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Special Issue Article

Longitudinal effects and environmental moderation of *ALDH2* and *ADH1B* gene variants on substance use from age 14 to 40

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Abstract

Alcohol use and dependence are strongly affected by variation in aldehyde dehydrogenase (*ALDH2*) and, to a lesser extent, alcohol dehydrogenase (*ADH1B*) genes. We use this genetic variation with an adoption design to test the causal role of alcohol use on other drug use, as well as the moderating role of adoptive parent, sibling, and peer alcohol use. Longitudinal models were run on 412 genotyped adopted individuals of East Asian ancestry with multiple assessments between ages 14 and 40. We found robust associations between alcohol frequency, quantity, and maximum drinks and *ALDH2*, but not *ADH1B*, status. The magnitude of the *ALDH2* protective effect increased with age, particularly for maximum drinks, though estimates were smaller than previously reported in ancestrally similar individuals in East/North-East Asian countries. These results suggest that sociocultural factors in Minnesota may reduce the protective effects of *ALDH2*. We found that peer alcohol use, but not parent or sibling use, predicted adopted offspring's use, and that these environmental influences did not vary by *ALDH2* status. Finally, we did not find strong evidence of associations between *ALDH2* status and tobacco, marijuana, or illegal drug use, contrary to expectation if alcohol serves as a gateway to use of other drugs.

Keywords: alcohol; development; longitudinal; Mendelian randomization; trajectory

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Alcohol use is increasingly common in the United States. Nearly 86% of individuals 18 and older report using alcohol at some point in their lives, with approximately 55% reporting use in the previous month (Substance Abuse and Mental Health Services Administration, 2020). By the end of high school, 59% of adolescents have reported consuming at least one alcoholic drink (Johnston et al., 2020). Alcohol use and problems are also heritable, with genetic variation accounting for approximately 50% of the variance in alcohol dependence (Verhulst et al., 2015). Similar heritability estimates ($h^2 = 30$ to 51%) have been found for other alcohol use behaviors like alcoholic drinks per week and maximum number of drinks (Grant et al., 2009). The influence of genetic factors on alcohol use behaviors, estimated in biometric studies of twin resemblance, appears to strengthen over time, increasing in magnitude from early adolescence to young adulthood before stabilizing (Kendler et al., 2008). This mirrors a decline in the influence of shared environmental factors (i.e., those environmental factors that are shared by individuals reared in the same home), from explaining approximately 40% of the variance in alcohol use at age 14 to near zero by the mid-20's.

Alcohol consumption also often co-occurs with use of other substances, like tobacco (Agrawal & Lynskey, 2009; Conway et al., 2017), marijuana (Vrieze et al., 2012), and other illegal drugs (Kessler et al., 2005; Krueger, 2002). Phenotypic correlations between symptom counts of alcohol, marijuana, and tobacco use disorders in a longitudinal study from ages 14 to 29 ranged from $r = 0.24$ to $r = 0.61$ (Vrieze et al., 2012). Correlations between measures of consumption are also similarly high across ages 14 to 40 (Zellers et al., 2022). These correlations declined in magnitude across developmental age, suggesting that young individuals tend to use substances broadly while older individuals show relatively more preferences for one substance over another.

There are several proposed theories to account for substance use comorbidity. For example, co-occurrence among multiple substances may be explained by an underlying externalizing factor that accounts for the associations between these substances, often termed the common liability model. In this case, a latent factor represents a general vulnerability to risk of substance use and development of substance use disorders (Iacono et al., 2008; Krueger, 2002). Indeed, several studies have provided evidence that a latent factor explains the majority of covariation between externalizing behaviors like substance dependence, conduct disorder, and ADHD (Hicks et al., 2004, 2013; Krueger, 2002). Moreover, this general vulnerability to externalizing behaviors was found to be highly heritable ($h^2 = 0.80$) (Hicks et al., 2004). Substance use co-occurrence is also consistent with the idea that use of one substance causally influences risk for use of other substances (i.e., the gateway hypothesis) (Kandel, 1975; Kandel & Kandel, 2015). In this case, it may be that after an individual uses one drug, they seek out other drugs from which to get a stronger high. The gateway hypothesis explains both the co-occurrence of use of different substances and developmental sequencing of drug use, where users of "hard" drugs (e.g., cocaine) almost always have first used other, legal drugs (e.g., alcohol or tobacco) (Degenhardt et al., 2010).

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In general, evidence for the gateway hypothesis has been limited and is often subject to methodological criticisms (Peele & Brodsky, 1997; Vanyukov et al., 2012). The more recent literature largely supports the common liability model, with consistent findings across multiple studies and age ranges (Hicks et al., 2007; Hicks BM et al., 2004; Krueger, 2002; van Leeuwen et al., 2011; Vrieze et al., 2013).

The gateway hypothesis is based on the premise that use of one drug causally influences use of others. One method for evaluating evidence for causal effects in observational studies is through Mendelian randomization. Mendelian randomization (MR) is a method frequently employed in epidemiology that uses a genetic variant(s) that is robustly associated with an exposure to evaluate the association with outcome (Lawlor et al., 2008). In other words, MR evaluates the relationship between genetic variants that are of known function and strongly associated with an exposure (e.g., alcohol use), and an outcome of interest (e.g., illegal drug use). MR takes advantage of the random assortment of genes during meiosis which is analogous to the randomization process in randomized control trials. In this way, MR offers a relatively strong test of causal effects in observational data. Using genetic data as a proxy for an environmental modifiable exposure like alcohol use minimizes the likelihood that the observed exposure–outcome association is due to unmeasured confounding and removes the possibility of reverse causality (Smith & Ebrahim, 2008; Smith & Hemani, 2014). Because genotypes are assigned randomly before birth, they are unlikely to be associated with factors that may confound the relationship between alcohol and other drug use (e.g., socioeconomic status) and because genotype is unchangeable, reverse causality is ruled out. If the gateway hypothesis is correct, we would expect the genetic variant to be associated with both alcohol use exposure and the use of other substances. This would provide support for a causal effect of alcohol use on risk for other substance use.

There are two genetic variants that have shown robust associations with alcohol use, and on which we focus in the present paper: *ALDH2* (rs671) and *ADH1B* (rs1229984) (Edenberg, 2007). Both variants influence alcohol consumption by affecting the way alcohol is metabolized in the body. After alcohol is consumed, alcohol dehydrogenase converts it into acetaldehyde. Aldehyde dehydrogenase then oxidizes acetaldehyde into acetate, which is ultimately cleared from the body. High concentrations of acetaldehyde in the body lead to significant physical discomfort. *ADH1B* acts to accelerate the conversion of alcohol to acetaldehyde, while *ALDH2* slows the conversion into acetate. Both genes cause a build-up of acetaldehyde in the body leading to severe discomfort and have been robustly associated with reduced alcohol consumption and dependence. The *ALDH2*2* allele, which is common only in populations of East/North-East Asian ancestry and is well tagged by a single variant, has the largest effect on alcohol consumption. A single copy of this variant can affect the breakdown of alcohol enough to cause severe symptoms. *ADH1B*2*, which is also common in these populations, has a weaker, but still robust, association with alcohol consumption. The structure of the *ADH1B* gene may be more complex than *ALDH2*, being defined by more than one genetic variant with weaker effects.

Much of the existing literature on the associations between *ALDH2* and *ADH1B* status and alcohol use are based on cross-sectional designs, including several genome-wide association studies (GWAS) of alcohol use and dependence. In a sample of Japanese individuals, the effect size of the *ALDH2* protective allele on drinks per week was estimated at −0.43 standard deviation (SD) units (Matoba et al., 2020). Other GWAS have estimated the effect of variants within *ADH1B* on drinks per week between −0.07 and −0.25 SD units (Brazel et al., 2019; Liu et al., 2019; Matoba et al., 2020). The few studies of longitudinal associations between these genetic variants and alcohol use patterns have found that the protective effect of *ALDH2*2* increases over developmental age (Irons et al., 2012; Kim et al., 2010). Irons et al. (Irons et al., 2012), using an earlier data freeze from the present study, found that the association between *ALDH2*2* and alcohol consumption was negligible around age 15 but increased to moderate in effect size by age 22. To our knowledge, there is one study of the longitudinal effect of *ADH1B* status on alcohol use, finding an interaction between gene status and age from early to mid-adolescence such that those with *ADH1B*2* increased their alcohol use less than those without (Cleveland et al., 2018). Increasing magnitudes of association between *ALDH2* and *ADH1B* variants and alcohol use over time would be consistent with the observed pattern of strengthening influence of genetic factors from adolescence to adulthood (Kendler et al., 2008). This may be explained by a reduction in the influence of the shared environment as offspring leave the rearing, parental home and begin to select their own environments, which itself may be partially influenced by genetic factors. It is not yet clear whether the strength of association between *ALDH2* and *ADH1B* variants and alcohol use continues to increase beyond early adulthood.

The effect of *ALDH2* and *ADH1B* status on alcohol consumption has also been found to vary in magnitude by environmental or cultural factors. For example, among those with the *ALDH2* and *ADH1B* polymorphisms there is substantial variability in mean alcohol intake levels within China (Millwood et al., 2019). Despite having two copies of the *ALDH2* protective allele (i.e., the same genotype; *ALDH2*2*), the mean alcohol intake differed by a factor of 24 across geographic areas of China, illustrating cultural effects on consumption patterns. The estimated magnitude of association between *ALDH2* status and alcoholic drinks per week in East Asian populations has also been shown to vary by country. Genetic effect sizes of *ALDH2* are 2.5 times larger when estimated in samples of East Asian individuals living in Japan (Matoba et al., 2020) compared to samples of East Asian individuals living in the United States (effect size of −0.43 in Japan versus −0.17 in the United States) (Jorgenson et al., 2017). Because the *ALDH2* variant is only polymorphic in East Asian populations, the vast majority of individuals in the United States, who are not of East Asian ancestry, do not have this genotype. Thus, mean alcohol consumption is higher in the United States compared to many East Asian countries, where the *ALDH2* deficiency is common, though this difference is decreasing. It may be that the protective effect of *ALDH2*2* (and potentially *ADH1B*2*) is weaker within cultures or environments like the United States with higher rates of alcohol consumption. Indeed, Higuchi et al. (Higuchi et al., 1994) found that the percentage of Japanese individuals suffering from alcoholism grew from 2.5% to 13% from 1979 to 1992, mirroring cultural shifts in alcohol consumption in Japan at that time. Environmental influences within families and amongst peers may also moderate the protective effects of *ALDH2*2* and *ADH1B*2*. Previous research has found that parent, sibling, and peer alcohol consumption patterns predict continued use in those with the *ALDH2* deficiency (Irons et al., 2007, 2012; Luk et al., 2017; O'Shea et al., 2017).

The current study makes use of a unique longitudinal adoption sample to extend the literature on the associations between *ALDH2/ADH1B* and alcohol consumption from adolescence to adulthood (ages 14 to 40) and to evaluate the causal relationship

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between alcohol exposure and other substance use outcomes. The sample consists of individuals who were adopted as infants from South Korea, primarily by White American adoptive parents living in Minnesota. Thus, they are reared in homes with genetically unrelated adoptive parents and siblings, allowing for exploration of important environmental influences on alcohol use behaviors, as well as adult outcomes hypothesized to be caused by alcohol exposure. The adoption and MR design provide quasi-experimental controls for genetic confounding in typical observational and family studies of closely related individuals. Our specific hypotheses are

1. Individuals with A*LDH2/ADH1B* protective alleles will drink less than those without and that this protective effect will increase in magnitude with age.
2. Sibling and parent alcohol use will moderate the protective effect of *ALDH2*2/ADH1B*2* and that this moderating effect, if present, will diminish with age. We made no explicit hypotheses about the environmental moderation of peer alcohol use as this was included based on reviewer feedback.
3. Alcohol use will not be associated with tobacco, marijuana, or other drug use in Mendelian randomization tests with *ALDH2/ADH1B* variants as instruments, inconsistent with the gateway hypothesis.

Methods

Sample

The current sample was taken from the Minnesota Center for Twin and Family Research (Wilson et al., 2019) Sibling Interaction and Behavior Study (SIBS) which includes 409 adoptive families. Adoptive families consisted of two genetically unrelated siblings and their rearing parents. Families were ascertained through infant placements at large, private adoption agencies in Minnesota. Eligible adoptive families included those in which the adopted adolescent was between the ages of 11 and 21 at the intake assessment, was placed in the adoptive home before 2 years of age, and had an adolescent sibling to which they were unrelated. While the sibling could not be genetically related to the adopted child, they could be genetically related to one or both of the parents, or could have also been adopted and placed before the age of 2 years. A more complete description of the SIBS sample is given elsewhere (McGue et al., 2007). SIBS participants have been followed longitudinally with up to four assessments. At the intake assessment, both parents and offspring were assessed. The first follow-up, approximately 3.5 years after intake, included an assessment and collection of DNA samples. The second and third follow-up assessments occurred approximately 7 and 17 years after the intake assessment, respectively.

The sample used in the current analysis was restricted to families with at least one offspring adopted from East/North-East Asia who had also provided genetic data. This results in N = 412 East Asian adopted participants, all of whom were born in South Korea, from N = 259 families. Adopted offspring had a mean age of 15.1 years (SD = 1.9 years) at intake, 18.3 years (SD = 2.1) at the first follow-up, 22.3 years (SD = 1.8) at the second follow-up, and 32.2 years (SD = 2.6) at the third follow-up. Offspring data used in the present study were taken from the first, second, and third follow-up assessments. Table 1 shows demographic information for adopted offspring. At the intake assessment N = 239 mothers and N = 241 fathers reported their own alcohol use. All adult participants gave informed consent, and minor participants gave assent with their parents providing consent.

Phenotypic measures

All substance use measures were collected using either the Substance Abuse Module (SAM) (Robins et al., 1987) of the Composite International Diagnostic Interview (CIDI) (Robins LN et al., 1988) or the Computerized Substance Use Questionnaire (CSU), depending on the age of the participant.

Alcohol-related outcomes

Quantitative alcohol-related measures, covering the time since the prior assessment, included frequency of alcohol use (scored from 6 = every day to 1 = less than once per month), usual quantity of alcohol use (measured as number of drinks), and maximum number of drinks in a 24-hour period. A single lifetime alcohol-related measure included the age of first alcoholic drink without parental permission. The physical effects of *ALDH2* and *ADH1B* deficiency are largely dependent on the amount of alcohol consumed, so the protective effect of these genes may be greater for measures of consumption than for frequency of use. For this reason, we included multiple measures of alcohol-related behaviors that index different aspects of consumption instead of creating a composite measure of alcohol use. Sibling frequency and quantity of use were measured in the same way as adopted offspring. At the intake assessment, parents reported lifetime number of DSM-IV alcohol dependence symptoms. We used the mid-parent mean symptom count as the measure of parental alcohol problems. Peer alcohol use was measured at the first and second follow-up assessments and was based on a single question of how many friends use alcohol (scored from 1 = none of my friends to 4 = all of my friends).

Other substance use outcomes

Lifetime binary measure of substance use included ever used tobacco regularly, ever used marijuana before the age 22 assessment, and ever used illegal drugs before the age 22 assessment. Quantitative outcomes, covering the time since the prior assessment, included number of cigarettes per day amongst regular smokers, number of marijuana uses amongst ever users, and a count of the number of illegal drug types used (e.g., cocaine, opiates, amphetamines, etc.). Tobacco, marijuana, and drug use data were taken only from the age 18 and age 22 assessments (follow-ups 1 and 2).

Genotyping

Genotype data (obtained from offspring at the first follow-up assessment) was used to identify relevant variants within the *ALDH2* (rs671; chromosome 12) and *ADH1B* (rs1229984; chromosome 4) genes in adoptees. Participants were genotyped on 527,829 single nucleotide polymorphism (SNP) markers using Illumina's Human660W-Quad array (Miller et al., 2012). Genotypes were then imputed to 1000 Genomes using the Michigan imputation server (Das et al., 2016). Both *ALDH2* and *ADH1B* were imputed with imputation qualities (expected squared correlations) of $r^2 = 0.48$ and $r^2 = 0.82$, respectively, in N = 365 individuals. Despite the modest expected quality of the rs671 variant, the *ALDH2* gene was also directly assayed in N = 354 individuals of East Asian ancestry. Here, we use the directly assayed genotype whenever available. The correlation between the directly assayed *ALDH2* genotype and the imputed *ALDH2* genotype was .89, suggesting the expected imputation quality calculated from the

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Table 1. Sample characteristics of East/North-East Asian adoptive offspring

	Full sample		Group comparison by genotype	
	N	M (SD)	***ALDH2* (*p*-value)**	***ADH1B* (*p*-value)**
Female (%)	412	61.7	.25	.87
Age				
Follow-up 1	405	18.3 (2.1)	.55	.96
Follow-up 2	392	22.3 (1.8)	.30	.86
Follow-up 3	247	32.2 (2.6)	.49	.71
Familial/peer alcohol use				
Mid-parent dependence symptoms	383	.43 (0.8)	.19	.69
Sibling frequency	389	2.8 (1.0)	.95	.46
Sibling quantity	387	3.8 (2.8)	.72	.90
Peer use	411	2.7 (0.6)	.24	.94

Note. Both *ALDH2* and *ADH1B* groups include those with at least one copy of the protective A allele. Comparisons of means (or proportions) were made within genotype (e.g., comparison of means between those with at least one *ALDH2* protective allele and those without) and were based on a *t* statistic for continuous variables or χ^2 statistic for binary variables. Parents reported lifetime DSM-IV alcohol dependence symptoms at the intake assessment. Sibling alcohol quantity and frequency was defined as the mean across all available assessments. Peer use was based on a single question of how many friends use alcohol on an ordinal scale from 1 = none to 4 = all. M = mean; SD = standard deviation.

imputation procedure was downwardly biased. Further details on how *ALDH2* was assayed can be found in Irons et al. (Irons et al., 2007).

The *ALDH2* and *ADH1B* gene variants have three possible genotypes: A/A, A/G, and G/G. In both, the A allele is associated with the strongest protection against alcohol use problems. Because just a single copy of each allele has been shown to be protective against alcohol use (Luczak et al., 2006), genotypes for each variant were dichotomized and coded as 1 for A/A homozygotes and A/G heterozygotes (e.g., *ALDH2*1/*2* and *ALDH2*2/*2*) and 0 for G/G homozygotes (e.g. *ALDH2*1/*1*).

Statistical analysis

To address the first hypothesis, regression models were used to examine the associations between *ALDH2* and *ADH1B* polymorphisms and alcohol-related outcomes with a focus on how the effect sizes change from ages 14 to 40. Given the longitudinal nature of the data, mixed effects models were fit that included *ALDH2* genotype, *ADH1B* genotype, sex, and age at assessment (linear and quadratic terms) as fixed effects. These models also included an interaction between genotype and age to test whether the magnitude of the protective effects of *ALDH2*2* and *ADH1B*2* changed significantly over development. All models included a random intercept for each individual, to account for the correlated family structure, and random slope for age.

To address the second hypothesis, we fit similar mixed effects models adding predictors of parent, sibling, and peer alcohol use and their interaction with *ALDH2/ADH1B* status. In this way we evaluated whether parent, sibling, or peer alcohol use is associated with an adoptee's alcohol use and whether the magnitude of the effects differs by genotype. Finally, to test whether *ALDH2/ADH1B* status predicted tobacco, marijuana, and illegal drug use (hypothesis 3), we used mixed effects models (with sex, age at assessment, and genotype as fixed effects and random effects of age and individual), with each of these substances as the outcome. Given the multiple hypothesis tests performed here with correlated outcome variables, we calculated the effective number of tests using the correlations between substance-related outcomes (Derringer, 2018; Nyholt, 2004). This results in a corrected statistical significance threshold of $p < 0.005$.

Results

Descriptive statistics

Among 412 East/North-East Asian adopted participants, 301 (73.1%) were G/G homozygotes for *ALDH2*, 9 (2.2%) were A/A homozygotes, and 102 (24.8%) were heterozygotes. For *ADH1B*, 196 (53.7%) were G/G homozygotes, 26 (7.1%) were A/A homozygotes, and 143 (39.2%) were heterozygotes. For both variants, we combined those with at least one protective A allele (i.e., genotypes of A/A and A/G) into a single group which was then compared to those with G/G genotypes. This results in 111 individuals (26.9%) with at least one copy of the *ALDH2* A allele (i.e., *ALDH2*2/*2* or *ALDH2*1/*2*) and 169 (46%) with at least one *ADH1B* A allele (i.e., *ADH1B*2/*2* or *ADH1B *1/*2*).

Table 1 reports descriptive statistics of the full sample including comparisons between those with and without *ALDH2* and *ADH1B* protective alleles and indicates that those with the protective genotypes did not differ from those without in gender, age at follow-up, or family member alcohol use. Approximately 62% of the sample is female with no difference in proportions by genotype group. The large proportion of females reflects the gender imbalance of children adopted from East Asia at the time. The effects of sample attrition were evaluated by comparing participants and nonparticipants at a given assessment with their responses at the previous assessment. In general, we found no differences in alcohol or other substance-related outcomes between follow-up 2 participants and nonparticipants at their prior assessment (follow-up 1) or between follow-up 3 participants and nonparticipants at the prior follow-up 2 assessment. The single exception to this was for differences in usual alcohol quantity at both follow-up 1 and follow-up 2 assessments. Those who completed the follow-up 1 assessment, but not the second follow-up, had higher levels of usual alcohol quantity than those who participated at both assessments (standardized mean difference of 0.82, $p = 0.04$). While this is a large mean difference, we note that there were only N = 13 individuals who participated in the first, but not second, follow-up assessment. Those who completed the follow-up 2 assessment, but not the third follow-up, had higher levels of usual alcohol quantity than those who participated at both assessments (standardized mean difference of 0.28, $p = 0.02$).

ALDH2/ADH1B associations with alcohol use

Figure 1 shows the observed patterns of alcohol frequency, usual quantity, and maximum drinks from ages 14 to 40 for the full sample and split by *ALDH2* status. In general, alcohol consumption and frequency increased across adolescence, peaking in the early 20's, before stabilizing or declining slightly through age 40. We did not find an association with age at first alcohol drink and either *ALDH2* or *ADH1B* status (*p*-values of 0.76 and 0.72, respectively). This is expected given that an individual may not know their *ALDH2/ADH1B* status prior to first initiating alcohol use. Longitudinal models showed significant associations between *ALDH2*2*, but not *ADH1B*2*, with alcohol-related outcomes (shown in Table 2). Interactions between *ALDH2* genotype and

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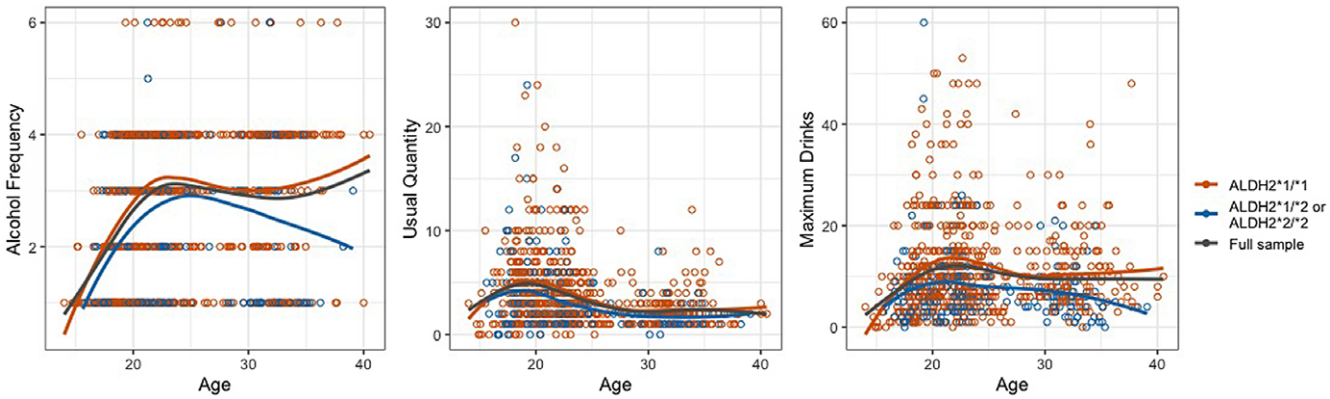

Figure 1. Plot of alcohol-related outcomes across age for the full sample and split by ALDH2 status. Loess curves were added to graphically display the relationship between alcohol behaviors and age by genotype. Confidence intervals around the loess lines were removed for clarity. Points are colored by genotype where orange indicates those with G/G genotype (ALDH2*1/*1) and blue indicates those with the protective A/A or A/G genotype (ALDH2*1/*2 or ALDH2*2/*2).

Table 2. Associations between *ALDH2/ADH1B* status and alcohol-related outcomes

		ALDH2		***ALDH2* × Age**		***ADH1B***		***ADH1B* × Age**	
	N	β (SE)	***p*-value**	β (SE)	***p*-value**	β (SE)	***p*-value**	β (SE)	***p*-value**
Alcohol frequency	786	−.14 (.06)	.0002*	−.04 (.04)	.29	−.06 (.04)	.11	.01 (.05)	.84
Usual quantity[a]	766	−.13 (.04)	.0006*	−.02 (.03)	.53	−.03 (.04)	.46	.04 (.04)	.28
Max drinks[a]	786	−.19 (.04)	<.0001*	−.10 (.03)	.0047*	−.05 (.05)	.22	.002 (.04)	.96

Note. All models included genotype, sex, age at assessment (linear and quadratic term), and a genotype × age interaction as fixed effects along with random intercept per individual and slope for age. For both *ALDH2* and *ADH1B*, genotypes of A/A or A/G (i.e., at least one protective allele) are coded as 1. β = standardized coefficient; SE = standard error; [a]denotes variables that were log transformed prior to analysis; *denotes *p*-values below the corrected threshold of 0.005.

age were all in the expected direction, though this effect was only significant for maximum drinks (p-value = 0.0047). These results indicate that while we find evidence for increasing effect size estimates across age the magnitude of this change is too small to detect at conventional levels of statistical significance in this sample.

Environmental influence of parent/sibling/peer alcohol use

Next, we evaluated the environmental influences of family and peer alcohol use on adoptee's own drinking behaviors and whether this effect differed by genotype. Because we did not find evidence for associations between *ADH1B* and alcohol-related outcomes, we focus here only on *ALDH2*. For each alcohol outcome, we fit separate models with sibling frequency of alcohol use, sibling usual quantity of alcohol use, mid-parent number of alcohol dependence symptoms, and peer use as predictors, along with an interaction between each predictor and adoptee *ALDH2* status. Sibling alcohol frequency and quantity were defined as the mean across all available follow-up assessments, while parental alcohol dependence symptoms were reported lifetime. Peer alcohol use was indexed by a single question at the adoptee's age 18 and 22 assessments. Full results are reported in Table 3. While nearly all estimated main effects of parent and sibling use were in the expected positive direction, only one estimate (the association between sibling usual alcohol quantity and adoptee's usual alcohol quantity) was significantly different from zero based on the corrected significance threshold of 0.005. Peer alcohol use was significantly associated with all measures of adoptee alcohol use, showing the largest effect sizes. None of the interactions between parent, sibling, or peer use and adoptee *ALDH2* status were significantly different from zero. This suggests evidence for environmental moderation of alcohol use through peer, but not familial, alcohol use and that these environmental effects do not differ as a function of *ALDH2* status.

ALDH2 associations with other substance use

The associations between *ALDH2* status and other substance use outcomes are shown in Table 4. We again focus only on *ALDH2* given the lack of association between *ADH1B* and alcohol use measures in the current sample. There is minimal evidence for an association between *ALDH2* status and any tobacco, marijuana, or illegal drug use measures. Associations with marijuana and illegal drug use were in the expected direction but effect sizes were small in magnitude, and all p-values were greater than 0.13. We note that sample sizes for the binary measures of substance use are substantially lower than for continuous measures because they are lifetime and not repeated measures.

Discussion

The current study extends the existing literature on the longitudinal protective effects of *ALDH2* and *ADH1B* gene variants. Using a unique adoption sample we evaluated whether parent, sibling, or peer alcohol use was associated with alcohol use behaviors and whether this effect differed by *ALDH2/ADH1B* status. This allowed for testing of some environmental effects on alcohol use unconfounded by genetic factors. We additionally evaluated the associations between *ALDH2* status and tobacco, marijuana, and illegal drug use in a test of the gateway hypothesis.

Across the full sample, we observed expected developmental patterns of alcohol use such that alcohol consumption and frequency increased across adolescence, peaking in the early 20's, before stabilizing or declining slightly through the early 30's

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Table 3. Associations between parent, sibling, and peer alcohol use and alcohol-related outcomes.

	Sibling alcohol frequency N = 809–830				*Sibling alcohol quantity* N = 804–825				*Mid-parent alcohol dependence symptoms* N = 783–800				*Peer alcohol use* N = 600–606			
	Main effect		Interaction		Main effect		Interaction		Main effect		Interaction		Main effect		Interaction	
	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value
Alcohol frequency	.07 (.04)	.09	−.02 (.11)	.84	.06 (.04)	.15	−.10 (.06)	.10	.01 (.04)	.79	.004 (.04)	.92	.22 (.04)	<.001*	−.01 (.12)	.92
Usual quantity[a]	.09 (.04)	.02	.07 (.11)	.55	.15 (.04)	.0003*	−.02 (.07)	.78	.03 (.04)	.49	.09 (.05)	.06	.25 (.04)	<.001*	.13 (.13)	.33
Maximum drinks[a]	.08 (.04)	.07	.04 (.12)	.73	.08 (.04)	.05	.00 (.07)	.96	−.01 (.04)	.79	.04 (.05)	.34	.30 (.04)	<.001*	.02 (.13)	.85

Note. All models included *ALDH2* status, sex, age at assessment (linear and quadratic term), and an *ALDH2* status × sibling, parent, or peer alcohol use interaction as fixed effects along with random intercept per individual and slope for age. β = standardized coefficient; SE = standard error; [a]denotes variables that were log transformed prior to analysis; *denotes *p*-values below the corrected threshold of 0.005.

Table 4. Associations between *ALDH2* status and other substance-related outcomes*.

		ALDH2		
	N	β/OR	SE	***p*-value**
Binary measures				
Ever regular smoker	320	0.98	0.32	0.95
Marijuana ever	357	0.66	0.18	0.13
Illegal drug ever	162	0.61	0.28	0.29
Continuous measures				
Cigarettes per day[a]	698	−0.05	0.06	0.41
Marijuana uses[a]	704	0.06	0.08	0.28
Illegal drug classes	284	0.02	0.09	0.86

Note. All models included *ALDH2* genotype, sex, age at assessment (linear and quadratic term; or year of birth for lifetime measures) as fixed effects along with random intercept per individual and slope for age. *ALDH2* genotypes of A/A or A/G (i.e., at least one protective allele) are coded as 1. β = standardized coefficient; OR = odds ratios; SE = standard error; [a]denotes variables that were log transformed prior to analysis; *denotes *p*-values below the corrected threshold of 0.005.

(Figure 1) (Saunders et al., 2016). Longitudinal models found robust associations between *ALDH2* status and all measures of alcohol use except age of alcohol initiation. Given that the adopted individuals in this sample are raised by White American parents, who themselves would not have the *ALDH2* polymorphism, they would likely have limited information on their own *ALDH2* status prior to initiating alcohol use. This is consistent with prior studies finding no association between *ALDH2* status and alcohol initiation or age at first intoxication (Irons et al., 2007; Wall et al., 2001). Results for all other alcohol-related outcomes showed that while those with and without the *ALDH2* polymorphism showed increases in alcohol consumption and frequency from adolescence through early adulthood, those with *ALDH2*2* protection had lower mean levels of consumption through age 40 than those without. Contrary to much of the existing literature we did not find evidence for significant associations between *ADH1B*2* and any of the alcohol-related phenotypes. The effect size estimates found here were generally lower than what has been previously reported (Liu et al., 2019; Matoba et al., 2020) but were in the expected direction.

Results showed an increasing protective effect of *ALDH2*2* against heavy alcohol consumption across developmental age, evidenced by interaction estimates in the negative direction. This is largely consistent with findings from other studies (Irons et al., 2012; Kim et al., 2010) and extends the existing literature by covering a much wider age well into adulthood. This effect was particularly pronounced for maximum number of drinks where we found a significant association between *ALDH2* status and age such that the protective effect of this variant increased over time. For other alcohol-related measures , the effect size of this interaction was relatively small. While in the expected direction, it may be that the current study is underpowered to detect such subtle interaction effects.

Effect sizes of *ALDH2*2* found here were similar in magnitude to previous studies of East Asian ancestry Americans (Jorgenson et al., 2017). That these estimates are smaller than those found in individuals living in East Asian countries supports the idea that sociocultural factors reduce the protective effects of *ALDH2*2* in a form of gene–environment interaction. This is further bolstered by observed differences in GWAS effect sizes estimates between a Japanese sample (Matoba et al., 2020) and a sample of nonadopted American individuals of East Asian ancestry (Jorgenson et al., 2017), as well as findings that the proportion of alcoholic *ALDH2*-deficient individuals has increased over time (Higuchi et al., 1994). This environmental effect may also explain the lack of association between our alcohol phenotypes and *ADH1B* status. Similar to *ALDH2*2*, the effect of *ADH1B*2* may be reduced in cultures in which alcohol use is prevalent. Indeed, the association between *ADH1B* status and alcohol dependence has been found to differ in magnitude between Europeans and East Asians (Whitfield, 2002). Because the current sample is composed of East Asian ancestry individuals raised in the United States, the association between *ADH1B* status and alcohol use may be small in magnitude.

Beyond sociocultural effects, prior work has suggested potential environmental moderation of parent, sibling, or peer alcohol use on offspring's own consumption patterns. In the current sample, many individuals with the *ALDH2* polymorphism, who likely experience significant physical discomfort after consuming alcohol, continue to drink frequently and, in some cases, heavily. We tested whether familial and peer alcohol use was associated with alcohol-related outcomes and whether these effects varied by *ALDH2* genotype. Because the current study is based on an adoption sample, parents and siblings are not genetically related to the adopted offspring. Thus, an association between parent or sibling alcohol use with adoptee alcohol use implies an

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environmental effect unconfounded by passive gene–environment correlation. Findings from the current study found strong effects of peer, but not parent or sibling, alcohol use. There was no evidence for significant interactions of these effects with adoptee genotype. This is generally consistent with prior work finding that close peer alcohol use is associated with alcohol use in those with and without *ALDH2*2* (Luk et al., 2017; O'Shea et al., 2017) though we do not find evidence that the associations differ in magnitude by *ALDH2* status as has been identified elsewhere (O'Shea et al., 2017). Our results are generally inconsistent, however, with the existing literature finding evidence for parental and sibling effects in both adoptive and nonadoptive families (Irons et al., 2007, 2012; McGue et al., 2014; Samek et al., 2018; Saunders et al., 2016). This disagreement may be due to different measures of parental use (i.e., alcohol consumption versus dependence symptoms) or would be more consistent if measures of familial alcohol use were more temporally linked to adoptee use (i.e., parent and sibling alcohol use was measured at the same time as adoptee use).

Finally, using a Mendelian randomization framework, we evaluated the association between *ALDH2* status and several tobacco, marijuana, and illegal drug use behaviors. The association between *ALDH2* status and marijuana and illegal drug initiation was consistent with the gateway effect, although not significant, and there was little evidence of association between *ALDH2* status and levels of use of marijuana and other substances. Taken together, this is generally inconsistent with the gateway hypothesis (Kandel & Kandel, 2015), suggesting that co-morbidity of substance use may be better explained in some other way, possibly by an underlying vulnerability to broad substance use and other externalizing behaviors. The recent literature largely supports the existence of a latent externalizing factor, with evidence in favor found across multiple studies and age ranges (Hicks et al., 2007; Hicks BM et al., 2004; Krueger, 2002; van Leeuwen et al., 2011; Vrieze et al., 2013). Evidence for the gateway hypothesis, in contrast, is limited and hindered by methodological concerns (Peele & Brodsky, 1997; Vanyukov et al., 2012). We do note, however, that odds ratios for the *ALDH2*2* effect on ever used marijuana or illegal drugs were 0.66 and 0.61, respectively, which, if robust and replicable, would be consistent with a gateway effect for initiation of use. While not significantly different from 1 here, larger sample sizes may lead to greater precision in effect size estimates. Understanding substance use comorbidity and reasons for their co-occurrence is important for informing prevention efforts. If it was the case that alcohol use causally influences risk for illegal drug use, then prevention efforts aimed at reducing alcohol consumption would also lead to reductions in illegal drug use. That we do not find strong evidence in support of causal effects of alcohol use on tobacco, marijuana, or illegal drug use suggests that efforts to prevent or reduce alcohol use may not lead to significant reductions in other drug use.

The results of the current study should be interpreted in the context of several limitations. The alcohol and other substance use measures included here are limited to normative substance use behaviors. We included a variety of measures that indexed initiation, quantity, and frequency of use but did not include measures of problematic use like dependence symptoms. Similarly, inclusion of broader familial environmental influences (i.e., other parent and sibling alcohol use measures) would provide additional information about potential moderating effects. For example, parental and sibling alcohol use, in the current study, was measured lifetime or averaged over assessments instead of at the same time as the adopted offspring. More temporally relevant measures of familial use may show stronger effects. Lastly, while our sample is uniquely informative in several ways, we note the modest sample size (N = 412) of individuals with *ALDH2/ADH1B* genotype data. This, coupled with smaller than expected effects sizes particularly in the case of *ADH1B*, may have led to somewhat underpowered analyses. Power analysis based on mixed effects models with a longitudinal sample size of N = 786, which is roughly the observed sample size and structure (see Table 2), and an alpha of 0.05 shows that we have 80% power to detect genetic effect sizes as small as −0.10 SD units in the outcome variable. It may be the case that we are insufficiently powered to detect small effects of *ADH1B* status on alcohol-related behaviors or of *ALDH2* on other substances of abuse.

In sum, the current study, based on a unique sample of East Asian adoptees, extended the existing literature describing the protective effects of *ALDH2*2* and *ADH1B*2* from adolescence to mid-adulthood. These findings support the idea that while the protective effect of *ALDH2*2* increases over development, the overall effects of both *ALDH2*2* and *ADH1B*2* may be diminished in cultures like the United States where alcohol use is prevalent compared to cultures of East Asian countries in which the *ALDH2* polymorphism is common and alcohol use is generally lower. We found evidence that environmental influences of peer, but not parent or sibling, alcohol use significantly predicted offspring's own alcohol use. These environmental effects did not vary by *ALDH2* status. This suggests that peer behaviors may be important for explaining alcohol use to a greater extent than familial use and that the influence of peer use operates similarly in those with and without *ALDH2* deficiency. We also do not find evidence for a potentially causal association between alcohol and tobacco, marijuana, or illegal drug use, which is largely inconsistent with the gateway hypothesis of drug use and provides important information for informing prevention efforts.

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References

Agrawal, A., & Lynskey, M. T. (2009). Tobacco and cannabis co-occurrence: Does route of administration matter? *Drug and Alcohol Dependence*, *99*(1), 240–247. https://doi.org/10.1016/j.drugalcdep.2008.08.007

Brazel, D. M., Jiang, Y., Hughey, J. M., Turcot, V., Zhan, X., Gong, J., Batini, C., Weissenkampen, J. D., Liu, M., CHD Exome+ Consortium, Consortium for Genetics of Smoking Behaviour, **Barnes, D. R., Bertelsen, S., Chou, Y.-L., Erzurumluoglu, A. M., Faul, J. D., Haessler, J., Hammerschlag, A. R., Hsu, C.,** ... **Vrieze, S.** (2019). Exome Chip meta-analysis fine maps causal variants and elucidates the genetic architecture of rare coding variants in smoking and alcohol use. *Biological Psychiatry*, *85*(11), 946–955. https://doi.org/10.1016/j.biopsych.2018.11.024

Cleveland, H. H., Schlomer, G. L., Vandenbergh, D. J., Wolf, P. S. A., Feinberg, M. E., Greenberg, M. T., Spoth, R. L., & Redmond, C. (2018). Associations between alcohol dehydrogenase genes and alcohol use across early and middle adolescence: Moderation × Preventive intervention. *Development and Psychopathology*, *30*(1), 297–313. https://doi.org/10.1017/S0954579417000633

Conway, K. P., Green, V. R., Kasza, K. A., Silveira, M. L., Borek, N., Kimmel, H. L., Sargent, J. D., Stanton, C., Lambert, E., Hilmi, N., Reissig, C. J., Jackson, K. J., Tanski, S. E., Maklan, D., Hyland, A. J., & Compton, W. M. (2017). Co-occurrence of tobacco product use, substance use, and mental health problems among adults: Findings from Wave 1 (2013–2014) of the Population Assessment of Tobacco and Health (PATH) Study. *Drug and*

https://doi.org/10.1017/S0954579422000712 Published online by Cambridge University Press

Alcohol Dependence, *177*, 104–111. https://doi.org/10.1016/j.drugalcdep.2017.03.032

Das, S., Forer, L., Schönherr, S., Sidore, C., Locke, A. E., Kwong, A., Vrieze, S. I., Chew, E. Y., Levy, S., McGue, M., Schlessinger, D., Stambolian, D., Loh, P.-R., Iacono, W. G., Swaroop, A., Scott, L. J., Cucca, F., Kronenberg, F., Boehnke, M., … Fuchsberger, C. (2016). Next-generation genotype imputation service and methods. *Nature Genetics*, *48*(10), 1284–1287. https://doi.org/10.1038/ng.3656

Degenhardt, L., Dierker, L., Chiu, W. T., Medina-Mora, M. E., Neumark, Y., Sampson, N., Alonso, J., Angermeyer, M., Anthony, J. C., Bruffaerts, R., de Girolamo, G., de Graaf, R., Gureje, O., Karam, A. N., Kostyuchenko, S., Lee, S., Lépine, J.-P., Levinson, D., Nakamura, Y., … Kessler, R. C. (2010). Evaluating the drug use "gateway" theory using cross-national data: Consistency and associations of the order of initiation of drug use among participants in the WHO World Mental Health Surveys. *Drug and Alcohol Dependence*, *108*(1), 84–97. https://doi.org/10.1016/j.drugalcdep.2009.12.001

Derringer, J. (2018). *A simple correction for non-independent tests*. https://doi.org/10.31234/osf.io/f2tyw

Edenberg, H. J. (2007). The genetics of alcohol metabolism: Role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Research & Health*, *30*(1), 5–13.

Grant, J. D., Agrawal, A., Bucholz, K. K., Madden, P. A. F., Pergadia, M. L., Nelson, E. C., Lynskey, M. T., Todd, R. D., Todorov, A. A., Hansell, N. K., Whitfield, J. B., Martin, N. G., & Heath, A. C. (2009). Alcohol consumption indices of genetic risk for alcohol dependence. *Biological Psychiatry*, *66*(8), 795–800. https://doi.org/10.1016/j.biopsych.2009.05.018

Hicks, B. M., Blonigen, D. M., Kramer, M. D., Krueger, R. F., Patrick, C. J., Iacono, W. G., & McGue, M. (2007). Gender differences and developmental change in externalizing disorders from late adolescence to early adulthood: A longitudinal twin study. *Journal of Abnormal Psychology*, *116*(3), 433–447. https://doi.org/10.1037/0021-843X.116.3.433

Hicks BM, Foster KT, Iacono WG, & McGue M. (2013). GEnetic and environmental influences on the familial transmission of externalizing disorders in adoptive and twin offspring. *JAMA Psychiatry*, *70*(10), 1076–1083. https://doi.org/10.1001/jamapsychiatry.2013.258

Hicks BM, Krueger RF, Iacono WG, McGue M, & Patrick CJ. (2004). Family transmission and heritability of externalizing disorders: A twin-family study. *Archives of General Psychiatry*, *61*(9), 922–928. https://doi.org/10.1001/archpsyc.61.9.922

Higuchi, S., Matsushita, S., Imazeki, H., Kinoshita, T., Takagi, S., & Kono, H. (1994). Aldehyde dehydrogenase genotypes in Japanese alcoholics. *The Lancet*, *343*(8899), 741–742. https://doi.org/10.1016/S0140-6736(94)91629-2

Iacono, W. G., Malone, S. M., & McGue, M. (2008). Behavioral disinhibition and the development of early-onset addiction: Common and specific influences. *Annual Review of Clinical Psychology*, *4*(1), 325–348. https://doi.org/10.1146/annurev.clinpsy.4.022007.141157

Irons, D. E., Iacono, W. G., Oetting, W. S., & McGue, M. (2012). Developmental trajectory and environmental moderation of the effect of ALDH2 polymorphism on alcohol use. *Alcoholism: Clinical and Experimental Research*, *36*(11), 1882–1891. https://doi.org/10.1111/j.1530-0277.2012.01809.x

Irons, D. E., McGue, M., Iacono, W. G., & Oetting, W. S. (2007). Mendelian randomization: A novel test of the gateway hypothesis and models of gene–environment interplay. *Development and Psychopathology*, *19*(4), 1181–1195. https://doi.org/10.1017/S0954579407000612

Johnston, L. D., Meich, R. A., O'Malley, P. M., Bachman, J. G., Schulenberg, J. E., & Patrick, M. E. (2020). *Monitoring the future national survey results on drug use 1975–2019: Overview, key findings on adolescent drug use*. Institute for Social Research, University of Michigan.

Jorgenson, E., Thai, K. K., Hoffmann, T. J., Sakoda, L. C., Kvale, M. N., Banda, Y., Schaefer, C., Risch, N., Mertens, J., Weisner, C., & Choquet, H. (2017). Genetic contributors to variation in alcohol consumption vary by race/ethnicity in a large multi-ethnic genome-wide association study. *Molecular Psychiatry*, *22*(9), 1359–1367. https://doi.org/10.1038/mp.2017.101

Kandel, D. (1975). Stages in adolescent involvement in drug use. *Science (New York, N.Y.)*, *190*(4217), 912–914. https://doi.org/10.1126/science.1188374

Kandel, D., & Kandel, E. (2015). The Gateway Hypothesis of substance abuse: Developmental, biological and societal perspectives. *Acta Paediatrica*, *104*(2), 130–137. https://doi.org/10.1111/apa.12851

Kendler, K. S., Schmitt, E., Aggen, S. H., & Prescott, C. A. (2008). Genetic and environmental influences on alcohol, Caffeine, Cannabis, and Nicotine use from early adolescence to middle adulthood. *Archives of General Psychiatry*, *65*(6), 674–682. https://doi.org/10.1001/archpsyc.65.6.674

Kessler, R. C., Chiu, W. T., Demler, O., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of rwelve-month DSM-IV disorders in the National Comorbidity Survey Replication (NCS-R). *Archives of General Psychiatry*, *62*(6), 617–627. https://doi.org/10.1001/archpsyc.62.6.617

Kim, S.-K., Lee, S.-I., Shin, C.-J., Son, J.-W., & Ju, G. (2010). The genetic factors affecting drinking behaviors of Korean young adults with variant aldehyde dehydrogenase 2 genotype. *Psychiatry Investigation*, *7*(4), 270–277. https://doi.org/10.4306/pi.2010.7.4.270

Krueger, R. F. (2002). Etiologic connections among substance dependence, antisocial behavior and personality: Modeling the externalizing spectrum. *Journal of Abnormal Psychology*, *111*(3), 411. https://doi.org/10.1037/0021-843X.111.3.411

Lawlor, D. A., Harbord, R. M., Sterne, J. A. C., Timpson, N., & Davey Smith, G. (2008). Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. *Statistics in Medicine*, *27*(8), 1133–1163. https://doi.org/10.1002/sim.3034

Liu, M., Jiang, Y., Wedow, R., Li, Y., Brazel, D. M., Chen, F., Datta, G., Davila-Velderrain, J., McGuire, D., Tian, C., Zhan, X., Choquet, H., Docherty, A. R., Faul, J. D., Foerster, J. R., Fritsche, L. G., Gabrielsen, M. E., Gordon, S. D., Haessler, J., … Vrieze, S. (2019). Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature Genetics*, *51*(2), 237–244. https://doi.org/10.1038/s41588-018-0307-5

Luczak, S. E., Glatt, S. J., & Wall, T. J. (2006). Meta-analyses of ALDH2 and ADH1B with alcohol dependence in Asians. *Psychological Bulletin*, *132*(4), 607–621. https://doi.org/10.1037/0033-2909.132.4.607

Luk, J. W., Liang, T., & Wall, T. L. (2017). Gene-by-environment interactions on alcohol use among Asian American College Freshmen. *Journal of Studies on Alcohol and Drugs*, *78*(4), 531–539. https://doi.org/10.15288/jsad.2017.78.531

Matoba, N., Akiyama, M., Ishigaki, K., Kanai, M., Takahashi, A., Momozawa, Y., Ikegawa, S., Ikeda, M., Iwata, N., Hirata, M., Matsuda, K., Murakami, Y., Kubo, M., Kamatani, Y., & Okada, Y. (2020). GWAS of 165,084 Japanese individuals identified nine loci associated with dietary habits. *Nature Human Behaviour*, *4*(3), 308–316. https://doi.org/10.1038/s41562-019-0805-1

McGue, M., Keyes, M., Sharma, A., Elkins, I., Legrand, L., Johnson, W., & Iacono, W. G. (2007). The environments of adopted and non-adopted youth: Evidence on range restriction from the Sibling Interaction and Behavior Study (SIBS). *Behavior Genetics*, *37*(3), 449–462.

McGue, M., Malone, S., Keyes, M., & Iacono, W. G. (2014). Parent–offspring similarity for drinking: A longitudinal adoption study. *Behavior Genetics*, *44*(6), 620–628. https://doi.org/10.1007/s10519-014-9672-8

Miller, M. B., Basu, S., Cunningham, J., Eskin, E., Malone, S. M., Oetting, W. S., Schork, N., Sul, J. H., Iacono, W., & McGue, M. (2012). The Minnesota Center for Twin and Family Research genome-wide association study. *Twin Research and Human Genetics*, *15*(6), 767–774.

Millwood, I. Y., Walters, R. G., Mei, X. W., Guo, Y., Yang, L., Bian, Z., Bennett, D. A., Chen, Y., Dong, C., Hu, R., Zhou, G., Yu, B., Jia, W., Parish, S., Clarke, R., Smith, G. D., Collins, R., Holmes, M. V., Li, L., … Chen, Z. (2019). Conventional and genetic evidence on alcohol and vascular disease Aetiology: A prospective study of 500 000 men and women in China. *The Lancet*, *393*(10183), 1831–1842. https://doi.org/10.1016/S0140-6736(18)31772-0

Nyholt, D. R. (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *American Journal of Human Genetics*, *74*(4), 765–769.

O'Shea, T., Thomas, N., Webb, B. T., Dick, D. M., Kendler, K. S., & Chartier, K. G. (2017). ALDH2*2 and peer drinking in East Asian college students. *The American Journal of Drug and Alcohol Abuse*, *43*(6), 678–685. https://doi.org/10.1080/00952990.2017.1314489

Peele, S., & Brodsky, A. (1997). Gateway to nowhere: How alcohol came to be scapegoated for drug abuse. *Addiction Research*, *5*(5), 419–425. https://doi.org/10.3109/16066359709004356

Robins, L., Babor, T., & Cottler, L. (1987). *Composite international diagnostic interview: Expanded substance abuse module*. Washington University.

Robins, L. N., Wing, J., Wittchen, H., Helzer, J. E., Babor, T. F., Burke, J., Farmer, A., Jablenski, A., Pickens, R., & Regier, D. A. (1988). The composite international diagnostic interview: An epidemiologic instrument suitable for use in conjunction with different diagnostic systems and in different cultures. *Archives of General Psychiatry*, *45*(12), 1069–1077. https://doi.org/10.1001/archpsyc.1988.01800360017003

Samek, D. R., Goodman, R. J., Riley, L., McGue, M., & Iacono, W. G. (2018). The developmental unfolding of sibling influences on alcohol use over time. *Journal of Youth and Adolescence*, *47*(2), 349–368. https://doi.org/10.1007/s10964-017-0703-7

Saunders, G. R. B., McGue, M., Iacono, W. G., & Elkins, I. J. (2016). Parent–offspring resemblance for drinking behaviors in a longitudinal twin sample. *Journal of Studies on Alcohol and Drugs*, *78*(1), 49–58. https://doi.org/10.15288/jsad.2017.78.49

Smith, G. D., & Ebrahim, S. (2008). Mendelian randomization: Genetic variants as instruments for strengthening causal inference in observational studies. In M. Weinstein, J. W. Vaupel, & K. W. Wachter (Eds.), *Biosocial surveys* (pp. 336–366). National Academies Press.

Smith, G. D., & Hemani, G. (2014). Mendelian randomization: Genetic anchors for causal inference in epidemiological studies. *Human Molecular Genetics*, *23*(R1), R89–R98.

Substance Abuse and Mental Health Services Administration. (2020). Key substance use and mental health indicators in the United States: Results from the 2019 national survey on drug use and health. *Security Research Hub Reports*. https://digitalcommons.fiu.edu/srhreports/health/health/32

van Leeuwen, A. P., Verhulst, F. C., Reijneveld, S. A., Vollebergh, W. A. M., Ormel, J., & Huizink, A. C. (2011). Can the gateway hypothesis, the common liability model and/or, the route of administration model predict initiation of Cannabis use during adolescence? A survival analysis—The TRAILS study. *Journal of Adolescent Health*, *48*(1), 73–78. https://doi.org/10.1016/j.jadohealth.2010.05.008

Vanyukov, M. M., Tarter, R. E., Kirillova, G. P., Kirisci, L., Reynolds, M. D., Kreek, M. J., Conway, K. P., Maher, B. S., Iacono, W. G., Bierut, L., Neale, M. C., Clark, D. B., & Ridenour, T. A. (2012). Common liability to addiction and "gateway hypothesis": Theoretical, empirical and evolutionary perspective. *Drug and Alcohol Dependence*, *123*, S3–S17. https://doi.org/10.1016/j.drugalcdep.2011.12.018

Verhulst, B., Neale, M. C., & Kendler, K. S. (2015). The heritability of alcohol use disorders: A meta-analysis of twin and adoption studies. *Psychological Medicine*, *45*(05), 1061–1072. https://doi.org/10.1017/S0033291714002165

Vrieze, S. I., Hicks, B. M., Iacono, W. G., & McGue, M. (2012). Decline in genetic influence on the co-occurrence of alcohol, Marijuana, and Nicotine dependence symptoms from age 14 to 29. *American Journal of Psychiatry*, *169*(10), 1073–1081. https://doi.org/10.1176/appi.ajp.2012.11081268

Vrieze, S. I., McGue, M., Miller, M. B., Hicks, B. M., & Iacono, W. G. (2013). Three mutually informative ways to understand the genetic relationships among behavioral disinhibition, alcohol use, drug use, nicotine use/dependence, and their co-occurrence: Twin biometry, GCTA, and genome-wide scoring. *Behavior Genetics*, *43*(2), 97–107. https://doi.org/10.1007/s10519-013-9584-z

Wall, T. L., Shea, S. H., Chan, K. K., & Carr, L. G. (2001). A genetic association with the development of alcohol and other substance use behavior in Asian Americans. *Journal of Abnormal Psychology*, *110*(1), 173–178. https://doi.org/10.1037//0021-843x.110.1.173

Whitfield, J. B. (2002). Alcohol dehydrogenase and alcohol dependence: Variation in genotype-associated risk between populations. *American Journal of Human Genetics*, *71*(5), 1247–1250.

Wilson, S., Haroian, K., Iacono, W. G., Krueger, R. F., Lee, J. J., Luciana, M., Malone, S. M., McGue, M., Roisman, G. I., & Vrieze, S. (2019). Minnesota center for twin and family research. *Twin Research and Human Genetics*, *22*(6), 746–752. https://doi.org/10.1017/thg.2019.107

Zellers, S. M., Iacono, W. G., McGue, M., & Vrieze, S. (2022). Developmental and etiological patterns of substance use from adolescence to middle age: A longitudinal twin study. *Drug and Alcohol Dependence*, *233*, 109378. https://doi.org/10.1016/j.drugalcdep.2022.109378