.38)[h]	1.83 (1.28-2.62)[g]
OR including ancestry (95% CI)[c]	2.13 (1.41-3.22)[g]	1.73 (1.27-2.37)[g]	1.89 (1.29-2.77)[g]
	Count Alcohol Phenotype		
	AD severity	**AUD severity**	**Consumption: Maxdrinks[d]**
		Mean	
Presence of protective allele (AA/AG; N=536)	1.01	1.25	4.19
Absence of protective allele (GG; N=593)	1.33	1.68	5.65
		Regression analysis[e]	
Risk ratio (RR; 95% CI)	1.31 (1.14-1.52)[g]	1.34 (1.15-1.55)[h]	1.33 (1.17-1.51)[h]
RR including ancestry (95% CI)[c]	1.27 (1.09-1.48)[f]	1.29 (1.11-1.51)[g]	1.32 (1.16-1.51)[h]

[a] At least weekly drinking of ≥5 drinks per day for men, ≥4 drinks per day for women

[b] Logistic regression, adjusting for age, sex, former Soviet Union status

[c] N=1,009

[d] Greatest lifetime number of drinks on a single day

[e] Overdispersed Poisson regression, adjusting for age, sex, former Soviet Union status

[f] p-values: ≤0.01;

[g] p-values: ≤0.001;

[h] p-values: ≤0.0001

Table 4

Relationship between alcohol-related phenotypes and *ADH1C*-rs698 among ever-drinkers (N=1,129)

	Binary Alcohol Phenotype		
	Alcohol dependence (AD)	**Any Alcohol use disorder (AUD)**	**Consumption: Risk drinking[a]**
	Prevalence (%)		
Presence of protective allele (AA/AG; N=1,059)	11.9	24.3	14.4
Absence of protective allele (GG; N=70)	21.4	38.6	20.0
	Regression analysis[b]		
Odds ratio (OR; 95% CI)	2.67 (1.38-5.16)[g]	2.32 (1.34-4.02)[g]	1.49 (0.78-2.83)
OR including ancestry (95% CI)[c]	2.30 (1.14-4.64)[f]	2.19 (1.20-3.89)[g]	--
	Count Alcohol Phenotype		
	AD severity	**AUD severity**	**Consumption: Maxdrinks[d]**
	Mean		
Presence of protective allele (AA/AG; N=1,059)	1.15	1.44	4.92
Absence of protective allele (GG; N=70)	1.60	2.09	5.61
	Regression analysis[e]		
Risk ratio (RR; 95% CI)	1.47 (1.13-1.90)[g]	1.51 (1.17-1.96)[g]	1.16 (0.91-1.48)
RR including ancestry (95% CI)[c]	1.37 (1.04-1.81)[f]	1.42 (1.08-1.87)[f]	--

[a] At least weekly drinking of ≥5 drinks per day for men, ≥4 drinks per day for women

[b] Logistic regression, adjusting for age, sex, former Soviet Union status

[c] N=1,009

[d] Greatest lifetime number of drinks on a single day

[e] Overdispersed Poisson regression, adjusting for age, sex, former Soviet Union status

[f] p-values: <0.05;

[g] p-values: ≤0.01

Table 5

Relationship between alcohol-related phenotypes and *ADH1C*-rs698/*ADH1B*-rs1229984 diplotypes among ever-drinkers (N=1,118)

	Binary Alcohol Phenotype		
	Alcohol dependence (AD)	**Any Alcohol use disorder (AUD)**	**Consumption: Risk drinking[a]**
		Prevalence (%)	
Low risk diplotypes (N=525)	8.6	19.8	11.1
Intermediate risk diplotypes (N=526)	15.4	28.5	17.9
High risk diplotype (N=67)	22.4	38.8	20.9
		Odds ratio (95% CI)[b]	
Intermediate vs. low	2.06 (1.37-3.08)[g]	1.69 (1.25-2.29)[g]	1.75 (1.21-2.53)[f]
High vs. low	4.31 (2.11-8.81)[h]	3.16 (1.76-5.69)[h]	2.12 (1.07-4.19)[e]
High vs. intermediate	2.09 (1.05-4.16)[e]	1.87 (1.05-3.33)[e]	1.21 (0.62-2.35)
	Count Alcohol Phenotype		
	AD severity	**AUD severity**	**Consumption: Maxdrinks[c]**
		Mean	
Low risk diplotypes (N=525)	1.01	1.24	4.23
Intermediate risk diplotypes (N=526)	1.29	1.63	5.65
High risk diplotype (N=67)	1.60	2.06	5.69
		Risk ratio (95% CI)[d]	
Intermediate vs. low	1.27 (1.09-1.48)[f]	1.30 (1.12-1.52)[g]	1.32 (1.16-1.50)[h]
High vs. low	1.67 (1.27-2.20)[g]	1.73 (1.31-2.29)[h]	1.36 (1.05-1.76)[e]
High vs. intermediate	1.31 (1.00-1.72)[e]	1.33 (1.01-1.75)[e]	1.04 (0.81-1.33)

[a] At least weekly drinking of ≥5 drinks per day for men, ≥4 drinks per day for women

[b] Logistic regression, adjusting for age, sex, former Soviet Union status

[c] Greatest lifetime number of drinks on a single day

[d] Overdispersed Poisson regression, adjusting for age, sex, former Soviet Union status

[e] p-values: <0.05;

[f] p-values: ≤0.01;

[g] p-values: ≤0.001;

[h] p-values: ≤0.0001

Definition of diplotype groups:
Low risk = A-A, A-A; A-G, A-A; A-A, G-G: presence of protective allele (A) for both SNPs
Intermediate risk = A-G, A-G; A-G, G-G: presence of protective allele for *ADH1C*-rs698, absence of protective allele for *ADH1B*-rs1229984
High risk = G-G, G-G: absence of protective allele for both SNPs