CHARACTERIZING SPECIFIC GENETIC AND ENVIRONMENTAL INFLUENCES ON ALCOHOL USE

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Matt McGue

September 2012

Acknowledgements

My endless thanks go to Matt McGue for his unflagging support and guidance. I thank Bill Oetting for his enthusiastic encouragement and advice. I am deeply grateful to all of the faculty, staff, and fellow students at the Minnesota Center for Twin and Family Research, as well as the Personality, Individual Differences, & Behavior Genetics program for their many years of work to produce and maintain the wonderfully rich resources and the stimulating and supportive environment that I have been lucky to be part of. In particular, the work described in this dissertation would not have been possible without the help of Mike Miller, Rob Kirkpatrick, Scott Vrieze, and Meg Keyes. My thanks go to Marcia Brott for sharing her technical expertise. To my family, my brothers Matt and Jonah, to Mindy, to Mary Ellen, to the Montgomery family and the Ostlund family: Thank you. Thank you.

Dedication

This dissertation is dedicated to my grandparents.

Abstract

Although both genetic and environmental influences, as well as the interplay between them, are clearly important to the development of alcohol use and related psychopathology, the effects of many of the particular genetic variants and environmental risk factors responsible have not yet been confirmed. We conducted three studies with the goal of moving beyond abstract estimates of genetic and environmental variance to the assessment of whether specified risk factors were causally implicated in the development of alcohol-related behaviors and problems. First, in a longitudinally assessed sample of 356 adopted adolescents and young adults of East Asian descent, we examined the progression over time of the relationship between a functional polymorphism in the alcohol metabolism gene aldehyde dehydrogenase 2 (ALDH2) and multiple measures of drinking behavior. We found that the protective effect of the less-functional ALDH2 variant increased between mid-adolescence and early adulthood, and that non-biological parental alcohol use, but not sibling alcohol use, nor deviant peer affiliation, moderated the effect of the gene. In a second study, using a community-based sample of 7224 individuals assessed in early and middle adulthood, we employed multiple methods to conduct a comprehensive examination of the effects of markers in GABA system genes on measures of alcohol use and related symptomatology. We tested not only the potential effects of individual markers, but also their effects in aggregate, and at the whole-gene and system-wide levels. None of these methods produced results indicative of an effect of GABA system variants on measures of alcohol use or misuse. We conducted a third study with a sample of 1512 twins, longitudinally assessed from early adolescence into adulthood, to determine whether adult alcohol use and misuse, as well as other adult outcomes, could be attributed to the causal effect of alcohol exposures in early adolescence. We used two separate techniques to adjust for potentially confounding factors. First, we used a propensity score design to adjust for the potentially confounding effects of a number of measured background covariates. Second, we used the cotwin control design to adjust for confounding due to unmeasured factors (including genetic influences) shared between twins in pairs discordant for early alcohol exposure. The

results of both methods applied in this third study were generally consistent with there being a causal effect of early alcohol exposures on the later development of adult alcohol problems and other related adult outcomes, but contrasting the two methods indicated that exposure effect estimates from the propensity score application were likely to be biased by unmeasured confounding variables. In summary, we have first substantially elaborated upon the effects of a genetic variant known to influence alcohol-related behaviors (in the ALDH2 gene); next, despite thorough investigation, we have found no evidence for the effects of a second set of purported genetic influences (GABA system genes); and finally, we provided evidence that early alcohol exposure likely exerts a genuinely causal influence on later alcohol-related problems and other adult outcomes.

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Chapter 1.

General Introduction

Alcoholism is common, and is harmful to the individuals who experience it, their families, their communities, and society. Alcoholism and problematic alcohol use run in families (Cotton, 1979; Sher, Walitzer, Wood, & Brent, 1991), and twin, adoption, and other family-based designs have shown that a large proportion of the variance in these and other alcohol-related behaviors is explained by genetic factors (Heath et al., 1997; Agrawal & Lynskey, 2008; Grant et al., 2009). Though they can provide an estimate of the magnitude of genetic effects, twin and other family-based designs can not alone be used to uncover the specific sources of genetic influence that contribute to variation in alcohol use behaviors. Linkage, candidate gene, and recently genome-wide studies have been used in attempts to pinpoint these variants (Goldman, Oroszi, & Ducci, 2005; Gelernter & Kranzler, 2009; Heath et al., 2011). Although a number of chromosomal regions and individual genes have been implicated by such studies, few findings have been replicated in subsequent follow-up investigations, and associations with individual variants do not account for a substantial portion of the genetic variance implied by twin and family studies.

Genes involved in the alcohol metabolic pathway, especially those coding for enzymes in the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) families, have been particularly implicated in measures of alcohol response and alcohol-related behaviors, including dependence. For example, multiple linkage studies suggest that a chromosome 4 region containing a cluster of acetaldehyde dehydrogenase (ADH)

genes is linked to variation in risk for alcohol dependence (Gelernter & Kranzler, 2009). One of the only candidate genes to be both consistently and strongly associated with variation in alcohol use behaviors is the aldehyde dehydrogenase 2 gene (ALDH2) on chromosome 12, which encodes an enzyme with a critical role in the metabolic pathway of alcohol. A functional variant in this gene, common only among East Asian populations, produces an enzyme with severely reduced metabolic efficacy (Crabb, et al., 2004) and as a result is consistently associated with lower risk for alcohol dependence (Edenberg, 2007).

In Chapter 2, I describe a study conducted in order to examine the effects of this ALDH2 polymorphism in a longitudinally assessed sample of adopted adolescents and young adults of East Asian descent. Measures of quantity and frequency of alcohol use, as well as alcohol-related psychopathology, were assessed from mid-adolescence to early adulthood, in order to determine whether the reduction in alcohol use associated with the less-active form of the gene was developmentally variable. Further, since earlier studies had suggested that social or cultural influences might moderate the effects of the less-active variant (Higuchi et al., 1994; Higuchi et al., 2007), we tested whether adopted parent alcohol use, adopted sibling alcohol use, or deviant peer behavior interacted with the ALDH2 polymorphism.

The functional ALDH2 polymorphism that we studied, as well as those in other ADH and ALDH alcohol metabolism genes, most likely influences alcohol use behaviors largely as a secondary product of physiological symptoms in the periphery, such as facial flushing, tachycardia, and nausea, produced due to acetaldehyde accumulation following

alcohol consumption when metabolism is disrupted or rendered less efficient by variants in these genes. These symptoms are experienced as aversive, and reduced alcohol use thus likely occurs substantially as a result of learning or other cognitive processes in response to the subjectively negative somatic experience of the alcohol sensitivity syndrome (Hendershot et al., 2011). However, many other genes affecting variation in alcohol use behaviors are likely to do so by directly affecting structures or functions in the central nervous system, so as to effect individual differences in alcohol-induced neurotransmission, susceptibility to the rewarding and reinforcing effects of alcohol or to addiction in general, or behavioral disinhibition, of which the elevated use of alcohol may be only one manifestation of many (Spanagel, 2009; Iacono, Malone, & McGue, 2003).

Many of the direct pharmacological effects of alcohol in the central nervous system are mediated by type A GABA receptors ($GABA_A$) (Kumar et al., 2009), and a large number of candidate gene studies have indicated that markers in $GABA_A$ subunit genes are associated with alcohol dependence and other alcohol-related behaviors (Cui, Seneviratne, Gu, & Li, 2012). However, most of the variation in alcohol use behaviors attributable to genetic influences, including that attributable to GABA system genes, is not likely caused by variants of relatively large effect like the functional polymorphism in ALDH2 among East Asians. Rather, drinking behaviors are probably influenced by a large number of variants in many genes, which collectively influence risk (Kendler et al., 2012). Because of the large number of tests involved which require statistical correction, genome-wide association studies that consider each marker in isolation are likely to often fail to detect many variants of genuine, but small, individual effect. Candidate gene

studies can help focus on genes that, because of their biological significance, might be more likely to be involved in alcohol related behaviors.

In the study described in Chapter 3, rather than just using a single candidate gene approach, since polymorphisms in a number of genes coding for $GABA_A$ receptor subunits have been associated with alcohol related behaviors, we studied SNPs in multiple GABA system genes. Furthermore, in addition to testing the effect of individual SNP markers on alcohol-related phenotypes, we computed a polygenic score reflecting the aggregated effects of multiple GABA system SNPs. We also used SNP-based estimates of pairwise genetic relatedness between participants to estimate the variance in alcohol use and abuse measures attributable to GABA system markers. Finally, we used gene-based tests to determine whether the test statistics of SNP markers, taken together in individual GABA genes, were likely to be significantly related to alcohol use and abuse measures.

Patterns of alcohol use in adulthood are the product of developmental processes that may begin in adolescence, or earlier. Twin studies indicate that environmental influences (both shared and non-shared) are more important to the initiation and early use of alcohol than the later development of alcohol dependence or other maladaptive alcohol use (Kendler, Schmitt, Aggen, & Prescott, 2008). Having used alcohol at an early age is one of the most straightforward risk factors for the development of maladaptive alcohol use in adulthood. Adult alcohol dependence and abuse are associated with earlier age at onset of alcohol use (Grant & Dawson, 1997; Dewit, Adlaf, Offord, & Ogborne, 2000), and a number of mechanisms might credibly mediate a causal effect of early use on adult

outcomes, including social feedback loops as a result of affiliation with peers who are themselves intoxicated or encouraging of alcohol use (Spear & Varlinskaya, 2000), or neurocognitive adaptation or neurotoxicity (Zeigler et al., 2005) that could lead to cognitive deficits, elevated addiction risk, or higher disinhibition in general.

However, in non-experimental observational studies, associations are not evidence for a causal relationship, though they are often nonetheless (either explicitly or implicitly) interpreted as such. Early alcohol use being associated with later alcohol use, and there existing reasonable mechanisms by which the former could provoke the latter, are not conclusive evidence of a causal effect; it is also possible that some additional confounding variable is a common cause of both the supposed exposure and the outcome, and thereby induces the association between the two.

The potential outcomes model provides a useful framework within which to formalize assumptions in order to allow for the valid conceptualization of causal relationships (Foster, 2010). The effect of an exposure on an outcome for a given individual can be thought of as the difference in outcomes between that individual having experienced the exposure and the same individual having not experienced the exposure. Since an individual cannot be both exposed and non-exposed, the value of the outcome for whichever level of the exposure the individual did not actually experience, called the counterfactual, is effectively missing data (McGue, Osler, & Christensen, 2010; Hernan & Robins, 2012).

In this framework, additional assumptions required to plausibly infer causal connections between putative exposures and outcomes are made explicit, and can be

examined or addressed as appropriate. One of the most critical assumptions required for unbiased causal inference of the effect of an exposure is that the counterfactual outcomes for individuals who received an exposure should be identical to the outcomes for individuals who actually did not receive the exposure; and likewise, the counterfactual outcomes for individuals who did not receive an exposure should be identical to the outcomes for their actually-exposed counterparts (Hofler, 2005). In order for this to hold true, all pre-exposure characteristics of the sample other than exposure status itself must be *balanced*, on average, between the exposed and non-exposed groups, so that these characteristics can not confound the relationship between the exposure and any outcomes that might arise from it. In practice, this can be accomplished using a number of different methods to adjust for confounding factors.

In the setting of an experiment with random assignment to groups, for example, randomization (in most circumstances) ensures that the probability of assignment to a particular treatment or exposure group is not dependent on pre-existing characteristics of the participants (that is, that the pre-existing characteristics are balanced across the randomly assigned exposure groups), so that the missing counterfactual outcomes may be considered to be generated at random (Hernan & Robins, 2012). Consequently, the average outcome values among the group that receives the treatment or exposure and the group that does not can be treated as counterfactuals to each other, with the effect of the exposure being the difference in the average outcomes between the two groups.

In chapter 4, I describe a study conducted using two separate methods to approximate the counterfactual in an observational, non-experimental context, in order to

estimate the longitudinal effects of alcohol exposures in early adolescence on measures of alcohol use and abuse in adulthood, as well as a variety of other adult outcomes.

The first approach made use of the propensity score, which is the predicted probability of experiencing the exposure conditional on a set of demographic and behavioral background variables. One of several ways to apply propensity scores is by weighting each individual by the inverse of their probability of their having experienced the level of the exposure that they actually experienced. In the resulting weighted sample, exposure is independent of the set of background variables included in the propensity score. If the variables in the propensity score include all sources of potential confounding, then in the weighted sample exposed and non-exposed individuals may be treated as counterfactual alternatives to each other, and used to estimate an unbiased average effect of the exposure on the outcomes (Hernan & Robins, 2012).

Our second approach used the cotwin control design, in which twins discordant for an exposure, because of the similarities of their genetic and shared environmental backgrounds, may be taken to approximate counterfactual counterparts to each other (McGue et al., 2010). The difference in the average outcome values between exposed and non-exposed twins therefore provides an estimate of causal effect which is unbiased with respect to shared genetic and shared environmental influences, but which may be biased by non-shared environmental factors and genetic influences not shared between the twins in a pair (ie: the 50% of segregating genetic material not shared by DZ twins, on average).

These two approaches each have important limitations, with propensity score-based adjustment potentially being biased by non-observed confounders, and cotwin control analysis potentially being biased by confounding factors not shared by members of a discordant twin pair. However, they may be used separately to cross-check each other, or simultaneously to supplement each other, each buttressing the other's limitations (Huibregtse et al., 2011).

Chapter 2.

Developmental Trajectory and Environmental Moderation of the Effect of ALDH2 Polymorphism on Alcohol Use

2.1 Introduction

Although developmental patterns of alcohol use vary between individuals (Brown et al., 2008), on average, alcohol use increases in accelerating fashion over the course of adolescence (Walden, et al., 2007), typically reaching a maximum during early adulthood (Sher, Grekin, & Williams, 2005), and declining thereafter (Moore et al., 2005). Twin studies, and studies using other genetically informative family designs, indicate that genetic factors account for a substantial proportion of variance in alcohol use (McGue, 1999), but the magnitude of genetic effects upon alcohol use may change over the course of development. Some studies have shown that shared environmental influences are important to the initiation and establishment of alcohol use during adolescence, but give way to increasing genetic influences upon alcohol use phenotypes through adolescence and into adulthood (Kendler, et al., 2008; Rose and Dick, 2005). The pattern of increasing genetic relationship to alcohol use-related phenotypes with age has also been observed in association with measured genetic polymorphisms in candidate genes. For example, the strength of the association observed between a polymorphism in the GABA-A receptor alpha 2 subunit gene *GABRA2* and alcohol dependence was shown to increase with age (Dick et al., 2006).

Twin, adoption, and other genetically informative studies designed to disentangle genetic factors from those of the social environment, suggest that heritable effects upon

adolescent alcohol use may be both magnified and attenuated by environmental factors (Dick, 2011; van der Zwaluw, 2009). Generally, in less constrained (Dick, 2011), or more adverse environments, such as when the alcohol use of family members and peers is high (Hicks, et al., 2009), variance attributable to genetic influences upon adolescent alcohol use and other externalizing behaviors increases. There is also evidence that environmental moderation may modulate the magnitude of the influence of specific measured genetic polymorphisms upon alcohol use. For instance, one study found a polymorphism in the dopamine transporter gene DAT1 to be associated with serious alcohol problems only among young men who also had alcoholic fathers (Vaske, et al., 2009).

The enzyme aldehyde dehydrogenase 2 (*ALDH2*) performs an essential step in the normal function of the pathway of ethanol metabolism, by mediating the oxidation of acetaldehyde to acetate in the mitochondria of the liver and other tissues (Bosron and Li, 1986). While very rare in other populations (Li et al., 2009), among East Asian populations a substantial proportion of the populace carries an ALDH2 gene variant rs671 resulting in a Glu504Lys amino acid change (Yoshida, et al., 1984; Li et al., 2009), which codes for an enzyme with greatly diminished oxidative efficacy (Crabb, et al., 2004). Reduced enzyme activity causes build-up of acetaldehyde in tissues after consumption of alcohol (Wall et al., 1997), which produces a number of dysphoric symptoms, including facial flushing, nausea, and headache (Eriksson, 2001). Consequently, it has been repeatedly observed that, relative to those homozygous for the allele producing a fully active enzyme (*ALDH2*1*), individuals carrying the reduced

activity ALDH2 variant (*ALDH2*2*) drink less (Hendershot et al., 2009), and are less likely to become alcohol dependent (Luczak, et al., 2006).

The relationship between *ALDH2*2* possession and lower alcohol use may increase during late adolescence and early adulthood. Doran, et al. (2007) assessed a sample of Asian American college students during the first year of college, and again in the second year. They found that the frequency of heavy drinking episodes was not related to *ALDH2* genotype during the freshman year, but was during the sophomore year. Possession of the *ALDH2*2* allele, the authors concluded, protected against progression towards heavy drinking from freshman to sophomore year, and made desistance from heavy drinking during this period more likely. Similarly, the association between *ALDH2* genotype and alcohol use outcomes in a sample of Korean college students in Korea increased between an initial freshman year assessment and follow-up 6 years later (Kim, et al., 2010).

Because *ALDH2* specifically affects alcohol use via a known mechanism of altered alcohol metabolism, it is not likely to contribute to the same largely genetic latent factor that accounts for a large proportion of the variance in both alcohol use and other externalizing behaviors (Krueger et al., 2002). Therefore, environmental moderators of genetic influences upon alcohol use shown in twin and other family-based designs, as well as in candidate gene studies involving polymorphisms in genes active within the nervous system, may not similarly moderate the effects of ALDH2 polymorphism. However, indirect evidence indicates that the strength of the effect of ALDH2 upon drinking may be influenced by the environment. Higuchi et al. (1994) noted that between

1979 and 1992, a progressively greater proportion of alcoholics in Japan possessed the *ALDH2*2* allele. This change is presumed to be the result of sociocultural shifts in Japan during the interim years, resulting in increased prevalence of alcohol use (Higuchi, 2007). These results imply, albeit indirectly, that the protection against maladaptive alcohol use afforded by possession of the *ALDH2*2* allele may be attenuated by environments where drinking is more common, or social pressures to drink are high.

In conducting the present study, we expected that possession of the *ALDH2*2* allele would be associated with lower levels of alcohol use, and that this association would become stronger over the course of adolescence and into early adulthood. We predicted, further, that the environmental effects of parent and elder sibling alcohol use, and level of deviant peer behavior, would each moderate the effect of *ALDH2* genotype, and that these interactive effects would also increase with the age of the target participants. By studying adopted individuals, we were able to assess the environmental effect of parent and sibling drinking upon *ALDH2* effect, unconfounded by shared genetic influences.

2.2 Materials and Methods

The Sibling Interaction and Behavior Study (SIBS) is a prospective longitudinal study of sibling pairs, including both adopted and non-adopted adolescents, and their parents (McGue, et al., 2007). Sibling participants and their parents attended an intake (IN) assessment when the siblings were in mid-adolescence, and the siblings were re-assessed at approximately 3.5 year intervals, in late adolescence (FU1), and again in early

adulthood (FU2). Participants visited the research facility for IN and FU1 assessments, but the FU2 assessment was conducted over the phone.

2.2.1 Sample

Because the SNP variant (rs671) resulting in reduced *ALDH2* enzymatic activity is almost exclusively found in East Asian populations, we genotyped only those SIBS participants of Korean descent, amounting to 356 participants. All participants had been adopted, with an average (SD) age at placement of .39 (.55) years. Phenotypic data were available for all 356 participants at IN and FU1 assessments, and 266 participants at FU2, because the second follow-up assessment is still ongoing. Age at IN was M(SD) = 14.81(1.81), at FU1: M(SD) = 18.13(1.99), and at FU2: M(SD) = 22.25(1.79). Of the genotyped participants, 59% were female.

Among the SIBS families from whom at least one child was genotyped for the *ALDH2* SNP rs671, all adoptive parents were white except for two step-parents of Asian descent, and one step-parent of mixed ethnicity. Alcohol use data were available for the elder non-biological siblings of 165 genotyped participants at IN, 160 elder siblings at FU1, and 128 elder siblings at FU2. As the SIBS study includes only sibling pairs, each genotyped participant had the possibility of only one elder sibling. Elder siblings were .22 to 4.73 years older than target siblings (M(SD) = 2.33(.91) years). Of these elder siblings, 17 were white, 145 were of Korean descent, and 3 were of mixed ethnic origin. Of the 145 elder siblings of Korean descent, 126 were themselves genotyped participants, while the other 19 non-genotyped elder siblings of Korean descent did not have available DNA samples.

2.2.2 Genotyping

Participants provided samples of either peripheral blood (n = 191) or buccal swab (n=165). The *ALDH2* Glu504Lys polymorphism (rs671) was genotyped using an Applied Biosystems TaqMan drug metabolism genotyping assay (Foster City, CA, USA). Additional details regarding the genotyping procedure have been previously published (Irons et al., 2007). Of 356 genotyped samples, 260 (73%) were homozygous *ALDH2*1/*1*, 87 (24.5%) were heterozygous *ALDH2*1/*2*, and 9 (2.5%) were homozygous *ALDH2*2/*2*. These genotype proportions did not deviate from Hardy Weinberg equilibrium, $X^2(1) = 0.28$, $p = 0.6$. The frequency of the *ALDH2*2* allele that we observed (14.8%) was approximately equivalent to previous studies of the polymorphism in samples of Korean descent (Li et al., 2009; Luczak, et al., 2006). *ALDH2* genotype did not vary by sex, $X^2(1) = 1.87$, $p = .17$.

2.2.3 Measures

All SIBS participants, both parents and children, underwent structured interviews including the assessment of DSM-IV diagnostic criteria, producing symptom counts and diagnostic status for alcohol abuse and alcohol dependence. Diagnostic data for parents (who were assessed at intake only), covered parental lifetime. Diagnostic data for sibling participants covered lifetime for assessment at intake, and covered the interval elapsing since their last assessment (typically over 3 years) at FU1 and FU2. To account for the possibility that symptoms may be forgotten when reporting on past disorder, participants were classified as alcohol dependent if they satisfied criteria for either definite (meeting all DSM-IV criteria) or probable (missing one symptom) alcohol dependence. Alcohol

abuse, which requires only one symptom, was diagnosed only when full DSM-IV criteria were met.

We also measured the alcohol use of parents and sibling participants (including both target siblings and their co-siblings) aged 16 or older at the time of assessment using a modified version of the Substance Abuse Module (SAM; Robins, et al., 1987), which was originally developed to supplement the World Health Organization's Composite International Diagnostic Interview (Robins et al., 1988). These measures were collected at all three assessment time points for sibling participants, but at intake assessment only for parents. To all sibling participants younger than 16 at the time of assessment, we administered a computerized substance use questionnaire, which included alcohol-use items re-scaled to be equivalent to those in the SAM. To index alcohol use for genotyped participants, older siblings, and parents, we used four items included on both the SAM and the computerized substance use questionnaire: frequency of drinking sessions over the year preceding the current assessment, average amount of alcohol consumed during drinking sessions over the year preceding the current assessment, maximum amount of alcohol consumed during a single drinking session over the participant's lifetime until the current assessment, and frequency of intoxication over the participant's lifetime until the current assessment. These items were combined, with equal weighting, to form a drinking index representing participants' overall drinking behavior.

Peer behavior was assessed at all three assessments using a nine item questionnaire regarding delinquent, antisocial, or otherwise deviant peer behaviors, asking sibling participants to evaluate statements such as "My friends get into trouble

with the police" or "My friends work hard to get good grades in school." Only a single item on this questionnaire referred directly to peer alcohol use ("My friends drink alcohol or beer."). Each item was scored on a four point scale for proportion of peers exhibiting the indicated behavior ("None of my friends", "Just a few of my friends", "Most of my friends", or "All of my friends"), and item scores were summed.

2.2.4 Analyses

Because possession of a single *ALDH2*2* allele is sufficient to produce substantially diminished enzyme activity (Crabb, et al., 1989), for analyses including *ALDH2* genotype, individuals both homozygous and heterozygous for the *ALDH2*2* allele were grouped together, and contrasted with individuals homozygous for the *ALDH2*1* allele. Age was centered for analysis by subtracting the minimum observed age (10.73 years) from each participant's age. Count data (not including age), considered both as predictors and outcomes, were log-transformed for analysis. Individuals who had never had a drink at the time of their latest assessment (n = 56) were excluded from analyses. Status as a lifetime never-drinker did not differ by possession of the *ALDH2*2* allele at the latest assessment for which data was available for each individual ($X^2(1) = .48$, $p = .49$) nor at IN ($X^2(1) = .06$, $p = .81$), FU1 ($X^2(1) < .01$, $p = .96$), or FU2 ($X^2(1) = .14$, $p = .70$). In assessing the main effects of *ALDH2* genotype, as well as effects of moderation by parental alcohol use and misuse, and peer deviance, all 300 genotyped participants who had ever had a drink by the time of their latest assessment were included in the analyses of all compared models. However, in assessing the potential moderating

effect of elder sibling alcohol use and misuse, analyses included only the 165 genotyped participants for whom elder sibling data were available.

Both the drinking index and the sum of the alcohol abuse and alcohol dependence symptom counts were each averaged across mothers and fathers, so that parental alcohol use represents maternal and paternal alcohol use in combination. Because their distributions were non-normal, for all participants the drinking index and the sum of alcohol dependence and alcohol abuse symptom counts were each log-transformed. For analysis, for parents and elder siblings separately, the log-transformed drinking index and the log-transformed sum of alcohol abuse and alcohol dependence symptom counts were standardized and averaged to form a combined alcohol problem index, which was itself then standardized. All standardization was calculated relative to the variable values of the total number of available SIBS parents (N=1164) and both adopted and non-adopted offspring (N=1232), including both genotyped and non-genotyped SIBS participants.

To account for intra-individual correlation across longitudinal observations, for counted data outcomes we used mixed modeling as implemented in SAS (SAS Institute, Inc., Cary, North Carolina) PROC MIXED (Bryk and Raudenbush, 1992; Singer, 1998) and generalized estimating equations (Liang and Zeger, 1986) for binary outcomes. Within each model comparison set, likelihood ratio testing (LRT) was used to compare the least complex model to each of the successive models that appended one or more additional terms. We identified the best fitting model within each model set as the one that minimized the Akaike's information criterion (AIC; Akaike, 1973) for quantitative outcomes, or the comparable quasi-likelihood function under the independence model

criterion (QICC; Pan, 2001) for binary outcomes. For binary outcomes, we also considered Wald tests to determine the significance of added terms, as more complex models were compared.

For each outcome, we first fit a best-fitting baseline model including fixed effects for sex, age, and polynomials of age up to the cubic term (for analyses of count data outcomes) or squared term (for analyses of binary outcomes). The best-fitting baseline model included a sex-by-age interaction for all continuous phenotypes, but not for the binary alcohol dependence or abuse phenotype. Preliminary model fitting did not support the inclusion of an interaction term between sex and *ALDH2* genotype, nor any role for elder sibling sex, for any alcohol use outcomes, so these terms were excluded from the baseline model and subsequent model comparisons. To the baseline model, we fit a series of models adding terms first for the effect of *ALDH2* genotype, then for the change in *ALDH2* effect over time (that is, an *ALDH2* by age interaction). Next, separately for the effects of parental alcohol use and misuse (ie: the parental combined alcohol problem index), elder sibling alcohol use and misuse (ie: the elder sibling combined alcohol problem index), and peer deviance, we fit a successive series of incrementing models including the change in the effect of each potential environmental moderator over time, the interaction of each with *ALDH2* genotype, and the change in the interaction of each with *ALDH2* genotype over time.

2.3 Results

Table 1 displays the non-transformed observed values of the descriptive statistics for genotyped participant ages, as well as the primary alcohol use outcomes (drinking

index, alcohol abuse and dependence symptom sum, and alcohol abuse or dependence diagnosis), at each assessment stage (IN, FU1, and FU2) by *ALDH2* genotype. Model fit comparisons are given in Table 2. For all three outcomes, likelihood ratio tests indicated that including the *ALDH2* main effect and the age by *ALDH2* interaction effect significantly improved model fit relative to the baseline model. Likewise, the best-fitting model by minimized AIC or QICC included the main effect of *ALDH2* as well as the interaction of *ALDH2* with age.

To understand the nature of the *ALDH2* by age interaction, table 3 provides best-fitting model estimated mean alcohol use phenotype values for each genotype group at three ages (15, 18, and 22), which correspond approximately to the sample mean age at each assessment. For every phenotype, alcohol use occurred with very low incidence at early ages, but increased over the course of adolescence and early adulthood before leveling off or decreasing near the end of early adulthood (figures 1-3). Effect sizes for continuous outcomes, and odds ratio for the discrete outcome, show that, as we expected, the protective effect of the *ALDH2*2* allele against all forms of alcohol use also increased with participant age (this trend may also be observed for each of the outcomes displayed on figures 1-3). For example, for the drinking index, the effect size for the estimated standardized mean difference between the *ALDH2* groups grew from d = -.07 at age 15, to .22 at age 18 and .40 at age 22. Likewise, the predicted probabilities of alcohol abuse or dependence diagnosis showed the protective effect of the *ALDH2*2* allele increased with age, from OR = 2.29 at age 15, to 1.10 at age 18 and .42 at age 22. Although the OR might suggest that *ALDH2*2* is associated with increased risk at age 15, it is important to

recognize that rates of diagnosis are very low at this age (approximately 2% for both genotypes), in which case the estimated OR is statistically unstable.

Results of the analysis of the effects of parental alcohol use and misuse are given in Tables 4A, 4B, and 4C; comparable results for the effects of elder sibling alcohol use and misuse and peer deviance are given in Supplementary tables S1 and S2, respectively. The means of potential environmental influences did not differ between *ALDH2* genotype groups (parent combined alcohol problem index: $t(898) = .06$, $p = .96$; elder sibling combined alcohol problem index $t(363) = -.7$, $p = .48$; peer deviance $t(826) = .98$, $p = .33$), indicating absence of gene-environment correlation. Birth order status (ie; being either a younger or elder sibling) also did not vary between *ALDH2* genotype groups, $X^2(1) = 0.04$, $p = 0.84$.

Our primary interest is in determining whether the *ALDH2* effect on drinking outcomes was moderated by each environmental indicator (as indicated by inclusion of the two-way interaction term in model F in the tables) and further whether this effect was moderated by age (by inclusion of the three-way interaction effect in model H). Regarding the parental combined alcohol problem index, for the drinking index and alcohol abuse and dependence symptom count outcomes, but not the binary alcohol abuse or dependence diagnosis outcome, the AIC-determined best-fitting model included the hypothesized interaction between the parental combined alcohol problem index and *ALDH2* polymorphism (tables 4A, 4B, and 4C; Model F for each outcome). However, by the likelihood ratio test, for the alcohol abuse and dependence symptom count outcome, models including the influence of the parental combined alcohol problem index did not

differ significantly from the best fitting model without the parental combined alcohol problem index (Model C*). In no case was there evidence for the three-way *ALDH2* by Parent combined alcohol problem index by Age interaction effect.

Regarding the effect of the elder sibling combined alcohol problem index, for the drinking index measure, the AIC-determined best-fitting model included the hypothesized interaction between the elder sibling combined alcohol problem index and *ALDH2* polymorphism (table 5A, Model G). However, contrary to hypotheses, there was no evidence for moderation of *ALDH2* effect by the elder sibling combined alcohol problem index upon the combined alcohol abuse and dependence symptom count (table 5B), nor the diagnosis of alcohol abuse or dependence (table 5C). Notably, for all three alcohol use outcomes, the magnitude of the positive relationship between the elder sibling combined alcohol problem index and the target sibling drinking index decreased over time. Similarly, we found no consistent evidence for interactions of *ALDH2* with deviant peer behavior (tables 6A, 6B, and 6C).

For the drinking index and alcohol abuse and dependence symptom count outcomes, estimation by best-fitting models generally reflects that, as expected, the magnitude of the protective effect afforded by *ALDH2*2* against alcohol had a negative relationship to the level of the parental combined alcohol problem index (tables 7A and 7B). The same pattern is shown in estimates by the best-fitting model for the moderation of *ALDH2* effect upon the drinking index outcome by the elder sibling combined alcohol problem index, and also upon alcohol abuse or dependence by peer deviance (table 8).

2.4 Discussion

Consistent with many previous studies, we found that adolescent and young adult participants who carried the *ALDH2*2* allele exhibited reduced alcohol use compared to *ALDH2*1* homozygotic participants. For every phenotypic outcome (drinking index, alcohol dependence and abuse symptom count, and alcohol dependence or abuse diagnosis), the best-fitting model included terms for the effect of *ALDH2* genotype (table 2). Furthermore, best-fitting models for each of these primary phenotypes included an age by *ALDH2* genotype interaction, such that the protective effect of *ALDH2*2* increased over the course of adolescence and young adulthood,. This pattern is in accordance with earlier research showing that *ALDH2* genotype is not related to the initiation of alcohol use, or to ever having been intoxicated (Wall, et al., 2001), and that evidence for association between the *ALDH2*2* allele and diminished alcohol use may not be apparent in young samples (Hendershot, et al., 2005) but may increase during young adulthood (Doran et al., 2007; Kim et al., 2010).

The influence of *ALDH2* polymorphism upon drinking behaviors has been previously shown to be partially mediated by cognitive factors (Hendershot et al., 2011). One possible mechanism by which the genetic effect of *ALDH2*2* upon drinking behavior might increase with age is via learning processes, whereby alcohol cognitions develop in response to alcohol sensitivity experienced during past drinking sessions. In this framework, the effect of *ALDH2*2* might be expected to increase over time, as individuals, following initial exposure to alcohol, learn to avoid ingesting larger amounts of alcohol, in order to avoid the adverse physiological effects that make up the alcohol sensitivity syndrome.

In the present study, we found that parental drinking (gauged by the parental combined alcohol problem index) was related to the strength of the effect of *ALDH2* genotype upon both the drinking index, and the alcohol abuse and dependence symptom count, such that the protective effect of *ALDH2*2* was stronger when parental drinking was lower, and weaker when parental drinking was higher (tables 7A and 7B). This finding is in accord with the previous observation that *ALDH2*2* apparently afforded reduced protection against alcohol dependence as environmental exposure to alcohol use increased (Higuchi et al., 1994)—although in our sample, parental moderation of *ALDH2* genotype effect did not extend to diagnosis of alcohol abuse and dependence per se.

Because genotyped participants in this study were adopted, mostly by white adoptive parents, into American families, our inference regarding the phenotypic effects of variation in *ALDH2* is enhanced. Caucasian adoptive parents do not carry the *ALDH2*2* allele themselves, but in non-adoptive families of *ALDH2*2* carriers, at least one biological parent must also carry *ALDH2*2*, and thus is likely to experience the protection against alcohol use associated with the allele. Therefore, a passive gene-environment correlation may develop when *ALDH2*2* carrying individuals are raised by their biological parents, whereby the influence of parental *ALDH2* genotype results in a family environment characterized by fewer models of adult drinking, and, to the extent that offspring drinking is environmentally influenced by parent drinking, this environmental influence may compound upon, and be confounded with, the purely biological effect of the offspring's own *ALDH2* genotype. Consequently, in families where *ALDH2*2* carrying offspring are raised by *ALDH2*2* carrying biological parents,

the protective effect associated with the *ALDH2*2* allele is likely to be amplified, because it will also include the environmentally-mediated influence of parental *ALDH2*2* genotypes (although it has not yet been demonstrated that this gene-environment correlation actually exists). In our sample, where white adoptive parents do not carry the *ALDH2*2* allele, differences in alcohol use associated with participants' *ALDH2* genotype should be exclusively a function of that genotype, and will not be confounded with parental *ALDH2* genotype. Similarly, although a large proportion of the elder siblings of genotyped target participants were themselves of Korean descent, they were not biological siblings to the target participants, so the environmental effect of elder sibling drinking was not confounded by a systematic relationship between target participant and elder sibling *ALDH2* genotypes.

For analyses involving potential moderation of the effect of *ALDH2* polymorphism by elder sibling alcohol use (gauged by the elder sibling combined alcohol problem index) and peer deviance (table 8), results were less consistent than those involving moderation by parental alcohol use. These inconsistencies may reflect limitations in the measurements available for elder sibling alcohol use and peer deviance. Notably, since not every participant had an older sibling, fewer observations were available for analyses assessing the effect of elder sibling drinking, reducing power to detect moderating effect upon the *ALDH2* polymorphism. Moreover, although alcohol-related peer influences have previously been shown to moderate the heritability of alcohol use (Dick et al., 2007; Agrawal et al., 2010), it is possible that measures of adopted familial alcohol use may exert a more readily apparent moderating influence

upon *ALDH2* genotype effect than peer deviance, because they are specific measures of environmental exposure to alcohol use, whereas the scale of deviant peer behavior that we used reflected the proportion of peers engaging in a wider variety of externalizing behaviors. Because this *ALDH2* polymorphism influences alcohol use via a known mechanism which specifically affects alcohol metabolism, *ALDH2* would not be expected to contribute to variation in the largely genetic latent factor which likely influences alcohol use and other externalizing behaviors in common (Krueger et al., 2002). Peer deviance in general, although highly correlated with adolescent alcohol use (Sher et al., 2005), may be too indirectly related to environmental alcohol exposure to moderate the influence of *ALDH2* genotype. SIBS study participants were residents of Minnesota, where alcohol use and binge drinking in adolescents and young adults are substantially above mean national levels (Substance Abuse and Mental Health Services Administration, 2010), so the omission of a sensitive and specific measure of peer alcohol use may be critical.

Genetic factors may influence the selection of peers, or may be otherwise correlated with peer identity or behavior. In fact, a longitudinal twin study found that genetic factors accounted for the entirety of the relationship between direct-report peer alcohol use and adolescent drinking (Hill, et al., 2008). However, in our sample, peer deviance did not vary by *ALDH2* genotype, suggesting that it is appropriate to consider its influence as a potential environmental moderator of *ALHD2* effect.

Meta-analyses of the association between alcohol dependence diagnosis and ALDH2 polymorphism suggest a rather large genetic effect (Luczak, et al., 2006;

Zintzaras, et al., 2006), although these meta-analyses largely comprise case-control studies in fully adult samples, unlike our own. In contrast, for the alcohol use outcomes we examined, the magnitude of the protection associated with possession of the ALDH2*2 allele ranged from small to moderate (table 3). There are multiple possible reasons why possession of the ALDH2*2 allele might afford only a small-to-moderate level of protection in our sample. First, as described above, the estimated effect of possession of the ALDH2*2 allele in this sample of adopted participants should be free from inflation due to confounding introduced by the environmental influence of a biological parent or parents who also possesses the allele. Further, the maximum *ALDH2*2* effect that we observed for each alcohol use phenotype occurred at the later ages included in the sample, so it is possible that the effect may continue to increase with age, beyond the ages at which our participants were assessed. Alternatively, though, rather than continuing to increase with age, the magnitude of the effect of *ALDH2* polymorphism may peak when alcohol use is typically highest – that is, during young adulthood (Sher et al., 2005). In fact, in this sample, the effect of *ALDH2*2* possession appears to level off or even diminish near the eldest observations. Although previous studies have noted increasing effect of *ALDH2* polymorphism across periods of one or more years during young adulthood (Doran, et al., 2007; Kim et al. , 2010), the development of the effect of *ALDH2*2* possession upon alcohol use between young adulthood and later adulthood has yet to be studied.

To conclude, in this longitudinal sample of adopted participants of Asian descent assessed in adolescence and young adulthood, we re-confirmed the observation

that possession of the *ALDH2*2* allele, which results in deficient *ALDH2* enzyme activity, was also associated with multiple measures of reduced alcohol use, and lower risk for alcohol-related psychopathology. We also observed that the reduction in alcohol use associated with *ALDH2*2* genotypes became more pronounced over the course of adolescence and into young adulthood – a period during which alcohol use also typically increases. Finally, we noted that parent and elder sibling alcohol use, as well as peer deviancy, may moderate the protective effect of *ALDH2*2* – although moderation by sibling alcohol use and peer deviancy was distinctly less certain than that by parent alcohol use, and none of the potential environmental moderators exhibited a strong and incontrovertible influence across all measures of target participant alcohol use.

While our observations support the notion that the effect of *ALDH2* polymorphism develops longitudinally and may be environmentally moderated, we did not determine the mechanisms by which these processes occur. This problem may be addressed in future research by including longitudinally-assessed measures of alcohol-related motives and cognitions, as well as more detailed measures of familial and social alcohol exposure, for example, household alcohol availability, and peer alcohol use and alcohol-oriented attitudes.

Chapter 3.

GABA System Genes – No Evidence for a Role in Alcohol Use and Abuse in a Community-Based Sample

3.1 Introduction

Twin and adoption studies indicate that genetic factors are likely to substantially influence alcohol-related behavioral phenotypes, including alcohol dependence (McGue, 1999), and quantitative measures of alcohol use (Heath & Martin, 1994). However, few individual common genetic variants have been consistently shown to have replicable effect upon alcohol use and dependence. One reason genetic association studies might fail to account for a substantial proportion of the genetic variance suggested by biometrical analyses is that the variants underlying variation in alcohol-related phenotypes are of such small individual effect that markers tagging them do not meet thresholds for significance after correction for multiple testing. Methods to investigate aggregated, whole-gene, and whole-system effects might be most fruitful in biologically-relevant systems that have already been repeatedly implicated in single marker and haplotype based research, such as γ -aminobutyric acid (GABA) system.

Many of alcohol's effects—subjective, soporific, anxiolytic, and motor-skill impairing, among others—are mediated by activity involving GABA, the neurotransmitter principally responsible for inhibitory neurotransmission in the central nervous system (Kumar, 2009; Barnard et al., 1998). In particular, ethanol's action is largely effected, both directly and indirectly, upon type A GABA ($GABA_A$) receptors—ligand-gated chloride channels that are the foremost form of GABA receptor—to mediate

many of its behavioral consequences**.** In mammals, $GABA_A$ receptors are mostly pentameres, with several combinations of nineteen known subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3) forming an ion pore (Barnard, et al., 1998)**.** The location and subunit composition of a $GABA_A$ receptor affects the nature and sensitivity of its response to ethanol exposure. In particular, the effects of low to moderate dose alcohol (levels of exposure typical of social drinking) are likely to be disproportionately mediated by tonic-acting extrasynaptic $GABA_A$ receptors containing a δ subunit, which are much more sensitive to alcohol than the more-common synaptic $GABA_A$ receptors that mediate the effects of most $GABA_A$ agonists and positive allosteric modulators, such as benzodiazepines (Olsen, Hanchar, Meera, & Wallner, 2007). Knockout, knockin, and other genetically modified murine models have shown that functional variation in GABAA receptor subunit genes can alter physiological and behavioral response to alcohol and other GABA-active drugs (Lobo & Harris, 2008). Alcohol administration also influences GABA-related activity and functioning by more indirect processes, for example by inducing presynaptic GABA release, elevating GABA-influencing neuroactive steroids, or altering the phosphorylation of $GABA_A$ receptors by protein kinase C (Kumar et al., 2009). Although the nature of ethanol's amplifying effects on GABAergic transmission is complex, and not yet fully understood (Enoch, 2008), ethanol's indirect GABAergic effects contribute substantially to the mediation of alcohol-related behavioral outcomes.

GABAergic activity is involved in mediating the effects of chronic alcohol exposure, and becomes altered with the development of alcohol tolerance and

dependence, and during withdrawal. Rats administered GABA agonists consume more alcohol, while those administered GABA antagonists consume less alcohol (Boyle, Segal, Smith, & Amit, 1993); but while acute alcohol exposure enhances GABA activity (Buck, 1996), $GABA_A$ receptors down-regulate with chronic exposure to ethanol, resulting in diminished efficacy of alcohol, GABA agonists, and GABA positive allosteric modulators (Grobin, Matthews, Devaud, & Morrow, 1998). Further, GABA agonists block the behavioral symptoms of alcohol withdrawal, while GABA antagonists exacerbate them (Koob, 2006). Chronic alcohol exposure also affects the expression and brain region localization of separate $GABA_A$ receptor subunits each differently, as well altering the subunit composition of the completed receptor (Enoch, 2008).

Type B GABA receptors ($GABA_B$), G-protein coupled receptors which regulate presynaptic GABA release, among other functions (Bettler, Kaupmann, Mosbacher, & Gassmann, 2004), are also involved in the biological effects of alcohol. Baclofen, a $GABA_B$ agonist, reduces craving for alcohol (Addolorato et al., 2002), and $GABA_B$ receptor mRNA expression is down-regulated in postmortem hippocampus of alcoholics and alcohol-preferring rats (Enoch et al., 2012).

$GABA_A$ receptor subunit genes lie in clusters on chromosomes 4p ($\gamma1$, $\alpha2$, $\alpha4$, $\beta1$), 5q ($\gamma2$, $\alpha1$, $\alpha6$, $\beta2$), 15 ($\beta3$, $\alpha5$, $\gamma3$), and X (ε, $\alpha3$, θ), as well as individually on chromosomes 1p (δ), 3q ($\rho3$), 5q (π, outside of the cluster), and 6q ($\rho1$, $\rho2$) (Enoch, 2008). Linkage and association studies have implicated variation in several $GABA_A$ subunit genes in a variety of behavioral phenotypes related to alcohol, including dependence diagnosis (Cui, Chamindi, Gu, & Li, 2012) and symptomatology (Lind et al.,

2008a), subjective intoxication and response (Lind et al., 2008b, Haughey et al., 2008) and electroencephalographic measures (Edenberg et al., 2004; Ghosh et al., 2003) among others. Among $GABA_A$ receptor subunit genes, markers and haplotypes in the a2 subunit gene GABRA2 have been the most frequently identified with variation in alcohol response and dependence (Cui et al., 2012) and phenotypes related to other psychoactive substances (Agrawal et al., 2006), as well as externalizing conduct (Dick et al., 2006). However, there have also been studies that were unable to confirm effects of GABRA2 polymorphisms on alcohol dependence (Drgon, D'Addario, & Uhl, 2006; Matthews, Hoffman, Zezza, Stiffler, & Hill, 2007; Onori et al., 2010).

The effects of variation in $GABA_B$ receptor genes GABBR1 and GABBR2 on alcohol use in humans have been comparatively infrequently appraised, although one study observing a non-significant trend for association with an allele in GABBR1 allowed the possibility that variation in GABAB genes may influence alcohol dependence (Sander et al., 1999). Furthermore, a number of other genes involved in GABAergic transmission but not coding for GABA receptors have also been shown to be associated with alcohol-related outcomes. For example, SNPs in one of the gene isoforms for the glutamate decarboxylase enzyme (GAD1), which is involved in GABA synthesis, have been associated with initial sensitivity to alcohol and age-of-onset of alcohol dependence (Kuo et al., 2009).

We used multiple methods to examine the influence of markers in GABA system genes on measures of alcohol use and alcohol abuse and dependence symptomatology. In addition to $GABA_A$ receptor subunit genes, markers in a number of which have been

previously associated with alcohol-related phenotypes, we also considered markers in and near genes involved in the synthesis, release, transport, and metabolism of GABA, as well as other activity related to GABA or GABA receptors.

First, because individual variants conveying risk for elevated alcohol use may be of such minute effect that markers in linkage with risk alleles may fail to exceed thresholds for significance in single-SNP analyses, we calculated a polygenic score reflecting variation in alcohol use phenotypes attributable to the combined set of linkage disequilibrium (LD) pruned GABA system SNPs, at several significance thresholds. Next, we derived an estimate of the phenotypic variance explained by the GABA SNPs in this set from a SNP-based estimate of genetic similarity between pairs of participants who are not close relatives. Finally, we examined the effect of individual GABA system genes using a gene-based test.

3.2 Materials and Methods

3.2.1 Sample

Participants were drawn from two studies at the Minnesota Center for Twin and Family Research (MCTFR; Iacono, McGue, & Krueger, 2006): the Minnesota Twin Family Study (MTFS; Iacono, Carlson, Taylor, Elkins, & McGue, 1999) comprising twins and their families, and the Sibling Interaction and Behavior Study (SIBS) (McGue et al., 2007), which includes adopted and biological sibling pairs and their families. Both studies are population based and longitudinal, with an initial assessment when twins and siblings are in adolescence, and follow-up assessments every three or four years thereafter. For this study, both offspring (twins and siblings) and their parents were

included in analyses. Parent data were collected at their family's first visit to the MCTFR, while for non-parental participants, data were taken from the nearest available assessment to age 17. Only white MCTFR participants were included in the sample, as determined by clustering in principal components calculated using EIGENSTRAT (Miller, et al., 2012). In all, genotypic and phenotypic data were available for 7224 participants (Table 1.).

3.2.2 Genotyping

GABA system SNPs used in this study were drawn from genome-wide genotyping using the Illumina 660w Quad array, which in the MCTFR sample yielded a total of 527,829 viable SNP markers after quality control filtering. Quality control procedures for SNP markers and DNA samples and have been previously described in detail (Miller et al., 2012). Briefly, the most common reasons for excluding markers were minor allele frequency less than 1%, more than two cross-family Mendelian inconsistencies, a call rate below 99%, and a significant deviation from Hardy-Weinberg equilibrium. For SNPs that remained in the analyses, missing genotypes were replaced with the mean genotypic value for each SNP. The most common reason for excluding DNA samples from analyses was genotype call failure for more than 5000 SNPs.

GABA system genes were selected based on their inclusion in any of three sources: a panel constructed to include candidate genes for addiction-related phenotypes (Hodgkinson et al., 2008), an expert-curated list of addiction-pertinent genes (Saccone et al., 2009), and a database devoted to organizing genes by biological system pathways (Kanehisa, 1996). Genes were selected if any of these sources listed them as being involved in GABA-related activity; in this way 36 genes were selected. We examined

markers within 5kb upstream (5' direction) and 1kb downstream (3' direction) of each gene. For two small genes, GABRD (chr. 1) and GABARAP (chr. 17), no markers within this region were available, so these genes were excluded from subsequent analyses. Because $GABA_A$ subunit genes in the chr. 15q cluster, GABRA5, GABRB3, and GABRG3 lie on an imprinted chromosomal region, in which only paternally-transmitted copies of the genes are expressed (Meguro et al., 1997), markers on these genes would be inappropriate to assess using methods that do not account for the identity of the parent from whom each allele was transmitted, and were therefore excluded from analyses. In all, 737 SNP markers in or near 31 genes (including 17 GABA receptor subunit genes) were retained (Table 2.).

3.2.3 Phenotypic Measures

We examined two measures related to alcohol use and related psychopathology. First, we computed an index of drinking behaviors using four items drawn from a customized form of the Substance Abuse Module (SAM; Robins, et al., 1987), an expansion to the World Health Organization's Composite International Diagnostic Interview (Robins et al., 1988). These were 1) frequency of alcohol use over the prior 12 months, 2) average number of drinks consumed per alcohol use occasion over the prior 12 months, 3) lifetime maximum number of alcoholic drinks ever consumed in a 24 hour period, and 4) lifetime number of times ever having been intoxicated. We calculated the drinking behavior index by standardizing these four items and combining them with equal weighting. Our second measure was a count of DSM-IIIR alcohol abuse and dependence symptoms. DSM-IIIR described the most current criteria at the time that

assessments were conducted. Both the drinking behavior index and the alcohol abuse and dependence symptom count were log transformed for analysis.

3.2.4 Statistical Analyses

3.2.4.1 Single SNP Analyses

Single SNP analyses were performed using a method incorporating a rapid feasible generalized least squares (RFGLS) model (Li, Basu, Miller, Iacono, & McGue, 2011), which accounts for correlations among family members attributable to both genetic relatedness and shared environmental effects. SNPs were modeled under assumption of additive effect, entered as number of minor alleles (0, 1, 2). For markers on chromosome X, genotypes for male participants who possessed one minor allele were set to two minor alleles for analysis. To account for genetic ancestry, the first 10 principal components from an EIGENSTRAT analysis were included as covariates (Price et al., 2006). Other covariates included in single SNP analyses were sex, age, birth year, generation (an indicator of whether an individual was a parent or child), a generation-by-age interaction, a generation-by-sex interaction, and a generation-by-birth-year interaction. We calculated the effective number of independent tests, accounting for LD patterns between the included markers in our sample, using the SimpleM method (Gao, Starmer, & Martin, 2008), which yielded an LD-inferred total of 485 effective independent tests, and therefore a Bonferroni-corrected significance threshold of .05/485 = .0001.

3.2.4.2 GABA System Polygenic Scoring

Genetic liability to alcohol dependence is likely to be substantially attributable to many variants, each contributing in only a small amount to the overall genetic risk. When many markers are examined separately for association with a complex trait, genuine genetic effects reflected by individual markers may be too small to overcome significance thresholds that account for multiple testing. However, the aggregated effects of multiple individually insignificant SNP markers combined into a single polygenic score may be associated with phenotypic variation (International Schizophrenia Consortium, 2009). A similar approach has been used to calculate a score from multiple SNPs in dopamine system genes, which accounted for a small but significant percentage of variance in cocaine dependence symptomatology (Derringer et al., 2012).

When calculating a polygenic score from markers in GABA system genes, each SNP was permitted to contribute to the score only if its individual effect was such that the p-value associated with the marker was below a particular cutoff. Scores were calculated at ten incrementally increasing p-value cutoffs ranging from $p < 0.1$ to $p <= 1.0$ (that is, at the final threshold, all SNPs were permitted to contribute to the polygenic score). At each p-value cutoff threshold, allowing more markers of smaller individual effect to contribute to the score potentially resulted in an increase in the number of markers reflecting minute but genuine genotypic influences, but also permitted the inclusion of more markers that had p-values below the cutoff merely due to chance.

We calculated polygenic scores for each individual by summing the product of the genotypes (the number of minor alleles) and the effects (beta weights) from single SNP analyses for all SNPs that were to be included in the score. However, because markers

within the same gene, or in proximal genes, may be in LD with each other, in order to ensure that markers contributing to the polygenic score reflected unique association signals, it was necessary to prune the results of single SNP analyses based on LD structure before calculating the GABA system polygenic score. We identified SNP pairs that were in substantial LD with each other, $r^2 > 0.5$, when only the founders of each family were considered (2079 males, 2449 females). At each p-value threshold, for every pair of SNPs with LD $r^2 > 0.5$ in which both SNPs in the pair were below the current p-value threshold, the effect of the SNP with the higher p-value was set to zero, so that the SNP did not contribute to the polygenic score. As a result, all SNPs that remained in the polygenic score after pruning were in low mutual LD ($r^2 < 0.5$).

We used 10-fold cross-validation to account for overfitting. That is, for each of 10 iterations, polygenic scores were first derived from LD-pruned estimates of single SNP effects in a training sub-sample comprising 90% of the overall sample. Training sample estimates were then used to predict the phenotypes of individuals in a separate testing sub-sample comprising the remaining 10% of the overall sample. Then, at each p-value threshold, polygenic score-based predictions were compared to the actual observed phenotypic values of individuals in the testing sub-sample using the coefficient of determination, averaged across all 10 iterations of the cross-validation procedure.

3.2.4.3 SNP-Based Genetic Relationship Variance Estimates

We employed an estimate of phenotypic variance in alcohol-related phenotypes attributable to a given set of SNPs (GCTA; Yang, Lee, Goddard, & Visscher, 2011). For both the set of GABA system SNPs, and the entire set of available genome-wide SNP

markers, separately, a SNP-based measure of genetic relatedness between each pair of individuals included in the analysis was computed. For each analysis, the matrix of the genetic relatedness estimates for all pairs of individuals was then included as random effects in a linear mixed model (along with the covariates as fixed effects), using restricted maximum likelihood estimation (REML), in order to estimate the proportion of phenotypic variance attributable to the SNPs used to compute inter-individual genetic relatedness. In order to derive an estimate of variance attributable solely to the SNPs included in the analysis, unburdened by the shared environment or other sources of phenotypic variance, we removed all but one biologically related member from each family. In order to maximize sample size, therefore, in families with data available for both parents, parents (being unrelated to each other) were retained preferentially to offspring. For both the set of GABA system SNPs and the full genome-wide set of SNPs, analyses were conducted separately for autosomal markers and markers on the X chromosome. This approach, based on a calculation of genetic relatedness from the simultaneous consideration of all of the SNPs in a particular set, does not suffer from the inaccuracy of prediction that affects polygenic scores due to error on the estimates of the effects of the individual SNPs that contribute to the score (Visscher, Yang, & Goddard, 2010).

3.2.4.4 Gene-Based Testing

Finally, we assessed the effect of individual GABA system genes using a gene-based test (VEGAS; Liu et al., 2010), which combines the test statistics from single-SNP analyses of all markers within a particular gene, then compares the resulting gene-based

test statistic to a large number of simulated multivariate normal-distributed gene-based test statistics, which are produced taking into account Hapmap (CEU) LD structure and gene length, and approximate the observed gene-based test statistic under the null hypothesis. The p-value resulting from this gene-based test is thus the proportion of simulated test statistics that exceed the observed test statistic. This form of analysis can reveal whether there are disproportionately many markers with low p-values in a given gene. We also performed a variation on the gene-based test which compares the top-ranked marker in each gene to the maximum element of the gene-based test statistic (the "Top-SNP" method).

3.3 Results

The p-value ranked top 10 results from the analyses of the 737 single SNP marker associations with both alcohol use phenotypes are displayed on Table 3. No single marker for either phenotype reached the LD-adjusted significance threshold of .0001, which corrects for the multiple testing.

The mean cross-validated correlations between polygenic score-predicted phenotypic values and observed phenotypic values at each of 10 p-value thresholds are shown in Table 4. The cross-validated squared correlation was uniformly small (in no case even approaching 1%) and not significant at any threshold for either phenotype.

Genetic correlation-based estimates of explained phenotypic variance (h^2_{SNP}) attributable to GABA system SNPs and all available SNPs, separately for autosomal and chromosome X markers, are shown in Table 5. Only the variance in the drinking index attributable to the full set of all available autosomal SNPs was significant, using a

likelihood test (LRT) of the null hypothesis that for each group of SNPs, $h^2_{SNP} = 0$. Neither autosomal nor chromosome X GABA system SNPs accounted for a significant proportion of the variance in either alcohol related phenotype.

The p-value ranked top 10 results of gene-based tests for both phenotypes are displayed in Table 6, and the top 10 results from the "Top-SNP" variation on the gene-based test are shown in Table 7. For neither phenotype did any gene or top-ranked SNP per gene meet the Bonferroni-corrected significance threshold of .0016.

3.4 Discussion

$GABA_A$ receptors are involved in mediating both the acute and chronic effects of alcohol (Kumar et al., 2009), and markers in GABA system genes have been associated in a number of studies with alcohol dependence and other alcohol-related phenotypes (Cui et al., 2012). We used multiple methods to interrogate the potential relationship between alcohol use and abuse phenotypes and variation in GABA system markers, either taken individually or aggregated using different methods, but no association was evident in any of them.

In analyses of individual SNPs, none approached the thresholds for significance determined by an appropriately stringent correction for multiple testing for either the drinking index or the count of alcohol abuse and dependence symptoms. It is perhaps worth noting that, in the drinking index, the three markers that most closely approach significance are all in the GABRA6 gene. In rat models, a functional GABRA6 variant modulates alcohol-evoked GABAergic neurotransmission (Senna et al., 2003), and α_6 subunit expression changes in response to chronic alcohol administration (Senna et al.,

2004), although behavioral measures of alcohol response, tolerance, and withdrawal were all unaffected by GABRA6 knockout (Homanics et al., 1998; Homanics et al., 1997). GABRA6 polymorphisms have also been previously associated with alcohol dependence in humans (Loh et al., 1999).

A polygenic score, which selectively retained and aggregated the GABA system markers of highest potential effect, was likewise unable to account for variation in either alcohol use phenotype. Since any true effects associated with the markers contributing to the polygenic score would be very small, it is possible that error on each of the individual estimates of single SNP effects result in the score failing to account for phenotypic variance (Visscher et al., 2010).

Similarly, estimates based on pairwise relationships between individuals, derived from all of the GABA system markers in the autosome and on the X chromosome, did not explain any of the variance in alcohol use or symptomatology. The proportion of variance in the drinking index (but not in alcohol dependence and abuse symptomatology) explained by all available autosomal SNPs (but not all available chromosome X SNPs) is appreciable, though less than most similar pedigree-based estimates (Slutske et al., 1999; Grant et al., 2009). This is likely because pairwise genetic correlations computed using this method only reflect the common variants that are tagged by the available genotyped SNPs, therefore a denser genotyping array including a greater number of markers might account for a larger proportion of the variance in alcohol use behaviors truly attributable to genetic factors across the entire autosome (Yang et al., 2011).

Gene-based tests did not indicate that SNPs with low p-values were significantly over-represented in any GABA system gene. As was apparent from the results of single marker analyses, SNPs with nominally low p-values for association with the drinking index were slightly over-represented in the GABRA6 gene; however, although GABRA6 was top-ranked among GABA system gene-based tests for the drinking index, it did not reach the threshold for significance after correction for multiple tests.

Many previous studies indicating a role for GABA system genetic variation in alcohol dependence were conducted using as cases individuals drawn from treatment programs for alcohol dependence, or other clinical settings (eg. Lappalainen et al., 2005); Some, such as those involving the samples from the Collaborative Studies on Genetics of Alcoholism (COGA) (Rice, et al., 2003), included participants belonging to families of probands with multiple alcohol-dependent first-degree relatives (Edenberg et al., 2004; Dick et al., 2004; Agrawal et al., 2006). Alcohol-dependent cases with severe phenotypes and family history of alcohol dependence may have an elevated genetic loading for the disorder, making the detection of genetic effects more likely. In one clinically-derived sample, associations between markers in GABRA2 and alcohol dependence increased when analyses were restricted to include as cases only individuals with indicators of severe or persistent alcohol dependence, or a family history of alcohol dependence (Fehr et al., 2006). Other studies suggest that GABRA2-related alcohol dependence vulnerability is limited to individuals with comorbid dependence on illicit drugs (Agrawal et al., 2006), or that GABRA2 markers are related to polysubstance abuse, but not alcohol dependence alone (Drgon et al., 2006). In contrast, the MTFS and SIBS

samples are community-based, reflecting psychopathology at rates and levels representative of the general population. However, there have been instances of GABRA2 SNPs being associated with alcohol dependence in samples drawn from the general population (eg. Covault, Gelernter, Hesselbrock, Nellissery & Kranzler, 2004)

Although GABAergic activity is clearly involved in the mediation of alcohol's effects, both acute and chronic, the exact mechanisms by which GABA system gene polymorphism might lead to variation in alcohol use behaviors are unknown. We therefore retained in our sample all white participants for whom genetic and phenotypic data were available, including a number of participants who had never used alcohol (1.6% of parents, 29.2% of offspring). If, for example, GABA system gene variants contribute to latent risk for substance use and externalizing behavior in general—as the broad range of behavioral phenotypes that have been associated with polymorphisms and haplotypes in GABRA2 and other $GABA_A$ receptor subunit genes might imply—then retaining these alcohol naïve individuals was appropriate. However, it may be reasonable to believe that any effects on alcohol use phenotypes attributable to GABA system genes are due specifically and solely to changes in the pharmacological response to the presence of ethanol by GABA system structures and functions. If that is the case, it may be less likely that GABA gene variation would be related to the initiation of alcohol use, and therefore participants who had never tried alcohol should be excluded from analyses. However, analyses conducted including only those participants who had ever tried alcohol (not shown) resulted in identical conclusions to the analyses conducted using the full sample.

The GABA system gene by far most frequently implicated in alcohol-related behaviors and other substance-related and externalizing behaviors is the $GABA_A$ receptor alpha-2 subunit gene GABRA2. The SNP rs279858, which lies in exon 5 of the gene but is a synonymous substitution (Covault et al., 2004), has been associated, either individually or as a member of multi-SNP haplotypes, with alcohol dependence and other alcohol related phenotypes such as alcohol sensitivity more often than any other marker in GABRA2 (Cui, Seneviratne, Gu, & Li, 2012). Although the genome-wide array upon which markers were genotyped for our study did not include rs279858, it did include markers in the same region of the gene (rs1808851, rs279856), which were in perfect LD with rs279858 in a HapMap reference panel of European descent (CEU) (Johnson et al., 2008), but neither of which were associated at even a nominal level with either the drinking index or alcohol abuse and dependence symptom count. It is possible, however, that the perfect LD previously observed in reference samples is not reflected in our sample, and that any variants with genuine phenotypic influence that might be tagged by rs279858 in previous studies are not likewise captured by GABRA2 variants that we genotyped. Synonymous SNPs can also affect protein functioning and expression via a number of different mechanisms (Hunt, Sauna, Ambudkar, Gottesman, and Kimchi-Sarfaty, 2009), so genotyping the exact SNP associated with alcohol-related phenotypes in previous studies may be critical.

Twin studies generally indicate that genetic influences on alcohol use tend to increase through adolescence and into early adulthood (Dick, 2011). The influence of polymorphisms in the GABA system may also change over time or across developmental

stages. In one study, a GABRA2 risk genotype was not associated with alcohol dependence in children, but was associated in an adult sample (Dick et al, 2006). Similarly, GABRA2 markers have been associated with membership in persistently elevated, rather than developmentally limited, developmental trajectories of externalizing behavior (Dick et al., 2009). We included both young adults and participants in middle adulthood in our analysis, but it is possible that GABA system gene-imparted variation in risk for alcohol use varies over time, and that not accounting for the potential for longitudinal genetic change masks the effect reflected by SNPs in GABA system genes.

Markers in GABA system genes have also been associated with less-complex biological markers such as Beta-frequency EEG (Edenberg et al., 2004) and event-related potentials (Winterer et al., 2000) that meet the criteria to be considered endophenotypes (Gottesman & Gould, 2003; Begleiter & Porjesz, 2003), more directly reflecting underlying genetic liability than their complex behavioral correlates. So many intermediary processes might separate alcohol abuse and dependence symptomatology, and even indexed quantity and frequency of alcohol consumption, from the genetic variants that contribute to their variation, that the causal distance might obscure true genetic effects. Association studies incorporating markers in GABA system genes may instead do better to continue to consider electrophysiological measures and other endophenotypes for complex alcohol-use behaviors instead.

Our analyses did not account for the possibility that genetic influences on alcohol use and related symptomatology may vary as a function of environmental factors, although there is evidence suggesting that the effect of GABA system genes on alcohol-

related phenotypes may be moderated by environmental factors. For example, a marker in GABRA2 was associated with alcohol dependence in married but not unmarried individuals (Dick et al., 2009). Though not specific to alcohol, GABRA2 polymorphisms also interacted with exposure to childhood trauma to influence substance addiction vulnerability (Enoch et al., 2010). If vulnerability to alcohol use attributable to variants in GABA system genes is dependent upon environmental context, then they will remain unobserved when using approaches that factor in only genetic main effects.

We did not include markers in the chromosome 15 $GABA_A$ receptor subunit gene cluster (GABRB3, GABRG3, and GABRA5) in our analyses because they belong to an imprinted region, in which only paternally-transmitted genes are expressed (Meguro et al., 1997). Studies using tests capable of differentiating between the parent-of-origin of alleles at each marker have found association between alleles at markers in all three chr. 15 cluster genes and alcohol dependence (Song et al., 2003; Dick et al., 2004), although the detected associations were not always exclusive to paternally-transmitted alleles. As variation in the chromosome 15 $GABA_A$ receptor subunit gene cluster may be related to risk for alcohol dependence, future studies should examine markers in these genes using tests informative as to allelic parent of origin.

We did not conduct tests for interaction among markers within and between GABA system genes, although research indicates that such interactions occur. Members of the same GABA receptor subunit clusters might be particularly likely to interact with each other; for example in GABRA6 knockout mice, expression of cluster-mates GABRA1 and GABRB2 was also reduced, even in brain regions where GABRA6 was

never expressed (Uusi-Oukari et al., 2000). Interactions may also occur between non-proximal GABA system genes. Linkage analysis of Beta 2 EEG waves identified epistatic interaction between significant linkage peaks on chromosomes 1, 4, and 15, all in regions containing GABAA receptor subunit genes, and only when alcohol dependence was not regressed out (Ghosh et al., 2003). Interactions might also occur at the level of the completed protein, if genetically-driven variations in multiple separate receptor subunits affect, in combination, a unique change in the function of the final receptor assemblage. Incorporating biological information with a view to greater nuance might help guide future hypotheses regarding GABA system candidates. For example, variants in genes coding for $GABA_A$ receptor subunits that are incorporated into alcohol sensitive extrasynaptic receptors (i.e. α4, α6, β3, and δ subunits; Meera et al., 2010) might in particular be of continued interest.

To conclude, we sought to determine whether polymorphisms in GABA system genes, including both $GABA_A$ receptor subunit genes and other genes involved in GABAergic structure or function, were related to variation in an index of quantity and frequency of alcohol use, or a measure of alcohol abuse and dependence symptomatology. Using multiple methods, we assessed the effect of GABA system gene markers individually, in aggregate, as they determined the strength of a correlation-based genetic relationship, and in a gene-based test. In no case were GABA system SNPs consistently related to alcohol use nor the symptomatology of alcohol-related psychopathology. Continued study is necessary to determine how GABA system variants might influence alcohol-related phenotypes.

Chapter 4.

Tests of the Effects of Adolescent Early Alcohol Exposures on Adult Outcomes

4.1 Introduction

Measures of early alcohol use have frequently been associated with adult psychopathology, maladaptive behaviors, and negative outcomes. A younger age at first use of alcohol is related to higher prevalence of alcohol abuse and dependence through young adulthood (Grant & Dawson, 1997; Dewit, Adlaf, Offord, & Ogborne, 2000), and early alcohol use also predicts adult use and abuse of other psychoactive substances, as well as antisocial behaviors (Ellickson, Tucker, & Klein, 2003; Flory, Lynam, Milich, Leukefeld, & Clayton, 2004). The perception that early alcohol exposure contributes to later alcohol problems and other negative outcomes has fueled policy and community-based efforts to prevent adolescent alcohol use (Hawkins, Catalano, & Arthur, 2002). Another possibility, however, is that there exist causal influences common to both early alcohol exposure and adult problems, which confound the relationship between the two and account for their association.

In particular, analyses in twins have suggested that the association between age at first drink and the later development of alcohol use disorders is strongly influenced by an underlying genetic liability (Prescott & Kendler, 1999; Sartor et al., 2009), which, further, likely evinces a more general genetically mediated relationship between problem behavior in early adolescence and a variety of forms of adult psychopathology (McGue, Iacono, & Krueger, 2006). Still, even if genetic influences account for the majority of the

covariance between early exposures and later outcomes, there may also exist smaller residual but significant effects of early alcohol exposures.

Ethical and practical considerations make it impossible to conduct randomized experimental study of many potential psychosocial risk factors, including adolescent substance exposure. A number of methods have been proposed to bolster causal interpretation of the observed relationship between putatively causal exposures and outcomes in non-experimental studies (Morgan & Winship, 2007). An increasingly popular approach involves adjusting for factors that may confound the relationship between an exposure and the outcomes that potentially arise from it by making use of the propensity score, which is the probability of receiving the exposure, conditional on a set of observed, potentially confounding, variables. Such variables may include any available measurement with the potential to influence both the exposure and the outcome, such as sex, or a family history of psychiatric disorder, among many others. After adjustment with the propensity score, if theoretical assumptions hold (most notably the assumption that there exist no confounding variables apart from those included in the propensity score model) the relationship between an exposure and later outcomes associated with it may have a causal interpretation (Rosenbaum & Rubin, 1983). One study, which used propensity scores estimated from a variety of personal and familial background covariates to match participants who had used alcohol or illicit drugs before age 15 with those who had not, found that after propensity score matching, early adolescent substance use retained its associations with several negative adult outcomes, including substance dependence, number of criminal convictions and others (Odgers et al., 2008).

Propensity score-based methods can adjust for bias attributable to measured covariates that are explicitly included in the propensity score model, but inference based on the use of propensity score methods relies on the key assumption that there exist no unmeasured confounding influences on the putative risk exposure and the outcome. It is not possible to use the propensity score to produce an unbiased estimate of treatment effect if there exist unmeasured confounding variables (Vanderweele, 2006). Yet, as described above, twin studies have shown that the association between early alcohol exposure and the development of adult alcohol use disorders, as well as a variety of other adult behaviors and outcomes, is likely subject to substantial genetic influence, which could confound estimates of the effects of early alcohol use if not taken into account.

Another approach to estimating the effects of specific environmental exposures, the cotwin control, or discordant-twin method, involves using the genetic similarity and shared rearing environment of twins discordant for exposure to an environmental risk factor to control for variables that might confound the association between the exposure and an outcome. Unlike methods that use propensity score adjustment, this method can be used to control for unknown genetic and shared environmental confounders, without having to measure or explicitly account for the potentially confounding variables. Although earlier twin pair analysis had suggested that the association between age at first drink and later development of alcohol dependence was non-causal, and likely attributable largely to common genetic effects (Prescott & Kendler, 1999), a more recent cotwin control study found that the relationship between early alcohol use and later alcohol dependence, as well as the use and abuse of other substances, may not be entirely

attributable to familial factors shared between twins, and were slightly but significantly influenced by non-shared environmental factors, consistent with, though not conclusively showing, an effect of early alcohol use mediated by the unique environment (Grant et al., 2006).

We sought to determine the extent to which the use of alcohol at an early age is directly implicated in the adult presentation of alcohol and other substance use and related problems, antisocial behavior, social maladjustment, and other negative life outcomes, rather than simply co-occurring as a result of common causal factors. In a community-based, prospective study of twins assessed through adolescence and into adulthood, we conducted both propensity score adjusted and cotwin control analyses. If there is a causal effect of early alcohol exposure on adult outcomes, we expected 1) that measures of early alcohol exposure would be associated with adult outcomes after propensity score adjustment, and 2) that in twin pairs discordant for measures of early alcohol exposure, the twins exposed to alcohol in early adolescence would, as adults, be more likely than their non-exposed cotwins to use and abuse alcohol, exhibit other externalizing behaviors, and experience negative outcomes. By comparing the results of the two analytic methods, we can use each to address the limitations of the other. Adjustment with the propensity score may help to identify imbalance in the distributions of measured variables that are not shared (or are incompletely shared) between discordant twins in cotwin control analyses, variables which could therefore confound the relationship between early alcohol exposures and adult outcomes. In turn, cotwin control

analysis may indicate whether the propensity score adjusted exposure effect estimate is biased by the existence of unmeasured shared genetic or environmental confounders.

4.2 Methods

4.2.1 Participants

The Minnesota Twin Family Study (MTFS) is a population-based, longitudinal study of twins born in Minnesota and their families (Iacono, McGue, & Krueger, 2006). Additional details regarding the MTFS sample, assessment procedures, and measurements have been previously published (Iacono, Carlson, Taylor, Elkins, & McGue, 1999). The current study includes same-sex twin pairs from an MTFS twin cohort first assessed at an approximate mean age of 12, then followed up with assessments at intervals of three or four years. This study uses measurements from the initial assessment (intake, IN; approximate age 12), the second assessment (follow-up one, FU1; approximate age 15), and the fifth assessment (follow-up four, FU4; approximate age 25). Additional sample characteristics are summarized on Table 1.

Twin zygosity was determined by parent report on a standard zygosity questionnaire, physical evaluation of twins by MTFS staff, and an algorithm including ponderal index, cephalic index, and fingerprint ridge count. Cases of uncertain zygosity were confirmed by genotyping using DNA samples extracted from blood or buccal swabs. Of the total sample, MZ twins numbered 972 (64%) and DZ twins numbered 540 (36%).

4.2.2 Measures

4.2.2.1 Background Covariate Measures for Propensity Score Estimation Model at Age 12

We took an inclusive approach to modeling the propensity score, recommended by some authors (Rubin & Thomas, 1996), whereby any variable thought to have the potential to confound the relationship between the exposure and the outcome is included in the propensity score model, because failure to include all confounders will bias the estimate of the effect of the exposure (Williamson, Morley, Lucas, & Carpenter, 2011). We included in the propensity score model a number of variables reflecting behavioral and demographic characteristics of the twin participants themselves, their parents, and their familial environment, with an emphasis on externalizing or disinhibited behavior. This inclusive approach runs the risk of including in the propensity score model covariates which may not actually confound the relationship between the exposure and an outcome. Including such variables will decrease the variance in estimates of the exposure effect if the variables are related to the outcome but not the exposure, and increase variance in estimates of the exposure effect if the variables are related to the exposure but not the outcome, but will in neither case increase the bias in the estimate (Brookhart et al., 2006).

The propensity score was derived from a model which included as predictors 29 variables measured at the initial intake assessment (Shown on Tables 2A and 2B). These reflect either individual twin measurements, which are unique to each twin in a pair, or parental and familial measurements, which hold the same value across both twins in a pair. All parental and familial variables were averaged across the reports of both parents,

except parental occupational status (Hollingshead, 1975), which was taken as the maximum of both parents' reports.

Full-scale IQ for parents was assessed using the Wechsler Adult Intelligence Scale – Revised (WAIS-R; Wechsler, 1981), and twin IQ was assessed with the Wechsler Intelligence Scale for Children – Revised (WISC-R; Wechsler, 1974).

Parental self-reported substance use was assessed with the Substance Abuse Module (SAM; Robins, Babor, & Cottler, 1987), an expansion to the Composite International Diagnostic Interview (CIDI; Robins et al., 1988). An index of parental alcohol use was calculated from SAM items reflecting past-year frequency of alcohol use, past-year amount of alcohol consumed per drinking session, past-year maximum number of alcoholic drinks consumed in a 24 hour period, and lifetime number of alcohol intoxications. SAM items were also used to calculate an index of past-year quantity and frequency of tobacco use, a binary indicator of whether the parent had ever used marijuana, a count of lifetime marijuana uses, and a sum of the number of distinct classes of psychoactive substances (other than alcohol and tobacco) that the parent had ever used.

Both parents reported on the history of occurrence of externalizing behavior disorders among their own biological parents and siblings. Relatives were counted as having experienced an externalizing behavior disorder if they had ever had seen or been treated by a medical or psychological professional for attention deficit hyperactivity disorder, oppositional defiant disorder, conduct disorder, abuse of or dependence upon alcohol or other substances, or other unspecified externalizing behavior disorder. From each parental report, familial history of externalizing behavior was indexed as the

proportion of the parent's biological family members who were reported to have been diagnosed or treated for at least one externalizing behavior disorder.

Parent and twin delinquent behavior was assessed with the Delinquent Behavior Inventory (DBI) (Gibson, 1967), which includes 36 items regarding delinquent or antisocial behaviors. Both parents completed self-ratings of their own delinquent behaviors, and one parent (nearly always the mother) rated twin delinquent behaviors. Twin delinquent behavior was taken as the mean of the twin's own report and his or her parent's report (Intraclass Correlation Coefficient (ICC) = 0.34).

Intrafamilial conflict was determined using a 12 item scale, which was completed by each twin and both parents. Each twin reported on conflict with each of his or her parents, separately. Each parent reported on his or her own conflict with each twin, as well as the other parent's conflict with each twin. An aggregate measure of parent-child conflict for each twin was obtained by first taking the mean of the twin's reported conflict with each parent, then taking the mean of each parent's report of self and other parent conflict with the twin, and finally taking the mean of the resulting three (twin, parent 1, and parent 2) reports (ICC = 0.45).

A structured clinical interview was conducted with each twin and parent, with parents reporting on both themselves and their twin offspring. Symptoms were recorded according to DSM-III-R (American Psychiatric Association, 1987) criteria, since it was the current version at the time of the assessment. Parent self-report of externalizing psychopathology included symptoms of conduct disorder and other antisocial behaviors, as well as symptoms of abuse or dependence upon alcohol or other psychoactive

substances. Symptoms of externalizing behavior in twins—attention deficit hyperactivity disorder, oppositional defiant disorder, and conduct disorder—were counted as present if either the twin or parent had reported the symptom.

Measures reflecting twin academic performance: GPA (ICC = 0.77), academic problems (ICC = 0.64), and academic motivation (ICC = 0.54), were taken as the mean of twin and parent report on equivalent items from an academic history questionnaire.

Adolescent physical maturity was assessed from twin self-report using a pubertal development scale (Petersen, Crockett, Richards, & Boxer, 1988). Because items were different on the male and female versions of the questionnaire, results were standardized in males and females separately before being included in analyses (Male $\sigma = 0.68$, Female $\sigma = 0.73$).

4.2.2.2 Early Adolescent Alcohol Exposure Measures at Age 15

Early alcohol exposure was indicated by two dichotomous measures: ever having had an alcoholic drink (without parental permission) by FU1, and ever having been intoxicated by FU1. Both items were based on twin self-report. Two FU1 sources, the SAM and the Diagnostic Interview for Children and Adolescents (DICA; Reich & Welner, 1988) contained equivalent items regarding alcohol use and intoxication; twins were counted as having had a drink or having ever been intoxicated if they responded in the affirmative to the relevant item on either instrument (Ever used alcohol $\kappa = 0.89$; Ever been intoxicated $\kappa = 0.80$). Exposure frequencies and frequencies of twin pair exposure discordance by zygosity are shown on Table 3.

4.2.2.3 Adult Outcome Measures at Age 25

Measures of adult twin alcohol and drug use, adult antisocial behavior, dependent stressful life events, family and interpersonal relationships, social engagement, and adult independence were taken at FU4. Indexes of alcohol and tobacco quantity and frequency of use were calculated using SAM items, the same as they were for parents as described above, and reflected alcohol and tobacco use over the four years preceding the assessment. The number of marijuana uses reported also reflected the preceding four years, but the reported number of drug classes ever used was a lifetime measure.

Twins underwent structured clinical interviews again at FU4, from which were drawn symptom counts of alcohol abuse and dependence, drug abuse and dependence, and adult antisocial behavior. Symptoms at FU4 were based on DSM-IV criteria (American Psychiatric Association, 2000), as it was the current version of the manual at the time of the assessment. Symptoms were reported for the three years preceding the FU4 assessment.

The dependent stressful life events measure consisted of yes-or-no items from the MTFS-developed Life Event Interview for Adolescents. 17 items were selected as being both undesirable, or stress-inducing, and dependent on the individual's own behavior, e.g. "Have you been in trouble with the police?". Dependent stressful life events from the four years preceding the assessment were reported.

Measurements of relationship quality and social functioning comprised three scales drawn from a social adjustment questionnaire, reflecting quality and closeness of relationships with other members of the twin's rearing family (12 items, $\alpha = 0.81$),

conflict and difficulty in interpersonal relationships (8 items, $\alpha = 0.70$), and quantity of friends and frequency of socialization (7 items, $\alpha = 0.74$).

Four dichotomous twin-reported items were summed to form an indicator of social and financial functioning as an independent adult: currently living apart from one's parents, currently receiving little or no financial assistance from one's parents, ever having been married or ever cohabited as if one was married, and currently being employed full time or attending a professional or graduate educational program full time ($\alpha = 0.52$).

4.2.3 Statistical Analyses

To address missing data, five datasets were imputed using the Amelia software package (Honaker, King, & Blackwell, 2009). All analyses were conducted separately in each of the five datasets, and results were combined following the method of Rubin (1987).

To ensure that variables assessed at intake were not influenced by extremely early alcohol use, participants who had experienced an alcohol exposure at a very young age, prior to the intake assessment (that is, before approximate age 12), were dropped from all subsequent analyses involving that exposure. Of the total sample, 2% (27 individuals) had used alcohol without their parents' permission by the intake assessment, and one individual reported having been intoxicated prior to the intake assessment. These cases were removed from subsequent analyses. Among those who remained, by the FU1 assessment, 36% had ever used alcohol without their parents' permission, and 15% had ever been intoxicated.

Positively skewed outcome variables were log-transformed before analyses. Log transformed outcomes included the tobacco use index, number of drug classes ever used, number of marijuana uses, alcohol abuse and dependence symptom count, drug abuse and dependence symptom count, and count of adult antisocial behavior symptoms. All outcomes were standardized to ease interpretation of the results.

4.2.3.1 Propensity Score Estimation, Weighting, and Exposure Effect Estimation

To estimate the propensity score, we used a logistic regression of each early alcohol exposure (ever used alcohol by FU1, or ever been intoxicated by FU1) on 29 variables reflecting intake assessment measurements of individual twins, their parents, and their familial environments (Tables 2A and 2B). Modeling the propensity score on covariates measured at an earlier time point than that at which the alcohol exposures were measured helps ensure that the covariates can not be affected by the exposures. The same covariates were included in the propensity score models used to predict both early alcohol use exposures. Covariates in the propensity score model with positive skew were log-transformed for analysis. Log-transformed variables included both parent and twin delinquent behaviors, both parent and twin externalizing disorder symptoms, parent alcohol abuse or dependence symptoms, parent drug abuse or dependence symptoms, parent tobacco use index, parent number of times used marijuana, parent number of drug classes ever used, and conflict between twin and parents. The estimated propensity scores for each individual, then, were that individual's model-fitted values from these logistic regression models—the predicted probability of having experienced each early alcohol use exposure, conditional on the model covariates.

One common method of adjustment using the propensity score is to weight each individual by the inverse of the probability of their receiving the exposure, as predicted by the propensity score model. That is, exposed individuals are weighted by 1/PS , while non-exposed individuals receive the weight 1/(1-PS) (Harder, Stuart, & Anthony, 2010). Weighting in this way produces a "pseudopopulation" in which the exposure is unconfounded by the measured variables included in the propensity score model (Robins, Hernan, & Brumback, 2000), which become balanced across those who have experienced the exposure and those who have not, so that in the weighted sample (again, assuming there are no unmeasured confounding variables), the association between the exposure and an outcome of interest can be considered the result of causal influence.

To estimate the effect of early alcohol exposure, the inverse probability of treatment weights were used as sample weights in regression analyses of adult behaviors and outcomes on early alcohol exposures. The models were fit using generalized estimating equations to obtain standard errors robust to twin-pair clustered observations.

4.2.3.2 Cotwin Control Application and Exposure Effect Estimation

The cotwin control approach makes use of data from twin pairs that are discordant for an environmental exposure of interest—that is, pairs in which one twin is exposed and the other is not. Members of MZ twin pairs share their entire genetic complement, while DZ twins share 50% of their segregating genetic material, on average. Both MZ and DZ twins may also be affected by environmental influences shared as a result of being reared in the same family. Therefore, members of twin pairs who are discordant for an environmental exposure can be compared to each other with respect to subsequent

outcomes potentially arising from the exposure, free from the confounding effects of shared genetic (100% for MZ twins, 50% for DZ twins) and shared environmental influences on the association between the exposure and the outcome.

We implemented a cotwin control analysis by decomposing the effects of exposure into between-pair, or family-level effects, which are represented in the model by the pair mean value for the exposure, and within-pair, or individual-level effects, which are represented by the difference between each individual twin's exposure value and the pair mean (Begg & Parides, 2003). We were primarily interested in the coefficient reflecting individual-level, within-pair effects—the association between the exposure and the outcome arising only from influences that are not shared between members of a twin pair, and consequently free from the potentially confounding effects of both shared environmental influences and either 50% (in DZ twin pairs) or 100% (in MZ twin pairs) of genetic influences. We again used generalized estimating equations to obtain standard errors robust to the pairwise intercorrelation of the observations. Because between-pair effects are included in the model, if there are no additional non-shared environmental confounders or other biases, then the within-pair effects are consistent with an interpretation as the causal effect of the exposure on the outcome (Frisell, Oberg, Kuja-Halkola, & Sjolander, 2012).

We conducted separate cotwin control analyses, first for all twins without consideration of zygosity, then with zygosity entered as a potential moderator of between- and within-pair effects. In analyses not including terms for moderation of treatment effects by zygosity, the shared influences reflected in the coefficients for

between-pair effects may arise from the twins' shared rearing environment, or from their common genetic background, but these two sources cannot be separated. However, when zygosity is allowed to moderate within-pair effects, differences in the within-pair estimates among discordant DZ twin pairs compared to within-pair estimates among discordant MZ twin pairs are likely to reflect the influence of the genetic factors which are not shared between the members of DZ twin pairs. Therefore, if genetic influences contribute to the differences between members of the same twin pair in the association between the exposure and the outcome, the within-pair coefficient for DZ twins will be expected to exceed that for MZ twins.

4.3 Results

4.3.1 Propensity Score and Inverse Probability of Treatment Weights

Tables 2A and 2B show the standardized difference between the exposed and non-exposed groups for early alcohol use (Table 2A) and early intoxication (Table 2B) across the background variables used to predict the propensity scores upon which the inverse probability of treatment weights were based. The standardized difference for each variable was calculated as the difference in means between the exposed and non-exposed groups, divided by the square root of the average of the variances. A small standardized difference suggests that the distribution of the variable is balanced across the exposed and non-exposed groups, and therefore unlikely to confound estimates of the effect of the exposure. A common criterion threshold for determining whether a variable is biased across exposed and non-exposed groups is whether the standardized difference exceeds 0.25 (Harder, et al., 2010).

In the full unweighted sample, of the 29 variables included in the propensity score model, 13 exceeded the standardized difference criterion threshold of 0.25 between those who had used alcohol by FU1 and those who had not, while 19 exceeded the criterion threshold between those who had been intoxicated by FU1 and those who had not. While in the unweighted sample, the mean of the absolute values of the standardized differences of the background variables between those who had ever had a drink by FU1 and those who had not was 0.23, the equivalent value in the weighted sample was reduced to 0.03. Bias was reduced in the weighted sample relative to the unweighted sample for every variable except for parent age, which was already balanced across the early drinking and non-early drinking groups in the unweighted sample. Likewise, the mean of the absolute values of the standardized differences comparing those who had ever been intoxicated by FU1 and those who had not decreased from 0.29 in the unweighted sample, to 0.07 in the weighted sample. Again, nearly every variable exhibited a reduction in bias as a result of weighting, except parent age and whether the participant had ever experienced the death of a close family member, both of which were already largely non-biased before weighting. Weights based on propensity score models for both early alcohol exposure measures therefore achieve balance across exposed and non-exposed individuals for all included covariates below the standardized difference criterion threshold of 0.25.

Tables 2A and 2B also show standardized differences for the subset of the variables included in the propensity score model that were individually varying—that is, variables which could take different values between the two members of a given twin pair—calculated between members of twin pairs discordant for early alcohol use and

early intoxication. Standardized differences are displayed separately for the entire set of exposure-discordant twin pairs without respect to zygosity, for MZ discordant twins, and for DZ discordant twins. All individual level variables are adequately balanced across discordant twin pairs, with the sole exception that the members of DZ twin pairs who had ever been intoxicated by FU1 reported experiencing a greater number of physical signs of puberty compared to their cotwins who had not been intoxicated by FU1.

4.3.2 Estimated Effects of Early Alcohol Exposures on Adult Outcomes

Tables 4A and 4B display means, standard deviations, and exposure effect sizes for adult outcomes among the exposed and non-exposed groups in the full original, unweighted sample. All measures of the use and abuse of alcohol and other substances, as well as symptoms of adult antisocial behavior and dependent stressful life events were substantially elevated among both those who had had a drink before FU1, and those who had been intoxicated before FU1. Measures of social functioning (family relationships, interpersonal problems, social engagement), and adult independence were not as distinct, varying only slightly or not at all between those exposed and those not exposed to alcohol in early adolescence.

Results of regression-based effect estimation are shown on Tables 5A and 5B. After weighting the sample with the inverse probability of treatment weights, the magnitude of the effects of both early alcohol use measures on substance related, antisocial, and dependent stressful life event FU4 outcomes were somewhat reduced, but largely still strongly significant. Effects of early alcohol exposure on adult independence

and interpersonal problems, which had been nominally significant in the unweighted sample, fell to insignificance in the weighted sample.

In cotwin control analyses among all discordant pairs without consideration of zygosity (Tables 5A and 5B), within-pair effects for both measures of early alcohol exposure were attenuated relative to exposure effects in both the full unweighted and weighted sample, but were still significant to at least the $P < 0.05$ level for alcohol and substance use variables, antisocial behavior, and dependent stressful life events, with the exception that early intoxication no longer significantly predicted symptoms of adult antisocial behavior.

Zygosity did not significantly moderate the within-pair effects of either early alcohol use or early intoxication on any of the FU4 adult outcomes; however, within-pair effects for several substance-related measures were significant among discordant DZ twins but not among discordant MZ twins, with a trend for the within-pair coefficient estimates to appear larger among discordant DZ pairs than among discordant MZ pairs. The exceptions to this pattern (as where the effect of early alcohol use on the FU4 alcohol quantity and frequency index and dependent stressful life events count are nominally significant among discordant MZ but not DZ twin pairs) are likely due to lower standard errors in MZ twin estimates due to there being a greater number of MZ twin pairs than DZ pairs in our sample. Only for the nominally significant effect of early intoxication on dependent stressful life events does the coefficient for discordant MZ twins appear larger than its nonsignificant counterpart among discordant DZ twins.

4.4 Discussion

We studied the effects of alcohol use and intoxication before age 15 on adult outcomes related to alcohol, other substances, antisocial behavior, dependent stressful life events, social functioning, and independent adult functioning, measured at age 25. We used two distinct approaches—propensity score based weighting and the cotwin control, or discordant-twin design—to adjust for potentially confounding influences on the association between early alcohol exposures and adult outcomes, in order to bolster an interpretation of the association as being consistent with a causal effect of early alcohol exposures on the adult outcomes.

In keeping with earlier research (Grant & Dawson, 1997; Dewit, Adlaf, Offord, & Ogborne, 2000; Ellickson, Tucker, & Klein, 2003; Flory, Lynam, Milich, Leukefeld, & Clayton, 2004), in both unweighted and weighted analyses using the full sample, early adolescent exposures to alcohol were associated with greater use and abuse of both alcohol and other substances in adulthood, as well as antisocial symptomatology. Early alcohol exposures also suggested elevated risk for stressful life events dependent on the individual's behavior. There was little evidence for impairment in measures of social functioning among those exposed to alcohol in early adolescence. Although both measures of early exposure were actually related to a slight but nominally significant increase in functioning as an independent adult in the unweighted sample, this effect disappeared in the weighted sample, suggesting that the apparent effect had been merely an artifact introduced by confounding.

Many of the background variables that were used to estimate the propensity scores, and which reflected behavioral and demographic features of the twins, their

parents, and their familial environment, were substantially unbalanced between those who used alcohol or had been intoxicated in early adolescence and those who had not, and thus had the potential to bias estimates of exposure effect. We showed that application of weights based on the propensity score, which is the estimated probability of exposure conditional on the background variables, successfully balanced the covariates, thereby allowing a causal interpretation of the subsequently estimated effects of early alcohol exposures, assuming that there no were no unmeasured sources of confounding.

Cotwin control analyses showed, however, that confounding factors other than those included in the propensity score model were in fact likely to exist. Within-pair effects estimated among twin pairs discordant for early alcohol exposures reflect only the influence of factors that are not shared between members of each twin pair, and thus provide an estimate free from potential confounders in the shared environment, and genetic influences, depending on the zygosity of the pair (100% for MZ pairs, 50% on average for DZ pairs). That cotwin control estimates in the entire set of discordant pairs (ignoring zygosity) were substantially attenuated relative to the estimates from the weighted sample indicates the presence of unmeasured confounders that were not included in the propensity score model.

In the cotwin control design, discordant MZ twin pairs provide the clearest estimate of the effect of non-shared factors between exposed and non-exposed twins. In our sample, estimates from MZ and DZ twins did not differ significantly from each other. However, the trend for several within-pair estimates of adult alcohol and drug-related

outcomes to be higher among discordant DZ pairs than among discordant MZ pairs suggests that the association between adolescent alcohol exposures and those adult outcomes among exposure-discordant DZ twin pairs is partially attributable to confounding by genetic factors. This would be in line with previous studies that have indicated that genetic factors substantially influence the relationship between early adolescent externalizing problems (including early alcohol use) and the later development of adult externalizing behaviors in general, including the use and abuse of alcohol and other substances as well as other disinhibited behaviors (McGue et al., 2006).

One of the primary limitations of the cotwin control method is its inability to distinguish true causal effects from other confounding influences that are not shared between exposure-discordant twins. In the present study, nearly all individually-varying predictors of early alcohol use and intoxication included in the propensity score model were also adequately balanced across the exposed and non-exposed members of discordant twin pairs. However, in other contexts, discordant twins may systematically differ on variables antedating the exposure or treatment for which they are discordant, so that these pre-exposure variables might confound the association between an exposure and outcomes potentially influenced by it (Stanek, Iacono, & McGue, 2011). In such cases, when the application of cotwin control methodology can be shown to have not achieved adequate balance across measured variables, thereby leaving open the possibility of confounding by those variables of the effect of the exposure on the outcome, adjustment with the propensity score might be used to achieve balance. Conversely, as we've described, comparison of effects estimated in discordant twin

designs to those estimated in propensity score adjusted models may act as a form of sensitivity analysis against the latter's assumption that variables that were not observed or not included in the propensity score model do not confound the relationship between the exposure and associated outcomes. An extra-rigorous form of simultaneous adjustment for both individually-varying measured variables (via propensity score application) and non-measured shared environmental and genetic variables (via cotwin control) has already been successfully applied (Huibregtse, Bornovalova, Hicks, McGue, & Iacono, 2011).

These methods do not speak to the mechanism by which early alcohol exposures, once experienced, might effect their impact upon later behaviors and outcomes. Possible vectors of the effects of early alcohol exposure include increased social affiliation with substance promoting or otherwise deviant peers, altered neurological sensitivity to the pharmacological effects of alcohol, leading to changes in substance-oriented behaviors (such as preference, or seeking), or ethanol-induced neurocognitive impairment fostering behavioral disinhibition in general (McGue et al., 2006; Sher et al., 2010), among others.

Causal effects of early alcohol exposure are also not necessarily distinct from genetic influences. Results from a biometrical twin study (Agrawal et al., 2009) indicate that the relationship between early age at first drink and later alcohol dependence symptomatology is partially attributable to a gene-by-environment interaction, whereby early age at first drink potentiates genetic influences on the symptoms of alcohol dependence, increasing their apparent heritability, even after accounting for the common

genetic influences affecting both age at first drink and alcohol dependence symptomatology.

Our propensity score-based and cotwin control analyses point to a similar conclusion: that there may be a causal effect of early alcohol use and intoxication leading to increased adult alcohol and drug related outcomes, antisocial behavior, and dependent stressful life events, so long as there does not exist confounding due to unmeasured variables (in the case of propensity score-based analyses) or non-shared genetic and environmental factors other than early alcohol exposure (in the case of cotwin control analyses). However, we observed no reliable evidence for effects of early alcohol exposures on measures of adult social functioning or adult independence. By comparison with estimates from cotwin control analyses, we found that propensity score adjustment was biased by unmeasured confounders, even though the propensity scores were based on a rich set of exposure-pertinent background covariates. Additional research is required to determine whether these apparent causal effects are authentic, and the mechanisms by which they might be mediated.

Chapter 5.

General Conclusions

The results of twin studies suggest that both genetic and environmental influences contribute substantially to the development of alcohol use behaviors (Agrawal & Lynskey, 2008), but the particular etiological bases of adult alcohol use and related problems are not entirely clear. The goal of this dissertation was to examine three sources of potential genetic and environmental risk: a functional polymorphism in the alcohol metabolism gene ALDH2, SNP markers in a number of GABA system genes, and exposure to alcohol in early adolescence, in order to determine the extent of their involvement in the development of alcohol use and abuse, as well as other related adult outcomes.

In a sample of adopted individuals of East Asian descent assessed longitudinally from adolescence into adulthood, the first study showed that the association between possession of the low-activity variant of a functional polymorphism in the alcohol metabolism gene ALDH2 and reduced alcohol use and abuse increased with age and was moderated by the alcohol use of adopted parents such that high parental use reduced the protective effect, while low parental use enhanced the protection; but the effect of the polymorphism was not consistently influenced by adopted sibling alcohol use nor affiliation with deviant peers. This study thus demonstrated that the functional polymorphism in ALDH2, rs671, follows the same developmental pattern of genetic influence increasing with age across adolescence and into adulthood that had previously been observed in twin studies of alcohol use and dependence (Dick, 2011). This study

also provided evidence in support of the previously hypothesized moderation of ALDH2 effects by social influences (Higuchi et al., 1994).

In a second study, using data from a community based sample of young and middle-aged adults, despite accounting for potentially cumulative or combined effects using a polygenic score, whole-gene, and whole-system tests, we found no evidence for association between GABA system markers and alcohol use or related psychopathology. The findings of this study therefore depart from those of previous studies of GABA system genes, most of which examined the individual or haplotype-based effects of markers in $GABA_A$ receptor subunit genes, and were largely supportive of a role for GABA system variants in alcohol dependence and a number of other externalizing behaviors (Cui et al., 2012; Dick et al., 2009).

Beyond the "low hanging fruit" of functional polymorphisms in alcohol metabolizing genes, identifying the sources of genetic influence underlying alcohol use has proved difficult. Because the variants influencing alcohol-related behaviors are likely to be so numerous and of such small effect, molecular genetic studies have, so far, often been stymied. Novel approaches to genetic analysis (e.g., Yang et al., 2012) and larger samples, as may be achieved by combining genotyped samples from multiple independent sites and studies, may aid in identifying these variants.

A full elucidation of the gene networks underlying alcohol related behaviors is likely to be substantially more complex than simply identifying a small number of genes belonging to any single biologically relevant system, such as alcohol metabolism genes or $GABA_A$ receptor subtype genes. Genes may interact with each other in non-obvious

ways to influence complex phenotypes. Bioinformatic databases integrating multiple public sources of genomic or other molecular-level data to document or predict functional networks may be useful in selecting candidate genes for further study (Farris & Miles, 2012).

In our third study, both propensity score adjustment via sample weighting and the cotwin control method suggested that early alcohol exposure is likely to elevate risk for adult alcohol use and abuse, and other negative adult outcomes related to behavioral disinhibition. Previous research using similar designs had been equivocal regarding the role of early alcohol exposures in contributing to adult outcomes (Prescott & Kendler, 1999; Grant et al., 2006), but in the present study the results of both methods were generally consistent with a causal effect of early alcohol exposures on adult outcomes related to alcohol and other substances, as well as dependent stressful life events, though not measures of social functioning.

Because we had the advantage of working with data from a genetically informative sample of twins, we were able to use the cotwin control method to check the assumption of the propensity score weighting method that there existed no confounding variables apart from those included in the propensity score model. In fact, we found that, because estimates of exposure effect based on the total sample of discordant twin pairs were substantially reduced compared to the propensity score weighted estimates, it was indeed likely that the propensity score model was biased by the exclusion of unmeasured confounding variables. However, it should be noted that, since it relies on information from twin pairs discordant for an exposure, who are in the minority, the cotwin control

approach has less power than adjustment using the propensity score. This problem is exacerbated when testing for moderation of the exposure effect by zygosity. There is therefore a tradeoff between sensitivity to detect exposure effects and ability to quantify and rule out confounding specifically due to genetic, as opposed to shared environmental, influences. If the causal effect of an exposure is of primary research interest, the ideal sample for cotwin control analysis would be a large sample of discordant MZ twin pairs.

Alcohol use behaviors emerge from a complex interplay of genetic and environmental influences, which develop over a non-constant longitudinal trajectory. The findings of the studies described in this dissertation reflect risk factors that might now be considered more valid targets for treatment or prevention (for example, by keeping alcohol out of the hands of adolescents), but they encompass only a small part of the total etiological influences contributing to alcohol-related behavior.

Table 1. Descriptive Statistics for Age and Alcohol Use Outcomes by Assessment Stage

			Stage		
Age	**M (SD)**		**IN**	**FU1**	**FU2**
		*ALDH2*1* homozygotes	14.8 (1.9)	18.1 (2.1)	22.3 (1.9)
		*ALDH2*2* group	14.8 (1.6)	18.1 (1.8)	22.1 (1.6)
Drinking index	**M (SD)**				
		*ALDH2*1* homozygotes	1.30 (2.92)	5.62 (5.32)	10.02 (4.92)
		*ALDH2*2* group	1.14 (2.86)	4.53 (4.72)	7.65 (4.77)
Alcohol abuse and dependence symptom sum	**M (SD)**				
		*ALDH2*1* homozygotes	.11 (.76)	.51 (1.34)	.91 (1.56)
		*ALDH2*2* group	.11 (.62)	.41 (1.43)	.49 (1.27)
Alcohol abuse or dependence diagnosis	**(%)**				
		*ALDH2*1* homozygotes	2.3	14.6	32.1
		*ALDH2*2* group	2.1	10.4	19.5

Table 2. Model Comparisons Evaluating Change in Alcohol Use Outcomes as a Function of ALDH2 Genotype and Age

Drinking index

Model	Predictors	-2LL	-2LL change	df	LRT p-value	AIC
A	Sex, Sex*Age, Age, Age^2, Age^3	5458.5				5474.5
B	A + *ALDH2*	5453.8	-4.7	1	**.03**	5471.8
C	B + *ALDH2**Age	5450.6	-7.9	2	**.02**	**5470.6**

Alcohol abuse and dependence symptom sum

Model	Predictors	-2LL	-2LL change	df	LRT p-value	AIC
A	Sex, Sex*Age, Age, Age^2, Age^3	5269.6				5285.6
B	A + *ALDH2*	5267.8	-1.8	1	.18	5285.8
C	B + *ALDH2**Age	5259.6	-10	2	**.01**	**5279.6**

Alcohol abuse or dependence diagnosis

Model	Predictors	Wald X^2 p-value for added term(s)	QICC
A	Sex, Age, Age^2		473.9
B	A + *ALDH2*	.29	473.7
C	B + *ALDH2**Age	**.02**	**471.0**

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 3. Estimated Alcohol Use Outcome Values and Effect Sizes for ALDH2 Genotype Groups at Selected Ages

Drinking index (log-transformed)	**M (SD)**		**Age 15**	**Age 18**	**Age 22**
		*ALDH2*1* homozygotes	0.43 (.07)	1.52 (.05)	2.64 (.07)
		*ALDH2*2* group	0.38 (.09)	1.38 (.07)	2.38 (.09)
		Effect size (d)	**0.07**	**0.22**	**0.40**
		(95% CI)	**(-.19, .33)**	**(-.04, .40)**	**(.14, .66)**

Abuse and dependence symptom sum (log-transformed)	**M (SD)**		**Age 15**	**Age 18**	**Age 22**
		*ALDH2*1* homozygotes	0.02 (.04)	0.31 (.03)	0.66 (.05)
		*ALDH2*2* group	0.05 (.05)	0.24 (.04)	0.46 (.07)
		Effect size (d)	**-0.08**	**0.16**	**0.49**
		(95% CI)	**(-.34, .18)**	**(-.10, .42)**	**(.23, .75)**

Probability of alcohol abuse or dependence diagnosis	**Probability (95% CI)**		**Age 15**	**Age 18**	**Age 22**
		*ALDH2*1* homozygotes	0.006 (.002, .016)	0.09 (.06, .14)	0.33 (.25, .43)
		*ALDH2*2* group	0.014 (.005, .035)	0.10 (.06, .17)	0.17 (.09, .31)
		***ALDH2*2* Odds Ratio**	**2.29**	**1.10**	**0.42**

Effect size is in the form of Cohen's d

Table 4A. Model Comparisons Evaluating Change in Drinking Index as a Function of the Parental Combined Alcohol Problem Index Covariate

Drinking index

Model	Predictors	-2LL	-2LL change	df	LRT p-value	AIC
C*	Model C including only participants with non-missing values for Parent combined alcohol problem index	5450.6				5470.6
D	C + Parent combined index	5445.9	4.70	1	**.03**	5467.9
E	D + Parent combined index*Age	5444.9	5.70	2	.06	5468.9
F	D + Parent combined index**ALDH2*	5442.2	8.40	2	**.01**	**5466.2**
G	D + Parent combined index*Age + Parent combined index **ALDH2*	5441.2	9.40	3	**.02**	5467.2
H	G + Parent combined index*Age**ALDH2*	5441.2	9.40	4	**.05**	5469.2

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT and Wald X^2 p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 4B. Model Comparisons Evaluating Change in Alcohol Abuse and Dependence Symptom Sum as a Function of the Parental Combined Alcohol Problem Index Covariate

Alcohol abuse and dependence symptom sum

Model	Predictors	-2LL	-2LL change	df	LRT p-value	AIC
C*	Model C including only participants with non-missing values for Parent combined alcohol problem index	5259.6				5279.6
D	C + Parent combined index	5258.9	0.70	1	.41	5280.9
E	D + Parent combined index *Age	5258.6	1.00	2	.61	5282.6
F	D + Parent combined index **ALDH2*	5255.5	4.10	2	.13	**5279.5**
G	D + Parent combined index *Age + Parent combined index **ALDH2*	5255.3	4.30	3	.23	5281.3
H	G + Parent combined index *Age**ALDH2*	5255.1	4.50	4	.48	5283.1

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT and Wald X^2 p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 4C. Model Comparisons Evaluating Change in Alcohol Abuse or Dependence Diagnosis as a Function of the Parental Combined Alcohol Problem Index Covariate

Alcohol abuse or dependence diagnosis

Model	Predictors	Wald X^2 p-value for added term(s)	QICC
C*	Model C including only participants with non-missing values for Parent combined alcohol problem index		**471.0**
D	C + Parent combined index	.39	472.6
E	D + Parent combined index *Age	.84	474.8
F	D + Parent combined index **ALDH2*	.07	472.2
G	D + Parent combined index *Age + Parent combined index **ALDH2*	.60 , .08	474.8
H	G + Parent combined index *Age**ALDH2*	**.02**	473.5

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT and Wald X^2 p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 5A. Model Comparisons Evaluating Change in Drinking Index as a Function of the Elder Sibling Combined Alcohol Problem Index Covariate

Drinking index

Model	Predictors	-2LL	-2LL change	df	LRT p-value	AIC
C*	Model C including only participants with non-missing values for Sibling combined index	2271.6				2291.6
D	C* + Sibling combined index	2256.2	15.40	1	**<.001**	2278.2
E	D + Sibling combined index*Age	2254.0	17.60	2	**<.001**	2278.0
F	D + Sibling combined index**ALDH2*	2254.2	17.40	2	**<.001**	2278.2
G	D + Sibling combined index*Age + Sibling combined index**ALDH2*	2251.4	20.20	3	**<.001**	**2277.4**
H	G + Sibling combined index*Age**ALDH2*	2251.2	20.40	4	**<.001**	2279.2

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 5B. Model Comparisons Evaluating Change in Alcohol Abuse and Dependence Symptom Sum as a Function of the Elder Sibling Combined Alcohol Problem Index Covariate

Alcohol abuse and dependence symptom sum

Model	Predictors	-2LL	-2LL change	df	LRT p-value	AIC
C*	Model C including only participants with non-missing values for Sibling combined index	2045.4				2065.4
D	C* + Sibling combined index	2045.2	0.20	1	.65	2067.2
E	D + Sibling combined index*Age	2041.3	4.10	2	.13	**2065.3**
F	D + Sibling combined index**ALDH2*	2044.9	0.50	2	.78	2068.9
G	D + Sibling combined index*Age + Sibling combined index**ALDH2*	2040.7	4.70	3	.20	2066.7
H	G + Sibling combined index*Age**ALDH2*	2040.7	4.70	4	.32	2068.7

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 5C. Model Comparisons Evaluating Change in Alcohol Abuse or Dependence Diagnosis as a Function of the Elder Sibling Combined Alcohol Problem Index Covariate

Alcohol abuse or dependence diagnosis

Model	Predictors	Wald X^2 p-value for added term(s)	QICC
C*	Model C including only participants with non-missing values for Sibling combined index		140.5
D	C* + Sibling combined index	.08	140.0
E	D + Sibling combined index*Age	**.001**	**133.7**
F	D + Sibling combined index**ALDH2*	.71	141.9
G	D + Sibling combined index*Age + Sibling combined index**ALDH2*	**.002 ,** .23	134.2
H	G + Sibling combined index*Age**ALDH2*	.13	135.2

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 6A. Model Comparisons Evaluating Change in Drinking Index as a Function of the Peer Deviance Covariate

T-value of log transformed drinking index

Model	Predictors	-2LL	-2LL change	df	LRT p-value	AIC
C*	Model C including only participants with non-missing values for Peer deviance	5334.7				5354.7
D	C* + Peer deviance	5140.7	194.00	1	**<.001**	**5162.7**
E	D + Peer deviance*Age	5140.5	194.20	2	**<.001**	5164.5
F	D + Peer deviance**ALDH2*	5140.5	194.20	2	**<.001**	5164.5
G	D + Peer deviance*Age + Peer deviance**ALDH2*	5140.3	194.40	3	**<.001**	5166.3
H	G + Peer deviance*Age**ALDH2*	5140.2	194.50	4	**<.001**	5168.2

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 6B. Model Comparisons Evaluating Change in Alcohol Abuse and Dependence Symptom Sum as a Function of the Peer Deviance Covariate

T-value of log transformed alcohol abuse and dependence symptom Sum

Model	Predictors	-2LL	-2LL change	df	LRT p-value	AIC
C*	Model C including only participants with non-missing values for Peer deviance	5148.8				5168.8
D	C* + Peer deviance	5087.8	61.00	1	**<.001**	5109.8
E	D + Peer deviance*Age	5049.9	98.90	2	**<.001**	**5073.9**
F	D + Peer deviance**ALDH2*	5087.3	61.50	2	**<.001**	5111.3
G	D + Peer deviance*Age + Peer deviance**ALDH2*	5048.9	99.90	3	**<.001**	5074.9
H	G + Peer deviance*Age**ALDH2*	5047.6	101.20	4	**<.001**	5075.6

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 6C. Model Comparisons Evaluating Change in Alcohol Abuse or Dependence Diagnosis as a Function of the Peer Deviance Covariate

Alcohol abuse or dependence diagnosis

Model	Predictors	Wald X^2 p-value for added term(s)	QICC
C*	Model C including only participants with non-missing values for Peer deviance		455.5
D	C* + Peer deviance	**<.001**	391.5
E	D + Peer deviance*Age	.55	393.6
F	D + Peer deviance**ALDH2*	**.05**	**391.3**
G	D + Peer deviance*Age + Peer deviance**ALDH2*	.82 , .07	393.4
H	G + Peer deviance*Age**ALDH2*	.14	394.3

-2LL = -2 Log Likelihood; LRT = Likelihood Ratio Test. LRT p-values < 0.05 are in bold. Lowest AIC or QICC among each model comparison set, representing best-fitting model, is in bold.

Table 7A. Estimated Drinking Index Values and Effect Sizes for ALDH2 Genotype Groups at Selected Ages as a Function of High and Low Parental Combined Alcohol Problem Index

Drinking index (log-transformed) M (SD)	Age 15	Age 18	Age 22
Parent combined alcohol problem index 1 SD above mean			
*ALDH2*1* homozygotes	0.45 (.07)	1.55 (.06)	2.67 (.08)
*ALDH2*2* group	0.55 (.11)	1.56 (.10)	2.56 (.11)
High parent combined alcohol problem index effect size (d)	**-0.15**	**-0.01**	**0.17**
(95% CI)	**(-.41, .11)**	**(-.27, .25)**	**(-.09, .43)**
Parent combined alcohol problem index 1 SD below mean			
*ALDH2*1* homozygotes	0.39 (.08)	1.48 (.07)	2.60 (.08)
*ALDH2*2* group	0.20 (.11)	1.20 (.09)	2.21 (.11)
Low parent drinking effect size (d)	**0.30**	**0.43**	**0.61**
(95% CI)	**(.04, .56)**	**(.17, .69)**	**(.35, .87)**

Results are displayed only for comparisons where best-fitting model includes environmental covariate.
Effect size is in the form of Cohen's d

Table 7B. Estimated Alcohol Abuse and Dependence Symptom Count Values and Effect Sizes for ALDH2 Genotype Groups at Selected Ages as a Function of High and Low Parental Combined Alcohol Problem Index

		Age		
Alcohol abuse and dependence symptom sum (log-transformed)	**M (SD)**	**15**	**18**	**22**
Parent combined alcohol problem index 1 SD above mean				
	*ALDH2*1* homozygotes	0.01 (.04)	0.30 (.04)	0.65 (.06)
	*ALDH2*2* group	0.13 (.07)	0.32 (.06)	0.55 (.08)
High parent combined alcohol problem index effect size (d)		**-0.30**	**-0.05**	**0.27**
	(95% CI)	**(-.56, -.04)**	**(-.31, .21)**	**(.01, .53)**
Parent combined alcohol problem index 1 SD below mean				
	*ALDH2*1* homozygotes	0.02 (.05)	0.31 (.04)	0.66 (.06)
	*ALDH2*2* group	-0.03 (.07)	0.16 (.06)	0.39 (.08)
Low parent combined alcohol problem index effect size (d)		**0.12**	**0.36**	**0.69**
	(95% CI)	**(-.14, .38)**	**(.10, .62)**	**(.42, .95)**

Results are displayed only for comparisons where best-fitting model includes environmental covariate.
Effect size is in the form of Cohen's d

Table 8. Estimated Alcohol Use Outcome Values and Effect Sizes for ALDH2 Genotype Groups at Selected Ages as a Function of High and Low Elder Sibling Combined Alcohol Problem Index and Peer Deviance

		Age		
Log-transformed drinking index	**M (SD)**	**15**	**18**	**22**
Elder sibling combined alcohol problem index 1 SD above mean				
*ALDH2*1* homozygotes		0.77 (.13)	1.63 (.10)	2.62 (.15)
*ALDH2*2* carriers		0.59 (.17)	1.45 (.12)	2.45 (.17)
High sibling combined alcohol problem index effect size (95% CI)		**0.30 (-.10, .70)**	**0.29 (-.10, .69)**	**0.28 (-.12, .68)**
Elder sibling combined alcohol problem index 1 SD below mean				
*ALDH2*1* homozygotes		0.17 (.11)	1.19 (.12)	2.41 (.22)
*ALDH2*2* carriers		0.33 (.14)	1.36 (.18)	2.59 (.31)
Low sibling combined alcohol problem index effect size (95% CI)		**-0.27 (-.67, .13)**	**-0.28 (-.68, .12)**	**-0.29 (-.68, .10)**
		Age		
Probability of alcohol abuse or dependence diagnosis (95% CI)		**15**	**18**	**22**
Peer deviance 1 SD above mean				
*ALDH2*1* homozygotes		0.018 (.01, .05)	0.151 (.09, .23)	0.427 (.32, .54)
*ALDH2*2* carriers		0.047 (.02, .13)	0.192 (.11, .33)	0.286 (.14, .49)
High peer deviance ALDH2*2 Odds Ratio		**2.67**	**1.35**	**0.54**
Peer deviance 1 SD below mean				
*ALDH2*1* homozygotes		0.002 (.00, .01)	0.015 (.01, .04)	0.058 (.03, .13)
*ALDH2*2* carriers		0.001 (.00, .01)	0.004 (.00, .02)	0.006 (.00, .02)
Low peer deviance *ALDH2*2* Odds Ratio		**0.49**	**0.25**	**0.10**

Results are displayed only for comparisons where best-fitting model includes environmental covariate.
Effect size is in the form of Cohen's d

Figure 1. Best-fitting model estimated drinking index by age (t-standardized and log-transformed). Lines represent ALDH2 genotype groups

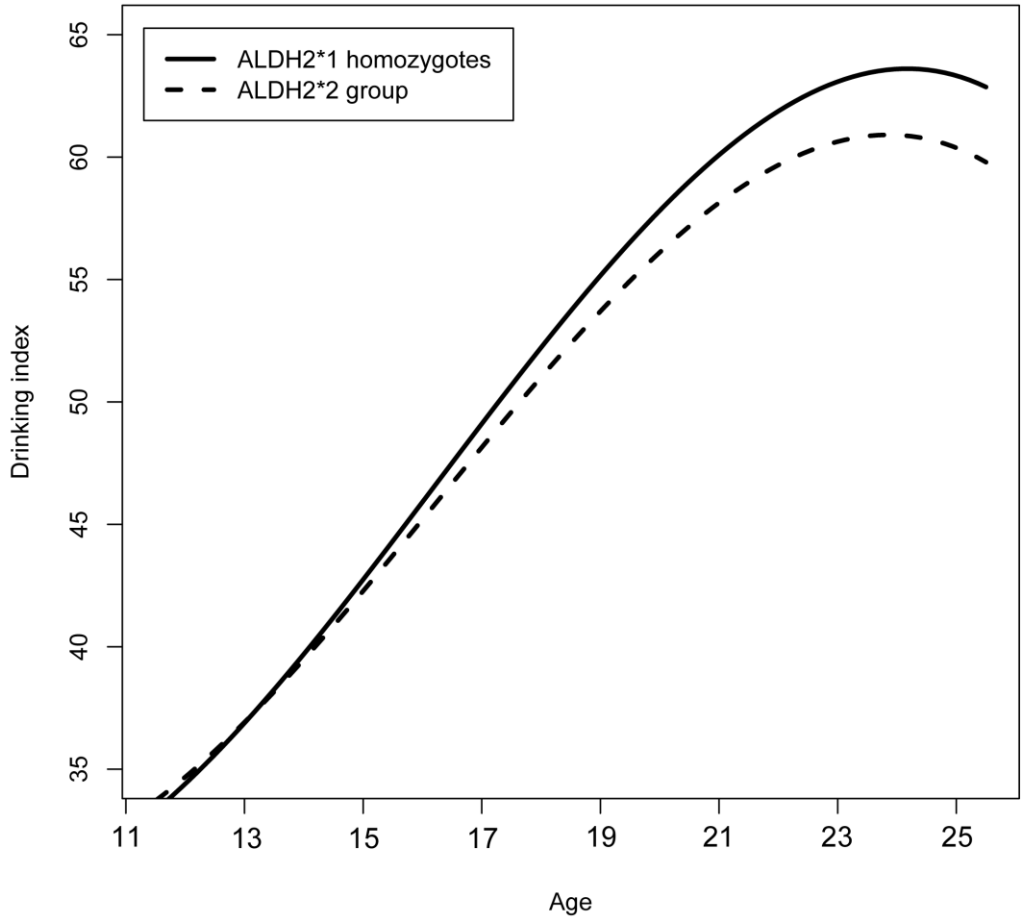

Figure 2. Best-fitting model estimated alcohol abuse and dependence symptom count by age (t-standardized and log-transformed). Lines represent ALDH2 genotype groups

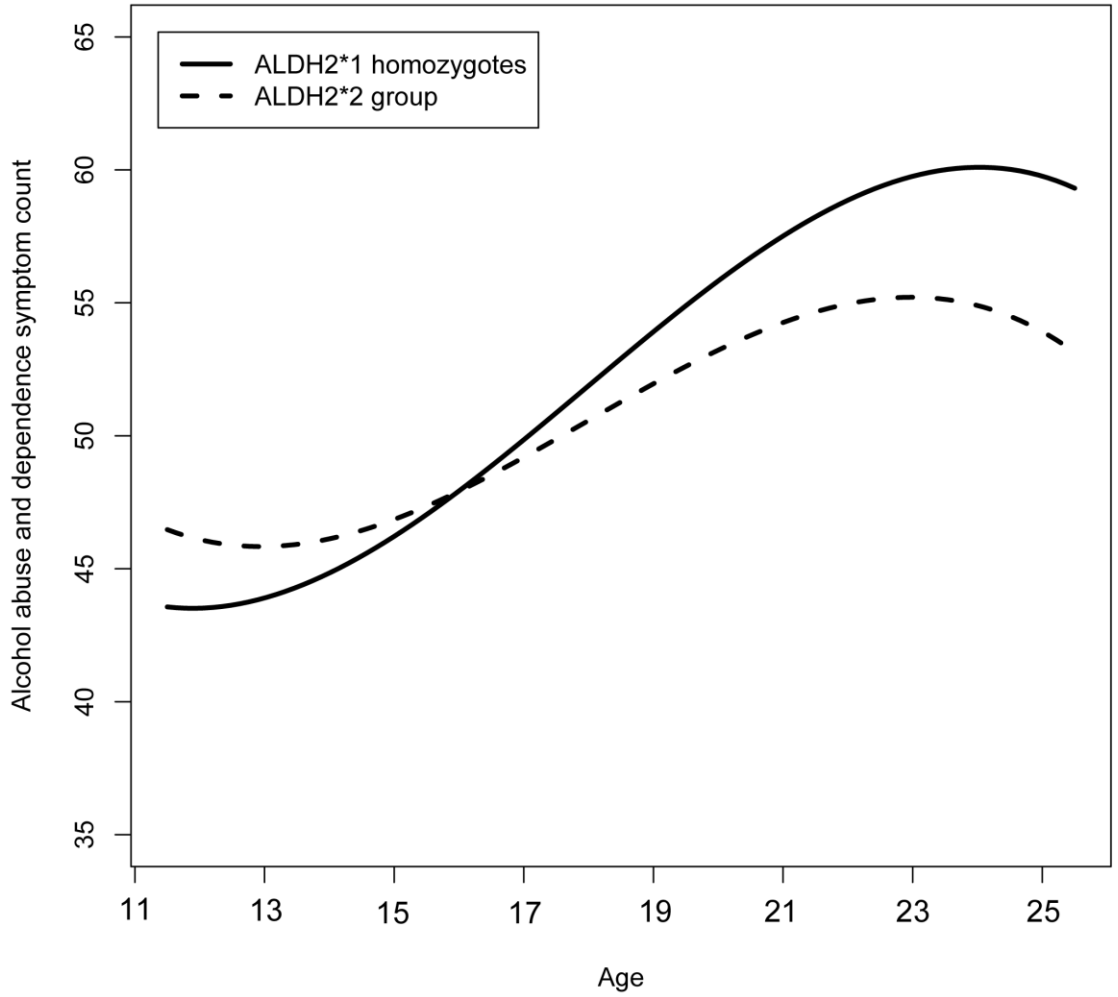

Figure 3. Best-fitting model estimated probability of alcohol abuse or dependence diagnosis by age. Lines represent ALDH2 genotype groups

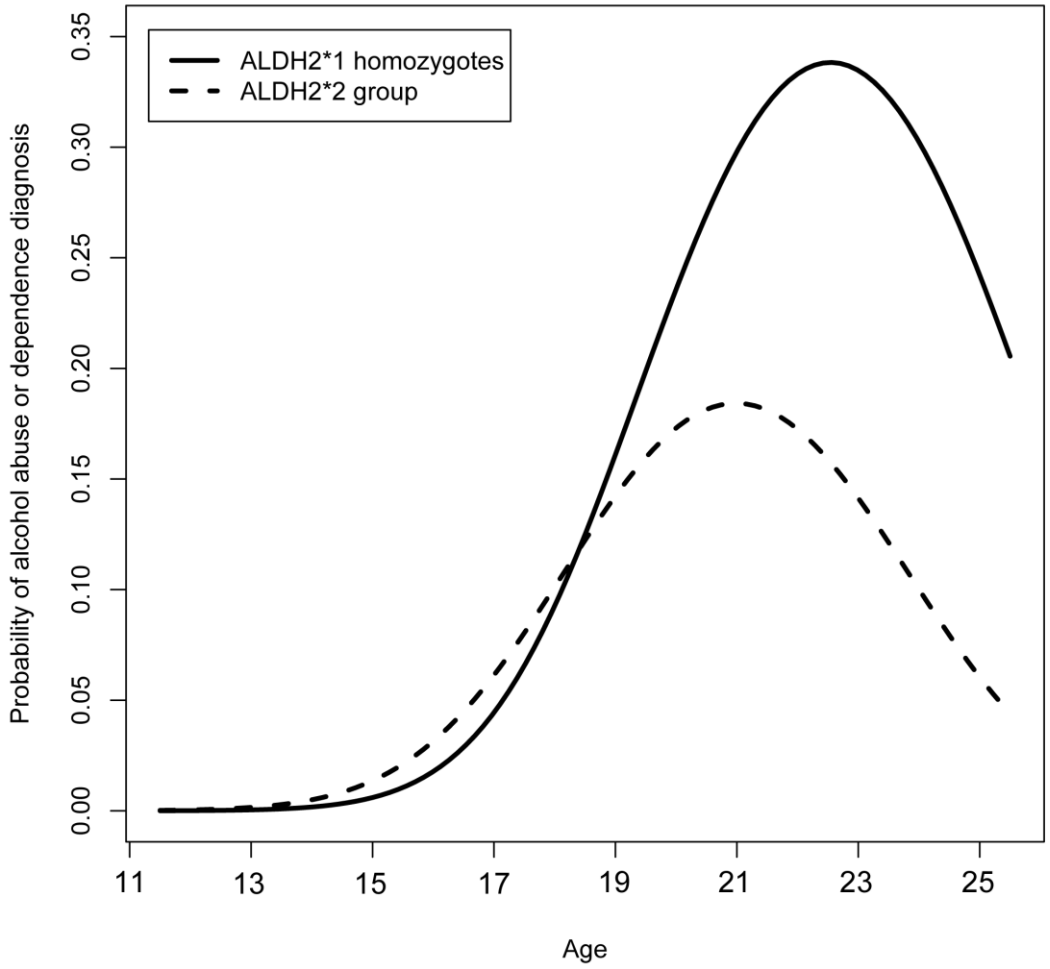

Table 1. Descriptive Statistics for Age and Alcohol Use and Abuse Phenotypes for Parent and Adolescent Participants

		Parents	Adolescents
N		3849	3375
Age	M (SD)	43.8 (5.6)	17.9 (0.8)
Female	(%)	53.9	53.3
Drinking index	M (SD)	10.04 (4.43)	5.91 (5.34)
Alcohol symptoms	M (SD)	1.13 (1.92)	0.53 (1.30)
Never tried alcohol	(%)	1.6	29.2

Table 2. Chromosomal Location and Size of GABA System Genes

Chromosome	Gene	SNPs	Gene 3' End + 1k	Gene 5' End + 5kb	Total Length (kb)
1	GABRD	0	1939627	1957052	17.4
2	DBI	6	119839973	119851592	11.6
2	GAD1	7	171380445	171430905	50.5
3	SLC6A11	51	10831916	10960146	128.2
3	SLC6A1	31	11008420	11060935	52.5
3	GNAI2	3	50247650	50276790	29.1
3	GABRR3	16	99187216	99241521	54.3
3	GPR156	14	121366018	121450832	84.8
4	GABRG1	13	45731543	45825839	94.3
4	GABRA2	12	45945338	46091813	146.5
4	GABRA4	16	46614673	46696181	81.5
4	GABRB1	73	46727051	47128204	401.2
5	SLC6A7	15	149548712	149575828	27.1
5	GABRB2	45	160647013	160912708	265.7
5	GABRA6	6	161044235	161067176	22.9
5	GABRA1	8	161205774	161264543	58.8
5	GABRG2	19	161426225	161520123	93.9
5	GABRP	12	170142300	170178628	36.3
6	GABBR1	10	29676983	29713941	37.0
6	GABRR1	31	89942941	89989215	46.3
6	GABRR2	27	90022957	90086686	63.7
9	GABBR2	147	100089184	100516300	427.1
10	GAD2	13	26544241	26638497	94.3
12	SLC6A12	8	168504	197874	29.4
12	SLC6A13	28	199049	247300	48.3
12	GABARAPL1	7	10255756	10271991	16.2
16	GABARAPL2	2	74156750	74174280	17.5
17	GABARAP	0	7083462	7091477	8.0
19	CACNA1A	82	13177256	13483274	306.0
20	SLC32A1	2	36785518	36796429	10.9
23	GABRE	11	150871251	150898807	27.6
23	GABRA3	20	151085289	151375487	290.2
23	GABRQ	2	151556292	151577481	21.2

Gene end point positions based on NCBI RefSeq release 36

Table 3. Top Ten Associations of Individual SNPs With Alcohol Use and Abuse Phenotypes

Drinking Index

SNP ID	Gene	Chromosome	Location (bp)	Beta	P-value
rs12515485	GABRA6	5	161048467	0.037	0.003
rs12518088	GABRA6	5	161048339	0.036	0.004
rs7700740	GABRA6	5	161063109	0.035	0.005
rs1967890	GABBR2	9	100457606	-0.046	0.006
rs7250783	CACNA1A	19	13237536	-0.027	0.010
rs13088757	GPR156	3	121384492	-0.024	0.014
rs3750348	GABBR2	9	100275144	0.054	0.014
rs2302080	CACNA1A	19	13217380	-0.023	0.020
rs7648922	GPR156	3	121428788	-0.023	0.024
rs12153421	GABRB2	5	160850163	-0.024	0.025

Alcohol symptoms

SNP ID	Gene	Chromosome	Location (bp)	Beta	P-value
rs4524525	GABRP	5	170141772	-0.031	0.004
rs12196758	GABRR2	6	90052018	0.048	0.004
rs2900964	CACNA1A	19	13413763	0.051	0.004
rs1994260	SLC6A11	3	10913584	0.033	0.005
rs2808534	GABBR2	9	100379768	-0.027	0.006
rs3805455	GABRP	5	170173072	-0.027	0.007
rs12206367	GABRR2	6	90031345	0.039	0.009
rs11946433	GABRA4	4	46641663	-0.047	0.009
rs7658410	GABRA4	4	46666081	-0.034	0.010
rs16916777	GABBR2	9	100320996	0.090	0.011

Table 4. Mean Cross-Validated Correlations Between Polygenic Score-Predicted Alcohol Use and Abuse Phenotypic Values and Observed Phenotypic Values at Each of 10 p-Value Thresholds

P-Value Threshold	Drinking Index Mean CV R^2	Alcohol Symptoms Mean CV R^2
<0.1	0	0.00016
<0.2	0	0.00002
<0.3	0	0.00019
<0.4	0	0.00024
<0.5	0	0.00019
<0.6	0	0.00009
<0.7	0	0.00001
<0.8	0	0.00004
<0.9	0	0
All SNPs included	0	0

Table 5. Genetic Correlation-Based Estimates of Explained Phenotypic Variance (h^2SNP) Attributable to GABA System SNPs and All Available SNPs, Separately for Autosomal and Chromosome X Markers

	Drinking index		**Alcohol Symptoms**	
	h^2_{SNP}	(SE)	h^2_{SNP}	(SE)
All Autosomal SNPs	0.169*	(.087)	0.120	(.088)
GABA Autosomal SNPs	0	(.006)	0	(.005)
All Chr. X SNPs	0	(.014)	0.011	(.014)
GABA Chr. X SNPs	0	(.002)	0	(.001)

* LRT $p < .05$

Table 6. Top Ten Associations with Alcohol Use and Abuse Phenotypes From Gene-Based Tests

Drinking index

Gene	Chromosome	p-value
GABRA6	5	0.00838
GPR156	3	0.08407
GABRB2	5	0.164
GNAI2	3	0.22
GABRA1	5	0.273
GABRR2	6	0.274
GABRA2	4	0.321
GABRR1	6	0.348
GABRR3	3	0.379
GABRB1	4	0.379

Alcohol symptoms

Gene	Chromosome	p-value
GABRP	5	0.01548
GABRR2	6	0.03851
GABRR3	3	0.07548
GABARAPL1	12	0.105
SLC6A11	3	0.111
GABRA4	4	0.1484
GABRA1	5	0.151
GABRR1	6	0.172
GABRA6	5	0.176
GABRB1	4	0.273

Table 7. Top Ten Associations with Alcohol Use and Abuse Phenotypes From "Top-SNP" Gene-Based Tests

Drinking index

Gene	Chromosome	Top SNP	p-value
GABRA6	5	rs12515485	0.01368
GPR156	3	rs13088757	0.08425
GAD1	2	rs16858988	0.133
GABRA1	5	rs6883877	0.182
GABRG1	4	rs16859084	0.278
GNAI2	3	rs11716295	0.327
GABBR2	9	rs1967890	0.366
GABRB2	5	rs12153421	0.417
CACNA1A	19	rs7250783	0.421
GABRR2	6	rs3777522	0.433

Alcohol symptoms

Gene	Chromosome	Top SNP	p-value
GABRP	5	rs4524525	0.02603
GABRR2	6	rs12196758	0.09414
GABRA4	4	rs11946433	0.09712
SLC6A11	3	rs1994260	0.128
GABRR3	3	rs937128	0.15299
GABARAPL1	12	rs11053685	0.156
GABRR1	6	rs12206367	0.175
GABRA1	5	rs4554269	0.176
CACNA1A	19	rs2900964	0.202
GABRA6	5	rs12515485	0.267

Table 1. Sample Descriptive Statistics

	N	Age M	(SD)	% female
Intake (IN)	1512	11.72	(.43)	50.5
Second assessment (FU1)	1404	14.80	(.53)	50.6
Fifth assessment (FU4)	1328	25.29	(.74)	51.4

Table 2A. Standardized Differences in the Means of Propensity Score Model Covariates Across Early Alcohol use Exposure Groups

Early alcohol use	Full sample				
Individual level variables	Unweighted sample	Weighted sample	All discordant twins	MZ discordant twins	DZ discordant twins
Twin IQ	-0.09	0.00	0.09	0.12	0.06
Twin menarche	0.10	0.01	-0.04	-0.02	-0.09
Twin puberty measure	**0.25**	0.01	0.07	0.03	0.13
Twin GPA	-0.21	-0.02	0.05	0.02	0.11
Twin academic problems	**0.31**	0.01	0.10	0.13	0.05
Twin academic motivation	**-0.38**	-0.01	-0.10	-0.04	-0.18
Twin externalizing disorder symptoms	**0.33**	0.00	0.20	0.19	0.21
Twin delinquent behaviors	**0.51**	0.04	0.06	0.05	0.05
Conflict between twin and parents	**0.29**	0.01	0.05	0.09	-0.02
Absolute mean of individual level variables	**0.27**	0.01	0.08	0.08	0.10
Family level variables					
Twin sex	-0.13	-0.03			
Twin age	**0.28**	0.02			
Twin ethnicity	-0.15	0.00			
Parent age	0.00	-0.02			
Parent IQ	-0.15	-0.04			
Parent years of education	-0.21	-0.01			
Parent occupational status	-0.14	-0.01			
Family income	-0.09	-0.02			
Family history of externalizing	0.14	0.01			
Parent delinquent behaviors	**0.32**	0.04			
Parent externalizing disorder symptoms	**0.29**	0.02			
Parent alcohol abuse or dependence symptoms	0.21	0.03			
Parent drug abuse or dependence symptoms	0.24	0.05			
Parent alcohol use quantity and frequency index	**0.39**	0.04			
Parent tobacco use quantity and frequency index	**0.45**	0.03			
Parent ever used marijuana	**0.25**	0.07			
Parent number of times used marijuana	0.24	0.06			
Parent number of drug classes ever used	**0.30**	0.06			
Parents ever separated or divorced	0.24	0.01			
Immediate family member of twins ever died	0.05	-0.01			
Absolute mean of all variables	0.23	0.03			

Standardized difference is calculated as

$$(M_{exposed} - M_{nonexposed}) / \sqrt{[(\sigma^2_{exposed} + \sigma^2_{nonexposed}) /2]}$$

Bolded values indicate standardized differences greater than 0.25

Table 2B. Standardized Differences in the Means of Propensity Score Model Covariates Across Early Intoxication Exposure Groups

Early Intoxication	Full sample				
Individual level variables	Unweighted sample	Weighted sample	All discordant twins	MZ discordant twins	DZ Discordant twins
Twin IQ	-0.14	-0.07	0.06	0.02	0.13
Twin menarche	0.11	0.04	0.01	0.06	-0.01
Twin puberty measure	**0.31**	0.02	0.16	0.05	**0.31**
Twin GPA	**-0.37**	-0.07	-0.03	-0.08	0.05
Twin academic problems	**0.46**	0.11	0.11	0.15	0.06
Twin academic motivation	**-0.46**	0.00	-0.10	-0.06	-0.14
Twin externalizing disorder symptoms	**0.44**	0.04	0.15	0.13	0.14
Twin delinquent behaviors	**0.79**	0.05	0.16	0.16	0.12
Conflict between twin and parents	**0.34**	0.05	0.02	0.05	-0.05
Absolute mean of individual level variables	**0.38**	0.05	0.09	0.08	0.11
Family level variables					
Twin sex	-0.14	0.02			
Twin age	**0.31**	0.07			
Twin ethnicity	-0.22	0.00			
Parent age	-0.01	-0.08			
Parent IQ	-0.11	-0.12			
Parent years of education	**-0.30**	-0.14			
Parent occupational status	-0.22	-0.05			
Family income	-0.10	-0.02			
Family history of externalizing	0.16	-0.03			
Parent delinquent behaviors	**0.37**	0.05			
Parent externalizing disorder symptoms	**0.30**	0.10			
Parent alcohol abuse or dependence symptoms	**0.26**	0.08			
Parent drug abuse or dependence symptoms	**0.29**	0.07			
Parent alcohol use quantity and frequency index	**0.44**	0.13			
Parent tobacco use quantity and frequency index	**0.42**	0.09			
Parent ever used marijuana	**0.28**	0.11			
Parent number of times used marijuana	**0.29**	0.11			
Parent number of drug classes ever used	**0.39**	0.10			
Parents ever separated or divorced	**0.30**	0.01			
Immediate family member of twins ever died	0.02	-0.05			
Absolute mean of all variables	**0.29**	0.07			

Standardized difference is calculated as $(M_{exposed} - M_{nonexposed}) / \sqrt{[(\sigma^2_{exposed} + \sigma^2_{nonexposed}) /2]}$

Bolded values indicate standardized differences greater than 0.25

Table 3 Frequency of Early Alcohol Exposures and Exposure-Discordant Twin Pairs

Early alcohol use		N	Individuals exposed		Pairs discordant	
	Total	1401	505	(36%)	156	(22%)
	MZ	901	321	(36%)	90	(20%)
	DZ	500	184	(37%)	66	(26%)
Early intoxication						
	Total	1401	212	(15%)	70	(10%)
	MZ	901	131	(15%)	41	(9%)
	DZ	500	81	(16%)	29	(12%)

Table 4A. Means, Standard Deviations, and Effect Sizes for Adult Outcomes Between Early Alcohol Use Exposure Groups in the Total, Unweighted Sample

Early alcohol use	Never used alcohol		Used alcohol		Effect size (d)
	M	(SD)	M	(SD)	
Alcohol use quantity and frequency index	9.7	(4.1)	12.4	(3.5)	**0.74**
Alcohol abuse or dependence symptoms	0.7	(1.4)	1.5	(2.0)	**0.44**
Tobacco use quantity and frequency index	3.9	(4.0)	6.8	(3.9)	**0.73**
Number of times used marijuana	40	(169)	140	(306)	**0.38**
Number of drug classes ever used	0.8	(1.2)	1.9	(1.9)	**0.65**
Drug abuse or dependence symptoms	0.3	(1.6)	1.4	(3.3)	**0.36**
Adult antisocial behavior symptoms	0.7	(0.9)	1.3	(1.2)	**0.53**
Dependent stressful life events	1.9	(1.7)	2.7	(2.0)	**0.37**
Family relationships	2.5	(0.4)	2.5	(0.4)	**-0.04**
Interpersonal problems	2.6	(0.3)	2.7	(0.4)	**0.16**
Social engagement	3.0	(0.4)	3.0	(0.5)	**0.09**
Adult independence	3.1	(1.0)	3.3	(0.9)	**0.16**

Means and standard deviations are displayed in non-transformed form for ease of interpretation, but effect sizes are calculated on log-transformed variables, where applicable

Table 4B. Means, Standard Deviations, and Effect Sizes for Adult Outcomes Between Early Intoxication Exposure Groups in the Total, Unweighted Sample

Early intoxication	Never been intoxicated		Has been intoxicated		Effect size (d)
	M	(SD)	M	(SD)	
Alcohol use quantity and frequency index	10.2	(4.1)	13	(3.4)	**0.79**
Alcohol abuse or dependence symptoms	0.8	(1.5)	1.8	(2.2)	**0.47**
Tobacco use quantity and frequency index	4.4	(4.1)	8	(3.5)	**1.00**
Number of times used marijuana	57	(202)	188	(346)	**0.40**
Number of drug classes ever used	1.0	(1.4)	2.4	(2.1)	**0.69**
Drug abuse or dependence symptoms	0.5	(1.9)	2.1	(4.2)	**0.41**
Adult antisocial behavior symptoms	0.8	(0.9)	1.5	(1.3)	**0.56**
Dependent stressful life events	2.1	(1.8)	3.0	(2.2)	**0.46**
Family relationships	2.5	(0.4)	2.5	(0.4)	**0.02**
Interpersonal problems	2.7	(0.3)	2.7	(0.4)	**0.16**
Social engagement	3.0	(0.5)	3.0	(0.4)	**-0.06**
Adult independence	3.1	(1.0)	3.3	(0.9)	**0.19**

Means and standard deviations are displayed in non-transformed form for ease of interpretation, but effect sizes are calculated on log-transformed variables, where applicable

Table 5A. Effect Estimates for Early Alcohol Use on Adult Outcomes

Ever used alcohol (FU1)	Unweighted Sample		Weighted sample		All discordant pairs		Discordant MZ pairs		Discordant DZ pairs	
	β_{expos}	(SE)	β_{expos}	(SE)	β_w	(SE)	β_w	(SE)	β_w	(SE)
Alcohol use quantity and frequency index	0.63	(0.06) [a]	0.57	(0.06) [a]	0.21	(0.09) [c]	0.22	(0.10) [c]	0.20	(0.14)
Alcohol abuse or dependence symptoms	0.46	(0.06) [a]	0.36	(0.07) [a]	0.24	(0.11) [c]	0.17	(0.14)	0.34	(0.16) [c]
Tobacco use quantity and frequency index	0.63	(0.06) [a]	0.49	(0.07) [a]	0.21	(0.09) [c]	0.09	(0.12)	0.39	(0.16) [c]
Number of times used marijuana	0.54	(0.06) [a]	0.43	(0.07) [a]	0.28	(0.10) [b]	0.22	(0.11)	0.38	(0.18) [c]
Number of drug classes ever used	0.73	(0.06) [a]	0.60	(0.07) [a]	0.44	(0.10) [a]	0.41	(0.12) [b]	0.50	(0.17) [b]
Drug abuse or dependence symptoms	0.51	(0.06) [a]	0.40	(0.07) [a]	0.27	(0.11) [c]	0.18	(0.14)	0.42	(0.17) [c]
Adult antisocial behavior symptoms	0.52	(0.07) [a]	0.38	(0.08) [a]	0.19	(0.09) [c]	0.08	(0.11)	0.37	(0.17) [c]
Dependent stressful life events	0.35	(0.07) [a]	0.26	(0.08) [b]	0.26	(0.10) [c]	0.26	(0.11) [c]	0.26	(0.19)
Family relationships	-0.10	(0.07)	-0.05	(0.08)	-0.08	(0.11)	-0.07	(0.14)	-0.09	(0.22)
Interpersonal problems	0.15	(0.06) [c]	0.08	(0.08)	0.03	(0.11)	0.10	(0.14)	-0.09	(0.21)
Social engagement	0.05	(0.06)	0.12	(0.07)	-0.06	(0.10)	-0.03	(0.13)	-0.12	(0.21)
Adult independence	0.16	(0.07) [c]	0.15	(0.08)	0.00	(0.12)	-0.02	(0.15)	0.03	(0.17)

β_{expos} is the coefficient for the effect of an FU1 alcohol exposure on an FU4 outcome.
β_w is the within-pair, individual-level coefficient for the effect of an FU1 alcohol exposure on an FU4 outcome in cotwin control analyses

[a] effect is statistically significant at P < .001
[b] effect is statistically significant at P < .01
[c] effect is statistically significant at P < .05

Table 5B. Effect Estimates for Early Intoxication on Adult Outcomes

Ever been intoxicated (FU1)	Unweighted Sample		Weighted sample		All discordant pairs		Discordant MZ pairs		Discordant DZ pairs	
	β_{expos}	(SE)	β_{expos}	(SE)	β_w	(SE)	β_w	(SE)	β_w	(SE)
Alcohol use quantity and frequency index	0.62	(0.08) [a]	0.57	(0.08) [a]	0.37	(0.13) [b]	0.26	(0.17)	0.54	(0.23) [c]
Alcohol abuse or dependence symptoms	0.53	(0.10) [a]	0.31	(0.11) [b]	0.29	(0.15) [c]	0.15	(0.19)	0.51	(0.25) [c]
Tobacco use quantity and frequency index	0.68	(0.07) [a]	0.50	(0.11) [a]	0.25	(0.12) [c]	0.16	(0.15)	0.38	(0.17) [c]
Number of times used marijuana	0.60	(0.10) [a]	0.31	(0.11) [b]	0.43	(0.17) [c]	0.27	(0.19)	0.66	(0.28) [c]
Number of drug classes ever used	0.84	(0.09) [a]	0.62	(0.10) [a]	0.50	(0.16) [b]	0.38	(0.15) [c]	0.68	(0.28) [c]
Drug abuse or dependence symptoms	0.68	(0.11) [a]	0.38	(0.14) [c]	0.47	(0.16) [b]	0.26	(0.21)	0.78	(0.24) [b]
Adult antisocial behavior symptoms	0.60	(0.08) [a]	0.43	(0.11) [a]	0.18	(0.16)	0.08	(0.16)	0.35	(0.26)
Dependent stressful life events	0.47	(0.10) [a]	0.40	(0.13) [b]	0.38	(0.15) [c]	0.43	(0.18) [c]	0.30	(0.21)
Family relationships	-0.08	(0.11)	0.05	(0.15)	0.07	(0.20)	0.09	(0.24)	0.03	(0.29)
Interpersonal problems	0.19	(0.10)	0.07	(0.13)	-0.07	(0.19)	-0.08	(0.25)	-0.05	(0.29)
Social engagement	-0.09	(0.09)	0.11	(0.12)	-0.06	(0.14)	0.07	(0.17)	-0.26	(0.25)
Adult independence	0.18	(0.09) [c]	0.18	(0.11)	0.01	(0.15)	0.02	(0.19)	-0.02	(0.25)

β_{expos} is the coefficient for the effect of an FU1 alcohol exposure on an FU4 outcome.
β_w is the within-pair, individual-level coefficient for the effect of an FU1 alcohol exposure on an FU4 outcome in cotwin control analyses

[a] effect is statistically significant at P < .001
[b] effect is statistically significant at P < .01
[c] effect is statistically significant at P < .05

Bibliography

Addolorato, G., Caputo, F., Capristo, E., Domenicali, M., Bernardi, M., Janiri, L., . . . Gasbarrini, G. (2002). Baclofen efficacy in reducing alcohol craving and intake: a preliminary double-blind randomized controlled study. *Alcohol and alcoholism (Oxford, Oxfordshire), 37*(5), 504-508.

Agrawal, A., Balasubramanian, S., Smith, E. K., Madden, P. A., Bucholz, K. K., Heath, A. C., & Lynskey, M. T. (2010). Peer substance involvement modifies genetic influences on regular substance involvement in young women. *Addiction, 105*(10), 1844-1853. doi: 10.1111/j.1360-0443.2010.02993.x

Agrawal, A., Edenberg, H. J., Foroud, T., Bierut, L. J., Dunne, G., Hinrichs, A. L., . . . Dick, D. M. (2006). Association of GABRA2 with drug dependence in the collaborative study of the genetics of alcoholism sample. *Behavior Genetics, 36*(5), 640-650.

Agrawal, A., & Lynskey, M. T. (2008). Are there genetic influences on addiction: evidence from family, adoption and twin studies. *Addiction, 103*(7), 1069-1081. doi: 10.1111/j.1360-0443.2008.02213.x

Agrawal, A., Sartor, C. E., Lynskey, M. T., Grant, J. D., Pergadia, M. L., Grucza, R., . . . Heath, A. C. (2009). Evidence for an interaction between age at first drink and genetic influences on DSM-IV alcohol dependence symptoms. *Alcoholism, clinical and experimental research, 33*(12), 2047-2056. doi: 10.1111/j.1530-0277.2009.01044.x

Akaike, H. (1973). Information theory and an extension of the maximum likelihood

principle. In B. N. Petrov, & Csaki, F. (Ed.), *Second International Symposium on Information Theory*. Budapest: Akademiai Kiado.

Association, A. P. (1987). *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition*. Washington, D.C.: American Psychiatric Association.

Association, A. P. (2000). *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*. Washington, D.C.: American Psychiatric Association.

Barnard, E. A., Skolnick, P., Olsen, R. W., Mohler, H., Sieghart, W., Biggio, G., . . . Langer, S. Z. (1998). International Union of Pharmacology. XV. Subtypes of gamma-aminobutyric acid(A) receptors: Classification on the basis of subunit structure and receptor function. *Pharmacological Reviews, 50*, 291-313.

Begg, M. D., & Parides, M. K. (2003). Separation of individual-level and cluster-level covariate effects in regression analysis of correlated data. *Statistics in medicine, 22*(16), 2591-2602. doi: 10.1002/sim.1524

Begleiter, H., & Porjesz, B. (2006). Genetics of human brain oscillations. *International journal of psychophysiology : official journal of the International Organization of Psychophysiology, 60*(2), 162-171. doi: 10.1016/j.ijpsycho.2005.12.013

Bergen, S. E., Gardner, C. O., & Kendler, K. S. (2007). Age-related changes in heritability of behavioral phenotypes over adolescence and young adulthood: a meta-analysis. *Twin research and human genetics : the official journal of the International Society for Twin Studies, 10*(3), 423-433. doi: 10.1375/twin.10.3.423

Bettler, B., Kaupmann, K., Mosbacher, J., & Gassmann, M. (2004). Molecular structure

and physiological functions of GABA(B) receptors. *Physiological reviews, 84*(3), 835-867. doi: 10.1152/physrev.00036.2003

Bornovalova, M. A., Huibregtse, B. M., Hicks, B. M., Keyes, M., McGue, M., & Iacono, W. (2012). Tests of a Direct Effect of Childhood Abuse on Adult Borderline Personality Disorder Traits: A Longitudinal Discordant Twin Design. *Journal of abnormal psychology*. doi: 10.1037/a0028328

Bosron, W. F., & Li, T. K. (1986). Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. *Hepatology, 6*(3), 502-510.

Boyle, A. E., Segal, R., Smith, B. R., & Amit, Z. (1993). Bidirectional effects of gabaergic agonists and antagonists on maintenance of voluntary ethanol intake in rats. *Pharmacology, Biochemistry and Behavior, 46*, 179-182.

Brody, G. H., Beach, S. R., Philibert, R. A., Chen, Y. F., Lei, M. K., Murry, V. M., & Brown, A. C. (2009). Parenting moderates a genetic vulnerability factor in longitudinal increases in youths' substance use. *Journal of consulting and clinical psychology, 77*(1), 1-11. doi: 10.1037/a0012996

Brown, S. A., McGue, M., Maggs, J., Schulenberg, J., Hingson, R., Swartzwelder, S., . . . Murphy, S. (2008). A developmental perspective on alcohol and youths 16 to 20 years of age. *Pediatrics, 121 Suppl 4*, S290-310. doi: 10.1542/peds.2007-2243D

Bryk, A., & Raudenbush, S. (1992). *Hierarchical linear models for social and behavioral research: Applications and data analysis methods*. Newbury Park, CA: Sage.

Buck, K. J. (1996). Molecular genetic analysis of the role of GABAergic systems in the

behavioral and cellular actions of alcohol. *Behavior Genetics, 26*, 313-323.

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: Understanding AIC and BIC in model selection. *Sociological Methods & Research, 33*, 261-304.

Cleveland, H. H., & Wiebe, R. P. (2003). The moderation of genetic and shared-environmental influences on adolescent drinking by levels of parental drinking. *Journal of studies on alcohol, 64*(2), 182-194.

Cleveland, H. H., Wiebe, R. P., & Rowe, D. C. (2005). Sources of exposure to smoking and drinking friends among adolescents: a behavioral-genetic evaluation. *The Journal of genetic psychology, 166*(2), 153-169.

Cotton, N. S. (1979). The familial incidence of alcoholism: a review. *Journal of studies on alcohol, 40*(1), 89-116.

Covault, J., Gelernter, J., Hesselbrock, V., Nellissery, M., & Kranzler, H. R. (2004). Allelic and haplotypic association of GABRA2 with alcohol dependence. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 129B*, 104-109.

Crabb, D. W., Edenberg, H. J., Bosron, W. F., & Li, T. K. (1989). Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity. The inactive ALDH2(2) allele is dominant. *The Journal of clinical investigation, 83*(1), 314-316. doi: 10.1172/JCI113875

Crabb, D. W., Matsumoto, M., Chang, D., & You, M. (2004). Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. *The Proceedings of the Nutrition Society,*

63(1), 49-63.

Cui, W. Y., Seneviratne, C., Gu, J., & Li, M. D. (2012). Genetics of GABAergic signaling in nicotine and alcohol dependence. *Human genetics, 131*(6), 843-855. doi: 10.1007/s00439-011-1108-4

DeLucia, C., Belz, A., & Chassin, L. (2001). Do adolescent symptomatology and family environment vary over time with fluctuations in paternal alcohol impairment? *Developmental psychology, 37*(2), 207-216.

Derringer, J., Krueger, R. F., Dick, D. M., Aliev, F., Grucza, R. A., Saccone, S., . . . Bierut, L. J. (2012). The Aggregate Effect of Dopamine Genes on Dependence Symptoms Among Cocaine Users: Cross-Validation of a Candidate System Scoring Approach. *Behavior genetics*. doi: 10.1007/s10519-012-9531-4

DeWit, D. J., Adlaf, E. M., Offord, D. R., & Ogborne, A. C. (2000). Age at first alcohol use: a risk factor for the development of alcohol disorders. *The American journal of psychiatry, 157*(5), 745-750.

Dick, D. M. (2011). Developmental changes in genetic influences on alcohol use and dependence. *Child Development Perspectives, 5*(4), 223-230.

Dick, D. M. (2011). Gene-environment interaction in psychological traits and disorders. *Annual review of clinical psychology, 7*, 383-409. doi: 10.1146/annurev-clinpsy-032210-104518

Dick, D. M., Bierut, L., Hinrichs, A., Fox, L., Bucholz, K. K., Kramer, J., . . . Schuckit, M. (2006b). The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. *Behavior Genetics, 36*(4), 577-

590.

Dick, D. M., Bierut, L., Hinrichs, A., Fox, L., Bucholz, K. K., Kramer, J., . . . Foroud, T. (2006). The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. *Behavior genetics, 36*(4), 577-590. doi: 10.1007/s10519-005-9041-8

Dick, D. M., Edenberg, H. J., Xuei, X., Goate, A., Kuperman, S., Schuckit, M., . . . Foroud, T. (2004). Association of GABRG3 with alcohol dependence. *Alcoholism: Clinical and Experimental Research, 28*, 4-9.

Dick, D. M., Latendresse, S. J., Lansford, J. E., Budde, J. P., Goate, A., Dodge, K. A., . . . Bates, J. E. (2009). Role of GABRA2 in trajectories of externalizing behavior across development and evidence of moderation by parental monitoring. *Archives of General Psychiatry, 66*, 649-657.

Dick, D. M., Pagan, J. L., Viken, R., Purcell, S., Kaprio, J., Pulkkinen, L., & Rose, R. J. (2007). Changing environmental influences on substance use across development. *Twin research and human genetics : the official journal of the International Society for Twin Studies, 10*(2), 315-326. doi: 10.1375/twin.10.2.315

Doran, N., Myers, M. G., Luczak, S. E., Carr, L. G., & Wall, T. L. (2007). Stability of heavy episodic drinking in Chinese- and Korean-American college students: effects of ALDH2 gene status and behavioral undercontrol. *Journal of Studies on Alcohol and Drugs, 68*(6), 789-797.

Drgon, T., D'Addario, C., & Uhl, G. R. (2006). Linkage disequilibrium, haplotype and association studies of a chromosome 4 GABA receptor gene cluster: Candidate

gene variants for addictions. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 141B*, 854-860.

Duranceaux, N. C., Schuckit, M. A., Luczak, S. E., Eng, M. Y., Carr, L. G., & Wall, T. L. (2008). Ethnic differences in level of response to alcohol between Chinese Americans and Korean Americans. *Journal of Studies on Alcohol and Drugs, 69*(2), 227-234.

Edenberg, H. J. (2007). The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism, 30*(1), 5-13.

Edenberg, H. J., Dick, D. M., Xuei, X., Tian, H., Almasy, L., Bauer, L. O., . . . Begleiter, H. (2004). Variations in GABRA2, encoding the α2 subunit of the $GABA_A$ receptor, are associated with alcohol dependence and with brain oscillations. *American Journal of Human Genetics, 74*, 705-714.

Ellickson, P. L., Tucker, J. S., & Klein, D. J. (2003). Ten-year prospective study of public health problems associated with early drinking. *Pediatrics, 111*(5 Pt 1), 949-955.

Eng, M. Y., Luczak, S. E., & Wall, T. L. (2007). ALDH2, ADH1B, and ADH1C genotypes in Asians: a literature review. *Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism, 30*(1), 22-27.

Enoch, M. (2008). The role of $GABA_A$ receptors in the development of alcoholism. *Pharmacology, Biochemistry and Behavior, 90*, 95-104.

Enoch, M. A., Hodgkinson, C. A., Yuan, Q., Shen, P. H., Goldman, D., & Roy, A.

(2010). The influence of GABRA2, childhood trauma, and their interaction on alcohol, heroin, and cocaine dependence. *Biological psychiatry, 67*(1), 20-27. doi: 10.1016/j.biopsych.2009.08.019

Enoch, M. A., Zhou, Z., Kimura, M., Mash, D. C., Yuan, Q., & Goldman, D. (2012). GABAergic gene expression in postmortem hippocampus from alcoholics and cocaine addicts; corresponding findings in alcohol-naive P and NP rats. *PloS one, 7*(1), e29369. doi: 10.1371/journal.pone.0029369

Eriksson, C. J. (2001). The role of acetaldehyde in the actions of alcohol (update 2000). *Alcoholism: Clinical and Experimental Research, 25*(5 Suppl), 15S-32S.

Farris, S. P., & Miles, M. F. (2012). Ethanol modulation of gene networks: implications for alcoholism. *Neurobiology of disease, 45*(1), 115-121. doi: 10.1016/j.nbd.2011.04.013

Fehr, C., Sander, T., Tadic, A., Lenzen, K. P., Anghelescu, I., Klawe, C., . . . Szegedi, A. (2006). Confirmation of association of the GABRA2 gene with alcohol dependence by subtype-specific analysis. *Psychiatric Genetics, 16*(1), 9-17.

Flory, K., Lynam, D., Milich, R., Leukefeld, C., & Clayton, R. (2004). Early adolescent through young adult alcohol and marijuana use trajectories: early predictors, young adult outcomes, and predictive utility. *Development and psychopathology, 16*(1), 193-213.

Foster, E. M. (2010). Causal inference and developmental psychology. *Developmental psychology, 46*(6), 1454-1480. doi: 10.1037/a0020204

Frisell, T., Oberg, S., Kuja-Halkola, R., & Sjolander, A. (2012). Sibling comparison

designs: bias from non-shared confounders and measurement error. *Epidemiology (Cambridge, Mass.), 23*(5), 713-720. doi: 10.1097/EDE.0b013e31825fa230

Gao, X., Starmer, J., & Martin, E. R. (2008). A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genetic epidemiology, 32*(4), 361-369. doi: 10.1002/gepi.20310

Gelernter, J., & Kranzler, H. R. (2009). Genetics of alcohol dependence. *Human genetics, 126*(1), 91-99. doi: 10.1007/s00439-009-0701-2

Ghosh, S., Begleiter, H., Porjesz, B., Chorlian, D. B., Edenberg, H. J., Foroud, T., . . . Reich, T. (2003). Linkage mapping of Beta 2 EEG waves via non-parametric regression. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 118B*, 66-71.

Gibson, H. B. (1967). Self-reported delinquency among schoolboys, and their attitudes to the police. *British Journal of Social and Clinical Psychology*(6), 168-173.

Ginsberg, G., Smolenski, S., Hattis, D., & Sonawane, B. (2002). Population distribution of aldehyde dehydrogenase-2 genetic polymorphism: implications for risk assessment. *Regulatory Toxicology and Pharmacology, 36*(3), 297-309.

Goldman, D., Oroszi, G., & Ducci, F. (2005). The genetics of addictions: uncovering the genes. *Nature reviews. Genetics, 6*(7), 521-532. doi: 10.1038/nrg1635

Gottesman, II, & Gould, T. D. (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *The American journal of psychiatry, 160*(4), 636-645.

Grant, B. F., & Dawson, D. A. (1997). Age at onset of alcohol use and its association

with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *Journal of substance abuse, 9*, 103-110.

Grant, J. D., Agrawal, A., Bucholz, K. K., Madden, P. A., Pergadia, M. L., Nelson, E. C., . . . Heath, A. C. (2009). Alcohol consumption indices of genetic risk for alcohol dependence. *Biological psychiatry, 66*(8), 795-800. doi: 10.1016/j.biopsych.2009.05.018

Grant, J. D., Scherrer, J. F., Lynskey, M. T., Lyons, M. J., Eisen, S. A., Tsuang, M. T., . . . Bucholz, K. K. (2006). Adolescent alcohol use is a risk factor for adult alcohol and drug dependence: evidence from a twin design. *Psychological medicine, 36*(1), 109-118. doi: 10.1017/S0033291705006045

Grobin, A. C., Matthews, D. B., Devaud, L. L., & Morrow, A. L. (1998). The role of $GABA_A$ receptors in the acute and chronic effects of ethanol. *Psychopharmacology, 139*, 2-19.

Harada, S., Agarwal, D. P., & Goedde, H. W. (1981). Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese. *Lancet, 2*(8253), 982. doi: S0140-6736(81)91172-7 [pii]

Harder, V. S., Stuart, E. A., & Anthony, J. C. (2010). Propensity score techniques and the assessment of measured covariate balance to test causal associations in psychological research. *Psychological methods, 15*(3), 234-249. doi: 10.1037/a0019623

Haughey, H. M., Ray, L. A., Finan, P., Villanueva, R., Niculescu, M., & Hutchison, K. E.

(2008). Human gamma-aminobutyric acid A receptor alpha2 gene moderates the acute effects of alcohol and brain mRNA expression. *Genes, brain, and behavior, 7*(4), 447-454. doi: 10.1111/j.1601-183X.2007.00369.x

Heath, A. C., Bucholz, K. K., Madden, P. A., Dinwiddie, S. H., Slutske, W. S., Bierut, L. J., . . . Martin, N. G. (1997). Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. *Psychological medicine, 27*(6), 1381-1396.

Heath, A. C., & Martin, N. G. (1994). Genetic influences on alcohol consumption patterns and problem drinking: results from the Australian NH&MRC twin panel follow-up survey. *Annals of the New York Academy of Sciences, 708*, 72-85.

Heath, A. C., Whitfield, J. B., Martin, N. G., Pergadia, M. L., Goate, A. M., Lind, P. A., . . . Montgomery, G. W. (2011). A quantitative-trait genome-wide association study of alcoholism risk in the community: findings and implications. *Biological psychiatry, 70*(6), 513-518. doi: 10.1016/j.biopsych.2011.02.028

Hedges, L. V. (1982). Estimation of effect size from a series of independent experiments. *Psychological Bulletin, 92*(2), 490-499. doi: 10.1037/0033-2909.92.2.490

Helzer, J. E., Canino, G. J., Yeh, E. K., Bland, R. C., Lee, C. K., Hwu, H. G., & Newman, S. (1990). Alcoholism--North America and Asia. A comparison of population surveys with the Diagnostic Interview Schedule. *Archives of general psychiatry, 47*(4), 313-319.

Hendershot, C. S., Collins, S. E., George, W. H., Wall, T. L., McCarthy, D. M., Liang, T., & Larimer, M. E. (2009). Associations of ALDH2 and ADH1B genotypes

with alcohol-related phenotypes in Asian young adults. *Alcoholism, clinical and experimental research, 33*(5), 839-847. doi: 10.1111/j.1530-0277.2009.00903.x

Hendershot, C. S., MacPherson, L., Myers, M. G., Carr, L. G., & Wall, T. L. (2005). Psychosocial, cultural and genetic influences on alcohol use in Asian American youth. *Journal of studies on alcohol, 66*(2), 185-195.

Hendershot, C. S., Witkiewitz, K., George, W. H., Wall, T. L., Otto, J. M., Liang, T., & Larimer, M. E. (2011). Evaluating a cognitive model of ALDH2 and drinking behavior. *Alcoholism, clinical and experimental research, 35*(1), 91-98. doi: 10.1111/j.1530-0277.2010.01325.x

Hernan, M. A., & Robins, J. M. (2012). *Causal Inference*. Harvard University Retrieved from http://www.hsph.harvard.edu/faculty/miguel-hernan/causal-inference-book/

Hicks, B. M., South, S. C., Dirago, A. C., Iacono, W. G., & McGue, M. (2009). Environmental adversity and increasing genetic risk for externalizing disorders. *Archives of general psychiatry, 66*(6), 640-648. doi: 10.1001/archgenpsychiatry.2008.554

Higuchi, S., Matsushita, S., Imazeki, H., Kinoshita, T., Takagi, S., & Kono, H. (1994). Aldehyde dehydrogenase genotypes in Japanese alcoholics. *Lancet, 343*(8899), 741-742.

Higuchi, S., Matsushita, S., Maesato, H., & Osaki, Y. (2007). Japan: alcohol today. *Addiction, 102*(12), 1849-1862. doi: 10.1111/j.1360-0443.2007.01902.x

Hill, J., Emery, R. E., Harden, K. P., Mendle, J., & Turkheimer, E. (2008). Alcohol use in adolescent twins and affiliation with substance using peers. *Journal of abnormal*

child psychology, 36(1), 81-94. doi: 10.1007/s10802-007-9161-0

Hodgkinson, C. A., Yuan, Q., Xu, K., Shen, P. H., Heinz, E., Lobos, E. A., . . . Goldman, D. (2008). Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol and Alcoholism, 43*(5), 505-515. doi: 10.1093/alcalc/agn032

Hofler, M. (2005). Causal inference based on counterfactuals. *BMC medical research methodology, 5*, 28. doi: 10.1186/1471-2288-5-28

Hollingshead, A. B. (1975). *Four factor index of social status*. Yale University. New Haven, Connecticut.

Homanics, G. E., Ferguson, C., Quinlan, J. J., Daggett, J., Snyder, K., Lagenaur, C., . . . Firestone, L. L. (1997). Gene knockout of the alpha 6 subunit of the gamma-aminobutyric type A receptor: Lack of effect on responses to ethanol, pentobarbital, and general anesthetics. *Molecular Pharmacology, 51*, 588-596.

Homanics, G. E., Le, N. Q., Kist, F., Mihalek, R., Hart, A. R., & Quinlan, J. J. (1998). Ethanol tolerance and withdrawal responses in GABA(A) receptor alpha 6 subunit null allele mice and in inbred C57BL/6J and strain 129/SvJ mice. *Alcoholism: Clinical and Experimental Research, 22*(259-265).

Honaker, J., King, G., & Blackwell, M. (2011). Amelia II: A program for missing data. *Journal of Statistical Software, 45*(7), 1-47.

Huibregtse, B. M., Bornovalova, M. A., Hicks, B. M., McGue, M., & Iacono, W. (2011). Testing the role of adolescent sexual initiation in later-life sexual risk behavior: a longitudinal twin design. *Psychological science, 22*(7), 924-933. doi:

10.1177/0956797611410982

Hunt, R., Sauna, Z. E., Ambudkar, S. V., Gottesman, M. M., & Kimchi-Sarfaty, C. (2009). Silent (synonymous) SNPs: should we care about them? *Methods in molecular biology (Clifton, N.J.), 578*, 23-39. doi: 10.1007/978-1-60327-411-1_2

Iacono, W. G., Carlson, S. R., Taylor, J., Elkins, I. J., & McGue, M. (1999). Behavioral disinhibition and the development of substance-use disorders: findings from the Minnesota Twin Family Study. *Development and psychopathology, 11*(4), 869-900.

Iacono, W. G., Malone, S. M., & McGue, M. (2003). Substance use disorders, externalizing psychopathology, and P300 event-related potential amplitude. *International journal of psychophysiology : official journal of the International Organization of Psychophysiology, 48*(2), 147-178.

Iacono, W. G., McGue, M., & Krueger, R. F. (2006). Minnesota Center for Twin and Family Research. *Twin research and human genetics : the official journal of the International Society for Twin Studies, 9*(6), 978-984. doi: 10.1375/183242706779462642

International Schizophrenia Consortium, Purcell, S. M., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., . . . Sklar, P. (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature, 460*(7256), 748-752. doi: 10.1038/nature08185

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. doi: 10.1017/S0954579407000612

Jacob, T., Waterman, B., Heath, A., True, W., Bucholz, K. K., Haber, R., . . . Fu, Q. (2003). Genetic and environmental effects on offspring alcoholism: new insights using an offspring-of-twins design. *Archives of general psychiatry, 60*(12), 1265-1272. doi: 10.1001/archpsyc.60.12.1265

Johnson, A. D., Handsaker, R. E., Pulit, S. L., Nizzari, M. M., O'Donnell, C. J., & de Bakker, P. I. (2008). SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics (Oxford, England), 24*(24), 2938-2939. doi: 10.1093/bioinformatics/btn564

Kanehisa, M. (1996). Toward pathway engineering: A new database of genetic and molecular pathways. *Science & Technology Japan, 59*, 34-38.

Kendler, K. S., Chen, X., Dick, D., Maes, H., Gillespie, N., Neale, M. C., & Riley, B. (2012). Recent advances in the genetic epidemiology and molecular genetics of substance use disorders. *Nature neuroscience, 15*(2), 181-189. doi: 10.1038/nn.3018

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. doi: 10.1001/archpsyc.65.6.674

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. doi: 10.4306/pi.2010.7.4.270

Koob, G. F. (2006). A role for GABA in alcohol dependence. *Advances in pharmacology (San Diego, Calif.), 54*, 205-229.

Koopmans, J. R., & Boomsma, D. I. (1996). Familial resemblances in alcohol use: genetic or cultural transmission? *Journal of studies on alcohol, 57*(1), 19-28.

Krueger, R. F., Hicks, B. M., Patrick, C. J., Carlson, S. R., Iacono, W. G., & McGue, M. (2002). Etiologic connections among substance dependence, antisocial behavior, and personality: modeling the externalizing spectrum. *Journal of Abnormal Psychology, 111*(3), 411-424.

Kumar, S., Porcu, P., Werner, D. F., Matthews, D. B., Diaz-Granados, J. L., Helfand, R. S., & Morrow, A. L. (2009). The role of $GABA_A$ receptors in the acute and chronic effects of ethanol: A decade of progress. *Psychopharmacology, 205*, 529-564.

Lappalainen, J., Krupitsky, E., Remizov, M., Pchelina, S., Taraskina, A., Zvartau, E., . . . Kranzler, H. R. (2005). Association between alcoholism and γ□□□□□□□□□□□□□□□□□□□□α□□□□□□□□□□□□□□□□□□□□□ □□□□□□□□□□□□□□□□□□□□□□. *Alcoholism: Clinical and Experimental Research, 29*(4), 493-498.

Lee, H. C., Lee, H. S., Jung, S. H., Yi, S. Y., Jung, H. K., Yoon, J. H., & Kim, C. Y. (2001). Association between polymorphisms of ethanol-metabolizing enzymes and susceptibility to alcoholic cirrhosis in a Korean male population. *Journal of*

Korean Medical Science, 16(6), 745-750. doi: 200112745 [pii]

Li, H., Borinskaya, S., Yoshimura, K., Kal'ina, N., Marusin, A., Stepanov, V. A., . . . Kidd, K. K. (2009). Refined geographic distribution of the oriental ALDH2*504Lys (nee 487Lys) variant. *Annals of human genetics, 73*(Pt 3), 335-345. doi: 10.1111/j.1469-1809.2009.00517.x

Li, X., Basu, S., Miller, M. B., Iacono, W. G., & McGue, M. (2011). A rapid generalized least squares model for a genome-wide quantitative trait association analysis in families. *Human heredity, 71*(1), 67-82. doi: 10.1159/000324839

Liang, K. Y., & Zeger, S. L. (1986). Longitudinal data analysis using generalized linear models. *Biometrika, 73*.

Lind, P. A., Macgregor, S., Agrawal, A., Montgomery, G. W., Heath, A. C., Martin, N. G., & Whitfield, J. B. (2008a). The role of GABRA2 in alchol dependence, smoking, and illicit drug use in an Australian population sample. *Alcoholism: Clinical and Experimental Research, 32*(10), 1721-1731.

Lind, P. A., Macgregor, S., Montgomery, G. W., Heath, A. C., Martin, N. G., & Whitfield, J. B. (2008b). Effects of GABRA2 variation on physiological, psychomotor and subjective responses in the alcohol challenge twin study. *Twin Research and Human Genetics, 11*(2), 174-182.

Liu, J. Z., McRae, A. F., Nyholt, D. R., Medland, S. E., Wray, N. R., Brown, K. M., . . . Macgregor, S. (2010). A versatile gene-based test for genome-wide association studies. *American journal of human genetics, 87*(1), 139-145. doi: 10.1016/j.ajhg.2010.06.009

Lobo, I. A., & Harris, R. A. (2008). GABA(A) receptors and alcohol. *Pharmacology, biochemistry, and behavior, 90*(1), 90-94. doi: 10.1016/j.pbb.2008.03.006

Loh, E., Smith, I., Murray, R., McLaughlin, M., McNulty, S., & Ball, D. (1999). Association between variants at the $GABA_A\beta 2$, $GABA_A\alpha 6$ and $GABA_A\gamma 2$ gene cluster and alcohol dependence in a Scottish population. *Molecular Psychiatry, 4*, 539-544.

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

Luczak, S. E., Wall, T. L., Cook, T. A., Shea, S. H., & Carr, L. G. (2004). ALDH2 status and conduct disorder mediate the relationship between ethnicity and alcohol dependence in Chinese, Korean, and White American college students. *Journal of Abnormal Psychology, 113*(2), 271-278. doi: 10.1037/0021-843X.113.2.271

Lynskey, M. T., Agrawal, A., & Heath, A. C. (2010). Genetically informative research on adolescent substance use: methods, findings, and challenges. *Journal of the American Academy of Child and Adolescent Psychiatry, 49*(12), 1202-1214. doi: 10.1016/j.jaac.2010.09.004

Matthews, A. G., Hoffman, E. K., Zezza, N., Stiffler, S., & Hill, S. Y. (2007). The role of the GABRA2 polymorphism in multiplex alcohol dependence families with minimal comorbidity: Within-family association and linkage analyses. *Journal of Studies on Alcohol and Drugs, 68*, 625-633.

McGue, M. (1999). The behavioral genetics of alcoholism. *Current Directions in*

Psychological Science, 8(4), 109-115.

McGue, M., & Iacono, W. G. (2008). The adolescent origins of substance use disorders. *International journal of methods in psychiatric research, 17 Suppl 1*, S30-38. doi: 10.1002/mpr.242

McGue, M., Iacono, W. G., & Krueger, R. (2006). The association of early adolescent problem behavior and adult psychopathology: a multivariate behavioral genetic perspective. *Behavior genetics, 36*(4), 591-602. doi: 10.1007/s10519-006-9061-z

McGue, M., Iacono, W. G., Legrand, L. N., & Elkins, I. (2001). Origins and consequences of age at first drink. II. Familial risk and heritability. *Alcoholism, clinical and experimental research, 25*(8), 1166-1173.

McGue, M., Iacono, W. G., Legrand, L. N., Malone, S., & Elkins, I. (2001). Origins and consequences of age at first drink. I. Associations with substance-use disorders, disinhibitory behavior and psychopathology, and P3 amplitude. *Alcoholism, clinical and experimental research, 25*(8), 1156-1165.

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. doi: 10.1007/s10519-007-9142-7

McGue, M., Osler, M., & Christensen, K. (2010). Causal Inference and Observational Research: The Utility of Twins. *Perspectives on psychological science : a journal of the Association for Psychological Science, 5*(5), 546-556. doi: 10.1177/1745691610383511

McGue, M., Sharma, A., & Benson, P. (1996). Parent and sibling influences on adolescent alcohol use and misuse: evidence from a U.S. adoption cohort. *Journal of Studies on Alcohol and Drugs, 57*(1), 8-18.

Meera, P., Olsen, R. W., Otis, T. S., & Wallner, M. (2010). Alcohol- and alcohol antagonist-sensitive human GABAA receptors: tracking delta subunit incorporation into functional receptors. *Molecular pharmacology, 78*(5), 918-924. doi: 10.1124/mol.109.062687

Meguro, M., Mitsuya, K., Sui, H., Shigenami, K., Kugoh, H., Nakao, M., & Oshimura, M. (1997). Evidence for uniparental, paternal expression of the human GABAA receptor subunit genes, using microcell-mediated chromosome transfer. *Human molecular genetics, 6*(12), 2127-2133.

Miller, M. B., Basu, S., Cunningham, J., Eskin, E., Malone, S. M., Oetting, W., . . . McGue, M. (2012). The Minnesota Center for Twin and Family Research genome-wide association study.

Moore, A. A., Gould, R., Reuben, D. B., Greendale, G. A., Carter, M. K., Zhou, K., & Karlamangla, A. (2005). Longitudinal patterns and predictors of alcohol consumption in the United States. *American journal of public health, 95*(3), 458-465. doi: 10.2105/AJPH.2003.019471

Morgan, S. L., & Winship, C. (2007). *Counterfactuals and Causal Inference*. Cambridge: Cambridge University Press.

Odgers, C. L., Caspi, A., Nagin, D. S., Piquero, A. R., Slutske, W. S., Milne, B. J., . . . Moffitt, T. E. (2008). Is it important to prevent early exposure to drugs and

alcohol among adolescents? *Psychological science, 19*(10), 1037-1044. doi: 10.1111/j.1467-9280.2008.02196.x

Olsen, R. W., Hanchar, H. J., Meera, P., & Wallner, M. (2007). GABAA receptor subtypes: the "one glass of wine" receptors. *Alcohol, 41*, 201-209.

Onori, N., Turchi, C., Solito, G., Gesuita, R., Buscemi, L., & Tagliabracci, A. (2010). GABRA2 and alcohol use disorders: no evidence of an association in an Italian case-control study. *Alcoholism, clinical and experimental research, 34*(4), 659-668. doi: 10.1111/j.1530-0277.2009.01135.x

Pagan, J. L., Rose, R. J., Viken, R. J., Pulkkinen, L., Kaprio, J., & Dick, D. M. (2006). Genetic and environmental influences on stages of alcohol use across adolescence and into young adulthood. *Behavior genetics, 36*(4), 483-497. doi: 10.1007/s10519-006-9062-y

Pan, W. (2001). Akaike's information criterion in generalized estimating equations. *Biometrics, 57*(1), 120-125.

Penninkilampi-Kerola, V., Kaprio, J., Moilanen, I., & Rose, R. J. (2005). Co-twin dependence modifies heritability of abstinence and alcohol use: a population-based study of Finnish twins. *Twin research and human genetics : the official journal of the International Society for Twin Studies, 8*(3), 232-244. doi: 10.1375/1832427054253095

Petersen, A., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: Reliability, validity, and initial norms. *Journal of Youth and Adolescence, 17*(2), 117-133.

Prescott, C. A., & Kendler, K. S. (1999). Age at first drink and risk for alcoholism: a noncausal association. *Alcoholism, clinical and experimental research, 23*(1), 101-107.

Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., & Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics, 38*(8), 904-909. doi: 10.1038/ng1847

Quertemont, E., & Didone, V. (2006). Role of acetaldehyde in mediating the pharmacological and behavioral effects of alcohol. *Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism, 29*(4), 258-265.

Reich, W., & Welner, Z. (1988). *Diagnostic Interview for Children and Adolescents - Revised: DSM-III-R version (DICA-R)*. St. Louis: Washington University.

Robins, L. M., Babor, T., & Cottler, L. B. (1987). *Composite International Diagnostic Interview; Expanded Substance Abuse Module*. St. Louis: Authors.

Robins, L. M., Wing, J., Wittchen, H. U., Babor, T. F., Burke, J., Famer, A., . . . Towle, L. H. (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*, 1069-1077.

Robins, L. N., Babor, T., & Cottler, L. B. (1987). *Composite International Diagnostic Interview: Expanded Substance Abuse Module*. St. Louis.

Robins, L. N., Wing, J., Wittchen, H. U., Helzer, J. E., Babor, T. F., Burke, J., . . . et al. (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.

Robins, L. N., Wing, J., Wittchen, H. U., Helzer, J. E., Babor, T. F., Burke, J., . . . Towle, L. H. (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.

Rose, R. J., & Dick, D. M. (2005). Gene-environment interplay in adolescent drinking behavior. *Alcohol Reasearch & Health, 28*(4), 222-229.

Rose, R. J., & Dick, D. M. (2010). Commentary on Agrawal et al. (2010): Social environments modulate alcohol use. *Addiction, 105*(10), 1854-1855.

Rosenbaum, P. R., & Rubin, D. B. (1983). The central role of the propensity score in observational studies for causal effects. *Biometrika, 70*(1), 41-55.

Rubin, D. B. (1987). *Multiple Imputation for Nonresponse in Surveys*. New York: J. Wiley & Sons.

Rubin, D. B., & Thomas, N. (1996). Matching using estimated propensity scores: relating theory to practice. *Biometrics, 52*(1), 249-264.

Saccone, S. F., Bierut, L. J., Chesler, E. J., Kalivas, P. W., Lerman, C., Saccone, N. L., . . . Rutter, J. L. (2009). Supplementing high-density SNP microarrays for additional coverage of disease-related genes: addiction as a paradigm. *PloS one, 4*(4), e5225. doi: 10.1371/journal.pone.0005225

Sander, T., Samochowiec, J., Ladehoff, M., Smolka, M., Peters, C., Riess, O., . . .

Schmidt, L. G. (1999). Association analysis of exonic variants of the gene encoding the GABAB receptor and alcohol dependence. *Psychiatric genetics, 9*(2), 69-73.

Sanna, A., Congeddu, E., Saba, L., Porcella, A., Marchese, G., Ruiu, S., . . . Pani, L. (2004). The cerebellar $GABA_A$ α_6 subunit is differentially modulated by chronic ethanol exposure in normal (R100R) and mutated (Q100Q) sNP rats. *Brain Research, 998*, 148-154.

Sartor, C. E., Lynskey, M. T., Bucholz, K. K., Madden, P. A., Martin, N. G., & Heath, A. C. (2009). Timing of first alcohol use and alcohol dependence: evidence of common genetic influences. *Addiction (Abingdon, England), 104*(9), 1512-1518. doi: 10.1111/j.1360-0443.2009.02648.x

Sartor, C. E., Lynskey, M. T., Heath, A. C., Jacob, T., & True, W. (2007). The role of childhood risk factors in initiation of alcohol use and progression to alcohol dependence. *Addiction (Abingdon, England), 102*(2), 216-225. doi: 10.1111/j.1360-0443.2006.01661.x

Sher, K. J. (1991). Psychological characteristics of children of alcoholics. Overview of research methods and findings. *Recent developments in alcoholism : an official publication of the American Medical Society on Alcoholism, the Research Society on Alcoholism, and the National Council on Alcoholism, 9*, 301-326.

Sher, K. J. (2007). Road to alcohol dependence: a commentary on Sartor et al. (2007). *Addiction (Abingdon, England), 102*(2), 185-187; discussion 189-190. doi: 10.1111/j.1360-0443.2006.01735.x

Sher, K. J., Grekin, E. R., & Williams, N. A. (2005). The development of alcohol use disorders. *Annual review of clinical psychology, 1*, 493-523. doi: 10.1146/annurev.clinpsy.1.102803.144107

Sigvardsson, S., Bohman, M., & Cloninger, C. R. (1996). Replication of the Stockholm Adoption Study of alcoholism. Confirmatory cross-fostering analysis. *Archives of general psychiatry, 53*(8), 681-687.

Singer, J. D. (1998). Using SAS PROC MIXED to fit multilevel models, hierarchical models, and individual growth models. *Journal of Educational and Behavioral Statistics, 24*, 323-355.

Slutske, W. S., True, W. R., Scherrer, J. F., Heath, A. C., Bucholz, K. K., Eisen, S. A., . . . Tsuang, M. T. (1999). The heritability of alcoholism symptoms: "indicators of genetic and environmental influence in alcohol-dependent individuals" revisited. *Alcoholism, clinical and experimental research, 23*(5), 759-769.

Song, J., Koller, D. L., Foroud, T., Carr, K., Zhao, J., Rice, J., . . . Edenberg, H. J. (2003). Association of $GABA_A$ receptors and alcohol dependence and the effects of genetic imprinting. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics), 117B*, 39-45.

Spanagel, R. (2009). Alcoholism: a systems approach from molecular physiology to addictive behavior. *Physiological reviews, 89*(2), 649-705. doi: 10.1152/physrev.00013.2008

Spear, L. P. (2000). Neurobehavioral changes in adolescence. *Current Directions in Psychological Science, 9*(4), 111-114.

Stanek, K. C., Iacono, W. G., & McGue, M. (2011). Returns to education: what do twin studies control? *Twin research and human genetics : the official journal of the International Society for Twin Studies, 14*(6), 509-515.

Substance Abuse and Mental Health Services Administration. (2010). State estimates of substance use from the 2007-2008 national surveys on drug use and health (Vol. NSDUH Series H-37). Rockville, MD: Office of Applied Studies.

Uusi-Oukari, M., Heikkila, J., Sinkkonen, S. T., Makela, R., Hauer, B., Homanics, G. E., . . . Korpi, E. R. (2000). Long-range interactions in neuronal gene expression: Evidence from gene targeting in the $GABA_A$ receptor β2-α6-α1-γ2 subunit gene cluster. *Molecular and Cellular Neuroscience, 16*, 34-41.

van der Zwaluw, C. S., & Engels, R. C. (2009). Gene-environment interactions and alcohol use and dependence: current status and future challenges. *Addiction, 104*(6), 907-914. doi: 10.1111/j.1360-0443.2009.02563.x

VanderWeele, T. (2006). The use of propensity score methods in psychiatric research. *International journal of methods in psychiatric research, 15*(2), 95-103. doi: 10.1002/mpr.183

Vaske, J., Beaver, K. M., Wright, J. P., Boisvert, D., & Schnupp, R. (2009). An interaction between DAT1 and having an alcoholic father predicts serious alcohol problems in a sample of males. *Drug and alcohol dependence, 104*(1-2), 17-22. doi: 10.1016/j.drugalcdep.2009.01.020

Viken, R. J., Kaprio, J., Koskenvuo, M., & Rose, R. J. (1999). Longitudinal analyses of the determinants of drinking and of drinking to intoxication in adolescent twins.

Behavior genetics, 29(6), 455-461.

Visscher, P. M., Yang, J., & Goddard, M. E. (2010). A commentary on 'common SNPs explain a large proportion of the heritability for human height' by Yang et al. (2010). *Twin research and human genetics : the official journal of the International Society for Twin Studies, 13*(6), 517-524. doi: 10.1375/twin.13.6.517

Walden, B., Iacono, W. G., & McGue, M. (2007). Trajectories of change in adolescent substance use and symptomatology: impact of paternal and maternal substance use disorders. *Psychology of addictive behaviors : journal of the Society of Psychologists in Addictive Behaviors, 21*(1), 35-43. doi: 10.1037/0893-164X.21.1.35

Walden, B., McGue, M., Iacono, W. G., Burt, S. A., & Elkins, I. (2004). Identifying shared environmental contributions to early substance use: the respective roles of peers and parents. *Journal of Abnormal Psychology, 113*(3), 440-450. doi: 10.1037/0021-843X.113.3.440

Wall, T. L., Peterson, C. M., Peterson, K. P., Johnson, M. L., Thomasson, H. R., Cole, M., & Ehlers, C. L. (1997). Alcohol metabolism in Asian-American men with genetic polymorphisms of aldehyde dehydrogenase. *Annals of Internal Medicine, 127*(5), 376-379.

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.

Wechsler, D. (1974). *Wechsler Intelligence Scale for Children-Revised*. San Antonio: Psychological Corporation.

Wechsler, D. (1981). *Wechsler Adult Intelligence Scale-Revised*. San Antonio: Psychological Corporation.

Winterer, G., Smolka, M., Samochowiec, J., Mulert, C., Ziller, M., Mahlberg, R., . . . Gallinat, J. (2000). Association analysis of $GABA_A$ β_2 and γ_2 gene polymorphisms with event-related prefrontal activity in man. *Human Genetics, 107*, 513-518.

World Health Organization. (2011). *Global status report on alcohol and health*. Geneva: WHO Press.

Yang, J., Ferreira, T., Morris, A. P., Medland, S. E., Madden, P. A., Heath, A. C., . . . Visscher, P. M. (2012). Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nature genetics, 44*(4), 369-375, S361-363. doi: 10.1038/ng.2213

Yang, J., Lee, S. H., Goddard, M. E., & Visscher, P. M. (2011). GCTA: a tool for genome-wide complex trait analysis. *American journal of human genetics, 88*(1), 76-82. doi: 10.1016/j.ajhg.2010.11.011

Yoshida, A., Huang, I. Y., & Ikawa, M. (1984). Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. *Proceedings of the National Academy of Sciences of the United States of America, 81*(1), 258-261.

Zeigler, D. W., Wang, C. C., Yoast, R. A., Dickinson, B. D., McCaffree, M. A., Robinowitz, C. B., & Sterling, M. L. (2005). The neurocognitive effects of

alcohol on adolescents and college students. *Preventive medicine, 40*(1), 23-32. doi: 10.1016/j.ypmed.2004.04.044

Zintzaras, E., Stefanidis, I., Santos, M., & Vidal, F. (2006). Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? *Hepatology, 43*(2), 352-361. doi: 10.1002/hep.21023