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Interactions Between Alcohol Metabolism Genes and Religious Involvement in Association With Maximum Drinks and Alcohol Dependence Symptoms

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ABSTRACT. Objective: Variations in the genes encoding alcohol dehydrogenase (ADH) enzymes are associated with both alcohol consumption and dependence in multiple populations. Additionally, some environmental factors have been recognized as modifiers of these relationships. This study examined the modifying effect of religious involvement on relationships between *ADH* gene variants and alcohol consumption–related phenotypes. **Method:** Subjects were African American, European American, and Hispanic American adults with lifetime exposure to alcohol (N = 7,716; 53% female) from the Collaborative Study on the Genetics of Alcoholism. Genetic markers included *ADH1B*-rs1229984, *ADH1B*-rs2066702, *ADH1C*-rs698, *ADH4*-rs1042364, and *ADH4*-rs1800759. Phenotypes were maximum drinks consumed in a 24-hour period and total number of alcohol dependence symptoms according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.* Religious involvement was defined by self-reported religious services attendance. **Results:** Both religious involvement and *ADH1B*-rs1229984 were negatively associated with the number of maximum drinks consumed and the number of lifetime alcohol dependence symptoms endorsed. The interactions of religious involvement with *ADH1B*-rs2066702, *ADH1C*-rs698, and *ADH4*-rs1042364 were significantly associated with maximum drinks and alcohol dependence symptoms. Risk variants had weaker associations with maximum drinks and alcohol dependence symptoms as a function of increasing religious involvement. **Conclusions:** This study provided initial evidence of a modifying effect for religious involvement on relationships between *ADH* variants and maximum drinks and alcohol dependence symptoms. (*J. Stud. Alcohol Drugs, 77,* 393–404, 2016)

ALCOHOL DEHYDROGENASE (*ADH*) enzyme genes are consistently associated with both alcohol consumption and dependence (Hurley & Edenberg, 2012). Generally, those variants associated with a higher rate of ethanol metabolism to acetaldehyde are associated with decreased alcohol consumption and lower risk for alcohol dependence. The hypothesized mechanism is that enhanced negative reactions to ethanol (e.g., flushing, higher levels of sedation) are associated with a buildup of acetaldehyde in the body, which tends to limit heavy alcohol consumption (Hurley & Edenberg, 2012; Macgregor et al., 2009; McCarthy et

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al., 2010). Social factors also influence drinking and risks for alcohol dependence, and are recognized to play a role in varying the genetic susceptibility or protection for this disease. The current study examined the joint associations between religious involvement, as measured by religious services attendance, and *ADH* gene variants in predicting two alcohol consumption–related phenotypes. Services attendance is negatively associated with alcohol use and is protective against alcoholism (Borders et al., 2010; Edlund et al., 2010). Lower drinking may occur through several recognized mechanisms, including integration into a positive social network, better behavioral self-control, and religious drinking norms (DeWall et al., 2014; Ford, 2006; Ford & Kadushin, 2002). Prior models of gene-by-environment relationships indicate that environments characterized by more social restrictions on alcohol consumption—as with higher religiosity—are associated with reduced genetic influences (Dick & Kendler, 2012; Shanahan & Hofer, 2005). The influence of *ADH* genes on developing alcohol dependence requires some exposure to alcohol; thus, the genetic effect is susceptible to environmental variation.

Variants in the *ADH1B, ADH1C,* and *ADH4* genes have been studied in association with alcohol consumption–related phenotypes in multiple samples, including those represented in the current sample (African ancestry, European American, and Hispanic populations). There is strong evidence for a negative association between the *ADH1B*2* allele (i.e., A allele at rs1229984) and alcohol dependence and alcohol consumption (Ehlers et al., 2012; Konishi et al., 2004; Li et al., 2011; Macgregor et al., 2009), as well as evidence for the *ADH1B*3* allele (i.e., T allele at rs2066702) (Edenberg et al., 2006; Ehlers et al., 2007). Bierut et al. (2012) reported reduced risk for alcohol dependence for the A allele at rs1229984 versus the G/G genotype in a combined sample of European Americans and African Americans. Gelernter et al.'s (2014) genome-wide association study reported associations with alcohol dependence at rs1229984 in European Americans and at rs2066702 in African Americans. In Hispanic Americans, the *ADH1C*2* allele (i.e., G allele at rs698) was associated with an increased risk for alcohol dependence compared with the *ADH1C*1* or A allele (Konishi et al., 2003, 2004). The association for *ADH1C*2* may be attributable to its high linkage disequilibrium with *ADH1B*1* (Konishi et al., 2004; Osier et al., 1999).

ADH4 gene variants also correlate with the liability for alcohol dependence (Edenberg et al., 2006), including rs1042364 and rs1800759. In individuals of European descent, rs1042364 was associated with a greater risk for alcohol dependence (Luo et al., 2006; Preuss et al., 2011; Turchi et al., 2012). The C/T genotype (corresponding with genotype G/A) at rs1042364 was protective for alcohol dependence in a combined African and European American sample (Luo et al., 2005). Preuss et al. (2011), in their study of Europeans, showed a higher frequency of the rs1042364 A allele in alcohol-dependent cases compared with controls. Edenberg et al. (1999) reported that the *ADH4*-rs1800759 C allele has lower promoter activity than the A allele, suggesting a greater risk for alcohol dependence for C allele carriers. Rs1800759 has been associated with alcohol dependence in some samples (Guindalini et al., 2005; Luo et al., 2006; Preuss et al., 2011) but not in others (Edenberg et al., 2006; Luo et al., 2005). This could be because of differences in the characteristics for the samples studied (e.g., case–control samples recruited from clinical populations versus family-based samples; Preuss et al., 2011). Taken together, this evidence, across the three populations, provides support for a similar biological mechanism via *ADH* enzyme genes for alcohol dependence susceptibility or protection, although the specific variants may differ by population.

These genetic influences on alcohol consumption and dependence depend, in part, on the environmental context. Studies of the environment in association with genetic influences and alcohol consumption-related phenotypes have generally included two lines of research, examining the roles of access to alcohol and levels of experienced stress (Dick & Kendler, 2012). Environments characterized by reduced social control and increased access to alcohol or by more severe exposure to stressful events are hypothesized to enable (in the case of access) or induce (in the case of stress) alcohol use in individuals who are genetically vulnerable. For example, an early study by Higuchi et al. (1994) reported decreasing effects for the *ALDH2*2* protective flushing allele against alcohol dependence in successive Japanese cohorts. This change over time was hypothesized to reflect increased per capita alcohol use in Japan and less restrictive social norms for drinking in younger cohorts. Hasin et al. (2002) reported weakened effects for the rs1229984 protective allele in immigrants to Israel from heavy drinking cultures. Meyers et al. (2015), also using an Israeli sample, showed stronger effects for the rs1229984 G/G risk genotype in individuals who experienced childhood adversity. These two studies examined environmental contexts, characterized by, respectively, relaxed cultural drinking norms and increased exposure to stressful life events.

For the current study, we evaluated the environmental context of religious involvement in a Gene × Environment interaction analysis. We expected that the associations between *ADH* variants and alcohol consumption-related phenotypes would be modified by the level of religious involvement (e.g., a weaker effect for risk variants at higher vs. lower levels of religious involvement). Higher levels of religious involvement could act to weaken the effect of risk variants by exerting increased social controls on drinking. This protective relationship may occur because individuals with more frequent services attendance have less access to alcohol by being socially integrated into a low- or non-drinking reference group (Ford & Kadushin, 2002). Religious beliefs and practices could also serve to restrict individual

choices and behaviors (Shanahan & Hofer, 2005). DeWall et al. (2014), for example, found that religiousness, which included services attendance, related to lower alcohol use through greater self-control over personal thoughts, emotions, and behaviors. Some religious denominations also have norms that discourage drinking. Ford (2006) showed that lower drinking was associated with prohibitive norms against drinking for Protestants but with greater social integration for Catholics.

Different aspects of religion are associated with alcohol use, including and described above, religious services attendance, denomination, and drinking norms. Using prayer to cope, like services attendance, is a religious practice that is inversely associated with alcoholism (Borders et al., 2010). The combination of having both strong spiritual beliefs and greater religious involvement appears to provide a particularly strong protection against heavy drinking (Brechting et al., 2010; Hodge et al., 2007; Holt et al., 2015). In addition, there are some differences in religious practices by racial/ethnic groups. Most relevant to the current study are the higher rates of religious participation, including services attendance, for African Americans and sometimes Hispanic Americans compared with Whites (Brown et al., 2015; Chatters et al., 2009; Robinson et al., 2012).

Twin studies have examined the joint effects of religiosity and latent genetic factors on alcohol consumption, although these studies were primarily conducted in youth. Koopmans et al. (1999) found that a religious upbringing was associated with smaller effects for genetic influences in predicting risk for alcohol use initiation. Genetic effects on problem drinking were also attenuated with increasing levels of religiosity in adolescents but not for young adults (Button et al., 2010). We are aware of only one study that examined the relationship between a measured alcohol metabolism gene and religious involvement in predicting drinking. Findings from Luczak et al.'s (2003) study in Asian young adults suggested that services attendance is more protective against heavy drinking in individuals who do not carry the protective *ALDH2*2* flushing allele. Here we build on the evidence from *ALDH* and *ADH* variant and twin studies by examining, in adults, the Gene × Environment interaction between religious involvement and five measured *ADH* variants (*ADH1B* [rs1229984, rs2066702], *ADH1C* [rs698], and *ADH4* [rs1042364 and rs1800759]) and their joint influence on maximum drinks consumed in a 24-hour period and number of lifetime endorsed alcohol dependence symptoms.

Method

Sample

Data were from the Collaborative Study on the Genetics of Alcoholism (COGA). Subjects (N = 7,716) were ages 18 years and older, who self-reported non-Hispanic White (i.e., European American), non-Hispanic Black (i.e., African American), or Hispanic race/ethnicity and a lifetime exposure to alcohol (i.e., had consumed at least one drink of alcohol). COGA is a multisite extended family study of probands with alcohol dependence, their relatives, and comparison families. The design of the COGA study is described in more detail elsewhere (Begleiter et al., 1995). Data collection sites included Indiana University; State University of New York at Brooklyn; University of California, San Diego; University of Connecticut; Howard University; University of Iowa; and Washington University in St. Louis. The institutional review boards at all sites reviewed and approved the study protocol. Informed consent was obtained from all subjects by trained COGA research assistants.

Staff at study sites selected probands from consecutive admissions to inpatient and outpatient chemical dependency treatment centers; probands were required to meet lifetime criteria for both alcohol dependence (according to criteria from the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised* [DSM-III-R]; American Psychiatric Association, 1987) and Feighner definite alcoholism (Feighner et al., 1972) and to have at least two first-degree relatives available to participate. All available first-degree relatives of probands were invited to join the study. Community-based comparison families, with the same family structure, were recruited at each site through driver's license registries, dental clinics, university-based studies, and medical clinics. Alcohol dependence, other drug dependence, and psychiatric disorders were not exclusionary criteria for comparison subjects.

Subjects completed the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview. The SSAGA is a reliable and valid semi-structured interview designed to assess current and lifetime Axis I disorders, including alcohol use, other drug use, and smoking (Bucholz et al., 1994; Hesselbrock et al., 1999). DNA data were acquired through blood sampling. Single nucleotide polymorphisms (SNPs) in the *ADH* gene cluster were genotyped using standard Sequenom MassArray technology (Sequenom MassArray system; Sequenom, San Diego, CA). All SNP genotypes were checked for Mendelian inheritance using the program PEDCHECK (O'Connell & Weeks, 1998). Marker allele frequencies and heterozygosities were estimated in the European and African American samples, separately, using founders and computed in Plink (Purcell et al., 2007).

Measures

Two alcohol-related phenotypes were examined: (a) the maximum number of drinks consumed in a 24-hour period and (b) the total number of lifetime alcohol dependence symptoms endorsed according to DSM-IV (American Psychiatric Association, 1994) criteria. Subjects' responses for maximum drinks in 24 hours were capped at 60 drinks; those

TABLE 1. Tested alcohol dehydrogenase (*ADH*) gene variants

Marker	Chromosomal position[a]	Gene	Functionality/ placement	Alleles[b]	Minor allele frequency[c]	Genotype frequency[b] (*n*)		
						Homozygous for minor allele	Heterozygous	Homozygous for major allele
rs1229984	100239319	*AHD1B*	missense	**G**:A	0.031	18	449	7,204
rs2066702	100229017	*ADH1B*	missense	**C**:T	0.038	36	457	7,183
rs698	100260789	*ADH1C*	missense	A:**G**	0.345	978	3,313	3,360
rs1042364	100045574	*ADH4*	3′-UTR	G:**A**	0.247	519	2,778	4,371
rs1800759	100065509	*ADH4*	5′ promoter	**C**:A	0.487	1,928	3,452	2,272

Notes: UTR = Untranslated region. [a]Positions reference human genome assembly GRCh37.p13; [b]alleles are listed as major:minor allele with the high-risk allele (based on past studies) in **bold;** [c]minor allele and genotype frequencies are for the combined sample. Markers with significant interaction effects ($p < .05$) are in **bold.**

reporting greater than 60 drinks comprised 1.57% of the sample. Earlier studies document strong genetic influences for these two traits (Grant et al., 2009; Saccone et al., 2000).

We included SNPs in *ADH1B* (rs1229984; rs2066702), *ADH1C* (rs698), and *ADH4* (rs1042364; rs1800759). Genetic variants were coded as additive (0, 1, and 2) based on the minor allele count, with the exception of rs1229984 and rs2066702. These *ADH1B* variants were coded yes/no (carrier of the minor allele; 0, 1) because of the small number of subjects homozygous for the minor allele. Table 1 gives additional details for the *ADH* markers, including chromosomal position, functional status, alleles, and minor allele and genotype frequencies. Figure 1 shows the linkage disequilibrium between the markers.

We defined *religious involvement* by self-reported religious services attendance (i.e., the number of times attended services in the past 12 months before interview). This variable was coded for the analysis at four levels (0 times = *never,* 1–11 times = *less than monthly,* 12–51 times = *monthly,* and 52 or more times = *weekly or more*).

Control variables included age and gender, genetic ancestry, and religious affiliation. Age was measured in years at the time of interview and gender was male or female. Genetic ancestry was evaluated using a 96 SNP panel developed at the Rutgers University DNA and Cell Repository (RUID™). This panel was enriched with 64 ancestry informative markers, which were used in SNPrelate (Zheng et al., 2012), a library function in R, to estimate principal components. Several HapMap populations were included as reference samples (ASW, CEU, CHB, CHD, JPT, GIH, LWK, MEX, MKK, TSI, YRI) to aid in the assignment of three ancestry groups: European American, African American, and other (primarily Hispanic). This resulted in a 90% concordance with self-reported race/ethnicity. The current study used the first three principal components to capture maximum genetic variability in the European American, African American, and Hispanic American samples. Subjects reported their religious affiliation, categorized as follows: (a) unaffiliated, (b) Catholic affiliation, and (c) other affiliations (e.g., Protestant, fundamentalist Protestant, other Christian, Jewish, and Muslim). Both "having no religious affiliation" or a "Catholic affiliation" have been associated in adults with increased risk for alcoholism and heavier drinking (Heath et al., 1997; Michalak et al., 2007) and with lower levels of religious devotion (e.g., services attendance; Kendler et al., 1997) relative to other religious affiliations. For subjects who identified an affiliation, more than one third of those in three affiliations (i.e., Muslim 85%, fundamentalist Protestant 52%, and Christian, other 46%) reported strict religious rules against drinking alcohol compared with 21% (Protestant) to 0% (Jewish) for other affiliations. The three affiliations were, surprisingly, not different than the other groups in the "other

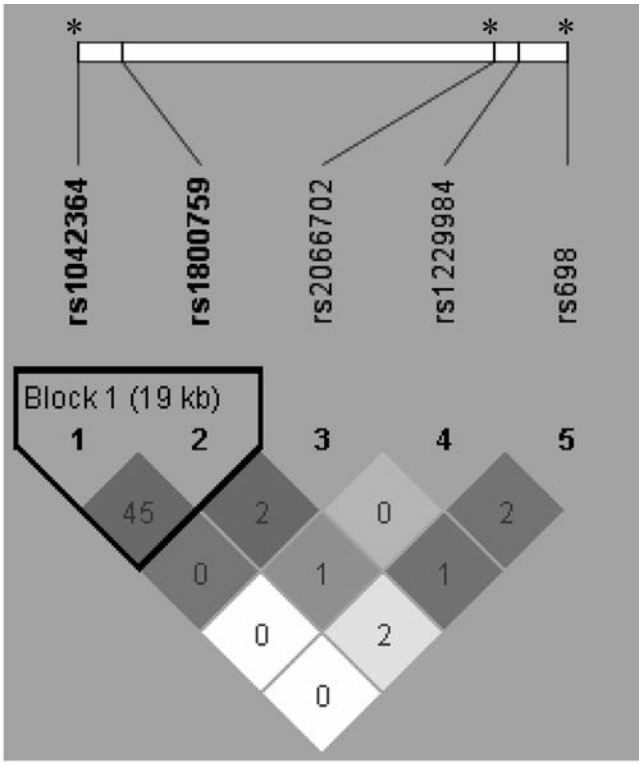

FIGURE 1. Location (top) and correlations (bottom) between the *ADH* markers. The numbers in diamonds are $r^2 \times 100$. The plot was generated using Haploview in 589 singletons and 385 trios from 1,338 families. Shading is the strength of association between pairs of markers; white represents $r^2 = 0$, shades of gray $0 < r^2 < 1$, and black $r^2 = 1$. The black triangle groups two *ADH4* markers in a highly correlated 19 kilobase (kb) block. The asterisks (*) at the top indicate markers that showed a significant interaction with religious involvement for maximum drinks and alcohol dependence symptoms.

affiliations" category on number of maximum drinks consumed (M [SD] = 14.76 [12.59] vs. 14.33 [14.02], $p > .05$) and frequency of alcohol dependence symptoms endorsed (1.70 [2.22] vs. 1.77 [2.23], $p > .05$). Based on these findings, we elected to maintain a combined "other affiliations" category.

Data analysis

Haploview (Barrett et al., 2005) was used to compute the degree of correlation between marker pairs as measured by r^2. Other analyses were conducted in SAS Version 9.3 (SAS Institute Inc., Cary, NC). Chi-square statistics tested the gene–environment associations between the *ADH* variants and religious involvement. Linear regression was performed and accounted for family clustering in the data. Dependent variables (i.e., our phenotypes) were modeled as continuous. Models were tested in two steps for each marker and religious involvement as follows: (a) variant and environment main effects and (b) main effects plus the Gene × Environment interaction term. Interactions were tested for a significant departure from additivity. All models controlled for age, gender, genetic ancestry using principal components, and religious affiliation. Primary analyses used a combined race/ethnicity sample; however, models were run separately in each racial/ethnic group because of population differences on *ADH* variant genotype frequencies. In additional analyses, genotype frequencies, religious involvement, and alcohol-related phenotypes were examined by racial/ethnic group using the chi-square statistic and analysis of variance with Tukey pairwise comparisons. Statistical significance was assessed at $p < .05$. Plots of raw data were constructed using IBM SPSS Statistics for Windows, Version 21 (IBM Corp., Armonk, NY) to assist in the interpretation of Gene × Environment interactions.

Results

Sample characteristics are presented in Table 2. Subjects were on average 38 years of age. Slightly more than half (53%) of the sample was female. Most self-reported European American or African American race/ethnicity, with fewer reporting Hispanic American race/ethnicity. In the past 12 months, most subjects attended religious services infrequently or never; however, approximately 40% of the sample attended at least once a month or more. Fifty-two percent of subjects reported a Catholic affiliation or no religious affiliation. On average, subjects reported consuming a maximum number of 15.6 drinks in a 24-hour period. They endorsed an average of 1.8 lifetime alcohol dependence symptoms; approximately 25% met lifetime criteria for DSM-IV alcohol dependence. No significant associations were found between services attendance and the *ADH* variants ($p > .147$; individual results not shown).

Table 2. Sample characteristics (N = 7,716)

Variable	M (SD) or %
Age, in years	37.94 (14.59)
Male, %	46.55
Race/ethnicity, %	
African American	18.87
European American	73.38
Hispanic American	7.75
Services attendance, %	
Never	21.82
<Monthly	36.07
Monthly	23.57
Weekly or more	18.55
Religious affiliation, %[a]	
None	17.86
Catholic	34.34
Protestant	21.67
Fundamentalist Protestant	10.23
Other Christian	13.30
Other	2.60
Maximum drinks in 24 hours	15.57 (13.04)
Alcohol dependence symptoms	1.81 (2.21)
Alcohol dependence diagnosis	25.49

[a]For regression models, religious affiliation was recoded into two dummy variables including no affiliation (0, 1) and Catholic affiliation (0, 1).

The regression results are provided in Table 3. In main effect models, *ADH1B*-rs1229984 (0 G/G vs. 1 A/G or A/A) was negatively associated with both the maximum drinks consumed and the number of alcohol dependence symptoms endorsed at $p < .0001$ (3rd and 9th columns). *ADH1B*-rs2066702 (0 C/C vs. 1 C/T or T/T) was also negatively associated with maximum drinks ($p = .0150$) and with alcohol dependence symptoms ($p = .0317$). The only positive significant association found was between *ADH1C*-rs698 (0 A/A, 1 A/G, and 2 G/G) and alcohol dependence symptoms ($p = .0385$). The main effects for *ADH4*-rs1042364 (0 G/G, 1 G/A, and 2 A/A) and *ADH4*-rs1800759 (0 C/C, 1 C/A, and 2 A/A) were nonsignificant. Conversely, higher religious involvement was consistently associated ($p < .0001$) with a lower number of maximum drinks and alcohol dependence symptoms (5th and 11th columns).

The interaction models (Table 3) showed significant joint effects with religious involvement for three of the five *ADH* markers (7th and 13th columns). The interaction of religious involvement with rs1042364 was significantly associated ($p \leq .0021$) with both maximum drinks and alcohol dependence symptoms. The interaction effects with rs2066702 and rs698 were also associated both with maximum drinks ($p < .05$) and with alcohol dependence symptoms ($p < .005$) (Figures 2 and 3). All three (rs2066702, rs698, and rs1042364) risk variants showed weaker associations with maximum drinks and alcohol dependence symptoms in the context of increasing religious involvement. Risk variants were associated with more maximum drinks and alcohol dependence symptoms than protective variants under low or no religious involvement. However, the associations for risk and protective variants with these phenotypes were no longer different under

TABLE 3. *ADH* gene variants, religious involvement, and associations with maximum drinks and dependence symptoms

	Maximum drinks						Alcohol dependence symptoms					
	Main effect of variant[b]		Main effect of environment[b]		Variant × Environment[c]		Main effect of variant[b]		Main effect of environment[b]		Variant × Environment[c]	
Marker	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*
ADH1B												
rs1229984[a]	-3.361	**<.0001**	-2.040	**<.0001**	-0.026	.9536	-0.705	**<.0001**	-0.363	**<.0001**	0.067	.4363
rs2066702[a]	-1.638	**.0150**	-2.072	**<.0001**	1.442	**.0197**	-0.242	**.0317**	-0.365	**<.0001**	0.269	**.0045**
ADH1C												
rs698	0.367	.0700	-2.054	**<.0001**	-0.394	**.0328**	0.075	**.0385**	-0.367	**<.0001**	-0.110	**.0010**
ADH4												
rs1042364	0.352	.1146	-2.055	**<.0001**	-0.653	**.0021**	0.063	.1106	-0.365	**<.0001**	-0.130	**.0005**
rs1800759	0.299	.1208	-2.053	**<.0001**	0.088	.6173	0.053	.1320	-0.367	**<.0001**	0.057	.0699

Notes: Significant effects ($p < .05$) are in **bold,** and all models controlled for age, gender, genetic ancestry using principal components, and religious affiliation. [a]*ADH1B* variants were coded yes/no for carrier of the minor allele; all other variants were coded additively based on the minor allele count; [b]main effects are from models including each main effect and control variables; [c]interaction effects are from models including each main effect, their interaction term, and control variables.

higher levels of religious involvement. The interaction effects for rs1800759 and rs1229984 with religious involvement were nonsignificant.

Additional analyses, by racial/ethnic groups, showed significant differences in genotype frequencies ($p < .0001$); these allele differences were primarily observed for European Americans and Hispanic Americans compared with African Americans. The *ADH1B*-rs1229984 A, *ADH1C*-rs698 G, and *ADH4*-rs1042364 A alleles were more common in European Americans (respectively, 6.71%, 63.46%, and 48.91%) and Hispanic Americans (8.08%, 51.70%, and 33.45%) than African Americans (2.83%, 29.05%, and 23.88%). Conversely, the *ADH1B*-rs2006702 T and *ADH1B*-rs1800759 A alleles were more common in African Americans (respectively, 28.98% and 92.63%) than in European Americans (0.37% and 63.7%) and Hispanic Americans (8.91% and 78.91%). For these groups, Hispanic Americans reported the lowest level of religious involvement. Hispanic Americans had higher rates of attending services never (25.05%) or less than monthly (42.13%) and lower rates of attending monthly (17.08%) or weekly or more (15.75%), compared with African Americans (respectively, for these levels: 22.36%, 37.47%, 21.72%, and 18.94%) and European Americans (21.33%, 35.06%, 24.86%, and 18.75%) ($p < .001$). Pairwise comparisons ($p < .05$) showed that European Americans (*M* [*SD*] = 16.02 [12.91]) reported more maximum drinks than African Americans (14.18 [13.63]) but not Hispanic Americans (14.73 [13.04]). European Americans (*M* [*SD*] = 1.86 [2.22]) also endorsed more alcohol dependence symptoms, this time compared with Hispanic Americans (1.58 [2.11]) but not African Americans (1.72 [2.20]). The rate of DSM-IV alcohol dependence, 23.62%–26.11%, was not different by population ($p = .122$).

Regression models stratified by racial/ethnic population showed trends that were consistent with those reported for the combined sample, although not always statistically significant. These results are presented here in a brief format. The exceptions included markers with low minor allele frequencies in a racial/ethnic group or the switched direction of an effect that was highly nonsignificant. For example, the associations between rs698 and maximum drinks and alcohol dependence symptoms in Hispanic Americans were negative and highly nonsignificant, and rs2066702 was not associated with either phenotype in Hispanic Americans. Hispanic Americans were the smallest subsample and their results were less stable. In all groups, rs1229984 was negatively associated with maximum drinks and/or alcohol dependence symptoms ($p < .05$). In African Americans and European Americans, but not in Hispanic Americans, negative main effects for religious involvement with maximum drinks and alcohol dependence symptoms were significant ($p < .0001$). Few interaction effects were statistically significant except in European Americans, which was the largest group. Consistent with the combined sample, the interaction effect between religious involvement and rs1042364 was significantly associated with maximum drinks and alcohol dependence symptoms ($p < .05$). Conversely, for alcohol dependence symptoms, the interaction with rs1229984 was positive and statistically significant in Hispanic Americans only; it was not significant in the combined sample. The interaction with rs2066702 in European Americans, a marker with a low minor allele frequency in this population, had a switched direction compared with the combined sample.

Discussion

This study provides initial evidence in adults of a modifying effect for religious involvement on relationships between *ADH* gene variants and the number of maximum drinks consumed as well as number of lifetime DSM-IV alcohol dependence symptoms endorsed. Under low or no religious involvement, *ADH1B*-rs2066702, *ADH1C*-rs698, and *ADH4*-rs1042364 risk variants were associated with higher maximum drinks and more dependence symptoms relative to

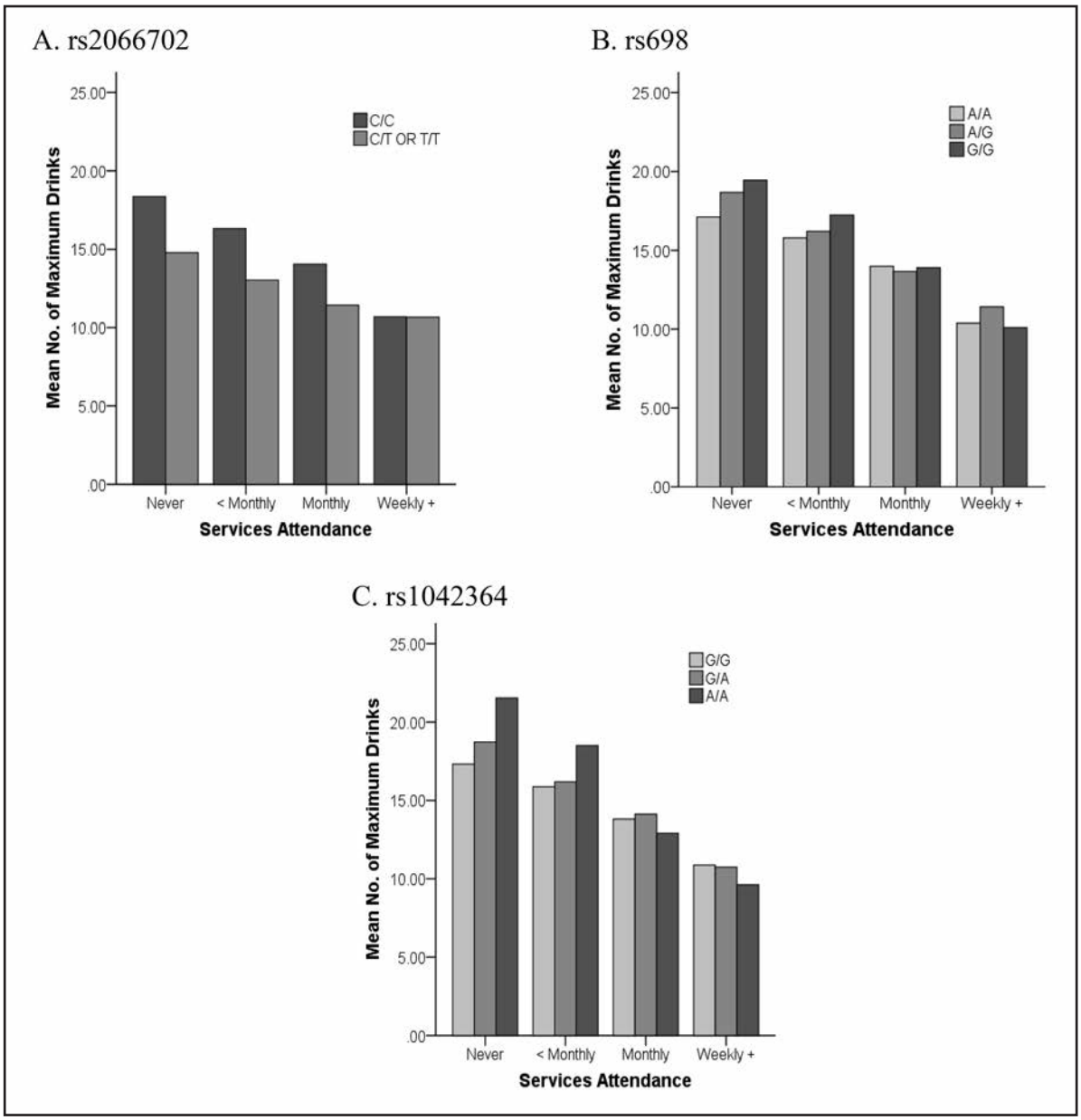

FIGURE 2. Plots of religious involvement with maximum drinks. Interaction effects showed higher maximum drinks consumed for *ADH1B*-rs2066702, *ADH1C*-rs698, and *ADH4*-rs1042364 risk versus protective variants in the context of lower or no religious services attendance. Risk variants are identified by darker shaded bars. No. = number.

protective variants. Conversely, risk and protective variants for these markers had similar associations with the phenotypes at higher religious involvement levels. The modifying effect of religious involvement was observed for three of the *ADH* gene markers examined (i.e., no significant interaction effects for *ADH1B*-rs1229984 and *ADH4*-rs1800759). Of the many possible reasons for this, none are entirely clear.

The presence or absence of Gene × Environment interactions may be partially related to differences in the strength of the associations of the *ADH* variants with alcohol dependence. The current evidence is inconsistent for the association of rs1800759 with alcohol-related phenotypes (Edenberg et al., 2006; Guindalini et al., 2005; Luo et al., 2005; Preuss et al., 2011), suggesting a weaker association for this variant. In our study, rs1229984 showed evidence of a main-effect-only relationship with maximum drinks and alcohol dependence symptoms. This may point to the stronger effect for rs1229984 in association with alcohol dependence, as previously reported by Bierut et al. (2012) in individuals of both European and African American ancestry. However, two studies by Meyers et al. (2015) and Sartor et al. (2014) found a significant interaction for rs1229984 and childhood

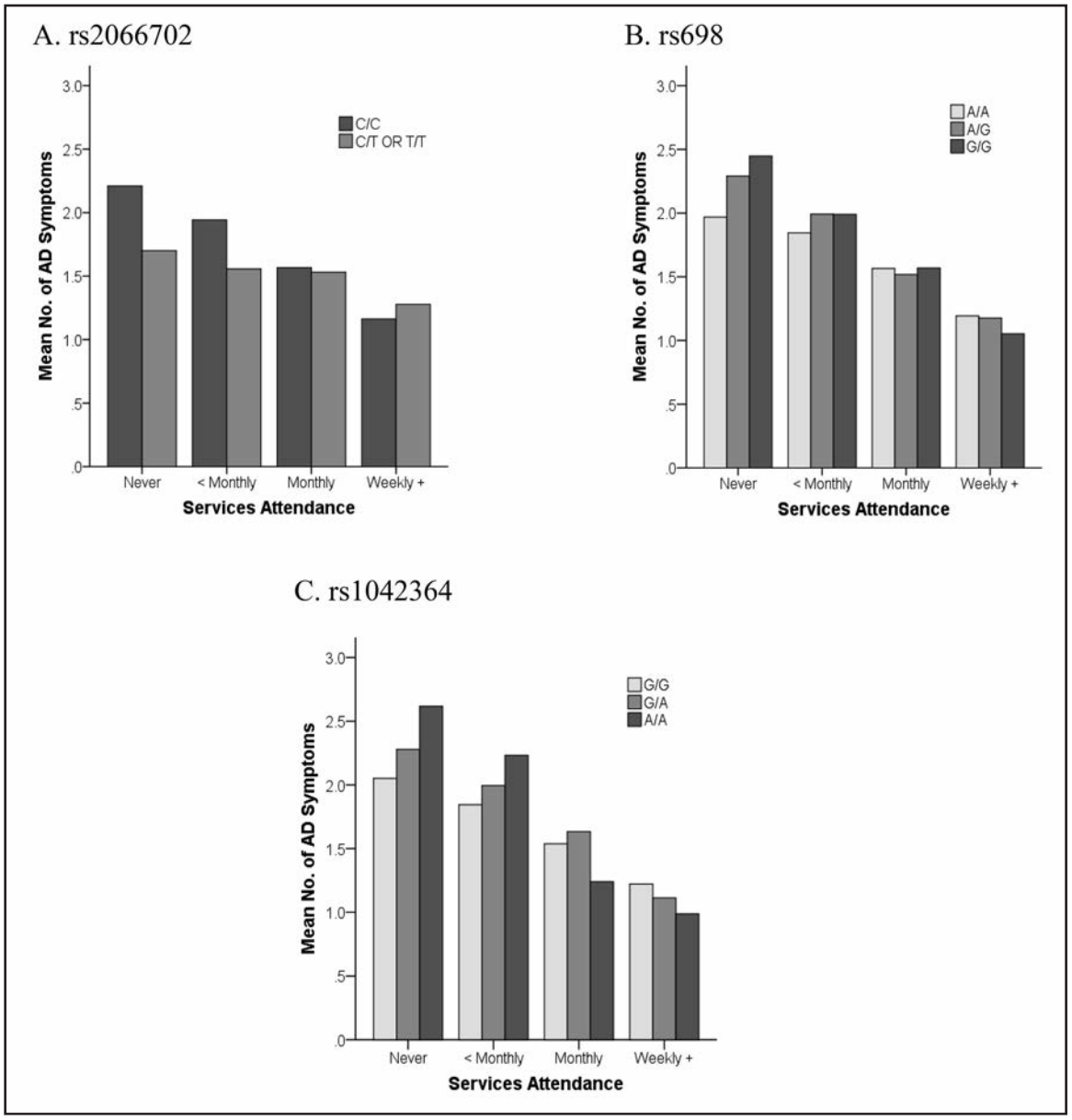

FIGURE 3. Plots of religious involvement with alcohol dependence symptoms. Interaction effects showed more alcohol dependence symptoms for *ADH1B*-rs2066702, *ADH1C*-rs698, and *ADH4*-rs1042364 risk versus protective variants at lower or no religious services attendance levels. Risk variants are identified by darker shaded bars. No. = number; AD = alcohol dependence.

adversity in predicting alcohol dependence symptoms. It may be that childhood adversity provides a stronger environmental exposure compared with religious involvement.

The functionality and location of the five markers do not appear to entirely explain why some variant-phenotype relationships were modified by religious involvement and others were not. Rs1229984, rs2066702, and rs698 are functional markers and encode different enzyme forms that metabolize ethanol at higher or lower rates (see Hurley & Edenberg, 2012). Rs1800759 is located in a 5′ promoter region and is associated with the expression of *ADH4* (Edenberg et al., 1999). Rs1042364, which had highly significant interaction effects with both phenotypes, is located in a 3′ untranslated mRNA encoding region (Edenberg et al., 2006). It is possible that the effect of *ADH4*-rs1042364 was modified by religious involvement (through restricted access to alcohol and social constraints) because of the contribution of the *ADH4* gene to alcohol metabolism at higher levels of consumption (Hurley & Edenberg, 2012).

Weekly or more frequent religious involvement was associated with a moderate level of protection against a higher number of maximum drinks and alcohol dependence

symptoms for both *ADH* risk and protective variant carriers. Our results suggest a gene-by-environment relationship that is characterized by negligible phenotype differences between genotypes in a more protective environment, but increased differences under less benign conditions (i.e., plots display a fan-shaped interaction; Dick & Kendler, 2012). Likewise, Beaver et al. (2009) showed this modifying effect for religiosity in association with the *DRD2* dopamine D2 receptor gene. High religiosity was protective for delinquent behaviors among both the *DRD2* A-1 and A-2 allele carriers, whereas under low religiosity, A-l carriers reported higher delinquent behaviors. Luczak et al. (2003) showed that, in Asians, greater religious services attendance was protective against heavy drinking in nonflushing allele carriers but not for carriers of the flushing allele (*ALDH2*2*). It is likely that the flushing response was already a strong deterrent against heavy drinking.

Religious involvement may function as a protective environment against more drinking and more alcohol dependence symptoms through several mechanisms, including more restrictive social drinking norms, by providing a positive, low/no drinking social network (Ford, 2006; Ford & Kadushin, 2002), and/or by improving behavioral self-control (DeWall et al., 2014). Under Shanahan and Hofer's (2005) social control model, religion is one source of societal constraint on individual choices and behaviors that was reported to modify genetic susceptibility. Twin studies indicate social control mechanisms for religion with other related phenotypes. For example, Koopmans et al. (1999) and Boomsma et al. (1999) showed that youths with a religious upbringing had reduced genetic influences on alcohol use initiation and disinhibition, respectively. Kendler et al. (1999) offered another putative mechanism for religion in a twin study of depression, indicating that religious involvement can be protective for depression against stressful life events. Religious involvement may, alternatively, serve as a positive environmental condition that enables individuals to effectively cope with stress, buffering the influence of risk alleles (i.e., an example of the gene–environment compensation model in Shanahan & Hofer, 2005).

To our knowledge, this is the first time that religious involvement has been examined in combination with *ADH* gene variants and their joint associations with alcohol-related phenotypes in adults. The current study's large, combined sample size provides adequate power for the analysis and may reduce potential false discoveries (Duncan & Keller, 2011). In addition, using principal components to identify genetic ancestry in a regression analysis performed well in accounting for the population structure in a sample that includes subjects from different ancestry populations (Bouaziz et al., 2011). Larger racial/ethnic subsamples are needed to facilitate a more detailed analysis of religiosity and *ADH* gene variants in each group. The population differences in this sample for genotype frequencies, religious involvement, and alcohol phenotypes may suggest different gene–environment relationships by racial/ethnic group. The significant interaction effect between rs1042364 and religious involvement, observed in European Americans, was consistent with results from the combined sample. It is possible that this interaction is population specific, but also that it was not observed in the African American and Hispanic American samples because of small sample sizes. Similarly, the interaction for rs2066702 was observed in the combined sample but not in the subsample of African Americans, who have both a higher minor allele count at this locus (Hurley & Edenberg, 2012) and higher rates of religious participation (Brown et al., 2015; Chatters et al., 2009; Robinson et al., 2012). It is possible that this interaction was missed because of low statistical power.

The COGA sample is family based and includes several subjects from densely affected families with many affected biological family members. This may limit the generalizability of our findings to other community samples or to the general population. The public health implications are very tentative. Borders et al.'s (2010) prospective study of religion and drinking suggested the possible benefit of promoting services attendance and prayer for at-risk drinkers. Our study might extend this suggestion to *ADH* risk allele carriers. However, prospective studies are needed to help clarify the relationships between religious involvement, *ADH* gene variants, and drinking and alcohol dependence symptoms because the current study is cross-sectional and religious involvement was measured at a single time point. Religious services attendance in the past 12 months before interview may not be representative of lifetime attendance, although there is some evidence that services attendance is relatively stable over time (Kendler et al., 1997; Koenig & Vaillant, 2009). Some studies identify reductions in religious attendance from adolescence to young adulthood (Button et al., 2011), which may be less relevant to this adult sample. It is also possible that the time order between religious involvement and alcohol problems is reversed; some subjects may increase or decrease their religious involvement as a result of having problems with alcohol. Kendler et al.'s (1997) study suggested that these alternative relationships are less likely, reporting a stronger relationship between earlier church attendance and later alcohol use rather than between earlier alcohol use and later attendance.

A strength of this study was our use of robust genetic, environmental, and phenotypic variables. The mechanisms that link *ADH* genes to alcohol consumption and dependence are relatively well understood (Edenberg et al., 1999; Macgregor et al., 2009; McCarthy et al., 2010). Although specific markers may differ by population (e.g., Gelernter et al., 2014), there is no evidence that the biological mechanisms associated with *ADH* variants are different. Previous literature supports the importance of religious involvement for examining drinking behavior. We studied one aspect of

religiosity (i.e., services attendance) as a limitation of our data set, but this is a complex construct, and other aspects of religion may offer additional information about this relationship. Kendler et al. (1997), for example, showed that the dimensions of religiosity are differentially associated with alcohol use; religious devotion (including services attendance) was associated with the ability to quit or maintain low levels of use, whereas traditional religious beliefs were associated with decisions to ever drink. We were not able to examine the role of spirituality. Earlier studies indicate that greater religious involvement in combination with strong spiritual beliefs provides strong protection against drinking (Brechting et al., 2010; Hodge et al., 2007; Holt et al., 2015). In addition, we investigated two phenotypes (i.e., maximum drinks and alcohol dependence symptoms), and our findings were reasonably consistent across both traits. However, some significant interactions for predicting maximum drinks ($p < .033$) would not be robust following corrections for multiple testing.

In summary, our findings suggest a modifying role for religious involvement, in that the associations of *ADH* gene variants with alcohol consumption–related phenotypes varied by the level of religious involvement that individuals reported. This relationship can be characterized by negligible phenotype differences between risk and protective genotypes under high religious involvement but larger differences at lower and no religious involvement levels. We hope this initial study will serve to generate other investigations of religious involvement, *ADH* and other genetic variants, and alcohol consumption and dependence. In particular, future investigations could be extended to include prospective study designs of at-risk individuals, other aspects of religiosity, and larger and separate samples of different racial/ethnic populations.

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