Published in final edited form as:
Alcohol Clin Exp Res. 2014 November ; 38(11): 2869–2877. doi:10.1111/acer.12560.

Genetic Influences on Alcohol Use Behaviors Have Diverging Developmental Trajectories: A Prospective Study among Male and Female Twins

Jacquelyn L. Meyers, Ph. D.[1], **Jessica E. Salvatore, Ph.D.**[2], **Eero Vuoksimaa, Ph.D.**[3], **Tellervo Korhonen, Ph.D.**[3,6,7], **Lea Pulkkinen, Ph.D.**[4], **Richard J. Rose, Ph.D.**[5], **Jaakko Kaprio, M.D., Ph.D.**[3,6], and **Danielle M. Dick, Ph.D.**[2]

[1]Columbia University Medical Center, New York [2]Virginia Commonwealth University, Richmond [3]University of Helsinki, Helsinki, Finland [4]University of Jyväskylä, Jyväskylä,Finland [5]Indiana University, Bloomington, Indiana [6]National Institute for Health and Welfare, Helsinki Finland [7]University of Eastern Finland, Kuopio, Finland

Abstract

Background—Both alcohol-specific genetic factors and genetic factors related to externalizing behavior influence problematic alcohol use. Little is known, however, about the etiologic role of these two components of genetic risk on alcohol-related behaviors across development. Prior studies conducted in a male cohort of twins suggest that externalizing genetic factors are important for predicting heavy alcohol use in adolescence, whereas alcohol-specific genetic factors increase in importance during the transition to adulthood. In this report, we studied twin brothers and sisters and brother-sister twin pairs to examine such developmental trajectories and investigate whether sex and co-twin sex effects modify these genetic influences.

Methods—We used prospective, longitudinal twin data collected between ages 12 and 22 within the population-based *FinnTwin12* cohort study (analytic n=1,864). Our dependent measures of alcohol use behaviors included alcohol initiation (age 12), intoxication frequency (ages 14 and 17), and alcohol dependence criteria (age 22). Each individual's genetic risk for alcohol use disorders (AUD-GR) was indexed by his/her parents' and co-twin's DSM-IV Alcohol Dependence (AD) criterion counts. Likewise, each individual's genetic risk for externalizing disorders (EXT-GR) was indexed with a composite measure of parents' and co-twin's DSM-IV Conduct Disorder and Antisocial Personality Disorder criterion counts.

Results—EXT-GR was most strongly related to alcohol use behaviors during adolescence while AUD-GR was most strongly related to alcohol problems in young adulthood. Further, sex of the twin and sex of the co-twin significantly moderated the associations between genetic risk and alcohol use behaviors across development: AUD-GR influenced early adolescent alcohol use behaviors in females more than in males, and EXT-GR influenced age 22 AD more in males than

Corresponding Author Jacquelyn L. Meyers, Ph.D. Columbia University Medical Center Department of Epidemiology 722 West 168th Street, 720 New York, New York 10032 Phone: 212-305-9412 Fax: 212-342-5168 jlm2261@columbia.edu.

in females. In addition, the associations of AUD-GR and EXT-GR with intoxication frequency were greater among 14 and 17 year old females with twin brothers.

Conclusions—We found divergent developmental trajectories for alcohol-specific and externalizing behavior-related genetic influences on alcohol use behaviors; in early adolescence, genetic influences on alcohol use behaviors are largely non-specific, and later in adolescence and young adulthood, alcohol specific genetic influences on alcohol use are more influential. Importantly, within these overall trajectories, several interesting sex differences emerged. We found that the relationship between genetic risk and problematic drinking across development is moderated by the individual's sex and his/her co-twin's sex. AUD-GR influenced adolescent alcohol outcomes in females more than in males and by age 22, EXT-GR influenced AD criteria more for males than females. In addition, the association between genetic risk and intoxication frequency was greater among 14 and 17 year old females with male co-twins.

Keywords

Developmental Twin study; Adolescence; Sex Differences; Alcohol Dependence; Externalizing

Introduction

Adolescence is typically the period of development when alcohol use is initiated and regular patterns of use are established (Swendsen et al., 2012). This period is characterized by rapid transitions in the degree to which individual differences in alcohol use is attributed to genetic or environmental factors, with environmental factors predominating in early adolescence, while genetic factors increase in importance over time (Dick et al. 2007; Kendler et al. 2008; Viken et al. 1999). Both alcohol-specific genetic factors (Kendler et al. 2007; Kendler et al. 2003; Macgregor et al. 2009; Hicks et al. 2004) and non-specific genetic factors that impact general externalizing behavior influence alcohol consumption and the risk for developing alcohol use disorders across adolescence into adulthood (Kendler et al. 2003; Krueger et al. 2002; Hicks et al. 2004). Although there is a substantial literature on the genetic influences on externalizing disorders in adolescence (Stallings et al. 2005; Stephens et al. 2012; Dick et al. 2009) and a separate literature on the genetic influences on alcohol use disorders in adulthood (Rietschel and Treutlein, 2013), little is known about the etiologic role of these two types of genetic risk on alcohol-related behaviors across development.

In a male cohort of the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (Kendler et al. 2006), Kendler and colleagues examined the influence of the two components of genetic risk across development (Kendler et al. 2011). Using retrospective reports of alcohol use across the lifespan, these authors found that the importance of non-specific genetic factors related to externalizing behavior in maximal alcohol consumption is greatest during early to mid-adolescence, peaking at ages 15–17 years and then declining slowly towards adulthood. In contrast, the influence of alcohol-specific genetic factors increased slowly through mid-adulthood. However, several important questions regarding the depth and limitations of these effects remain.

The first of these unanswered questions concerns potential sex differences. Rates of problematic drinking behavior are typically greater in males (Kendler et al. 2007), and exist even when rates of alcohol use do not differ between sexes. In related research, studies have reported sex differences in alcoholic subtypes, reporting an excess of women in internalizing subtypes and an excess of men in externalizing subtypes (Carpenter and Hasin, 2001; Epstein et al., 2002; Moss et al., 2007). Whether there are sex differences in the genetic influences on alcohol use disorders across development is less clear since studies conducted on one sex, or that examine mean levels of drinking collapsed across sexes, may mask these effects. Second, the men in Kendler's study made retrospective reports of their past drinking at a mean age of 40. Several studies suggest that there are important recall biases in self-reports of past drinking behavior (Engels et al. 1997.; Labouvie et al. 1997). Accordingly, examining these effects with prospective alcohol use data would circumvent the recall biases. Third, previous studies have also indicated that other aspects of familial transmission, including sibling relationships (Penninkilampi-Kerola et al., 2005) may modulate genetic influences on alcohol use behaviors, particularly in adolescence. Integrating these non-genetic family dynamics into models examining genetic risk for problematic alcohol use may elucidate important differences among those with same sex and opposite sex co-twins.

Finally, drinking cultures and related alcohol-control policies vary greatly both between and within countries, as well as over time. Thus, while overall rates of drinking problems are similar in Finland and the United States, there are important differences in drinking culture, and age of legal drinking (Bloomfield et al., 2010; Helasoja et al., 2007) which may impact how strongly genetic predispositions are expressed.

In the present study, we expanded the previous findings from Kendler et al. (2010) using longitudinal Finnish twin data from the population-based *FinnTwin12* cohort study. First, we sought to replicate previous findings in examining the impact of alcohol-specific and non-specific (general externalizing) genetic factors on alcohol use behaviors from early adolescence (age 12) through early adulthood (age 22) using prospective reports in both male and female twins from same-sex and opposite-sex twin pairs. However, our main goal was to extend this work by examining whether the influence of genetic risk for alcohol use behaviors was moderated by sex of the twin, given sex differences documented across alcohol use behaviors. Finally, our third goal was to examine whether the sex of the co-twin impacted genetic risk for alcohol use behaviors across development.

Methods

Sample

FinnTwin12 is a population based, longitudinal study that has followed five consecutive birth cohorts of twins born 1983-1987 identified through Finland's central population registry (n = 5,600 twins and 5,000 of their parents). The study was initially designed to examine genetic and environmental influences on health-related behaviors. Baseline questionnaire data were collected on twins just prior to their 12th birthdays. The twins were followed-up at ages 14.2, 17.5 years, and most recently at 22.2 years, on average (hereafter referred to as 14, 17, and 22 years). Zygosity was determined using a well-validated

questionnaire completed by both co-twins and their parent(s) at baseline (Rose et al. 2001) and later by DNA confirmation. Zygosity confirmation by genetic markers at wave four (age 22) revealed that 97% of same-sex pairs retained their original questionnaire zygosity classification (Knaapila et al., 2011).

Nested within this epidemiological sample is an intensive assessment of a subsample of 1,035 families (*n*= 2,070 individual twins), largely selected at random (72.3%, 748 families). The remainder of the subsample (27.7%, 287 families) was enriched with families where the participants have a family history of alcoholism based on baseline data. Details about the sub-sample have been described previously (Rose et al. 2001). We drew upon this subsample for the present analyses. In this subsample, both twins at age 14 and their biological parents were interviewed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA;(Bucholz et al. 1994)). The final sample included 1,852 interviewed twin individuals (51% male) and both of their parents when data was available (89%). Of these participants, 1,347 had follow-up SSAGA interviews completed at an average age of 22 years. The analytic study design and corresponding sample sizes are detailed in supplemental figure 1. Briefly, this subsample consisted of 34.7% monozygotic (MZ) twin pairs (50.5% female) and 65.3% dizygotic (DZ) twin pairs (47.9% female).

Measures

Alcohol use behaviors at ages 12, 14, 17, and 22—At age 12, participants were asked if they had ever used alcohol when they were not in the presence of an adult, coded 0 (no) or 1 (yes) (n = 1,864 individual twins). Alcohol use behavior was assessed at ages 14 and 17 by asking the participants how frequently they became intoxicated. On the age 14 questionnaire, the item included four response options: (1) Never, I don't drink alcohol; (2) Less than once a month; (3) About 1 to 2 times a month; and (4) Once or more a week. At age 14, a total of 1,852 participants responded to the item (1,463 of which had initiated alcohol use by age 14). On the age 17 questionnaire, the item included nine response options: (1) Daily; (2) A couple of times a week; (3) Once a week; (4) A couple of times a month; (5) About once a month; (6) About once every two months; (7) 2-4 times per year; (8); Once a year or less; (9) I don't drink any alcohol. The latter response options were collapsed into four categories to parallel the age 14 data; (1) Never, (2) Yearly, (3) Monthly, and (4) Weekly. At age 17, a total of 1,864 participants responded to the item (1,783 of which had initiated alcohol use by age 17). At age 22, the DSM-IV criteria (American Psychiatric Association 2004) for Alcohol Dependence, code 303.90 (AD) were collected on 1,347 participants (1,312 of which had initiated alcohol use by age 22). Scores totaling the number of criteria endorsed were computed (range 0-7). Throughout this manuscript, we describe the above variables as "alcohol use behaviors" for ease of presentation. We note, however, that the measure at age 12 is drinking initiation, at ages 14 and 17 is frequency of alcohol intoxication, and at age 22 is AD criteria.

Procedure

Calculation of the alcohol-specific genetic risk scores (AUD-GR)—Each participant's genetic risk for alcohol use disorders (AUDs) was indexed by his/her parents' and co-twin's alcohol dependence criterion counts (at age 22). The contribution of each

measure (parents' AD criterion count, and co-twin's AD criterion count) to the total AUD risk was based on a modified ridit score (Kendler et al. 2011). When data on both parents were available, the criterion count from the most severely affected parent (the father in 70% of families with data from both parents) was used in risk score calculation. In an effort to use the information gained from the twin study, scores from MZ co-twins were weighted twice as strongly as scores from DZ co-twins or parents. As this method equates the genetic relationship of parents and offspring and siblings, an additive model is assumed. AUD-GRs were computed on 1,312 participants assessed.

Calculation of the externalizing genetic risk scores (EXT-GR)—The non-specific genetic risk score for externalizing disorders was a composite measure of the parents' (at baseline) and co-twin's (at interview) criterion counts of Conduct Disorder (CD) (age 14) and Antisocial Personality Disorder (ASPD) (age 22). Throughout this manuscript, we will describe the adolescent antisocial criteria as CD and the adult criteria as Antisocial Behavior (ASB). As mentioned above, scores from monozygotic (MZ) co-twins were weighted twice as strongly as scores from dizygotic (DZ) co-twins or parents. Because this method equates the genetic relationship of parents and offspring and siblings, an additive model is assumed. EXT-GRs were computed on 1,312 participants.

Statistical Analysis

The distribution of the alcohol use behavior data was highly skewed. Preliminary analyses indicated that a log transformation was optimal for all variables, with the exception of the binary age 12 drinking outcome. The appropriate distribution for each outcome was determined using traditional fit statistics (Akaike's Information Criterion, difference in X^2) for the data modeled using normal, *Poisson*, negative binomial, and zero-inflated distributions. As the majority of participants had not yet initiated drinking at age 12, a zero-inflated negative binomial distribution fit the data best and was used to assess the association between the GR scores (i.e., AUD-GR and EXT-GR, separately) and age 12 initiation of drinking. A *Poisson* distribution best fit all other variables (age 14 and 17 frequency of intoxication and age 22 AD criteria) and was used to assess the association between the GR scores (i.e. AUD-GR and EXT-GR, separately) and age 14 frequency of intoxication, age 17 frequency of intoxication, and age 22 AD. The residual correlation within twin pairs was substantial and stronger in MZ twin pairs compared to DZ pairs; accordingly, regression models were run as hierarchical linear models using PROC MIXED and PROC GENMOD in SAS (SAS Institute, 2008), with twin pairs and individuals within twin pairs being treated as separate levels to account for the non-independence of twin-family data (Kendler et al. 2011). Because there were differences in mean levels of alcohol use, sex was used as a covariate in all analyses that collapsed data across males and females. To test for sibling influences on alcohol use behaviors, we examined the moderating effects of sex of the twin and sex of his/her co-twin. Accordingly, an ANOVA was carried out in SAS 9.3 (SAS Institute, 2008), with twin pairs and individuals within twin pairs being treated as separate levels. Further, differences between observed effects on alcohol use behavior outcomes across development, and by sex, were also examined using an ANOVA in SAS 9.3 (SAS Institute, 2008). We note that only those individuals who had initiated alcohol use were included in analyses of the alcohol use behaviors after age 12. Finally, all β

estimates presented throughout this manuscript have been standardized to allow for comparisons across models.

Results

Descriptive Statistics

Frequencies of alcohol use behaviors by sex are presented in Table 1. At age 12, most (93.2%) twins responded that they had never used alcohol outside the presence of an adult. At age 14, 35.0% had used alcohol, but of them, most (76.6%) reported that they had never been intoxicated. At age 17, 12% were not alcohol users. Among users, 9.1% of the sample reported that they had never been intoxicated, 48.4% less often than once a month, 34.4% about 1 or 2 times per month, and 8.2% once per week or more, respectively. At age 22, 2.7% (n=35) were abstainers, while of those who had ever used alcohol 19.1% endorsed no alcohol dependence criteria (M=1.09, SD=1.37), and 13.5% of the sample met the criteria for DSM-IV AD (3 or more AD symptoms).

Consistent with expectations for a population-based sample, the parents of the twins largely fell within sub-threshold ranges of AD criterion counts (range=0-7, M=1.03, SD=1.68), with 6.2% of the parents (10.0% of fathers, 4.0% of mothers) meeting the criteria for an AD diagnosis (3 or more AD criteria endorsed in a 12 month period). Of parents meeting AD criteria, more (57%) were fathers than mothers (43%). Further, there was a significant relationship between parents' AD criterion counts (Pearson correlation=0.32, $p<0.001$).

AUD-GR scores (based on parent and co-twin AD) ranged from 0-8 (M=1.16, SD=1.41). The majority of the parents were within sub-threshold ranges of CD criteria (range=0-7, M=0.65, SD=0.99), with 1.7% of the parents (2.7% of fathers, 0.93% of mothers) meeting the criteria for a CD diagnosis. Twins' CD criterion sum scores ranged from 0-8 (M=1.08, SD= 1.26), with 12.3% meeting CD diagnosis criteria. The majority of the twins' parents were within normative sub-threshold ranges of ASB (Range=0-6, M=1.12, SD=1.18), with <1% (n=26, 21 men) of the parents meeting the criteria for the adult portion of the ASPD diagnosis (ASB). There was no significant relationship between parents' ASB symptom counts. Twins' ASB sum scores ranged from 0-6 (M= 0.63, SD= 0.96), with 2.6% meeting criteria for the adult ASPD diagnosis. EXT-GR (based on parent and co-twin CD and ASB) scores ranged from 0-7 (M=0.87, SD=1.27).

Zero-order correlations among outcome variables are shown in Table 2. AUD-GR and EXT-GR were correlated at 0.38.

[Table 2. Correlations between Twins' Alcohol Problem Outcomes across Development]

Genetic Risk and Alcohol Use Behaviors across Development

The relationships between AUD-GR, EXT-GR, and the alcohol use behaviors over development are depicted in Figure 1 and detailed in Table 3 for the overall sample (i.e. males and females combined). Overall, the patterns for AUD-GR and EXT-GR differed significantly (p-value<0.001).

For alcohol use behaviors, the regression coefficient for EXT-GR was non-significant at age 12 (β= −0.016 [−0.035; 0.003], p-value=0.107), increased significantly (p<0.001) to a peak at age 14 (β=0.127 [0.078; 0.176], p-value<0.001), and had lower point estimates at ages 17 (β=0.063 [−0.002; 0.128], p-value=0.058) and 22 (β=0.078 [−0.030; 0.185], p-value=0.155); the differences between age 14 and later ages were non-significant. In contrast, AUD-GR started at a low value at age 12 (β= −0.018 [−0.033; −0.003], p-value=0.018) and then rose nominally from age 12-17, and reached a peak value at age 22 (β=0.190 [0.109; 0.271], p-value<0.001). Only the point estimates for ages 12 and 14 were significantly lower than the point estimates at ages 17 and 22 (p-values<0.001). [Table 3. Alcohol Specific and General Externalizing Genetic Risk Scores Predicting the Twins' Alcohol Use Behaviors across Development]

[Figure 1. Developmental Trajectories of Two Classes of Genetic Risk for Alcohol Use Behaviors]

Sex Effects

Next, we examined whether the relationship between AUD-GR, EXT-GR, and alcohol outcomes over development differed by sex. Overall the patterns for AUD-GR and EXTGR were different by sex (p-value<0.001).

In males, the regression coefficient for EXT-GR began low at age 12 (β= −0.005 [−0.037; 0.026], p-value=0.745), rose significantly (p-value<0.001) at age 14 (β=0.121 [0.050; 0.191], p-value<0.001), decreased nominally at age 17 (β= −0.021 [−0.120; 0.079], p-value=0.684), and then nominally rose to its peak at age 22 (β=0.166 [0.013; 0.319], p-value=0.034); the differences between age 14 and later ages were not significant. In contrast, AUD-GR started at a low value at age 12 (β=0.010 [−0.035; 0.015] p-value=0.435) and reached a peak value at age 22 (β=0.250 [0.131; 0.369], p-value<0.001); the differences between age 12 and age 22 were statistically significant (p-value<0.001), while other age comparisons were not significant. The relationship between AUD-GR, EXT-GR, and the alcohol use behaviors over development for males is depicted in Figure 2.

In females, the regression coefficient for EXT-GR began low at age 12 (β= −0.027 [−0.050; −0.004], p-value=0.024), rose to a peak at age 14 (β=0.134 [0.065; 0.202], p-value<0.001), and then decreased nominally from age 17 (β=0.126 [0.040; 0.212], p-value=0.004) through age 22 (β= −0.005 [−0.156; 0.147], p-value=0.953). Age 12 and age 22-point estimates were significantly different from age 14 and 17-point estimates only. In contrast, AUD-GR started at a similarly low value at age 12 (β= −0.025 [−0.041; −0.008], p-value=0.005) that increased significantly (p-value<0.001) at age 14 (β=0.075 [0.024; 0.126], p-value=0.004), and age 17 (β=0.130 [0.069; 0.191], p-value=0.001) and reached its peak at age 22 (β=0.140 [0.029; 0.251], p-value=0.014); the differences between age 14 and later ages were not significant. The relationship between AUD-GR, EXT-GR, and the alcohol use behaviors over development for females is depicted in Figure 2.

[Figure 2. Developmental Trajectories of Two Classes of Genetic Risk for Alcohol Use Behaviors by Sex]

Co-Twin Effects

We then examined whether drinking across development differed by co-twin sex. Among same sex twins, drinking outcomes did not differ by sex at ages 12 ($\beta = 0.005$, p=0.874), 14 ($\beta = 0.006$, p=0.794), 17 ($\beta = 0.041$, p=0.087), or 22 ($\beta = 0.005$, p=0.919). Among opposite sex twins, drinking outcomes differed by co-twin sex at ages 12 ($\beta = 0.124$, p<0.048) and 14 ($\beta = 0.133$, p<0.001), in that females with male co-twins were more likely to have initiated alcohol use (age 12) and drink more frequently (age 14) than males with female co-twins.

Next, we examined whether the associations between GR (AUD-GR and EXT-GR) and drinking outcomes were moderated by sex of the co-twin. The results indicated that among opposite-sex twins the relationships between GR (AUD-GR and EXT-GR) and frequency of intoxication at ages 14 and 17 were significantly moderated by co-twin sex (Table 4). Specifically, the association between GR and intoxication frequency was greater amongst females with male co-twins. These effects were not observed at age 12 or 22.

[Table 4. Co-twin Effects on the Alcohol Specific and General-Externalizing Genetic Risk Scores Predicting the Twins' Alcohol Use Behaviors]

Discussion

Previous studies have demonstrated that genetic influences on alcohol-related outcomes have both an alcohol-specific component and a general externalizing component of behavior (Kendler et al. 2003; Kendler et al. 2007). The relative importance of these two sets of genetic influences across development has been understudied. Further, differences in these developmental trajectories by sex remain unexamined. Using prospective reports and diagnostic interview data of alcohol use, AD, CD, and ASB from a population based, longitudinal sample of Finnish twins, our results indicate that specific and non-specific genetic influences on alcohol use behaviors have different development trajectories. Specifically, these results suggest a more robust prediction of alcohol outcomes (initiation of use at age 12 and frequency of intoxication at age 14) with genetic risk for externalizing behaviors earlier in adolescence (12-14), and in contrast, a more robust prediction of alcohol outcomes (frequency of intoxication at age 17 and AD symptoms at age 22) with genetic risk specific to alcohol use behaviors in later adolescence into young adulthood (17-22). Importantly, within these overall trajectories, several interesting sex differences emerged, effects that are masked when examining mean levels of male and female alcohol use behavior across development. We found that the relationship between genetic risk and problematic drinking across development is moderated by the individual's sex; AUD-GR influenced adolescent alcohol outcomes (i.e. early initiation of use, intoxication frequency) in females more than in males and by age 22, EXT-GR influenced AD criteria more for males than females. In addition, the association between genetic risk and intoxication frequency was greater among 14 and 17 year old females with male co-twins.

Results from this study converge with previous epidemiological (Kendler et al. 2011) and twin studies that indicate that in early adolescence, genetic influences on alcohol use behaviors are largely non-specific and may reflect largely adolescent-limited externalizing behaviors (Moffitt, 1993; Moffitt *et al.* 2002). Further, alcohol-specific genetic risk factors

become more important than non-specific (externalizing) genetic influences in early adulthood (Rose et al. 2003). Moreover, this shift in genetic influences maps onto typical developmental timing for the onset of serious alcohol problems (Schuckit et al., 1995). In this study, prospective reports from a population based, longitudinal sample of Finnish twins permitted several important comparisons to extend previous findings. The first is the age at which alcohol-specific genetic risk factors and externalizing genetic risk factors shift in their relative importance. The most dramatic shift in genetic influence on drinking frequency occurred at approximately age 21 in the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD;(Kendler et al. 2011)). However, this shift occurred earlier at an average age of 17.5 in the *FinnTwin12*. There are differences both in the legal drinking age and cultural norms regarding alcohol use between Finland and the United States (Bloomfield et al., 2010; Helasoja et al., 2007; Mäkelä et al., 2012). In Finland, the legal drinking age is 18, and underage drinking at age 17 is socially tolerated in the Finnish society. Conversely, the legal age to drink is 21 in the US, and greater measures are taken to prohibit underage drinking. However, the observed age differences in the shift in genetic influence may be attributable to recall biases in the retrospective reporting of past drinking by men at a mean age of 40.3 years [SD=9.0] in the VATSPSUD, whereas *FinnTwin12* reports were made prospectively. Additionally, the same measure of alcohol use behavior was used across development in the VATSPSUD, whereas different, but developmentally appropriate measures of problematic alcohol use behaviors were used in the present study, which could also contribute to observed differences across these studies.

Sex Effects

Because rates of problematic drinking behavior are typically greater in males, we examined whether sex differences moderated the relationship between AUD-GR, EXTGR, and alcohol outcomes across development. Overall, the relative influence of AUDGR and EXT-GR on alcohol use behaviors across development was maintained; in early adolescence, genetic influences on alcohol use and problems are largely non-specific and later in adolescence and young adulthood, alcohol specific genetic influences on alcohol use are more influential. However, within each of these trajectories, several interesting sex differences emerged. Most striking is the relatively early influence of AUD-GR on alcohol use behaviors in females. Twin studies have indicated that genetic influences on drinking frequency emerge in girls at a younger age than boys (Maes et al. 1999; Rose et al. 2001), which may be a result of increased early alcohol use, pubertal timing, and having a greater number of older friends who may provide access to drinking opportunities. Earlier access to alcohol and earlier evidence of heritability in drinking frequency may also be related to the earlier influence of alcohol specific risk for intoxication frequency in early adolescence. In addition, risk for alcohol dependence symptoms at age 22 for females is largely influenced by AUD-GR, with EXT-GR playing a minor role. Conversely, both AUD-GR and EXT-GR appear to substantively influence age 22 alcohol problems for males, with AUD-GR playing the larger role. Past studies have reported sex differences in alcoholic subtypes, including an excess of women in internalizing subtypes and an excess of men in externalizing subtypes (Carpenter and Hasin, 2001; Epstein et al., 2002; Moss et al., 2007). Findings from the present study support these sex differences given that alcohol dependence criteria are influenced more by EXT-GR for males than females.

Several studies examining the relative contributions of family and peer influences on adolescents' alcohol and other substance use have indicated that the magnitude of sibling influences is greater than that of parental influences (Ary et al., 1993; Fagan and Najman, 2003; Windle et al., 2009) and is similarly important to the influences of peers (Brook et al. 1990; Needle et al. 1986). Twin studies have demonstrated that an interdependent sibling relationship (reliance on co-twin) is an important modifier of drinking habits, and it appears to reduce the impact of inherited liabilities on alcohol-related behavior especially in adolescence (Penninkilampi-Kerola et al., 2005). A study of Australian twins found that females with an older brother reported greater intoxication frequency and lifetime AUD symptoms (Ellingson et al., 2013). Previous work in *FinnTwin12* found that twins from opposite-sex pairs were more likely to have initiated drinking at age 14 than age and cohort matched twins from same-sex pairs (Rose et al. 2001). Taken together, this literature suggests that aspects of the sibling relationship (e.g. sibling interdependence, opposite-sex sibling pairs) have effects on adolescent drinking. However, no study to our knowledge has examined whether sex of the co-twin has an independent effect on adolescent drinking. Therefore, we examined whether sex of the co-twin modified the influence on genetic risk on alcohol outcomes across development. Results indicated that the association between genetic risk (both AUD-GR and EXT-GR) and intoxication frequency was greater among 14 and 17 year old females with male co-twins. This effect was not observed in young adulthood, likely due to the decrease in interdependence and the increase of unique environmental influences that the twins begin to encounter at that age (Penninkilampi-Kerola et al., 2005).

These results should be interpreted in the context of several important limitations. First, hierarchical linear modeling was used rather than structural equations modeling in our analyses. We chose this method because it allowed us to easily incorporate and interpret data on parental psychopathology in our measures of genetic risk. Second, although the present study uses developmentally-appropriate problematic drinking measures across time, there were differences in how the question was posed to the subject and how the response options were made available, and both introduce the potential for measurement invariance. Further, each measure is confounded with age and/or period of development. Therefore, it is possible that non-specific genetic risk (EXT-GR) is important for initiation of drinking regardless of age/period of development. Likewise, it is possible that alcohol-specific genetic risk (AUD-GR) is important for AD regardless of age/developmental period, however risk for AD peaks later in development. We note that Kendler et al. (2011) examined the same maximal drinking (drinking frequency × drinking quantity) measure across development. Therefore, comparisons between the current study and the previous study (Kendler et al., 2011) should be interpreted with caution. As mentioned above, the low base rate of ASPD in the parent sample (<1% of parents met criteria for ASPD) was expected. However, this low base rate may affect the interpretation of these results in that the EXT-GR is largely influenced by the co-twin's ASPD criteria and less so by the parent's ASPD criteria. Finally, while internalizing disorders (e.g. anxiety and depression) clearly influence alcohol-related problems across adolescence and young adulthood (Wellman et al., 2014), evidence regarding the genetic influences shared amongst internalizing and alcohol-related behaviors is mixed, with a recent study finding evidence that the phenotypic comorbidity between

intoxication frequency and internalizing disorders among adolescence is due to shared environmental risk factors (Edwards et al., 2011). Therefore, we chose to focus on the established shared *genetic* covariance between substance use disorders and externalizing spectrum disorders.

In summary, the present study extends previous findings indicating that two components of genetic risk related to problematic alcohol use change across development. Results indicated that alcohol-specific genetic risk factors increase in importance across adolescence and are most important during early adulthood (age 22); in contrast, non-specific genetic influences related to externalizing behavior decrease in importance across this same developmental period and are most important during early adolescence (age 14). In an important extension of previous work on this topic, we found that the relationship between genetic risk and problematic drinking across development is moderated by the individual's sex and his/her co-twin's sex. AUD-GR influenced alcohol outcomes in females earlier in adolescence than in males and by age 22, AD criteria are influenced more by EXT-GR for males than females. In addition, the association between genetic risk and intoxication frequency was greater among 14 and 17 year old females with male co-twins. Together, these findings highlight the importance of assuming a developmental perspective on the role of genetic influences on alcohol use behaviors during adolescence and young adulthood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The FinnTwin studies have been supported by the National Institute of Alcohol Abuse and Alcoholism (grants AA-12502, AA-00145, and AA-09203 to RJR and AA15416 to DMD), the Academy of Finland (grants 141054, 265240, 263278 and, 264146 to JK), and the Academy of Finland Centre of Excellence Programme (to LP & JK). This work was also supported by K02AA018755 (DMD), T32MH20030-14 (JLM and JES), F32AA022269 (JES), and T32MH0200-30 and 2T32MH13043-41 (JLM) and the Academy of Finland grant 257075 (EV). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Reference List

Ary DV, Tildesley E, Hops H, Andrews J. The influence of parent, sibling, and peer modeling and attitudes on adolescent use of alcohol. The International journal of the addictions. 1993; 28:853–80. [PubMed: 8359945]

Association, A.P.. Diagnostic and Statistical Manual of Mental Disorders (4th ed.). 4th ed. Washington D.C: 2000.

Bloomfield K, Wicki M, Gustafsson N-K, Mäkelä P, Room R. Changes in alcohol-related problems after alcohol policy changes in Denmark, Finland, and Sweden. Journal of studies on alcohol and drugs. 2010; 71:32–40. [PubMed: 20105411]

Brook JS, Whiteman M, Gordon AS, Brook DW. The role of older brothers in younger brothers' drug use viewed in the context of parent and peer influences. The Journal of genetic psychology. 1990; 151:59–75. [PubMed: 2332760]

Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger JI, Reich T, Schmidt I, Schuckit MA. A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. Journal of studies on alcohol. 1994; 55:149–58. [PubMed: 8189735]

Carpenter KM, Hasin DS. Reliability and discriminant validity of the Type I/II and Type A/B alcoholic subtype classifications in untreated problem drinkers: a test of the Apollonian--Dionysian hypothesis. Drug and alcohol dependence. 2001; 63:51–67. [PubMed: 11297831]

Dick DM, Bernard M, Aliev F, Viken R, Pulkkinen L, Kaprio J, Rose RJ. The role of socioregional factors in moderating genetic influences on early adolescent behavior problems and alcohol use. Alcoholism: Clinical and Experimental Research. 2009; 33:1739–1748.

Dick DM, Pagan JL, Viken R, Purcell S, Kaprio J, Pulkkinen L, Rose RJ. Changing environmental influences on substance use across development. Twin research and human genetics the official journal of the International Society for Twin Studies. 2007; 10:315–326. [PubMed: 17564520]

Edwards AC, Larsson H, Lichtenstein P, Kendler KS. Early environmental influences contribute to covariation between internalizing symptoms and alcohol intoxication frequency across adolescence. Addictive behaviors. 2011; 36:175–82. [PubMed: 21051153]

Ellingson JM, Slutske WS, Richmond-Rakerd LS, Martin NG. Investigating the influence of prenatal androgen exposure and sibling effects on alcohol use and alcohol use disorder in females from opposite-sex twin pairs. Alcoholism, clinical and experimental research. 2013; 37:868–76.

Engels RC, Knibbe RA, Drop MJ. n.d. Inconsistencies in adolescents' self-reports of initiation of alcohol and tobacco use. Addictive behaviors. 22:613–23. [PubMed: 9347063]

Epstein EE, Labouvie, Erich, McCrady BS, Jensen NK, Hayaki J. A multi-site study of alcohol subtypes: classification and overlap of unidimensional and multi-dimensional typologies. Addiction (Abingdon, England). 2002; 97:1041–53.

Fagan AA, Najman JM. Sibling influences on adolescent delinquent behaviour: an Australian longitudinal study. Journal of adolescence. 2003; 26:547–59. [PubMed: 12972268]

Helasoja V, Lahelma E, Prättälä R, Petkeviciene J, Pudule I, Tekkel M. The sociodemographic patterning of drinking and binge drinking in Estonia, Latvia, Lithuania and Finland, 1994-2002. BMC public health. 2007; 7:241. [PubMed: 17854484]

Hicks BM, Krueger RF, Iacono WG, McGue M, Patrick CJ. Family transmission and heritability of externalizing disorders: a twin-family study. Archives of general psychiatry. 2004; 61:922–8. [PubMed: 15351771]

Kendler KS, Gardner C, Dick DM. Predicting alcohol consumption in adolescence from alcohol-specific and general externalizing genetic risk factors, key environmental exposures and their interaction. Psychological medicine. 2011; 41:1507–16. [PubMed: 20942993]

Kendler KS, Gardner CO, Prescott CA. Toward a comprehensive developmental model for major depression in men. The American journal of psychiatry. 2006; 163:115–24. [PubMed: 16390898]

Kendler KS, Myers J, Prescott CA. Specificity of genetic and environmental risk factors for symptoms of cannabis, cocaine, alcohol, caffeine, and nicotine dependence. Archives of General Psychiatry. 2007; 64:1313–1320. [PubMed: 17984400]

Kendler KS, Prescott CA, Myers J, Neale MC. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. Archives of general psychiatry. 2003; 60:929–37. [PubMed: 12963675]

Kendler KS, Schmitt E, Aggen SH, Prescott CA. Genetic and environmental influences on alcohol, caffeine, cannabis, and nicotine use from early adolescence to middle adulthood. Archives of general psychiatry. 2008; 65:674–82. [PubMed: 18519825]

Knaapila A, Silventoinen K, Broms U, Rose RJ, Perola M, Kaprio J, Tuorila HM. Food neophobia in young adults: genetic architecture and relation to personality, pleasantness and use frequency of foods, and body mass index--a twin study. Behavior genetics. 2011; 41:512–21. [PubMed: 20953688]

Krueger RF, Hicks BM, Patrick CJ, Carlson SR, Iacono WG, McGue M. Etiologic connections among substance dependence, antisocial behavior, and personality: modeling the externalizing spectrum. Journal of abnormal psychology. 2002; 111:411–24. [PubMed: 12150417]

Labouvie E, Bates ME, Pandina RJ. Age of first use: its reliability and predictive utility. Journal of studies on alcohol. 1997; 58:638–43. [PubMed: 9391924]

Macgregor S, Lind PA, Bucholz KK, Hansell NK, Madden PAF, Richter MM, Montgomery GW, Martin NG, Heath AC, Whitfield JB. Associations of ADH and ALDH2 gene variation with self

report alcohol reactions, consumption and dependence: an integrated analysis. Human molecular genetics. 2009; 18:580–93. [PubMed: 18996923]

Maes HH, Woodard CE, Murrelle L, Meyer JM, Silberg JL, Hewitt JK, Rutter M, Simonoff E, Pickles A, Carbonneau R, Neale MC, Eaves LJ. Tobacco, alcohol and drug use in eight- to sixteen-year-old twins: the Virginia Twin Study of Adolescent Behavioral Development. Journal of studies on alcohol. 1999; 60:293–305. [PubMed: 10371255]

Mäkelä P, Tigerstedt C, Mustonen H. The Finnish drinking culture: change and continuity in the past 40 years. Drug and alcohol review. 2012; 31:831–40. [PubMed: 22690900]

Moss HB, Chen CM, Yi H. Subtypes of alcohol dependence in a nationally representative sample. Drug and alcohol dependence. 2007; 91:149–58. [PubMed: 17597309]

Needle R, McCubbin H, Wilson M, Reineck R, Lazar A, Mederer H. Interpersonal influences in adolescent drug use--the role of older siblings, parents, and peers. The International journal of the addictions. 1986; 21:739–66. [PubMed: 3781689]

Penninkilampi-Kerola V, Kaprio J, Moilanen I, Rose RJ. 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. 2005; 8:232–44. [PubMed: 15989750]

Rietschel M, Treutlein J. The genetics of alcohol dependence. Annals of the New York Academy of Sciences. 2013; 1282:39–70. [PubMed: 23170934]

Rose RJ, Dick DM, Viken RJ, Kaprio J. Gene-environment interaction in patterns of adolescent drinking: regional residency moderates longitudinal influences on alcohol use. Alcoholism: Clinical and Experimental Research. 2001a; 25:637–643.

Rose RJ, Dick DM, Viken RJ, Pulkkinen L, Kaprio J. Drinking or abstaining at age 14? A genetic epidemiological study. Alcoholism, clinical and experimental research. 2001b; 25:1594–604.

Rose RJ, Viken RJ, Dick DM, Bates JE, Pulkkinen L, Kaprio J. It does take a village: nonfamilial environments and children's behavior. Psychological science. 2003; 14:273–7. [PubMed: 12741753]

Schuckit MA, Anthenelli RM, Bucholz KK, Hesselbrock VM, Tipp J. The time course of development of alcohol-related problems in men and women. Journal of studies on alcohol. 1995; 56:218–25. [PubMed: 7760569]

Stallings MC, Corley RP, Dennehey B, Hewitt, John K, Krauter KS, Lessem JM, Mikulich-Gilbertson SK, Rhee SH, Smolen A, Young SE, Crowley TJ. A genome-wide search for quantitative trait Loci that influence antisocial drug dependence in adolescence. Archives of general psychiatry. 2005; 62:1042–51. [PubMed: 16143736]

Stephens SH, Hoft NR, Schlaepfer IR, Young SE, Corley RC, McQueen MB, Hopfer C, Crowley T, Stallings M, Hewitt J, Ehringer MA. Externalizing behaviors are associated with SNPs in the CHRNA5/CHRNA3/CHRNB4 gene cluster. Behavior genetics. 2012; 42:402–14. [PubMed: 22042234]

Swendsen J, Burstein M, Case B, Conway KP, Dierker L, He J, Merikangas KR. Use and abuse of alcohol and illicit drugs in US adolescents: results of the National Comorbidity Survey-Adolescent Supplement. Archives of general psychiatry. 2012; 69:390–8. [PubMed: 22474107]

Viken RJ, Kaprio J, Koskenvuo M, Rose RJ. Longitudinal analyses of the determinants of drinking and of drinking to intoxication in adolescent twins. Behavior genetics. 1999; 29:455–61. [PubMed: 10857250]

Wellman RJ, Contreras GA, Dugas EN, O'Loughlin EK, O'Loughlin JL. Determinants of Sustained Binge Drinking in Young Adults. Alcoholism, clinical and experimental research. 2014

Windle M, Spear LP, Fuligni AJ, Angold A, Brown JD, Pine D, Smith GT, Giedd J, Dahl RE. Transitions into underage and problem drinking: summary of developmental processes and mechanisms: ages 10-15. Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism. 2009; 32:30–40. [PubMed: 23104445]

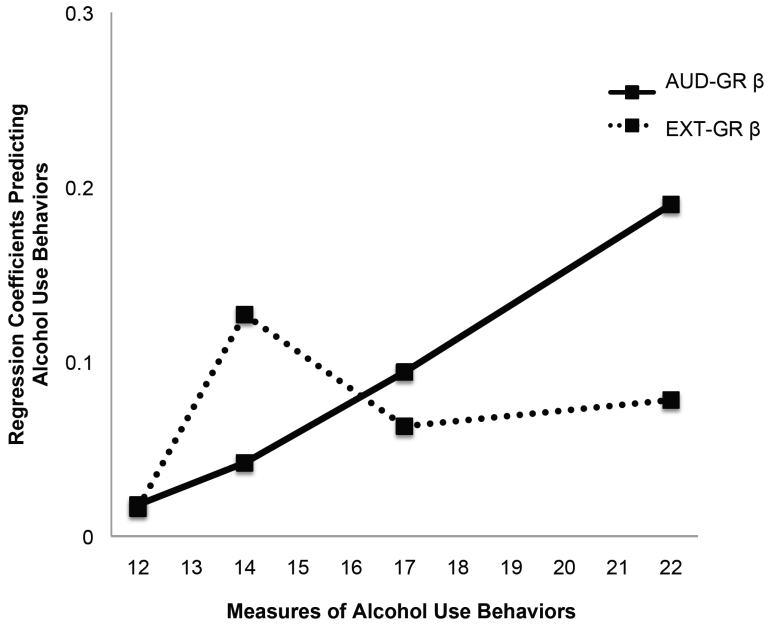

Figure 1.
Developmental Trajectories of Two Classes of Genetic Risk for Alcohol Use Behaviors

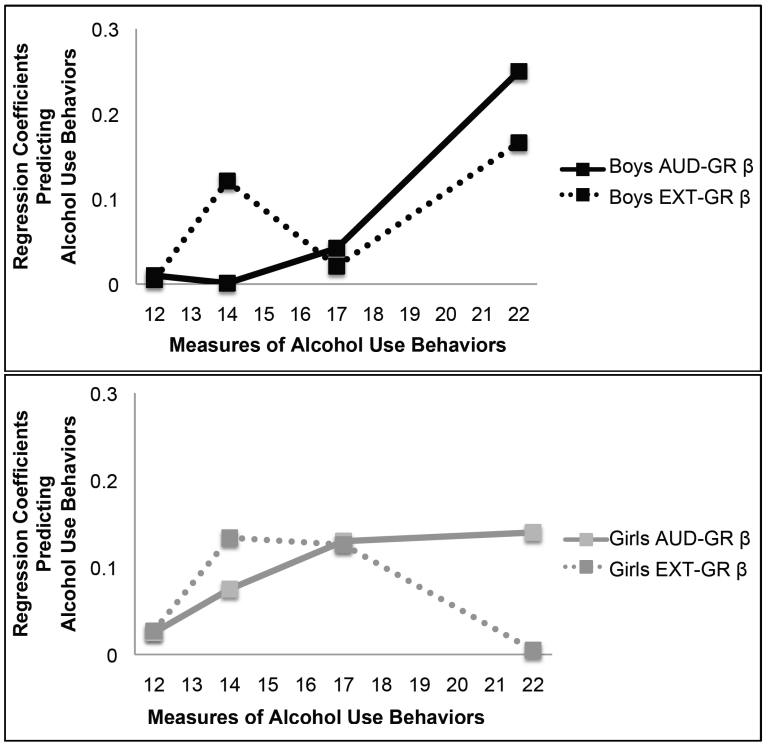

Figure 2.

Sex Differences in the Developmental Trajectories of Two Classes of Genetic Risk for Alcohol Use Behavior

Table 1

Participants' Alcohol Use Behaviors across Development by Sex

		Frequency (%) or Mean (Standard Deviation (SD))		
Alcohol Use Behavior	**Sample Size**	**All**	**Males**	**Females**
Age 12 Drinking Initiation (%)	1,864	6.8%	7.6%**	5.1%
"Age 14 Drinking Intoxication Frequency (Mean (SD))	1,463	2.3 (0.5)	2.2 (0.4)	2.4 (0.6)**
(1) Never, I don't drink alcohol (%)	--	33.6%	40.8%**	27.3%
(2) Less than once a month (%)	--	47.7%	45.4%	48.7%
(3) About 1 to 2 times a month (%)	--	16.3%	12.7%	20.7%**
(4) Once or more a week (%)	--	2.2%	1.2%	3.3%**
"Age 17 Drinking Intoxication Frequency (Mean (SD))	1,783	2.6 (0.7)	2.6 (0.7)	2.5(0.6)
(1) Never, I don't drink alcohol (%)	--	9.1%	8.6%	9.8%
(2) Less than once a month (%)	--	48.4%	44.9%	52.2%
(3) About 1 to 2 times a month (%)	--	34.4%	37.2%**	31.4%
(4) Once or more a week (%)	--	8.2%	9.4%**	6.8%
"Age 22 DSM-IV AD Symptom Sum Score (Mean (SD))	1,312	1.1 (1.4)	1.3 (1.4)**	0.9 (1.3)

** Significant sex differences ($p<0.05$);

"indicates that sample is restricted to those who have initiated alcohol use (i.e. abstainers excluded).

Table 2

Correlations between Participants' Alcohol Use Behaviors across Development

Pearson Correlations	**Measure of Alcohol Use Behavior**				
	N	**Age 12**	**Age 14**	**Age 17**	**Age 22**
Age 12 Drinking Initiation	1,864	-	0.203**	0.199**	0.155**
"Age 14 Drinking Intoxication Frequency	1,463	0.196**	-	0.259**	0.031
"Age 17 Drinking Intoxication Frequency	1,783	0.199**	0.300**	-	0.172**
"Age 22 DSM-IV AD Symptom Sum Score	1,312	0.030	0.075	0.132	-

** Note: Correlation is significant at p<0.01;

" indicates that sample is restricted to those who have initiated alcohol use (i.e. abstainers excluded); Correlations presented for males in the upper triangle and for females in the lower triangle

Table 3

Alcohol Specific and General Externalizing Genetic Risk Scores Predicting Participants' Alcohol Use Behaviors across Development

Alcohol Use Behaviors	***Sample Size***	***AUD-GR***		***EXT-GR***	
Males and Females	**N**	**β [95% C.I.]**	***p-value***	**β [95% C.I.]**	***p-value***
Age 12 Drinking Initiation	1,864	−0.018 [−0.033;−0.003]	0.018	−0.016 [−0.035; 0.003]	0.107
”Age 14 Drinking Intoxication Frequency	1,463	0.042 [0.003; 0.080]	0.034	0.127 [0.078; 0.176]	<0.001
”Age 17 Drinking Intoxication Frequency	1,783	0.094 [0.046; 0.142]	<.0001	0.063 [−0.002; 0.128]	0.058
”Age 22 DSM-IV AD Symptom Sum Score	1,312	0.190 [0.109; 0.271]	<.0001	0.078 [−0.030; 0.185]	0.155
Males		***AUD-GR***		***EXT-GR***	
Age 12 Drinking Initiation	998	0.010 [−0.035; 0.015]	0.435	−0.005 [−0.037; 0.026]	0.745
”Age 14 Drinking Intoxication Frequency	699	0.001 [−0.057; 0.059]	0.974	0.121 [0.050; 0.191]	<0.001
”Age 17 Drinking Intoxication Frequency	865	0.042 [−0.034; 0.117]	0.276	−0.021 [−0.120; 0.079]	0.684
”Age 22 DSM-IV AD Symptom Sum Score	627	0.250 [0.131; 0.369]	<0.001	0.166 [0.013; 0.319]	0.034
Females		***AUD-GR***		***EXT-GR***	
Age 12 Drinking Initiation	866	−0.025 [−0.041;−0.008]	0.005	−0.027 [−0.050; −0.004]	0.024
”Age 14 Drinking Intoxication Frequency	764	0.075 [0.024; 0.126]	0.004	0.134 [0.065; 0.202]	<0.001
”Age 17 Drinking Intoxication Frequency	918	0.130 [0.069; 0.191]	<.0001	0.126 [0.040; 0.212]	<0.004
”Age 22 DSM-IV AD Symptom Sum Score	685	0.140 [0.029; 0.251]	0.014	−0.005 [−0.156; 0.147]	0.953

”Note: indicates that sample is restricted to those who have initiated alcohol use (i.e. abstainers excluded); AUD-GR: alcoholspecific genetic risk score, indexed by his/her parents' and co-twin's alcohol dependence symptom counts; EXT-GR: externalizing genetic risk score, the non-specific genetic risk score for externalizing disorders was a composite measure of the parents' and cotwin's self-reported symptom count of Conduct Disorder (CD) (age 14) and Antisocial Personality Disorder (ASPD); AD: DSM-IV Alcohol Dependence symptom count.

Table 4

The Effects of Co-twin Sex on the Alcohol Specific and General-Externalizing Genetic Risk Scores Predicting Participants' Alcohol Use Behaviors

	Measure of Alcohol Use Behaviors				
	Sample Size	**Age 12 Drinking Initiation**	**"Age 14 Intoxication Frequency**	**"Age 17 Intoxication Frequency**	**"Age 22 DSM-IV AD Symptom Sum Score**
	(N)	**β**	**β**	**β**	**β**
AUD-GR					
Opposite-Sex twins with a:					
male co-twin	568	0.067	0.222*	0.361*	0.016
female co-twin	652	0.077	0.010	0.085	0.090
EXT-GR					
Opposite-Sex twins with a:					
male co-twin	568	0.047	0.208*	0.295*	0.146
female co-twin	652	0.089	0.069	0.122	0.051

*Note: indicates p<0.01;

"indicates that sample is restricted to those who have initiated alcohol use (i.e. abstainers excluded); AUDGR: alcohol-specific genetic risk score, indexed by his/her parents' and co-twin's alcohol dependence symptom counts; EXT-GR: externalizing genetic risk score, the non-specific genetic risk score for externalizing disorders was a composite measure of the parents' and co-twin's self-reported symptom count of Conduct Disorder (CD) (age 14) and Antisocial Personality Disorder (ASPD).