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*ADH*1B*3 and Response to Alcohol in African Americans

Denis M. McCarthy, Ph.D.,
University of Missouri

Sarah L. Pedersen, M.A.,
University of Missouri

Elizabeth A. Lobos, Ph.D.,
Washington University, School of Medicine

Richard D. Todd, MD, Ph.D., and
Washington University, School of Medicine

Tamara L. Wall, Ph.D.
University of California, San Diego

Abstract

Background—Variations in the alleles for the alcohol metabolizing enzymes have been shown to influence risk for alcohol dependence. One variant, *ADH*1B*3, is observed almost exclusively in populations of African ancestry and has been shown to be associated with reduced rates of alcohol dependence. We conducted an alcohol challenge study to test whether *ADH*1B*3 is associated with differences in subjective and physiological response to alcohol.

Method—We administered a moderate dose of alcohol (0.72g/kg for males, 0.65g/kg for females) to a sample of African American young adults (n = 91; ages 21–26). Participants were genotyped for *ADH*1B, as well as additional polymorphisms that might contribute to alcohol response. Breath alcohol concentration, self-reported sedation and stimulation, and pulse rate were assessed prior to alcohol administration and for 2.5 hours following administration.

Results—*ADH*1B*3 was associated with higher levels of sedation and a sharper increase in pulse rate immediately following alcohol consumption.

Conclusions—These findings suggest that the lower rates of alcohol dependence in those with *ADH*1B*3 alleles may be due to differences in alcohol response, particularly increased sedation.

Introduction

Genetic differences in the alcohol metabolizing enzymes have been shown to make a significant contribution to alcohol dependence risk (Luczak et al., 2006; Li, 2000). One

Corresponding Author: Denis M. McCarthy, 210 McAlester Hall, Columbia, MO 65211, Phone: 573-882-0426 Fax: 573-882-7710, mccarthydm@missouri.edu.
Author Note
Denis M. McCarthy and Sarah L. Pedersen, Department of Psychological Sciences, University of Missouri; Elizabeth A. Lobos and Richard D. Todd, Department of Psychiatry, Washington University; Tamara L. Wall, Department of Psychiatry, University of California, San Diego.

hypothesized mechanism by which these genetic differences influence alcohol use disorders is through differences in response to alcohol. Considerable research has demonstrated that individual differences in response to alcohol are genetically influenced (Heath et al., 1999) and related to risk for heavy alcohol use and alcohol dependence (e.g., Conrod et al., 2001; Schuckit & Smith, 2001).

Alleles for the alcohol metabolizing enzymes have been found to occur at different frequencies in different ethnic groups. A variant of one of the alcohol dehydrogenase genes, *ADH*1B*3, has been found almost exclusively in populations of African ancestry (Osier et al., 2002) and has been shown to be associated with lower rates of alcohol dependence (Edenberg et al., 2006; Wall et al., 2003). The present study is the first to test subjective and physiological response to alcohol as a potential mechanism of the protective effect of this allele.

ADH and ALDH Polymorphisms and Alcohol Dependence Risk

Following consumption, alcohol is primarily metabolized by alcohol dehydrogenase (ADH) enzymes in the liver, which oxidize ethanol into acetaldehyde. There are seven known genes that code for *ADH* enzymes. The majority of research has examined the class I *ADH* genes (*ADH*1A, *ADH*1B, *ADH*1C). Two of these (*ADH*1B and *ADH*1C) have been found to exhibit variants that encode for enzymes with different kinetic properties (Edenberg, 2007). Acetaldehyde is, in turn, broken down into acetate by aldehyde dehydrogenase (ALDH). There are also several molecular forms of *ALDH*. The gene for mitochondrial *ALDH* (*ALDH*2) has been found to have two allele variants and three variants have been identified in the promoter region of the cytosolic isoform *ALDH*1A1. Genes that encode for *ADH* enzymes that more rapidly metabolize alcohol, or for *ALDH* enzymes that are less efficient in the breakdown of acetaldehyde, have been found to be associated with lower risk for alcohol dependence and heavy drinking. The hypothesized mechanism for this protection is higher transient levels of acetaldehyde, which can produce a stronger response to alcohol, including a “flushing reaction” (see Wall, 2005 for a review). Although acetaldehyde levels are difficult to directly measure in humans, animal models have demonstrated that faster elimination of alcohol, due in part to high *ADH* activity, can create a short-term acetaldehyde “burst” which is associated with reduced alcohol intake (Quintanilla et al., 2007).

Significant variability in frequencies of *ALDH* alleles have been observed across ethnic groups. *ALDH*2*2 alleles have been found at moderate rates (20–50%) in samples of northeast Asian heritage (Goedde et al., 1992), and have been found to be associated with lower risk for the development of alcohol dependence (Luczak et al., 2006). Ethnic differences also have been identified in the frequency of the three variants in the *ALDH*1A1 promoter region: *ALDH*1A1*1, *ALDH*1A1*2, and *ALDH*1A1*3. *ADLH*1A1*2 alleles have been found at low frequencies (<4%) in Asian, Jewish, Caucasian, Native American, and African American samples; *ADLH*1A1*3 alleles have been identified at low frequency (≈3%) in samples of Native American and African ancestry (Ehlers et al., 2004; Moore et al., 2007; Spence et al., 2003). There is some evidence that *ALDH*1A*3 is associated with alcohol dependence in African Americans (Spence et al, 2003), while the association

between *ADLH*1A1*2 and alcohol dependence is mixed (Ehlers et al., 2004; Moore et al., 2007; Spence et al., 2003). The effect of *ALDH*1A1 variants on alcohol metabolism and alcohol response is unclear.

Studies of *ADH*1C variants have demonstrated decreased risk for alcohol dependence in those homozygous for *ADH*1C*1 compared to those with at least one *ADH*1C*2 allele in Asian populations (Higuchi et al., 1996; Shen et al., 1997). Modest associations between *ADH*1C*2 and increased alcohol dependence risk have also been demonstrated in European populations (Whitfield, 1997). Studies of linkage disequilibrium have indicated that the association between *ADH*1C*2 and alcohol dependence may be due instead to variants of *ADH*1B in some ethnic groups (Osier et al. 1999), although other studies have demonstrated independent effects of *ADH*1C*2 (Luo et al., 2006). It may be that *ADH*1C*2 has a larger effect on alcohol dependence in populations where *ADH*1B*2 is infrequent, such as Europeans and African Americans (Whitfield, 1997).

*ADH*1B*2 alleles have been identified at high frequencies (>60%) in east-Asian populations and at low frequencies (<13%) in European and North African populations (Osier et al., 2002). Meta-analytic studies have indicated a significant association between *ADH*1B*2 alleles and reduced risk for alcohol dependence in both Asian (Luczak et al., 2006) and European samples (Whitfield, 2002).

The *ADH*1B*3 allele has been found primarily in people of African descent (Bosron & Li, 1987) from almost all regions of Africa (Osier et al., 2002). This allele has also been identified at low frequency (≈ 6%) in certain Native American groups (Wall et al., 1997), although this may be due to population admixture. Up to one-third of African Americans possess an *ADH*1B*3 allele (Ehlers et al., 2001; Li et al., 2001; McCarver et al., 1997). In African Americans, the presence of at least one *ADH*1B*3 allele has been associated with reduced drinking behavior (Ehlers et al., 2001) and lower risk for alcohol dependence (Ehlers et al., 2007; Edenberg et al., 2006; Luo et al., 2006). *ADH*1B*3 has also been found to be negatively associated with family history of alcohol dependence (Ehlers et al., 2001) and associated with decreased risk of alcohol-related birth defects (McCarver et al., 1997). *ADH*1B*3 alleles have also been associated with reduced likelihood of alcohol dependence and heavy consumption in Native Americans (Wall et al., 2003).

ADH1B*3 and Response to Alcohol in African Americans

Epidemiological data suggest that, compared to Caucasians, African Americans have lower lifetime prevalence of alcohol use disorders (Breslau et al., 2006). African American adolescents show slower increases in rates of drinking (Warheit et al., 1996) and have higher abstention rates (Substance Abuse and Mental Health Services Administration, 2003). African American adolescents (Wallace et al., 2003; Bachman et al., 1991) and college students (O'Malley & Johnston, 2002) also engage in less heavy drinking than Caucasians.

The current study was designed to test the association between *ADH*1B polymorphisms and alcohol response in an African American sample. Prior studies have demonstrated that *ADH*1B*3 alleles are associated with faster elimination of alcohol (Thomasson et al., 1995), and in vitro studies support greater ethanol-oxidizing activity in enzymes encoded by

*ADH*1B*3 compared to *ADH*1B*1 (Lee et al., 2004). However, as Scott and Taylor (2007) have noted, research on alcohol metabolism in African-Americans is limited. To date, no study has examined differences in response to alcohol as the mechanism for the protective effect of this allele.

The current study utilized an alcohol challenge paradigm to test subjects with and without *ADH*1B*3 alleles for differences in their subjective and physiological response to alcohol. We administered a moderate dose of alcohol, designed to produce a peak breath alcohol concentration (BrAC) of approximately 0.075 to 0.080%, to a sample of African American young adults (ages 21–26). Measures of alcohol response (including self-reported sedation, stimulation, and pulse rate) were assessed at baseline, every 15 minutes during the first hour following ingestion, and every 30 minutes for 90 minutes thereafter. Participants were genotyped for polymorphisms of *ADH*1B, *ADH*1C, and the *ALDH*1A1 promoter region.

We hypothesized that participants with at least one *ADH*1B*3 allele would exhibit a stronger response to alcohol, indicated by increased sedation, stimulation, and pulse rate following consumption, than those with two *ADH*1B*1 alleles. Although *ADH*1B*2 has been shown to be associated with lower risk for alcohol dependence as well (Whitfield, 2002), this variant is rare in populations of African ancestry (Osier et al, 2002). *ADH*1C*2 has also been found to be associated with increased alcohol dependence risk (Whitfield, 1997), and is thought to be associated with slower metabolism of alcohol (Edenberg, 2007). We tested whether subjects with *ADH*1C*2 alleles exhibited lower response to alcohol than those with *ADH*1C*1. As it is unclear whether *ADH*1B and *ADH*1C exert independent effects on alcohol metabolism (Luo et al., 2006; Osier et al., 1999), analyses were conducted separately for *ADH*1B and *ADH*1C.

Materials and Methods

Participants

The full sample consisted of 116 young adult African Americans. The sample was 42% male and had a mean age of 21.9 years (*SD* = 1.15, range 21–26). While all study participants were of African descent, 10% of the sample described themselves as mixed race, and 3% identified their ethnicity as Hispanic. For those reporting mixed race, study inclusion criteria required that they have at least one parent of African ancestry. The majority of the sample (77%) had some college education and 20% reported being college graduates. Participants were required to be between the ages of 21 and 26 years and to be current drinkers. Participants were required to report at least one drinking episode in the past six months where their consumption was consistent with the amount they would receive in the present study (e.g., 3–4 drinks for a 165 pound male). Participants were excluded if they were currently abstaining from alcohol because of an alcohol use disorder, had significant medical or psychiatric illness (e.g., psychotic disorders, past head injury with loss of consciousness > 5 minutes), or were currently taking medication for which use of alcohol is contraindicated.

Procedures

Study procedures were approved by the University of Missouri and Washington University Institutional Review Boards, and written informed consent was obtained prior to each session. Participants were recruited from the University of Missouri, the city of Columbia, Missouri, and the surrounding area. Fliers were placed at various locations on campus and at local businesses. Potential participants were screened by phone to determine eligibility for the study. Participants who met eligibility criteria were scheduled for an in-person interview conducted by a trained research assistant in a private office. Participants also completed questionnaire measures, including self-report measures of alcohol use. Buccal brush samples were taken from each participant for genotyping. Participants received $40 for their participation in the interview.

Participants were scheduled for an alcohol challenge session approximately one week after the initial interview. Participants were given an information packet prior to their laboratory appointment, which instructed them to refrain from alcohol for 24 hours before the session and to refrain from other drug use for 48 hours. They were instructed to refrain from eating or drinking caffeinated or dairy beverages for 8 hours prior to their session (starting at 12 midnight the prior evening). Participants arrived at the laboratory at 8:00 a.m. A questionnaire was administered to verify compliance with pre-session instructions. A breath alcohol test was used to verify abstinence from alcohol. Females were given a urine pregnancy test and excluded from the study if they tested positive. A standard low-fat breakfast (bagel and juice) was provided.

The alcohol administration and assessment were conducted in a private office, with a semi-recumbent chair, separate from that used for interviews. This office was equipped with a vital signs monitor and computer. Participants were assessed prior to beverage consumption, in 15 minute intervals for the first hour following consumption, and 30 minute intervals thereafter (i.e., 15, 30, 45, 60, 90, 120, and 150 minutes). Between 8:30 and 9:00 a.m., baseline measures were taken. At 9:00 a.m., participants received an alcoholic beverage. Participants received a dose of alcohol equivalent to 0.72g/kg of alcohol for males and 0.65g/kg for females. This dose was designed to produce a peak BrAC of approximately 0.075 to 0.080 mg% for both males and females (Sher & Walitzer, 1986). The alcohol drinks were made using 50% alcohol (vodka), in 20% solution with non-caffeinated soda (tonic). Beverages were consumed over a 15-minute period. At approximately noon, each participant was provided lunch.

To minimize risk, the following procedures outlined in the NIAAA Recommended Council Guidelines on Ethyl Alcohol Administration in Human Experimentation were used (NIAAA, 2005). Participants were not allowed to leave the laboratory until their observable behavior had returned to normal and until their BrAC fell below 0.02mg%. Each participant was also required to travel home by taxi (provided by the study), or with a friend. Participants were required to state in writing that he or she would not drive a car or operate other machinery for three hours after leaving the laboratory. They were reimbursed $100 for participation in the session.

Measures

Alcohol Use Behavior—Participant alcohol involvement was assessed through both interview (i.e., the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-II; Bucholz et al., 1994)) and self-report (e.g., Drinking Styles Questionnaire (DSQ: Smith et al., 1995)). Self-report of past month quantity and frequency of alcohol use are included in the current analyses.

BrAC—Breath alcohol readings were taken using a breathalyzer device (Intoximeters, Inc.) at baseline and at all measurement points after consumption of the beverage.

Subjective Feelings of Intoxication—Subjective feelings of intoxication were evaluated at baseline and at all measurement points following beverage consumption, using the Biphasic Alcohol Effects Scale (BAES: Martin et al., 1993). This measure assesses separate sedating and stimulating effects of alcohol on both the ascending and descending limb of the blood alcohol curve (Earleywine & Erblich, 1996).

Pulse Rate—Pulse rate was obtained using an automatic vital signs measurement system at baseline and at all measurement points after consumption of the beverage.

Genotyping

Buccal brush samples were sent to Washington University School of Medicine for genotype analysis. The *ADH*1B haplotypes were determined by separate PCR amplifications (primers from Osier et al, 2002) of the regions around exon 3 (Arg48His; rs1229984) and exon 9 (Arg370Cys; rs2066702) followed by restriction endonuclease digests (with *Msl* I and *Alw* NI, respectively). No samples were heterozygous at both sites, so the haplotypes *ADH*1B*1 (48Arg-370Arg), *ADH*1B*2 (48His-370Arg), and *ADH*1B*3 (48Arg-370Cys) were clearly indicated.

Likewise, the *ADH*1C haplotypes were determined by separate PCR amplifications of the regions around exon 6 (Arg272Gln; rs1693482; PCR primers from sequencing protocol of ss8819648) and exon 8 (Ile350Val; rs698; primers from Osier et al, 2002), followed by restriction endonuclease digests (with *Alu* I and *Ssp* I, respectively). These sites are known to be in strong linkage disequilibrium, and, indeed, all samples were either homozygous for both Arg (exon 6) and Ile (exon 8), homozygous for both Gln (exon 6) and Val (exon 8), or were heterozygous at both sites. Thus, haplotypes *ADH*1C*1 (272Arg-350Ile) and *ADH*1C*2 (272Gln-350Val) were determined.

The *ALDH*1A1 promoter region polymorphism (rs8187866; alleles *ALDH*1A*1, *ALDH*1A*2 (17 base-pair deletion) and *ALDH*1A1*3 (3 base-pair insertion)) was genotyped on the ABI 310 Genetic Analyzer using the A4-forward and A4-reverse primers of Spence and colleagues (2003), with a 5′-FAM-fluorescent dye added to the A4-forward primer. The size estimates for the alleles using the Applied Biosystems Genescan-500 size standard were about 6 basepairs larger (198, 215, 218 bp vs. 192, 209, 212 bp) than Spence and colleagues (2003).

Results

Genotype Frequencies and Descriptive Statistics

Table 1 presents *ADH*1B and *ADH*1C genotype frequencies for the full sample. Thirty-four percent of the sample had at least one *ADH*1B*3 allele, while 25% had at least one *ADH*1C*2 allele. Hardy-Weinberg equilibrium was not significant for *ADH*1B (rs2066702; χ^2 (1, N = 116) = 0.39, ns; rs1229984; χ^2 (1, N = 116) = 0.02, ns) or *ADH*1C, χ^2 (1, N = 116) = 0.07, ns; Rodriguez, Gaunt & Day, 2009). Chi-square analyses did not indicate a significant association between *ADH*1B and *ADH*1C genotype frequencies (χ^2 (6, N = 116) = 7.27, p = .30). The distribution of genotypes did not differ across gender or ethnic group (African American, mixed race, Hispanic/Latino).

Two participants had one *ADH*1B*2 allele, rare in samples of African ancestry. Seven participants were heterozygous for *ALDH*1A*2 (*ALDH*1A*1/*2) and four were heterozygous for *ALDH*1A*3 (*ALDH*1A*1/*3). *ADH*1B*2 has been shown to influence alcohol response (Cook et al., 2005; Duranceaux et al., 2006), while the influence of *ALDH*1A*2 and *ALDH*1A*3 alleles on response to alcohol is uncertain. Participants with these alleles (n = 13) were excluded from study analyses testing the effect of *ADH*1B and *ADH*1C on alcohol response[1].

Thirteen participants became ill (vomited) following alcohol consumption. Chi-square analyses indicated that those who became ill did not differ from the remainder of the sample in *ADH*1B or *ADH*1C status. These participants also did not differ in gender, weight, and past month frequency of alcohol consumption. However, there was a trend for those who became ill to report a lower quantity of past month alcohol consumption (t (114) = 1.87, p = .06; mean drinks per occasion: 3.05 vs. 2.33). As the research protocol was discontinued for these participants, they were excluded from study analyses. Excluding these participants, as well as participants with *ADH*1B*2, *ALDH*1A*2 or *ALDH*1A*3 alleles, resulted in a sample size of 91 for study analyses.

Table 1 also presents genotype frequencies for the final sample used in study analyses. For analytic purposes, *ADH*1B and *ADH*1C gene status was coded as two levels, comparing subjects with at least one copy of either *ADH*1B*3 or *ADH*1C*2 to those with no copies (homozygous for either *ADH*1B*1 or *ADH*1C*1). Analyses of *ADH*1B were conducted within subjects homozygous for *ADH*1C*1 (*ADH*1B*3(-)/*ADH*1C*1 compared to *ADH*1B*3(+)/*ADH*1C*1), while analyses of *ADH*1C were conducted within those homozygous for *ADH*1B*1.

Table 2 presents gender, weight, mean peak BrAC and self-reported past month quantity and frequency of alcohol consumption for participants included in study analyses separately by *ADH*1B and *ADH*1C allele status. There were no significant differences in these variables for the alleles of either *ADH*1B or *ADH*1C.

[1]As the influence of *ALDH*1A variants on alcohol response is unclear, we chose to conduct analyses excluding participants with *ALDH*1A*2 and *ALDH*1A*3 (n = 11). The main findings of the study do not change if these participants are included in analyses. The main effect of *ADH*1B on sedation is slightly stronger (F (1, 73) = 5.86, p < .05; partial η^2 = .09) and the time x pulse rate interaction remains significant (F (7, 73) = 2.08, p < .05; partial η^2 = .04).

ADH1B and Alcohol Response

We first tested whether changes in BrAC varied as a function of *ADH*1B gene status. A 7 (time) × 2 (gender) × 2 (*ADH*1B gene status) mixed factorial analysis of variance (ANOVA) was conducted. Baseline BrAC was not included, as it was a constant. Results indicated a main effect of time (F (6, 62) = 41.91, $p < .01$; partial $\eta^2 = .41$) and gender on BrAC (F (1, 62) = 4.46, $p < .05$; partial $\eta^2 = .07$). Both the main effect of *ADH*1B gene status (F (1, 62) = 1.07, $p = .31$; partial $\eta^2 = .02$) and the time x *ADH*1B interaction (F (6, 62) = 0.51, $p = .80$; partial $\eta^2 = .01$) were not significant. Follow-up ANOVAs indicated that BrAC did not differ by *ADH*1B gene status at any time point (see Figure 1).

A series of 8 (time) × 2 (gender) × 2 (*ADH*1B gene status) mixed factorial ANOVAs were then conducted to examine change in alcohol response measures (sedation, stimulation, and pulse rate) by *ADH*1B gene status. For these analyses, BrAC at the 60 minute time point was included as a covariate, as this time point represented the average peak BrAC for most participants.

Results for sedation indicated a main effect of *ADH*1B gene status (F (1, 62) = 4.23, $p < .05$; partial $\eta^2 = .07$), gender (F (1, 62) = 5.90, $p < .05$; partial $\eta^2 = .09$). There was also a significant time x gender interaction (F (7, 62) = 3.59, $p < .01$; partial $\eta^2 = .06$), with females reporting greater increases in sedation. Follow-up analysis of covariance (ANCOVA) was then used to compare the groups differentiated by allele status at each time point. BrAC for each time point was included as a covariate, except for at baseline, where it was a constant. Results indicated no difference in self-reported sedation across *ADH*1B groups at baseline (F (1, 66) = 1.10, $p = .30$; partial $\eta^2 = .02$). *ADH*1B*3 participants reported significantly higher sedation at the 15 minute (F (1, 66) = 4.84, $p < .05$; partial $\eta^2 = .07$), 60 minute (F (1, 66) = 4.65; $p < .05$; partial $\eta^2 = .07$), and 150 minute (F (1, 66) = 5.16; $p < .05$; partial $\eta^2 = .08$) time points, with a marginally significant difference at the 90 minute (F (1, 66) = 3.55; $p = .06$; partial $\eta^2 = .05$) time point. *ADH*1B groups did not significantly differ at the 30 minute (F (1, 66) = 2.86, $p = .10$; partial $\eta^2 = .04$), 45 minute (F (1, 66) = 1.26, p = .27; partial $\eta^2 = .02$) and 120 minute (F (1, 66) = 1.83, p = .18; partial $\eta^2 = .03$) time points. Figure 2 presents estimated means of BAES sedation separately by *ADH*1B allele status.

Results did not indicate significant *ADH*1B main effects or interactions for self-reported stimulation. For stimulation, results indicated a time x gender interaction (F (7, 62) = 2.50, $p < .05$; partial $\eta^2 = .04$), with males reporting a sharper increase in stimulation than females. No other main effects or interactions were significant.

For pulse rate, there was a significant main effect of time (F (7, 62) = 2.18, $p < .05$; partial $\eta^2 = .04$), and gender (F (1, 62) = 13.07, $p < .01$; partial $\eta^2 = .18$), as well as a time x *ADH*1B interaction (F (7, 62) = 2.16, $p < .05$; partial $\eta^2 = .04$). Examination of means indicated that males exhibited higher pulse rate than females. Although ANCOVA results did not indicate significant differences across *ADH*1B groups at any time point, as shown in Figure 3, participants with *ADH*1B*3 alleles experienced a sharper increase in pulse rate from the baseline to 15 minute assessments, and presents estimated means of pulse rate separately by *ADH*1B gene status.

ADH1C and Alcohol Response

A 7 (time) × 2 (gender) × 2 (*ADH*1B gene status) mixed factorial analysis of variance (ANOVA) was conducted to test whether BrAC varied as a function of *ADH*1C gene status. Baseline BrAC was not included, as it was a constant. Results indicated a main effect of time (F (6, 55) = 39.94, $p < .01$; partial $\eta^2 = .43$) on BrAC, but no significant main effect of *ADH*1C (F (1, 55) = 0.04, $p = .84$; partial $\eta^2 = .001$) or time x *ADH*1C interaction (F (6, 55) = 0.64, $p = .70$; partial $\eta^2 = .01$).

A parallel series of 8 (time) × 2 (gender) × 2 (*ADH*1C gene status) mixed factor ANOVAs were then conducted to examine change in alcohol response measures by *ADH*1C gene status. No significant main effects were observed for *ADH*1C gene status on sedation (F (1, 55) = 1.78, $p = .19$; partial $\eta^2 = .03$), stimulation (F (1, 55) = 0.95, $p = .34$; partial $\eta^2 = .02$), or pulse rate (F (1, 55) = 1.15, $p = .29$; partial $\eta^2 = .02$). Interactions involving *ADH*1C were also not significant.

Discussion

The *ADH*1B*3 allele, observed in up to one-third of participants in African American samples, has been found to be associated with reduced risk for alcohol dependence (Edenberg et al., 2006; Luo et al., 2006; Wall et al., 2003). One hypothesized mechanism for this is that the faster elimination of alcohol in carriers of this allele leads to higher levels of acetaldehyde and a stronger response to alcohol. Results of the present study provide the first test of subjective and physiological indicators of alcohol response as a function of *ADH*1B*3 alleles.

Despite similar recent alcohol use and post-consumption BrAC levels, individuals in this study with at least one *ADH*1B*3 allele reported experiencing greater sedation following a moderate dose of alcohol. This difference was most pronounced at the 60 minute assessment, when most participants were at their peak BrAC. A time x gene status interaction effect indicated that subjects with *ADH*1B*3 alleles also experienced a sharper increase in pulse rate immediately after consumption compared to those homozygous for *ADH*1B*1. An increase in pulse rate is consistently associated with alcohol-induced flushing and *ADH*/*ALDH* polymorphisms (Peng et al., 1999; Wall et al., 1992). The sharp elevation in pulse rate, followed by a rapid return, is consistent with the short-term acetaldehyde “burst” hypothesized to result from faster alcohol conversion (Quintanilla et al., 2007). The pattern of results is similar to studies comparing *ADH*1B*1 and *ADH*1B*2 in an Asian sample (Cook et al., 2005) as well as a mixed Caucasian and African American sample (Duranceaux et al., 2006). The isoenzymes encoded by *ADH*1B*2 and *ADH*1B*3 are fairly similar in their kinetic constants and should produce faster alcohol elimination rates compared to *ADH*1B*1 (Edenberg, 2007).

No significant differences were observed between those with two *ADH*1C*1 alleles and those with at least one *ADH*1C*2 allele. Findings have been inconsistent regarding whether *ADH*1C polymorphisms influence alcohol dependence risk over and above the effect of *ADH*1B alleles (Luo et al., 2006; Osier et al., 1999). Some studies have identified modest

differences between *ADH*1C*1 and *ADH*1C*2 in alcohol response (Duranceaux et al., 2006) and alcohol metabolism (Lee et al., 2006) after accounting for *ADH*1B status.

As noted, *ALDH*1A1 polymorphisms are also found to occur at low frequencies in African Americans. The association of *ALDH*1A1 variants with risk for alcohol dependence is unclear (Ehlers et al., 2004; Moore et al., 2007; Spence et al., 2003). Although *ALDH*1A1 is associated with alcohol metabolism, it is also unclear whether variations in the *ALDH*1A1 promoter region contribute to differences in alcohol metabolism or alcohol response. In the present study, the frequencies of *ALDH*1A1 variants (*ALDH*1A1*2 and *ALDH*1A1*3) were too low to allow for separate analysis, so participants with these variants were excluded from the analyses, so as not to confound their possible effects on alcohol metabolism with the effects of the tested alleles for *ADH*1B and *ADH*1C.

While accounting for *ADH*1C is most relevant to testing the effect of *ADH*1B*3, there is also evidence for the influence of other *ADH* genes on alcohol dependence risk, including *ADH*4 (Edenberg et al., 2006), *ADH*5, and *ADH*7 (Luo et al., 2006). Molecular genetic studies of alcohol dependence risk are increasingly incorporating a range of *ADH* genes to test their joint and unique effects (Edenberg, 2007). An important direction for future studies of genetic influences on behavioral and psychological factors, such as alcohol response, is to examine a broader range of *ADH* genes in order to examine the distinct contribution of each and test for potential gene-gene interactions.

While *ADH*1B*3 is thought to result in faster metabolism of alcohol and higher levels of acetaldehyde, participants in the present study with *ADH*1B*3 alleles did not differ in BrAC from those with *ADH*1B*1. This is consistent with prior studies using oral alcohol administration (Taylor et al., 2008). Studies using intravenous alcohol administration and a BrAC clamping method are better able to detect differences in alcohol elimination rate as a function of *ADH* polymorphisms (Neumark et al., 2004). In addition, more research is needed to determine if *ADH*1B*3 results in faster production of acetaldehyde.

Another limitation of the current study is that alcohol response was tested for a single dose level across all participants. There is some evidence that the kinetic properties of *ADH* enzymes in those with *ADH*1B*3 have greater activity at higher alcohol concentrations (Lee et al., 2006). Effect size results for the current study are relatively modest, with partial η^2 values ranging from .04-.08 for sedation main effects. It may be that greater differences in alcohol response would be observed at higher alcohol doses. The study also lacked a placebo condition, which prevents testing of potential expectancy effects. However, both the participants and experimenter were blind to the participants' genetic status, which makes expectancy effects an unlikely explanation for observed *ADH*1B*3 differences.

Although previous studies have observed differences in drinking behavior in those with at least one *ADH*1B*3 allele (Ehlers et al., 2001), no such differences were observed in the current study. This may be the result of eligibility requirements for the study. Participants were only enrolled in the alcohol challenge portion of the study if they reported recent drinking at quantities at least equal to the amount of alcohol administered in the study. The similarity in recent drinking behavior between those with *ADH*1B*1 and *ADH*1B*3 alleles

reduces the likelihood that *ADH*1B differences in alcohol response are due to differences in recent drinking history or alcohol tolerance.

Convergent evidence has suggested that the reduced risk for alcohol dependence seen in African Americans with *ADH*1B*3 alleles may be in part due to differences in alcohol metabolism (Scott & Taylor, 2007). Results of this study provide the first test of behavioral and physiological differences in those with *ADH*1B*3 alleles following alcohol consumption. Further research is required to more fully understand the role that *ADH*1B*3 and response to alcohol play in determining alcohol use topography and alcohol dependence risk in African Americans.

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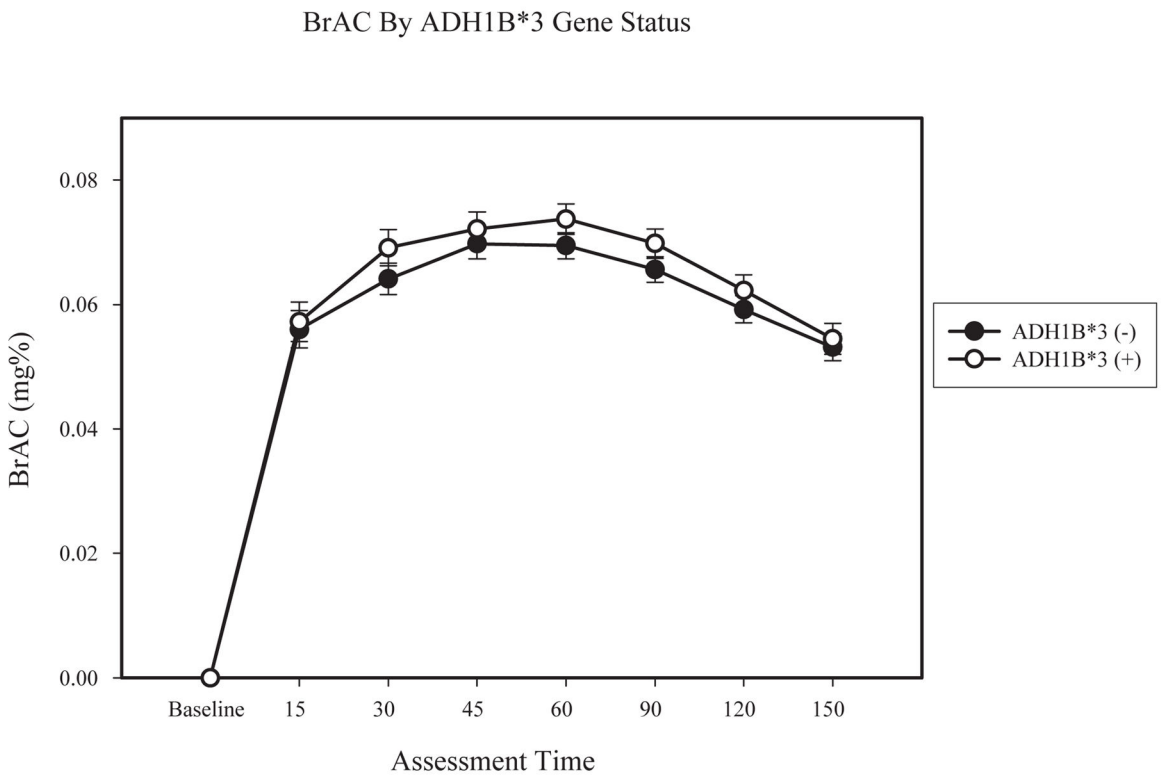

Figure 1.

Lines represent BrAC separately by *ADH*1B gene status, within participants homozygous for *ADH*1C*1. Error bars are based on the standard error of the mean.

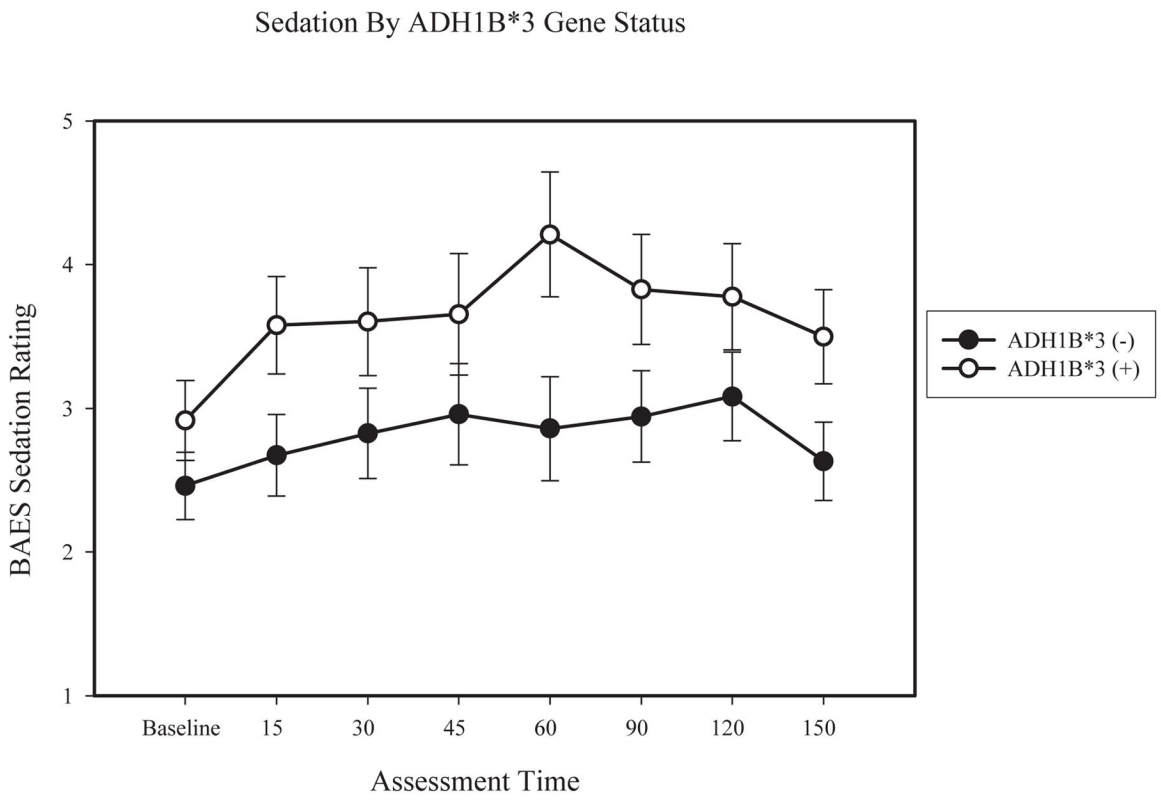

Figure 2.

Lines represent means of self-reported sedation from the BAES separately by *ADH*1B gene status, within participants homozygous for *ADH*1C*1, controlling for BrAC at 60 minutes. Error bars are based on the standard error of the mean.

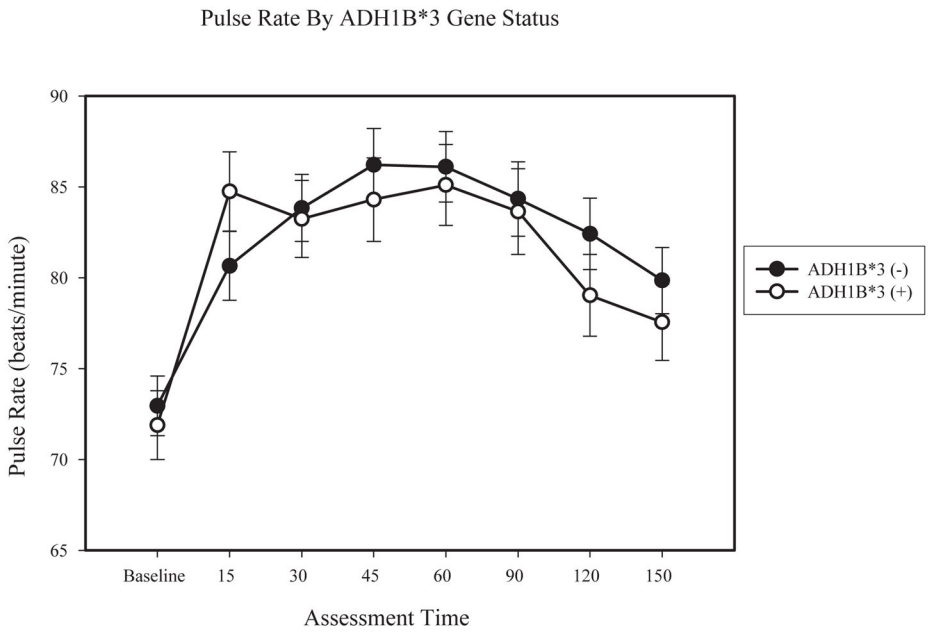

Figure 3.

Lines represent means of pulse rate separately by *ADH*1B gene status, within participants homozygous for *ADH*1C*1, controlling for BrAC at 60 minutes. Error bars are based on the standard error of the mean.

Table 1

ADH1B and ADH1C Genotype Frequencies.

***ADH*1B Genotype**	**Full Sample (*N* = 116)**	***ADH*1C*1/*1 (*n* = 85, 73%)**	***ADH*1C*1/*2 (*n* = 29, 25%)**	***ADH*1C*2/*2 (*n* = 2, 2%)**
*ADH*1B*1/*1	76 (66%)	50 (43%)	24 (21%)	2 (2%)
*ADH*1B*1/*2	2 (2%)	2 (2%)	0	0
*ADH*1B*1/*3	33 (28%)	28 (24%)	5 (4%)	0
*ADH*1B*3/*3	5 (4%)	5 (4%)	0	0
	Final Sample (n = 91)	*ADH*1C*1/*1 (*n* = 67, 74%)	*ADH*1C*1/*2 (*n* = 22, 24%)	*ADH*1C*2/*2 (*n* = 2, 2%)
*ADH*1B*1/*1	60 (66%)	39 (43%)	19 (21%)	2 (2%)
*ADH*1B*1/*3	26 (29%)	23 (25%)	3 (3%)	0
*ADH*1B*3/*3	5 (5%)	5 (6%)	0	0

Note: Values are number of participants or percent of the full or final sample (in parentheses).

Table 2

Gender, Weight, BrAC, and Alcohol Consumption by Gene Status.

	Final Sample	*ADH*1B		*ADH*1C	
	***n* = 91**	***ADH*1B*1 (n = 60)**	***ADH*1B*3 (n = 31)**	***ADH*1C*1 (n = 67)**	***ADH*1C*2 (n = 24)**
Female (%)	57%	58%	55%	55%	63%
Weight (pounds)	172.0 (42.26)	175.6 (45.63)	165.2 (34.48)	174.0 (45.57)	166.5 (31.41)
Peak BrAC (mg%)	.077 (.01)	.076 (.01)	.078 (.01)	.078 (.01)	.074 (.01)
Alcohol Frequency (days/month)	6.98 (7.47)	6.9 (6.56)	7.2 (9.10)	6.9 (7.71)	7.3 (6.92)
Alcohol Quantity (drinks/occasion)	2.92 (1.49)	3.0 (1.53)	2.7 (1.42)	3.0 (1.54)	2.7 (1.36)

Note: Values are percents, means, or standard deviations (in parentheses).