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Authors

Olfson, Emily
Edenberg, Howard J
Nurnberger, John
et al.

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An *ADH1B* Variant and Peer Drinking in Progression to Adolescent Drinking Milestones: Evidence of a Gene-by-Environment Interaction

Emily Olfson, Howard J. Edenberg, John Nurnberger Jr, Arpana Agrawal, Kathleen K. Bucholz, Laura A. Almasy, David Chorlian, Danielle M. Dick, Victor M. Hesselbrock, John R. Kramer, Samuel Kuperman, Bernice Porjesz, Marc A. Schuckit, Jay A. Tischfield, Jen-Chyong Wang, Leah Wetherill, Tatiana M. Foroud, John Rice, Alison Goate, and Laura J. Bierut

Background: Adolescent drinking is an important public health concern, one that is influenced by both genetic and environmental factors. The functional variant rs1229984 in alcohol dehydrogenase 1B (*ADH1B*) has been associated at a genome-wide level with alcohol use disorders in diverse adult populations. However, few data are available regarding whether this variant influences early drinking behaviors and whether social context moderates this effect. This study examines the interplay between rs1229984 and peer drinking in the development of adolescent drinking milestones.

Methods: One thousand five hundred and fifty European and African American individuals who had a full drink of alcohol before age 18 were selected from a longitudinal study of youth as part of the Collaborative Study on the Genetics of Alcoholism (COGA). Cox proportional hazards regression, with G × E product terms in the final models, was used to study 2 primary outcomes during adolescence: age of first intoxication and age of first DSM-5 alcohol use disorder symptom.

Results: The minor A allele of rs1229984 was associated with a protective effect for first intoxication (HR = 0.56, 95% CI 0.41 to 0.76) and first DSM-5 symptom (HR = 0.45, 95% CI 0.26 to 0.77) in the final models. Reporting that most or all best friends drink was associated with a hazardous effect for first intoxication (HR = 1.81, 95% CI 1.62 to 2.01) and first DSM-5 symptom (HR = 2.17, 95% 1.88 to 2.50) in the final models. Furthermore, there was a significant G × E interaction for first intoxication ($p = 0.002$) and first DSM-5 symptom ($p = 0.01$). Among individuals reporting none or few best friends drinking, the *ADH1B* variant had a protective effect for adolescent drinking milestones, but for those reporting most or all best friends drinking, this effect was greatly reduced.

Conclusions: Our results suggest that the risk factor of best friends drinking attenuates the protective effect of a well-established *ADH1B* variant for 2 adolescent drinking behaviors. These findings illustrate the interplay between genetic and environmental factors in the development of drinking milestones during adolescence.

Key Words: Gene–Environment Interaction, Adolescent, Alcohol Dehydrogenase, Peer Drinking.

B Y AGE 17, most U.S. adolescents (54 to 78%) have consumed alcohol, and a significant proportion (15%) meet the criteria for alcohol abuse (Merikangas et al., 2010; NSDUH, 2012; Swendsen et al., 2012). Patterns of alcohol use that begin in adolescence are important determinants for the development of alcohol use disorders during adulthood (Grant et al., 2006; Pitkanen et al., 2005). Therefore, understanding factors that contribute to early drinking behaviors is critical for disease prevention.

For decades, twin studies have recognized that both genetic and environmental factors influence individual risk for alcoholism (Heath et al., 1997; Kendler et al., 1994; Pickens et al., 1991; Prescott and Kendler, 1999). Recently, large-scale genetic studies have provided strong evidence for the contribution of specific genetic variants to alcohol use disorders in adults (Rietschel and Treutlein, 2013; Wang et al., 2012). An important next step in the translation of genetic findings identified in adults is to test whether these genetic variants also affect adolescent drinking behaviors and whether environmental risk factors moderate this role.

Among the most biologically well-understood genetic variants associated with alcohol use disorders is the polymorphism rs1229984 in the enzyme alcohol dehydrogenase 1B (*ADH1B*). The minor A allele (in the coding strand) of rs1229984 causes an amino acid change at position 48 by replacing arginine with histidine, which increases the activity of the *ADH1B* enzyme that oxidizes ethanol to acetaldehyde (Edenberg and Foroud, 2013; Hurley and Edenberg, 2012). After consuming alcohol, elevated *ADH1B* activity has been hypothesized to transiently increase the level of acetaldehyde, leading to unpleasant effects that limit further drinking. Meta-analysis of this variant in Asian populations, where the rs1229984 A allele is common (allele frequency = 0.7 in 1,000 Genomes) (Abecasis et al., 2012), has demonstrated strong effects on the risk of developing alcohol-related disorders (OR 0.45: $p = 7 \times 10^{-42}$) (Li et al., 2011). Recently, this polymorphism was shown to have a similar effect on risk of alcohol dependence in European and African Americans (African and European OR 0.34: $p = 6.6 \times 10^{-10}$; Bierut et al., 2012); European: $p = 1.17 \times 10^{-31}$; Gelernter et al., 2014), where the rs1229984 A allele is less common (European American frequency = 0.05; African American frequency = 0.02 in Exome Variant Server).

Other studies suggest that social environments that encourage drinking may diminish the protective genetic effects of alcohol-metabolizing variants (Hasin et al., 2002; Higuchi et al., 1994; Irons et al., 2007, 2012). However, to our knowledge, no study has explored the interplay of the *ADH1B* rs1229984 variant and the important social context of peer drinking during the critical developmental period of adolescence when alcohol use is initiated and drinking patterns are established. Peer drinking has long been recognized as a strong risk factor for adolescent drinking problems (Curran et al., 1997; Reifman et al., 1998), and recently, twin studies have provided evidence that peer drinking modifies heritable variation in adolescent alcohol involvement (Agrawal et al., 2010; Dick et al., 2007; Guo et al., 2009; Harden et al., 2008).

This study tests the interaction between a genome-wide significant functional *ADH1B* variant and the risk environment of peer drinking in the development of 2 adolescent drinking milestones: first intoxication and first DSM-5 alcohol use disorder symptom. Examining hypothesis-driven gene-by-environment (G × E) interactions using robust genetic and environmental risks during developmental transitions provides an important approach for untangling the complex etiology of alcohol use disorder.

MATERIALS AND METHODS

Collaborative Study on the Genetics of Alcoholism Sample Description

Study participants were enrolled in the Collaborative Study on the Genetics of Alcoholism (COGA), a large, multicenter, family study designed to identify genes that contribute to alcohol use disorders in high-risk (defined as recruited through alcohol-dependent probands) and community comparison families (Begleiter et al., 1995). Since 2005, the adolescent and young adult study in COGA has used a longitudinal design to examine the development of alcohol use disorders in young participants from these families. Individuals aged 12 to 22 were recruited from 6 sites across the United States and interviewed every 2 years. Institutional review boards at all sites approved the study design. Adult participants provided informed consent, parents provided consent for all children younger than 18, and children provided assent.

Assessment of Phenotypes

Interview assessment was performed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) to gather reliable and valid information on alcohol use behaviors (Bucholz et al., 1994, 1995; Hesselbrock et al., 1999). Participants 18 years and older were assessed with the Phase IV SSAGA, and those <18 years were assessed with an age appropriate adolescent version called the Phase IV C-SSAGA (Kuperman et al., 2001).

Drinking Milestones

Two adolescent drinking milestones were used as primary outcomes among adolescent ever-drinkers: age of first intoxication, a common and clinically relevant variable, and first DSM-5 alcohol use disorder symptom, a heritable characteristic associated with future alcohol-related problems (Rhee et al., 2003; Young et al., 2006). These outcomes commonly occur during adolescence and therefore coincide with the environment of adolescent peer drinking. Age of first intoxication was derived from responses to the question "How old were you the first time you got drunk, that is, your speech was slurred or you were unsteady on your feet?" Age of first DSM-5 symptom was developed from examining the youngest age that individuals first experienced 1 of the 11 symptoms of alcohol use disorder. Given the longitudinal design of this study with multiple assessments over time, the earliest interview in which the participant endorsed first intoxication or first DSM-5 symptom was selected to assign the age of onset.

Peer Drinking

The environment of adolescent peer drinking was derived from participant responses to questions addressing the proportion of best friends who drink. With the longitudinal design of the study, 88% (1,366/1,550) of participants received at least 1 adult SSAGA assessment at age 18 years or older. Assignment of the level of peer drinking in these participants was determined from the first adult SSAGA interview with the question "When you were 12 to 17, how many of your best friends used alcohol?" and the 4 possible answers of none, few, most, or all. For participants who had not reached age 18 at the last assessment, peer drinking was evaluated with the maximum value from all C-SSAGA answers to the question "How many of your best friends use alcohol?" For the primary analyses, peer drinking was dichotomized into low peer drinking (few or no best

friends drink) and high peer drinking (most or all best friends drink) as performed in the previous studies (Kuperman et al., 2013). The 4-level peer drinking variable (none, few, most, or all best friends) was also investigated in secondary analyses to assess a possible dose–response, but interaction effects are not presented because of the small number of individuals in some groups.

To assess the concordance of the retrospective SSAGA interview peer drinking responses for ages 12 to 17 with current peer drinking reported in C-SSAGA assessments, we compared the first adult SSAGA response and the maximum value from all C-SSAGA assessments among individuals with at least 1 adult and 1 child questionnaire. For the 996 participants with both adult and child interviews, 73% of peer drinking assignments had the same dichotomous variable (none/few vs. most/all best friends). This concordance demonstrates that our retrospective approach of using the first SSAGA interview when available is a reasonable strategy to assess peer drinking across adolescence. It also shows that for the 12% of participants without a single adult SSAGA assessment, using the maximum value from C-SSAGA assessments reasonably estimates the proportion of best friends drinking from ages 12 to 17.

Genotyping

Blood samples were obtained for genetic analysis. The *ADH1B* rs1229984 variant was genotyped with Sequenom MassArray technology (Sequenom, San Diego, CA) following standard procedures. Several quality control measures were employed. Genetic variants had a genotyping rate of >99% and were in Hardy–Weinberg equilibrium in both the European and African American groups. The program PEDCHECK (O'Connell and Weeks, 1998) was used to examine Mendelian inheritance, and only individuals with no Mendelian inconsistencies were included in the rs1229984 genotyped sample ($n = 2,580$; Fig. 1).

A set of 64 ancestry informative markers was genotyped as part of a 96 SNP Biorepository Panel by the Rutgers University Cell and DNA Repository. These markers were used in SNPrelate, a function in R, to assign ancestry groups. HapMap populations were included as reference groups. There was high concordance (97%) between self-reported and genetically determined ethnicity for European and African American individuals, and only concordant individuals were used in the analyses.

Sample Selection

In the COGA adolescent and young adult study, 2,580 individuals with a first interview age of 12 to 22 were genotyped for the *ADH1B* rs1229984 variant, and participants for the analyses were drawn from this group (Fig. 1). Focusing on European and African American subjects and excluding individuals with missing or unreliable data left 2,410 individuals (entire sample described in Table 1). The samples used for the primary analyses of first intoxication and first DSM-5 symptom consisted of 1,550 ever-drinkers before age 18 (also described in Table 1). Ever-drinkers were targeted because the *ADH1B* variant is only expected to exhibit a protective effect in response to alcohol consumption. Because the peer drinking variable examined the age range of 12 to 17, the primary analyses focused on events that occurred during this time.

Data Analysis

Data were analyzed using the statistical analysis system (SAS 9.3, Cary, NC). Cox proportional hazards regression (SAS PROC PHREG) was used to model drinking milestones, and all individuals
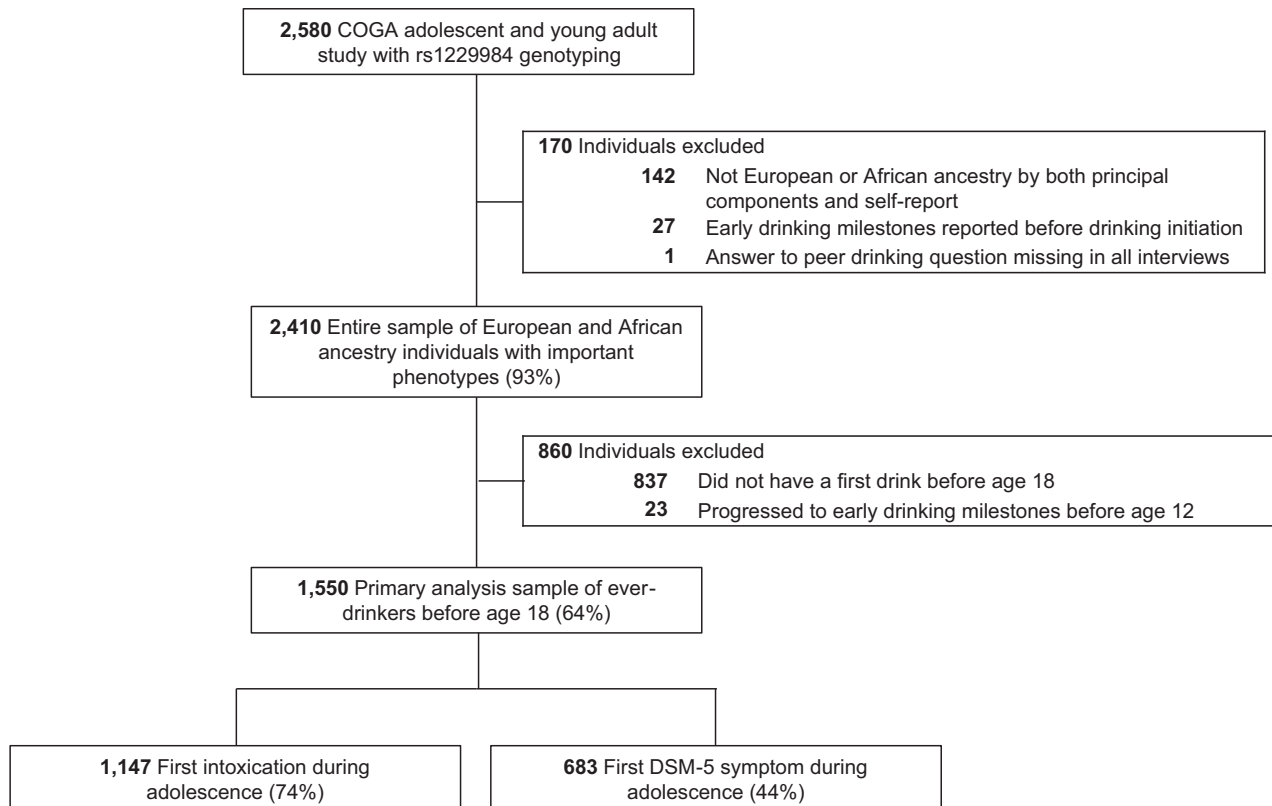

Fig. 1. European and African American adolescent ever-drinkers with *ADH1B* rs1229984 genotyping were drawn from the Collaborative Study on the Genetics of Alcoholism (COGA) for the primary analyses of 2 early drinking milestones.

Table 1. Characteristics of Samples Used in Analyses

Characteristic	Entire sample (n = 2,410)	Ever-drinkers before age 18 (n = 1,550)
Ancestry, n (%)		
European	1,648 (68.4)	1,130 (72.9)
African	762 (31.6)	420 (27.1)
Sex, n (%)		
Males	1,182 (49.1)	784 (50.6)
Females	1,228 (51.0)	766 (49.4)
Age at first interview, years		
Mean ± SD	16.3 ± 3.2	16.7 ± 3.0
Range	12 to 22	12 to 22
No. of interviews		
Mean ± SD	3.2 ± 1.1	3.2 ± 1.1
Range	1 to 5	1 to 5
Family status, n (%)		
From high-risk families	2,096 (87.0)	1,384 (89.3)
From comparison families	314 (13.0)	166 (10.7)
No. of extended families	781	645
No. of nuclear families		
Only full-siblings	1,629	1,151
Including half-siblings	1,438	1,044
No. of individuals per extended family, median (range)	2 (1 to 24)	2 (1 to 17)
Drinking milestones reached before age 18, n (%)		
First drink	1,573 (65.3)	1,550 (100.0)
First intoxication	1,170 (48.6)	1,147 (74.0)
First DSM-5 symptom	702 (29.1)	683 (44.1)
Among those who exhibit a first intoxication before age 18		
Mean age ± SD	15.3 (1.5)	15.4 (1.4)
Age range	8 to 17	12 to 17
Among those who exhibit a first DSM-5 symptom before age 18		
Mean age ± SD	15.6 (1.3)	15.6 (1.2)
Age range	10 to 17	12 to 17
rs1229984, n (%)		
GG	2,270 (94.2)	1,452 (93.7)
GA	137 (5.7)	96 (6.2)
AA	3 (0.1)	2 (0.1)
Reported proportion of best friends who use alcohol between ages 12 to 17, n (%)		
None	746 (31.0)	239 (15.4)
Few	981 (40.7)	708 (45.7)
Most	513 (21.3)	453 (29.2)
All	170 (7.1)	150 (9.7)

who did not experience an event in adolescence were censored at their age of last interview or 18. Participants with rs1229984 GA genotype (n = 96) and AA genotype (n = 2) were collapsed into 1 group for comparison with the GG genotype participants (n = 1,452), as performed in previous studies (Bierut et al., 2012). Models were checked for violations of the proportional hazards assumption, and Schoenfeld residuals were examined. The option COVSANDWICH (AGGREGATE) was used to statistically adjust for the non-independence of correlated familial data in all analyses, as performed in previous studies (Kuperman et al., 2013).

Models in Primary Analyses

Main effects of the *ADH1B* variant and peer drinking were examined in univariate and multivariate models of age of first intoxication and first DSM-5 symptom in the sample of adolescent ever-drinkers (n = 1,550, called *univariate model set* and *multivariate model set*; Table 2). All models presented in the tables employed STRATA statements for gender and ethnicity to adjust for differences in baseline hazards in these groups. The interplay between the *ADH1B* variant and peer drinking was assessed by adding product interaction terms to models of drinking milestones (called *interaction model set*, Table 2). This final proportional hazards model was $\lambda(t) = \lambda(t)\exp(\beta_1*(rs1229984) + \beta_2*(peer\_drinking) + \beta_3*(rs1229984*peer\_drinking))$. The possibility of a gene–environment correlation between *ADH1B* rs1229984 and peer drinking was also assessed because genetic factors influence selection of peers who drink (Fowler et al., 2007) and inadequate control of this correlation could produce false interactions. Using logistic regression, the outcome peer drinking was modeled with the variables of the *ADH1B* variant, gender, and ethnicity.

Secondary Analyses

Secondary analyses were performed to test the robustness of our primary findings. First, association of the *ADH1B* rs1229984 variant with the milestone of age of drinking initiation was examined in the entire sample, which included adolescent never-drinkers (n = 2,410). Second, analyses stratified by ancestry were performed to examine the main and interaction effects within the subpopulations of European and African Americans.

Table 2. Cox Proportional Hazards Regression Models of Adolescent Drinking Milestones

	Drinking milestones in ever-drinkers before age 18 (n = 1,550)			
	Models of first intoxication		Models of first DSM-5 symptom	
	Hazard ratio (95% CI)	χ^2 p-value	Hazard ratio (95% CI)	χ^2 p-value
Univariate model set				
rs1229984[a]	0.72 (0.56 to 0.91)	0.006	0.69 (0.50 to 0.94)	0.02
Peer drinking[b]	1.89 (1.70 to 2.10)	<0.0001	2.27 (1.98 to 2.60)	<0.0001
Multivariate model set				
rs1229984	0.76 (0.61 to 0.96)	0.02	0.73 (0.54 to 0.97)	0.03
Peer drinking	1.88 (1.69 to 2.09)	<0.0001	2.26 (1.97 to 2.60)	<0.0001
Interaction model set				
rs1229984	0.56 (0.41 to 0.76)	0.0002	0.45 (0.26 to 0.77)	0.004
Peer drinking	1.81 (1.62 to 2.01)	<0.0001	2.17 (1.88 to 2.50)	<0.0001
rs1229984*peer drinking	2.10 (1.32 to 3.32)	0.002	2.29 (1.21 to 4.30)	0.01
Examination of G × E term in interaction model set				
None/few best friends drink (GA/AA vs. GG)	0.56 (0.41 to 0.76)	0.0002	0.45 (0.26 to 0.77)	0.004
Most/all best friends drink (GA/AA vs. GG)	1.16 (0.82 to 1.65)	0.39	1.03 (0.73 to 1.45)	0.87

[a]Reference *ADH1B* rs1229984 genotype GG was compared to GA/AA.
[b]Reference peer drinking status none/few best friends drink was compared to most/all best friends drink; All models adjusted for gender and ethnicity.

RESULTS

Participant Characteristics

Demographic, behavioral, and genotypic characteristics of the study samples are presented in Table 1. The sample of ever-drinkers before age 18 used in the primary analyses consisted of 1,550 individuals from 1,151 nuclear families (defined by full-siblings) and 645 extended families. The mean first interview age was 17, 49% were female, and the majority came from high-risk families (89%) and were European American (73%). Before age 18, 74% had a first intoxication and 44% experienced a first DSM-5 symptom of alcohol use disorder. From ages 12 to 17, 39% reported that most or all of their best friends drank alcohol. Consistent with the expected population frequencies of the *ADH1B* variant, 6% carried at least 1 copy of the protective A allele (8% in European Americans and 3% in African Americans).

Effect of Peer Drinking

Most/all best friends drinking compared to none/few best friends drinking between ages 12 to 17 was associated with a main hazardous effect in univariate and multivariate models of early drinking behaviors (Table 2). In the final *interaction model set* with G $\times$ E product terms, self-reported peer drinking had a robust effect on first intoxication (hazards ratio [HR] = 1.81, 95% CI 1.62 to 2.01) and first DSM-5 symptom (HR = 2.17, 95% CI 1.88 to 2.50). In secondary analyses examining all 4 responses for best friends drinking (none, few, most, all), an increase in the number of best friends drinking was similarly related to the first intoxication (*multivariate model set* with *none* as the reference; *few* HR = 1.72, 95% CI 1.44 to 2.05; *most* HR = 2.65, 95% CI 2.20 to 3.18; *all* HR = 3.69, 95% CI 2.93 to 4.64), and first DSM-5 symptom (*multivariate model set* with *none* as the reference; *few* HR = 2.43, 95% CI 1.77 to 3.33; *most*

HR = 4.29, 95% CI 3.12 to 5.92; *all* HR = 5.84, 95% CI 4.16 to 8.21). These results indicate a "dosage effect" where the reported proportion of best friends drinking was positively associated with higher risk for developing adolescent drinking milestones.

Effect of ADH1B *rs1229984 Variant*

During adolescence, presence of the *ADH1B* variant (GA/AA genotypes) was associated with a protective main effect among ever-drinkers for first intoxication and first DSM-5 symptom in univariate and multivariate models (Table 2). In the final *interaction model set* with G $\times$ E product terms, the effect of the *ADH1B* variant was strong for both first intoxication (HR = 0.56, 95% CI 0.41 to 0.76) and first DSM-5 symptom (HR = 0.45, 95% CI 0.26 to 0.77). In secondary analyses of the entire sample that included never-drinkers, the presence of the variant exhibited no effect on drinking initiation (HR in univariate model = 1.12, 95% CI 0.92 to 1.36), consistent with the mechanism of the variant of only exhibiting an effect in response to alcohol consumption.

Interaction Between ADH1B *rs1229984 and Peer Drinking*

The interaction between the *ADH1B* variant and peer drinking was tested by adding the G $\times$ E product term to models of drinking milestones in adolescent drinkers (*n* = 1,550), which illustrated a significant statistical interaction for first intoxication (*p* = 0.002) and first DSM-5 symptom (*p* = 0.01) (Table 2). Among individuals who reported none/few best friends drinking, the *ADH1B* GA/AA genotypes had a strong protective effect for first intoxication (HR = 0.56, 95% CI 0.41 to 0.76) and first DSM-5 symptom (HR = 0.45, 95% CI 0.26 to 0.77). In individuals who reported most/all best friends drinking, however, this protective effect was not observed for either first intoxication
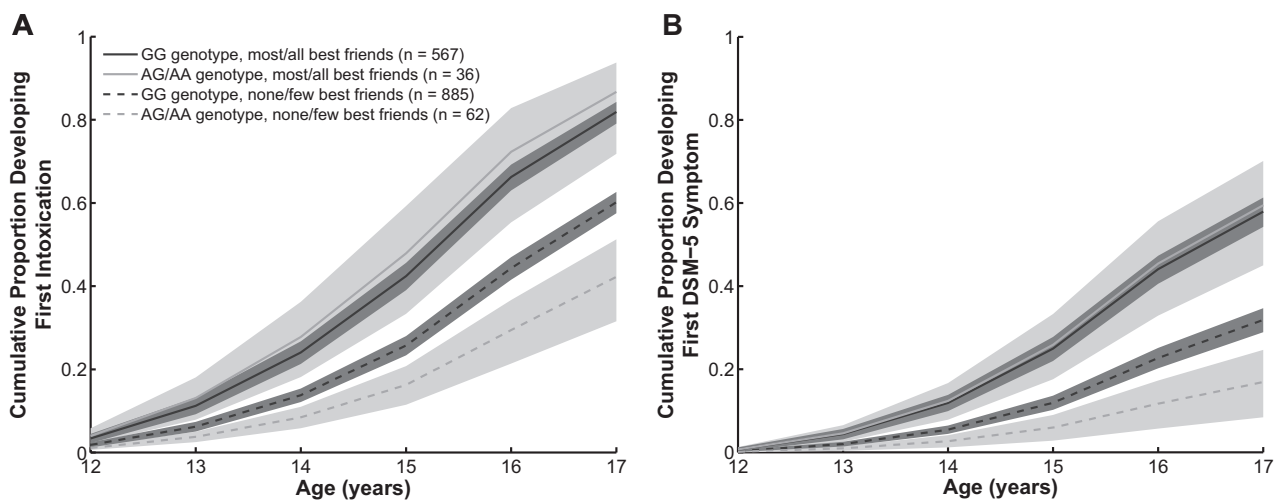

Fig. 2. Cox proportional hazards regression survival estimates of (**A**) first intoxication and (**B**) first DSM-5 alcohol use disorder symptom in adolescent ever-drinkers (*n* = 1,550) with the variables of *ADH1B* genotype, best friends drinking, and G $\times$ E interaction term.

(HR = 1.16, 95% CI 0.82 to 1.65) or first DSM-5 symptom (HR = 1.03, 95% CI 0.73 to 1.45), as illustrated by the point estimates close to 1. Figure 2 more clearly illustrates this G × E interaction by presenting the survival estimates.

Association Between ADH1B *Variant and Peer Drinking*

No evidence of a gene–environment correlation between the *ADH1B* variant and peer drinking was observed. Specifically, the independent variable of the *ADH1B* rs1229984 variant was not significant in the logistic regression model of perceived peer drinking controlling for sex and ethnicity as covariates (most/all vs. none/few best friends drink, odds ratio = 1.19, 95% CI 0.78 to 1.83).

Assessment of Robustness of Results

The proportional hazards assumption was satisfied in first DSM-5 symptom models. Violations were noted in a subset of first intoxication analyses. Examination of Schoenfeld residuals indicated that the group of 17-year-olds was driving this violation, perhaps reflecting important transitions at this age. Censoring at age 17 instead of 18 satisfied the proportional hazards assumption without substantially altering the parameter estimates, supporting our conclusions.

Ancestry-stratified analyses demonstrated consistent main and interaction effects in the European American subpopulation (*n* = 1,130). In the *interaction model set* for European American individuals, peer drinking had a hazardous effect on first intoxication (HR = 1.87, 95% 1.66 to 2.11) and first DSM-5 symptom (HR = 2.23, 95% CI 1.89 to 2.63); rs1229984 had a protective effect on first intoxication (HR = 0.60, 95% CI 0.44 to 0.82) and first DSM-5 symptom (HR = 0.47, 95% CI 0.27 to 0.82); and interaction terms were significant (*p* < 0.02). The *ADH1B* GA/AA genotypes were protective among individuals reporting none/few best friends drinking, but not among those reporting most/all best friends drinking, corroborating our findings in the overall sample.

Stratified analyses of African Americans (*n* = 420) provided trending evidence of main effects. In the *interaction model set* with G × E product terms, peer drinking had a hazardous effect on first intoxication (HR = 1.62, 95% 1.27 to 2.08) and first DSM-5 symptom (HR = 1.98, 95% CI 1.50 to 2.61); rs1229984 had a trending protective effect on first intoxication (HR = 0.32, 95% CI 0.08 to 1.27) and first DSM-5 symptom (HR = 0.35, 95% CI 0.05 to 2.28); and interaction terms were insignificant (*p* > 0.7). The limited sample size of African Americans combined with the low frequency of the rs1229984 minor allele limits power to detect interactions in this analysis. Nonetheless, the robust effect of peer drinking in both ancestry groups and the well-established role of rs1229984 across ancestry groups lends support for our conclusions drawn from the combined sample.

DISCUSSION

Alcohol use behaviors established during adolescence are important contributing factors for the later progression to alcohol dependence (Grant et al., 2006; Pitkanen et al., 2005). These data provide an example of the important interplay of genetic and environmental risks in the development of drinking milestones during this critical period of adolescence. Using a longitudinal sample of European and African American adolescent drinkers, we demonstrate that the *ADH1B* rs1229984 minor A allele is associated with a protective effect for early drinking behaviors, and in the environmental high-risk context of most or all best friends drinking, this genetic protection is negated.

The observation that the *ADH1B* variant is associated with a decreased risk of first intoxication and first DSM-5 symptom during adolescence (Table 2) extends previous findings that this variant protects against alcohol-related health problems in adulthood (Bierut et al., 2012; Gelernter et al., 2014; Li et al., 2011). Despite having an early role in the trajectory of drinking behaviors, the *ADH1B* variant was not associated with drinking initiation, consistent with the hypothesized mechanism of action that requires alcohol exposure (Edenberg and Foroud, 2013; Hurley and Edenberg, 2012). This specific example of a genetic variant that influences early drinking milestones, but not initiation, builds on twin and adoption study findings that genetic factors contribute to the development of adolescent alcohol-related problems, and environmental factors more strongly drive drinking initiation (Hopfer et al., 2003; Lynskey et al., 2010).

Beyond demonstrating an early protective role of the *ADH1B* GA/AA genotypes in the development of these drinking behaviors, the results illustrate that reporting most or all best friends drinking was associated with attenuation of this genetic protection (Fig. 2). The observation that social context modifies the effect of an *ADH1B* variant extends previous studies on alcohol-metabolizing variants. Higuchi and colleagues (1994) found that the proportion of alcohol-dependent adults in Japan with 1 copy of a protective aldehyde dehydrogenase 2 (*ALDH2*) variant increased between 1979 and 1992, following the increased cultural pressure to drink alcohol. Similarly, Irons and colleagues (2007) reported that the high-risk environment of sibling substance use was associated with a diminished effect of this *ALDH2* variant in east Asian adolescent adoptees, and more recently, this group demonstrated that high parental alcohol use and misuse reduced the effect of the *ALDH2* protective allele (Irons et al., 2012). For the *ADH1B* rs1229984 variant, Hasin and colleagues (2002) observed a weaker protective role in certain groups, which was hypothesized to reflect differences in environmental exposure to heavy drinking. Our findings expand on these earlier observations by demonstrating that the critical high-risk social context of adolescent peer drinking is associated with the loss of the protective genetic

effect of the *ADH1B* variant in European and African Americans.

Previous studies of metabolizing variants have focused on Asian populations where the *ADH1B* rs1229984 A allele is common, and only recently was this variant associated with alcoholism at a genome-wide level in an European and African American sample ($p = 6.6 \times 10^{-10}$) (Bierut et al., 2012). A recent genome-wide association study of alcohol dependence further supports a strong effect of this variant in European Americans ($p = 1.17 \times 10^{-31}$) (Gelernter et al., 2014). To our knowledge, this study is the first to examine the effect of the *ADH1B* rs1229984 variant on adolescent drinking behaviors and incorporate environmental moderation in European and African Americans.

One challenge of studying the influence of the *ADH1B* rs1229984 variant in populations of European and African ancestry is the low frequency of the protective A allele. Although over 1,500 adolescent drinkers were examined in this analysis, only 98 (6%) carried an A allele (of which 36 reported most/all best friends drinking). Nonetheless, the influence of this variant and the G × E interaction was persistently strong in models of first intoxication and first DSM-5 symptom (Table 2). Secondary ancestry-stratified analyses also demonstrated consistent main and interaction effects in the European American subpopulation ($n = 1,130$) and provided trending evidence of main effects in the African American subpopulation ($n = 420$), where power was limited. These analyses, combined with previous studies supporting the protective role of rs1229984 across ancestry groups as well as the moderating effect of social environments, support our conclusion that this variant is associated with a protective effect for early drinking behaviors in European and African Americans, but this genetic protection may be eliminated by adolescent peer drinking.

The findings reported here have several limitations. First, studying a specific genetic variant provides limited information on the general genetic underpinnings of complex diseases such as alcohol use disorder (Dick and Kendler, 2012). Nevertheless, examination of specific robust variants provides important insight into underlying biological mechanisms that are not assessed by traditional studies of latent genetic influences. Second, other genetic variants may influence associations between *ADH1B* rs1229984 and drinking behaviors (Meyers et al., 2013; Toth et al., 2011). Third, self-reported peer drinking was viewed as an environmental risk factor in this study, but research suggests that genetic factors contribute to peer alcohol involvement (Fowler et al., 2007). Gene–environment correlations can arise when an individual's heritable behavior evokes an environmental response (evocative rGE) or when an individual possesses a heritable propensity to select an environment (active rGE). In this study, the *ADH1B* rs1229984 variant was not associated with self-reported peer drinking, supporting our interpretation that peer drinking acts as an environmental modifier, but other gene–environment correlations may still contribute

to the observed effects. Fourth, the temporal ordering of peer drinking and the onset of drinking behaviors could not be assessed in this study (Table 1). It is possible that other risk factors correlated with peer drinking, such as parental monitoring or genetic risk for antisocial behavior, may account for the observed associations. Fifth, peer drinking was assessed by respondent report and may not reflect the actual proportion of best friends drinking. Finally, the majority of participants were from high-risk families, which may limit the generalizability of the findings. It is possible that only individuals at high risk for alcohol use disorders lose the protective effect of the *ADH1B* rs1229984 variant under environments that encourage drinking. Replication of these findings in independent samples is a critical next step.

Despite these limitations, this study has several strengths. First, the analysis focused on a genetic variant with strong statistical and biological evidence for alcohol-related measures, which addresses common criticisms of G × E studies (Duncan and Keller, 2011; Joober et al., 2007; Risch et al., 2009). Second, focusing on a youth population and employing a longitudinal study design reduced recall bias, enabling more accurate assessment of drinking behaviors during the critical period of adolescence. Third, the robust environment of respondent report of best friends drinking from ages 12 to 17 coincided with the timing of the primary outcomes under study. This analysis focused on drinking behaviors that are common in adolescence and therefore are more likely to be directly influenced by peer drinking during this period. Finally, studying adolescent drinking milestones facilitated the characterization of the unfolding of genetic and environmental risks across development. Recent studies further support the discovery potential of examining genetic variants during important behavioral transitions in at-risk youth (Belsky et al., 2013; Dick et al., 2013). Future research on alcohol use disorders may benefit from similar hypothesis-driven study designs that examine well-established genes and environments during critical developmental periods.

From a public health perspective, this study provides a genetic argument in support of early social interventions to decrease affiliation with peer drinkers. Specifically, these findings support the use of a screening tool for practitioners to identify at-risk youth, developed by the National Institute on Alcohol Abuse and Alcoholism and the American Academy of Pediatrics, in which the first question addresses friends' drinking (NIAAA, 2011). Under the high-risk environment of best friends drinking, all adolescents were at increased risk for early drinking problems, and particularly, those at lower genetic risk experienced the greatest added risk. This study serves as a model for how understanding the interplay between genes and environments may increase etiological knowledge of alcohol use disorders and potentially inform interventions that aim to disrupt progression to alcoholism.

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DISCLOSURES

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