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ALDH2 and *ADH1B* Interactions in Retrospective Reports of Low-Dose Reactions and Initial Sensitivity to Alcohol in Asian American College Students

Susan E. Luczak, **Danielle Pandika**, **Shoshana H. Shea**, **Mimy Y. Eng**, **Tiebing Liang**, and **Tamara L. Wall**
Department of Psychology, University of Southern California, Los Angeles, California (SEL), Department of Psychiatry, University of California, San Diego, San Diego, California (SEL, DP, SHS, TLW), Veterans Affairs San Diego Healthcare System, San Diego, California (SHS, TLW), Veterans Medical Research Foundation, San Diego, California (MYE, TLW), and Departments of Medicine and Pharmacology, Indiana University, Indianapolis, IN (TL).

Abstract

Background—A mechanistic model has been proposed for how alcohol metabolizing gene variants protect individuals from the development of alcohol use disorders, with heightened sensitivity to alcohol being an early step (endophenotype) in this model. The present study was designed to determine whether possession of two alcohol metabolizing genes variations, the aldehyde dehydrogenase *ALDH2*2* allele and the alcohol dehydrogenase *ADH1B*2* allele, was associated with self-reported sensitivity to alcohol at low doses and at initial use.

Methods—Asian-American college students (N = 784) of Chinese and Korean descent were genotyped at the *ALDH2* and *ADH1B* loci and assessed for lifetime alcohol symptoms following one or two drinks and level of response to alcohol during the first five lifetime drinking episodes.

Results—Participants who had an *ALDH2*2* allele were more likely to report experiencing all six low-dose symptoms and having heightened initial response to alcohol. An interaction was found between *ALDH2*2* and *ADH1B*2*, with *ADH1B*2* being associated with heightened self-reported sensitivity to alcohol only in individuals who also possessed one *ALDH2*2* allele.

Conclusions—These findings suggest the effects of *ADH1B*2* may be felt more strongly in Asians who already have some heightened sensitivity to alcohol from possessing one *ALDH2*2* allele, but who are not too sensitized to alcohol from possessing two *ALDH2*2* alleles. These results offer additional insight into the discrepant findings that have been reported in the literature for the role of *ADH1B*2* in response to alcohol and the development of alcohol-related problems.

Keywords

ALDH2; *ADH1B*; alcohol metabolizing genes; gene-gene interaction; alcohol sensitivity; subjective response to alcohol

INTRODUCTION

The etiology of alcohol use disorders (AUDs; alcohol abuse and dependence) is complex, with approximately 50 to 60% of the risk being attributed to genetic factors (Heath et al., 1997; Prescott et al., 1999). Polymorphic genes, including the aldehyde dehydrogenase gene

Address correspondence to Tamara L. Wall, Ph.D., Psychology Service (116B), Veterans Affairs San Diego Healthcare System, 3350 La Jolla Village Drive, San Diego, CA 92161. Telephone: 858-552-8585 extension 2485, Fax: 858-642-6201, twall@ucsd.edu. .

ALDH2 (12q24) and the alcohol dehydrogenase gene *ADH1B* (4q22), have been proposed as particularly compelling alcohol-specific genes associated with AUDs (Li, 2000).

In the primary pathway of alcohol metabolism, alcohol is initially converted to acetaldehyde by alcohol dehydrogenase, and then to acetate by aldehyde dehydrogenase. The *ALDH2* (rs671) gene codes for the primary liver isoenzyme involved in conversion of acetaldhyde to acetate. *ALDH2* has two allelic forms, the wild type *ALDH2*1* (*ALDH2*Glu487*) and the variant *ALDH2*2* (*ALDH2*Lys487*). *ALDH2*2* is extremely rare in non-Asians, but present in about 30-50% of northeastern Asians (Chinese, Koreans, Japanese) with a small percentage (about 5%) homozygous for this allele (Goedde et al., 1992). *ALDH2*2* has been consistently and strongly associated with decreased rates of alcohol problems and AUDs. In a meta-analysis, having one *ALDH2*2* allele was associated with a four-to-five-fold reduction in alcohol dependence (odds ratio, OR = 0.22) and having two *ALDH2*2* alleles was associated with an eight-to-nine-fold reduction in alcohol dependence (OR = 0.12; Luczak et al., 2006).

The alcohol dehydrogenase *ADH1B* (rs1229984) gene codes for the primary isoenzyme involved in conversion of alcohol to acetaldehyde. In Asians, *ADH1B* has two allelic forms, the wild type *ADH1B*1* (*ADH1B*47Arg*) and the variant *ADH1B*2* (*ADH1B*47His*). *ADH1B*2* is found in nearly 90% of northeast Asian populations and in lower prevalence in Caucasians (about 5-10%, although in higher rates in Eastern European Jews and Russians; Borras et al., 2000; Goedde et al., 1992; Ogurtsov et al., 2001). *ADH1B*2* also has consistently been associated with lower rates of alcohol dependence. This relationship is found after controlling for *ALDH2*2* in Asian subgroups (Luczak et al., 2006) and in European Caucasian subgroups (Borras et al., 2000; Ogurtsov et al., 2001; Wall et al., 2005; Whitfield, 2002. In a meta-analysis of the effect of *ALDH2*2* and *ADH1B*2* alleles in combination in Asians (Luczak et al., 2006), *ALDH2*1/*1* individuals with one *ADH1B*2* allele had about one fourth (OR = 0.26) and those with two *ADH1B*2* alleles had about one fifth (OR = 0.20) the risk of alcohol dependence compared with individuals with no *ADH1B*2* alleles. In *ALDH2*1/*2* individuals, those with one *ADH1B*2* allele had about one sixth (OR = 0.17) and those with two *ADH1B*2* alleles had about one eleventh (OR = 0.09) the risk of alcohol dependence compared with individuals with no *ADH1B*2* alleles. In an earlier meta-analysis, Whitfield (2002) found similar protective effects of *ADH1B*2* in Asians, but in Caucasians the protection from possessing one *ADH1B*2* allele was only about half (OR = 0.47). These results suggest *ALDH2* and *ADH1B* each contribute unique protective effects on alcohol dependence, and the level of protection may be even stronger in conjunction than alone.

A mechanistic pathway has been proposed for how possessing *ALDH2*2* and *ADH1B*2* alleles may reduce an individual's risk for alcohol dependence (Eriksson, 2001; Li, 2000; Thomasson et al., 1991; see Wall, 2005). Based on their kinetic properties, *ADH1B*2* should lead to faster production of acetaldehyde than *ADH1B*1* (Bosron and Li, 1986) and *ALDH2*2* should lead to slower elimination of acetaldehyde than *ALDH2*1* (Wilken, 1981). These increased acetaldehyde levels are hypothesized to lead to heightened responses to alcohol, which in turn are predicted to discourage heavy alcohol consumption. Lower alcohol consumption is then proposed to reduce the likelihood of an individual developing an AUD. Although there is much evidence to support this mechanism for *ALDH2*2*, the data supporting this mechanism for *ADH1B*2* has been less consistent (Eriksson, 2001; see Wall, 2005). The present study was undertaken to better understand early steps of the mechanistic pathway relating *ALDH2* and *ADH1B* polymorphisms to lower rates of AUDs. The primary objective was to clarify associations of *ALDH2* and *ADH1B* variations with low-dose reactions and initial sensitivity to alcohol.

*ALDH2*2* has been related to alcohol sensitivity as measured by retrospective self-reports of flushing and other symptoms (e.g., headaches, heart palpitations, drowsiness) following low doses of alcohol (Chen et al., 1998; Matsuo et al., 2006; Takeshita and Morimoto, 1999; Takeshita et al., 2001), as well as by retrospective self-ratings of heightened level of response to alcohol (Hendershot et al., 2009; Wall et al., 1999). These data are consistent with alcohol administration studies, which have found *ALDH2*2* relates to heightened physical (e.g., flushing, pulse rate, hormonal changes, EEG reactivity) and subjective (e.g., feeling great, drunk, dizzy) responses to alcohol (Cook et al., 2005; Luczak et al., 2002; Minami et al., 2002; Wall and Ehlers, 1995; Wall et al., 1992; 1994). These heightened responses to alcohol are found even at low doses of alcohol, with possession of two *ALDH2*2* alleles resulting in even more intense responses than possession of one *ALDH2*2* allele (Peng et al., 1999; 2002).

Findings for the proposed relationship between *ADH1B*2* and increased sensitivity to alcohol have been less consistently supported by data. Some studies of Asians (Chen et al., 1998; Matsuo et al., 2006), Jews (Carr et al., 2002), and non-Jewish Caucasians (Macgregor et al., 2009; Wall et al., 2005) find individuals with *ADH1B*2* alleles retrospectively self-report more intense reactions to alcohol (e.g., flushing, headaches, nausea, palpitations, and other alcohol-related symptoms) than participants without an *ADH1B*2* allele, but other studies do not relate *ADH1B*2* to self-reports of a heightened response to alcohol (Shea et al., 2001; Takeshita et al., 1994). Similarly, an alcohol challenge study of non-Asians (Caucasians and African Americans) supported *ADH1B*2* leading to heightened responses to alcohol (Duranceaux et al., 2006), but others alcohol challenge studies of Asians (Peng et al., 2002) and Caucasians (Heath et al., 1999) have not found significant effects of *ADH1B*2* on measures of intoxication and flushing. Several studies of Asians using alcohol challenge, retrospective self-report, and skin patch tests have reported *ADH1B*2* is associated with increased alcohol-related symptoms (e.g., facial flushing, vomiting), but that this relationship is only signfiicant in individuals who also possess an *ALDH2*2* allele (Cook et al., 2005; Takeshita et al., 1996; 2001; Yokoyama et al., 1999; 2003; although see Chen et al., 1998), suggesting it may be the combined effects of *ALDH2*2* and *ADH1B*2* that lead to more apparent associations of these alleles with response to alcohol.

In the current study, we used retrospective self-report measures to assess low-dose and initial sensitivity to alcohol in Asian participants with varying *ALDH2* and *ADH1B* genotypes. We first examined the effects of *ALDH2*2* alone, then after testing *ALDH2-ADH1B* interactions we controlled for *ALDH2* to determine whether the intensity of response to alcohol also differed across *ADH1B* genotypes. It was predicted that *ALDH2*2* would be associated with higher rates of alcohol-related symptoms. In addition, it was predicted that if *ADH1B* polymorphisms were related to a more intense response to alcohol (i.e., alcohol sensitivity), then participants homozygous for *ADH1B*2* would report the most alcohol-related symptoms while participants heterozygous for *ADH1B*2* would report more alcohol-related symptoms than those without the variant allele. Similar to previous reports (e.g., Chen et al., 1999; Cook et al., 2005; Luczak et al., 2006 Takeshita et al., 1996; 2001; Yokoyama et al., 1999; 2003), we expected the effects of *ADH1B* on alcohol sensitivity might be stronger in individuals who also possess an *ALDH2*2* allele. These findings would support the step of the hypothesized pathway that proposes an association between *ADH1B* variations and responses to alcohol, and would contribute some validity and clarity to the proposed mechanism of action for how these variants lead to protection against the development of AUDs.

METHODS

Participants were 784 Chinese- (n = 406; 50% women) and Korean-American (n = 378; 52% women) students at the University of California, San Diego (UCSD) who were 21 to 26 years old (M = 21.8, SD = 1.14) and reported all four of their grandparents were entirely of Chinese or Korean ethnicity. These participants were recruited through advertisements on campus and in the school newspaper that did not specify the nature of the study. To increase the likelihood of accurate reporting, participants were informed of the multiple procedures used to protect subject confidentiality, including the Certificate of Confidentiality obtained from the U.S. Department of Health and Human Services that restricts access to individual data by subpoena. Informed written consent for participation was obtained. This study was approved by the UCSD Human Research Protections Program.

Participants completed the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA; Bucholz et al., 1994; Hesselbrock et al., 1999) with a trained research interviewer. As part of this interview, participants were asked whether one or two drinks had ever caused them to (a) flush or blush; (b) feel very sleepy; (c) have headaches or head pounding/ throbbing; (d) have nausea; (e) have heart palpitations; or (f) break out into hives. A total score was created by summing the number of symptoms endorsed (range 0-6).

Participants also completed the Self-Rating of the Effects of Alcohol (SRE) form (Schuckit et al., 1997a) with a trained research interviewer. The SRE asks subjects to report the number of standard drinks required for each of four effects (beginning to feel intoxication, slurring speech, having an unsteady gait, and falling asleep when they did not want to) at several points in their lives. A standard drink was defined as 12 oz beer, 5 oz of wine, or one shot (1-1.5 oz) of 80-proof alcohol. The SRE has been shown to have one-year retest reliabilities as high as 0.8 and correlations with alcohol challenge results ranging from 0.4 to 0.6 (Schuckit et al., 1997a; 1997b). A higher score on the SRE indicates a lower level of response to alcohol. In this study we examined the SRE score for the first five lifetime drinking episodes (SRE_5) as a retrospective measure of initial sensitivity to alcohol.

A blood sample was collected by fingertip puncture and sent to the Alcohol Research Center at Indiana University for genotyping of *ALDH2* (rs671, Lys487Glu) and *ADH1B* (rs1229984, Arg47His) loci. Genotyping was conducted by one of two methods (1) using enzymatic amplification of genomic DNA and allele-specific oligonucleotides (Crabb et al., 1989; Xu et al., 1988) or (2) isolating genomic DNA with the “HotSHOT” method (Truett et al., 2000) using TaqMan probes for allelic discrimination (Applied Biosystems, Foster City, CA; see Hendershot et al., 2009 for additional details). Approximately 30 samples were run using both methods when the laboratory was converting from genotyping by the first method to the second method to verify results matched across protocols. Quality control was maintained by several methods, including a) sample and data management to avoid entering mistakes, b) dedicated equipment and supplies for DNA preparation and robot-assist PCR preparation, and c) the inclusion of eight controls on each 96-well plate: two no template controls, two heterozygous samples, and two of each of the homozygous samples. From previously repeated genotyping results using banked samples, we found the SNP calling to be highly reliable (>99.9%). Any undetermined samples were repeated or sequenced until genotypes were obtained.

Chi-square and logistic regression analyses were used to test each alcohol-related symptom separately and ANOVA, t-test, and linear regression analyses were used to test the total symptom and SRE_5 scores. Analyses were first performed to assess whether *ALDH2* genotype was significantly associated with alcohol-related symptoms. We then tested the combined effects of *ALDH2* and *ADH1B* genotypes in multivariate regression analyses that

included interaction terms. Based on the results of these analyses, we stratified the data by *ALDH2* to test the effects of *ADH1B*. We also conducted all analyses with ethnicity included as a covariate in the models given the issue of population stratification and our previous findings of differences in level of response to alcohol across Chinese and Korean ethnic groups as measured by the total SRE, which includes assessment of current and heaviest drinking periods (Duranceaux et al., 2008); results were consistent with and without ethnicity included in the models so we present the data without ethnicity included (data available upon request).

RESULTS

ALDH2 and *ADH1B* were in Hardy Weinberg equilibrium (*p*'s > .27), indicating no selection bias of this sample based on these genes. Symptom data were not available for 20 participants, including 10 participants who had never consumed alcohol. Two participants were also missing responses to the nausea symptom item and their total symptoms scores were calculated without this item included (total scores of 1 for both). An additional 29 individuals did not complete the SRE_5, including 14 individuals who had never had a full drink and 26 who had never been regular drinkers (i.e., consumed alcohol once a month for at least six months). This resulted in a sample size of 764 for symptom analyses and 735 for SRE_5 analyses.

ALDH2 Analyses

Table 1 shows the percentages of participants who endorsed each of the six alcohol-related symptoms by *ALDH2* genotype. *ALDH2* was significantly associated with all six symptoms, including flushing or blushing (χ^2 = 245.57, 2 *df*, *p* < .001), feeling very sleepy (χ^2 = 57.11, 2 *df*, *p* < .001), having headaches, head pounding or throbbing (χ^2 = 113.32, 2 *df*, *p* < .001), having nausea (χ^2 = 80.77, 2 *df*, *p* < .001), having heart palpitations (χ^2 = 184.14, 2 *df*, *p* < .001), and breaking out into hives (χ^2 = 30.77, 2 *df*, *p* < .001). Post-hoc analyses indicated significant differences across all three *ALDH2* genotypes for prevalence rates of sleepiness, headaches, nausea, and heart palpitations (*p*'s < .01). The other two symptoms, flush or blush and hives, were only significantly different across those who had no *ALDH2*2* alleles and those who had one or two *ALDH2*2* alleles (*p*'s < .001).

The bottom portion of Table 1 shows the means and standard deviations for total symptoms and SRE_5 across *ALDH2* genotype. Total symptoms significantly differed across *ALDH2* genotypes (*F* = 186.09, 2/761 *df*, *p* < .001), with post-hoc analyses indicating all three genotypes significantly differed from one another (Tukey HSD *p*'s < .001). Individuals with two *ALDH2*2* alleles endorsed the highest average number of symptoms (3.74) followed by those with one *ALDH2*2* allele (2.05) and those with no *ALDH2*2* alleles (0.73). SRE_5 also significantly differed across *ALDH2* genotype (*F* = 43.15, 2/732 *df*, *p* < .001). Post-hoc analyses indicated individuals with no *ALDH2*2* alleles had significantly higher SRE_5 scores (3.38) than individuals with one (2.22) and two (1.65) *ALDH2*2* alleles (Tukey HSD *p*'s < .001).

ADH1B Analyses

We next assessed the combined effects of *ADH1B* and *ALDH2*. We tested for interactive and additive effects of these two genes by entering the *ALDH2* and *ADH1B* genotypes in step 1 and their interaction term in step 2 of logistic regressions for individual symptoms and linear regressions for total symptoms and SRE_5.

For individual low-dose symptoms, the *ADH1B*-*ALDH2* interaction was statistically significant at the .05 level for sleepy (step χ^2 = 10.58, 4 *df*, *p* = .032). The second row of

Table 2, however, shows that the pattern for sleepy for *ADH1B* in *ALDH2*1/*1* is in the opposite direction from that predicted (and that seen in *ALDH2*1/*2*), with individuals with no *ADH1B*2* alleles having the highest rate (26%) of endorsing sleepy and those with two *ADH1B*2* alleles having the lowest rate (15%); this effect may be spurious, due in part to the small sample size of *ADH1B*1/*1* in *ALDH2*1/*1* (n = 39). No other interaction terms approached significance (p's > .11) so only main effects were considered. With both *ALDH2* and *ADH1B* genotypes entered, only *ALDH2* genotype was significantly associated with individual alcohol related symptoms. Details on these analyses are not presented given *ALDH2* associations have already been described above and are shown in Table 1. The percentages of participants who endorsed each of the six low-dose symptoms across *ADH1B* genotype when stratified by *ALDH2* genotype, however, are shown in Table 2 to provide additional details; only one participant had *ALDH2*2/*2* and *ADH1B*1/*1* so this column is not included in the table.

Linear regression analyses indicated an interaction of *ALDH2* and *ADH1B* in their association with total symptoms and SRE_5. For total symptoms, the *ALDH2-ADH1B* interaction term entered in step 2 was significant (F change = 8.05, p = .005). For SRE_5, the interaction approached significance (F change = 3.25, p = .072). Because the *ALDH2-ADH1B* interaction terms reached or approached significance for both of these variables, we next stratified the data by *ALDH2* genotype and tested for mean differences in total symptoms and SRE_5 across *ADH1B* genotype.

The bottom portion of Table 2 shows the means and standard deviations of total symptoms and SRE_5 across *ADH1B* genotype stratified by *ALDH2* genotype. We performed ANOVA analyses to examine mean differences across *ADH1B* genotypes in the *ALDH2*1/*1* and *ALDH2*1/*2* groups and t-tests to assess mean differences between *ADH1B*1/*2* and *ADH1B*2/*2* in the *ALDH2*2/*2* group. *ADH1B* genotype was not significantly associated with total symptoms or SRE_5 in the *ALDH2*1/*1* or *ALDH2*2/*2* groups, but was significantly associated in the *ALDH2*1/*2* group (F = 3.89, 2/262 df, p = .022 for total symptoms; F = 5.55, 2/254 df, p = .004 for SRE_5). In the *ALDH2*1/*2* group, individuals with two *ADH1B*2* alleles had higher total symptom scores than individuals with only one *ADH1B*2* allele (2.24 vs. 1.80, Tukey HSD p = .041), and had significantly lower SRE_5 scores than individuals with no *ADH1B*2* alleles (2.05 vs. 3.15, Tukey HSD p = .004); the difference between those with one versus no *ADH1B*2* alleles approached but did not reach significance for SRE_5 (2.32 vs. 5.15, Tukey HSD p =.050). Thus, only a few of the stratified comparisons yielded significant differences across genotypes, but it can be seen from Table 2 that there is a consistent trend in both total symptoms and SRE_5 across the *ALDH2-ADH1B* genotype combinations. As the number of variant alleles increases for both genes, the total symptoms score increases and the SRE_5 score decreases, indicating increased sensitivity to alcohol at low levels of consumption and during initial lifetime consumption for individuals who possess either variant allele.

DISCUSSION

Findings from this study suggest an interaction of *ALDH2* and *ADH1B* on alcohol sensitivity. This gene-gene interaction emerged as significant only in the two continuous dependent measures, retrospective reports of total number of alcohol-related symptoms from one or two drinks and initial level of response to alcohol, with a significant effect of *ADH1B* found only in individuals who were heterozygous for *ALDH2*. These findings suggest the effects of *ADH1B*2* may be felt more strongly in Asians who already have some heightened sensitivity to alcohol from possessing one *ALDH2*2* allele, but who are not too sensitized to alcohol from possessing two *ALDH2*2* alleles. This pattern of results is consistent with previous studies of retrospective self-reported facial flushing in Asians that find the

strongest effect of *ADH1B*2* alleles in *ALDH2* heterozygotes (Takashita et al., 1996; 2001; Yokoyama et al., 1999). Similarly, an alcohol challenge study found Asians with two *ADH1B*2* alleles had greater objective (e.g., vomiting) and subjective (e.g., feeling less "great") responses to a moderate dose of alcohol than individuals with one *ADH1B*2* allele, but that this occurred only in *ALDH2* heterozygotes (Cook et al., 2005). Our finding is also consistent with the associations of *ALDH2* and *ADH1B* with risk for alcohol dependence, where the protective effects of these genes are stronger for both variants alleles in conjunction (Chen et al. 1999; Luczak et al., 2006). Thus, the effects of *ADH1B*2* may be stronger (and thus emerge as significant in statistical analyses) when the allele is possessed along with other genetic variants (e.g., *ALDH2* in a gene-gene interaction) or as has been shown in previous reports when in association with certain alcohol behaviors (e.g., heavier drinking in a gene-environment interaction; Higuchi et al., 1996).

These results are largely supportive of our hypotheses. As expected and consistent with previous reports (Chen et al., 1998; Matsuo et al., 2006; Takeshita and Morimoto, 1999; Takeshita et al., 2001; Wall et al., 1999), *ALDH2*2* was associated with heightened response to alcohol for all low-dose symptom and initial sensitivity variables examined in this study. Furthermore, possessing two *ALDH2*2* alleles resulted in stronger effects than possessing one *ALDH2*2* allele for all alcohol variables with the exception of two individual alcohol-related symptoms that had relatively low (7-14%, hives) and relatively high (91-92%, flushing) endorsement rates in both genotypes.

Our prediction that individuals homozygous for *ADH1B*2* would report the most alcohol-related symptoms and heterozygotes would report more low-dose symptoms than those without the variant allele was partially supported by the trends in the data across the *ADH1B*-*ALDH2* genotype combinations (as seen in the bottom two rows of Table 2); although only significant in the *ALDH2* heterozygote group, sensitivity to alcohol increased within each *ALDH2* genotype as the number of *ADH1B*2* alleles increased. In a study of 4,597 Caucasian Australian twins who did not possess *ALDH2*2*, *ADH1B*2* was associated with self-reported flushing and endorsing any of the six alcohol-related symptoms that were assessed in the current study (Macgregor et al., 2008). Thus, it seems reasonable that *ADH1B*2* alone may heighten sensitivity to low doses of alcohol, and that this effect may emerge as significant in larger samples or in samples that have a greater proportion of individuals with no *ADH1B*2* alleles. There is also evidence that the effects of *ADH1B*2* on sensitivity may differ across type of alcohol (see Linneberg et al., 2009) and on alcohol dependence may differ across ethnic groups (e.g., Whitfield, 2002), which suggests the importance of continuing to examine *ADH1B* across ethnic groups and in conjunction with other genetic, physiological, and environmental factors.

This is the first study to examine the relationship between alcohol metabolizing genes and retrospective reports of level of response to alcohol during the first five lifetime drinking episodes. Initial sensitivity to alcohol as measured by the SRE_5 has been prospectively associated with later alcohol consumption, dependence symptoms, and AUDs in adolescent, college student, and adult samples (Morean and Corbin, 2008; Schuckit et al. 2007; 2008). Our findings are consistent with previous research using the total SRE score that demonstrated high response to alcohol in individuals with an *ALDH2*2* allele when assessed across multiple periods of drinking (Wall et al., 1999); our results further indicate the heightened response to alcohol seen in individuals with *ALDH2*2* and *ADH1B*2* alleles is apparent from the very onset of alcohol use (SRE_5 score) and may be indicative of an innate sensitivity rather than a rapid tolerance response. Importantly, *ADH1B*2* being associated with initial lifetime responses to alcohol in *ALDH2* heterozygotes offers another line of data to support *ADH1B* having an effect on sensitivity to alcohol, which has not been consistently reported in the literature.

The pattern of enhanced response to alcohol in individuals with *ALDH2*2* and *ADH1B*2* alleles is supportive of the hypothesized mechanistic pathway for how these variant alleles protect individuals from heavy drinking and alcohol-related problems. The results from this study indicate individuals with *ALDH2*2* and *ADH1B*2* alleles have more intense, and perhaps more aversive, responses to alcohol from the very onset of drinking and that this heightened response occurs even from small amounts of alcohol. These findings provide new support for one of the initial steps of the mechanistic model, the heightened subjective response to alcohol in individuals with these variant alleles. In the proposed mechanistic model, the endophenotype alcohol sensitivity is more proximal to the gene effects than the phenotype of alcohol dependence, and thus may better reflect the etiologic process by which genetic expression gives rise to differential vulnerability for alcohol dependence (Gottesman & Gould, 2003). Increased knowledge of the effects of alcohol metabolizing genes on early and low dose effects of alcohol may help researchers form a better understanding of how these variant alleles ultimately protect against the development of alcohol-related problems and AUDs.

We must consider these findings in light of several limitations to the present study. Participants were all 21 to 26 year old Chinese- and Korean-American college students so the conclusions reached from this sample may not generalize to other ethnic subgroups (e.g., Macgregor et al., 2009; Whitfield, 2002), age groups, educational levels, or samples selected for alcohol dependence or cancers (e.g., Yokoyama et al. 1999; 2003). In addition, our endorsement rates for flushing was higher than reported in several previous studies, which may be due to differences in study methodology, including level of alcohol consumption referenced (1-2 standard drinks vs. 0.25 to 1.0 standard drinks), symptom definitions (general flushing/blushing of the body vs. facial flushing), time frames (ever vs. first year or two of drinking, current, or always), and assessment method (interview self-report vs. questionnaire self-report, patch test, laboratory alcohol consumption, or observer rated); such differences likely result in different endorsement rates of symptoms that make it difficult to directly compare across studies. It is also important to note that retrospective self-reports may be biased in ways that differ across individuals with varying *ALDH2* genotypes, drinking histories, or family history of alcohol problems. The consistency of the findings for low doses of alcohol and for initial drinking episodes, however, as well as the trends in the data across *ADH1B-ALDH2* genotype combinations, suggest these findings may be robust. Regardless of whether the genetic findings are due to actual differences in low-dose and initial responses to alcohol or to biased reports, we can conclude that individuals with *ALDH2*2* and *ADH1B*2* remember experiencing stronger responses to alcohol in these situations, which may affect their alcohol expectancies and in turn alter their alcohol consumption behaviors. The retrospective self-reports used in the present study, however, preclude testing of this possibility and of the causal pathways proposed by the mechanistic model, which would require prospective data.

Although it is important to consider these limitations, this study reports several findings to support the initial steps of the proposed mechanistic pathway for both *ALDH2* and *ADH1B*, with low and initial alcohol use showing stronger responses in those with variant alleles. The present study also offers additional insight into why some studies have found a significant association of *ADH1B* with alcohol symptoms and behaviors whereas other reports have not. By teasing apart these genetic contributions to AUD protection, especially those early in the proposed mechanistic pathway, we may be able to better identify the necessary steps for protecting individuals against the development of alcohol problems.

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Table 1

Percentages, Odds Ratios, and Means of Alcohol-Related Symptoms by *ALDH2* Genotype.

Alcohol-Related Symptoms	***ALDH2*1/*1*** **(*n* = 461)**		***ALDH2*1/*2*** **(*n* = 265)**		***ALDH2*2/*2*** **(*n* = 38)**	
	%	**OR**	**%**	**OR (CI)**	**%**	**OR (CI)**
Flush or blush	34[a]	1.0	91[b]	19.82 (12.49-31.46)	92[b]	23.03 (6.97-76.07)
Sleepy	18	1.0	28	1.74 (1.22-2.49)	71	11.18 (5.33-23.44)
Headaches	9	1.0	35	5.12 (3.43-7.65)	66	18.23 (8.71-38.15)
Nausea [c]	7	1.0	14	2.10 (1.27-3.47)	55	16.48 (7.92-34.32)
Heart palpitations	4	1.0	31	11.70 (6.75-20.28)	74	73.13 (30.65-174.46)
Hives	1[a]	1.0	7[b]	6.65 (2.44-18.12)	16[b]	17.10 (4.95-59.08)
	M	*SD*	*M*	*SD*	*M*	*SD*
Total symptoms	0.73	1.01	2.05	1.39	3.74	1.54
SRE_5[d]	3.38[a]	2.00	2.22[b]	1.43	1.64[b]	1.27

[a] *Notes*. Symptom endorsement rates and means significantly differed across all three genotypes for all variables ($p < .001$) except those subscripted

[b] which significantly from one another ($p < .001$).

[c] $n = 762$ for nausea.

[d] $n = 735$ for SRE_5. OR = odds ratio, fixed at 1.0 for *ALDH2*1/*1* as the reference group.

Table 2

Percentages and Means of Alcohol-Related Symptoms by *ADH1B* Genotype Stratified by *ALDH2* Genotype

Alcohol-Related Symptoms	ALDH2*1/*1 ADH1B			ALDH2*1/*2 ADH1B			ALDH2*2/*2 ADH1B	
	***1/*1** ***n* = 39**	***1/*2** ***n* = 183**	***2/*2** ***n* = 239**	***1/*1** ***n* = 20**	***1/*2** ***n* = 89**	***2/*2** ***n* = 156**	***1/*2** ***n* = 16**	***2/*2** ***n* = 21**
	%	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Flush or blush	31	37	31	85	90	92	88	95
Sleepy	26	20	15	20	24	31	50[a]	86[b]
Headaches	10	10	9	25	28	40	63	67
Nausea[c]	5	7	7	0	8	19	63	52
Heart palpitations	3	3	5	30	28	33	69	76
Hives	0	1	1	5	2	10	13	19
	M (SD)	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*	*M (SD)*
Total symptoms	0.74 (1.07)	0.77 (1.00)	0.69 (1.02)	1.65 (1.04)	1.80[a] (1.22)	2.24[b] (1.49)	3.44 (1.71)	3.95 (1.43)
SRE_5[d]	3.01 (1.94)	3.44 (2.15)	3.39 (1.89)	3.15[a] (1.79)	2.32 (1.55)	2.05[b] (1.24)	1.78 (1.21)	1.60 (1.37)

[a] *Notes*. significantly differ from one another ($p < .05$).

[b] *Notes*. significantly differ from one another ($p < .05$).

[c] $n = 459$ for *ALDH2*1/*1* for nausea.

[d] $n = 446$ for SRE_5.