

Genetic influences on alcohol use across stages of development: *GABRA2* and longitudinal trajectories of drunkenness from adolescence to young adulthood

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ABSTRACT

Longitudinal analyses allow us to understand how genetic risk unfolds across development, in a way that is not possible with cross-sectional analyses of individuals at different ages. This has received little attention in genetic association analyses. In this study, we test for genetic effects of *GABRA2*, a gene previously associated with alcohol dependence, on trajectories of drunkenness from age 14 to 25. We use data from 1070 individuals who participated in the prospective sample of the Collaborative Study on the Genetics of Alcoholism, in order to better understand the unfolding of genetic risk across development. Piecewise linear growth models were fit to model the influence of genotype on rate of increase in drunkenness from early adolescence to young adulthood (14–18 years), the change in drunkenness during the transition to adulthood (18–19 years) and the rate of change in drunkenness across young adulthood (≥ 19 years). Variation in *GABRA2* was associated with an increase in drunkenness that occurred at the transition between adolescence and adulthood. The genotypic effect was more pronounced in females. These analyses illustrate the importance of longitudinal data to characterize how genetic effects unfold across development. The findings suggest that transitions across important developmental periods may alter the relative importance of genetic effects on patterns of alcohol use. The findings also suggest the importance of considering gender when evaluating genetic effects on drinking patterns in males and females.

Keywords Alcohol, COGA, *GABRA2*, genetic association, longitudinal, trajectories.

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INTRODUCTION

Most large-scale genetic association studies have tested for genetic effects on lifetime clinical diagnoses of substance use and psychiatric disorders. For example, there are large-scale efforts underway to identify genes involved in alcohol dependence (Bierut *et al.* 2010; Edenberg *et al.* 2010), schizophrenia (Schizophrenia Psychiatric Genome-Wide Association Study Consortium 2011; Lee *et al.* 2012), major depression (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium 2012; Wray *et al.* 2012), bipolar disorder (Chen *et al.* 2011), autism (Chahrour *et al.* 2012; Sanders *et al.* 2012) and attention-deficit hyperactivity disorder (Elia

et al. 2012; Stergiakouli *et al.* 2012; Williams *et al.* 2012). There has also been interest in expanding gene identification efforts to include phenotypes additional to clinical diagnoses. This has involved studying behavioral components related to psychiatric and substance use disorders (e.g. drinking patterns) (Dick *et al.* 2006c); subtypes that may have more homogeneous etiologies (e.g. early onset alcohol dependence) (Edenberg *et al.* 2007); and broader phenotypes that may jointly reflect a shared etiology (e.g. by studying general externalizing or internalizing disorders) (Hettema *et al.* 2006; Dick *et al.* 2008). The study of endophenotypes, or intermediary phenotypes thought to be closer to the underlying genetic architecture than clinical diagnoses, has also received

great attention (Almasy & Blangero 2001; Gottesman & Gould 2003; Cannon & Keller 2006; Dick *et al.* 2006b). However, one area that has remained relatively neglected is the evaluation of longitudinal phenotypes, that is, patterns of change in behavior and physiology over specified time periods. Here, we report analyses that use longitudinal measures of alcohol-related outcomes to characterize how genetic risk unfolds across developmental stages.

We focus on *GABRA2*, because polymorphisms in *GABRA2* have been associated with adult alcohol dependence across multiple independent studies (Covault *et al.* 2004; Edenberg *et al.* 2004; Fehr *et al.* 2006; Enoch 2008; Soyka *et al.* 2008). However, the two reports that have tested for association with alcohol dependence in younger samples have not found evidence for this association (Dick *et al.* 2006a; Sakai *et al.* 2010). These findings are consistent with twin data in which there is significant heritability for alcohol dependence in adulthood (in the range of 50–60%) (Dick *et al.* 2009); but little evidence of genetic influence on alcohol dependence symptoms in early adolescence (< age 15) (Rose *et al.* 2004; Knopik *et al.* 2009). A major limitation of the literature on *GABRA2* is that no studies of which we are aware of have used longitudinal data to explicitly test the unfolding of risk associated with *GABRA2* across development. In the current analyses, we used longitudinal data from individuals with up to three assessments between the ages of 14 and 25 in order to examine the effects of *GABRA2* on trajectories of drunkenness from adolescence to young adulthood. We used drunkenness as an index of risky drinking behavior, as we hypothesized that genetic effects may be evident for drinking patterns/subclinical indices of drinking problems earlier in the developmental history than for diagnostic level alcohol dependence problems. Accordingly, understanding how genetic influences impact changes in drunkenness across adolescence and into young adulthood may help us understand the unfolding of risk across this critical transitional period.

METHODS

Sample

The data presented here are from the Phase IV Prospective Study of the subjects in the Collaborative Study of the Genetics of Alcoholism (COGA) sample. COGA is a large family study with the goal of identifying genes affecting alcohol dependence and related phenotypes; the Prospective Study is focused on understanding how genetic risk unfolds across adolescence into young adulthood. COGA families were identified through probands in inpatient or outpatient alcohol treatment programs at six sites across

the United States. The institutional review boards of all participating centers approved the study and written consents were obtained from all study participants. Additional ‘community comparison’ families were obtained through a variety of sources such as driver’s license registries and dental clinics; alcohol dependence and other psychiatric disorders were not exclusionary criteria for the comparison families. More details about the basic COGA study have been published previously (Begleiter *et al.* 1995; Foroud *et al.* 2000).

Recruitment for the Prospective Study began in December 2004 among adolescents (age between 12 and 17 years) and young adults (age between 18 and 21 years) who were part of a current COGA family. Recruited participants had at least one parent who was interviewed in one of the previous phases of COGA. Individuals were interviewed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-IV), for adults 18 years or older, and using its adolescent version (C-SSAGA-IV) for subjects under 18 years (Reich *et al.* 1982). Here, we focus on responses to the item ‘How often did you get drunk during the last 12 months?’ Responses are grouped into 13 categories, from ‘never’ to ‘every day’, and the responses were converted into numeric values by taking the midpoint of each response category. For example, the category ‘everyday’ corresponds to 365/year, and the category ‘2 days per week (100–149 days)’ corresponds to 124.5/year in the converted variable.

After the initial assessment, the participants are followed up at approximately biennial intervals. To avoid potential effects associated with population stratification, we limited our analyses here to the European American subset of the sample. Individuals who answered ‘No’ to the screening question about lifetime drinking at all assessments were excluded from the analysis, as were individuals who had missing information on drunkenness at all assessments. We used data from ages 14 to 25 due to sparseness of data at earlier/later timepoints. This yielded a final N of 1070 individuals (49% male). Because data collection remains ongoing, 545 individuals had three assessments (51%) at the time analyses were conducted, 378 had two assessments (35%) and 147 had one assessment. Individuals contribute to the mean and trajectory parameters that encompass the age period for which their data are available; this allows all individuals to contribute to the analyses.

Genotyping

We tested six SNPs in *GABRA2* (major and minor alleles, respectively, shown in parentheses, along with minor allele frequencies): rs497068 (T-C; 0.42), rs279871 (A-G; 0.43), rs279867 (T-G; 0.43), rs279858 (A-G;

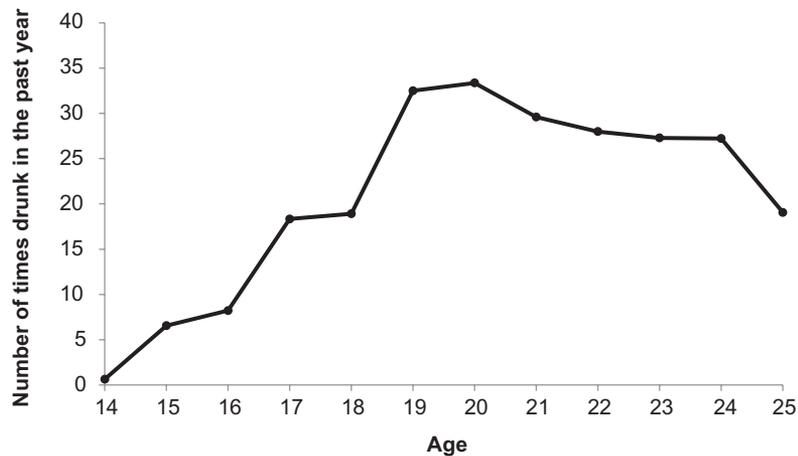


Figure 1 Means and standard deviations of drunkenness (days per year)

Age	14	15	16	17	18	19	20	21	22	23	24	25
N ^a	84	141	171	224	283	246	302	291	228	168	111	82
Mean	0.63	6.55	8.21	18.33	18.91	32.49	33.36	29.59	27.99	27.29	27.22	19.05
SD	2.44	21.27	24.56	38.45	40.63	55.64	56.54	48.50	45.63	47.23	51.83	34.03

^aNumber of subjects contributing data at each age; due to the longitudinal nature of the study design, these are not independent observations.

0.43), rs279845 (T-A; 0.45), and rs279836 (T-A; 0.43). These SNPs were selected for being among the most significantly associated with alcohol dependence in previous COGA analyses (Edenberg *et al.* 2004). The SNPs are highly correlated with each other, with r^2 values ranging from 0.82 to 0.99, mean = 0.89; accordingly, we expect consistency of results across SNPs. Genotyping was performed at the Genome Technology Access Center at Washington University School of Medicine in St. Louis (<http://gtac.wustl.edu/>) using an Illumina GoldenGate custom array as part of a larger set of 384 SNPs. All six SNPs had a genotyping rate greater than 98% and were in Hardy-Weinberg equilibrium. Because of the relatively few number of SNPs genotyped, we used both PLINK and PREST to examine IBD relationship.

Statistical model

We fit a piecewise linear growth model to evaluate different growth trajectories for the adolescent (≤ 18 years) and adult (≥ 19 years) periods by estimating different intercepts and slopes for each period. The transition from adolescence to adulthood is a period of considerable theoretical interest with important developmental changes associated with the attainment of adult status. Therefore, we modeled the transition from adolescence to adulthood as the discrepancy between the expected values at 19 based on the growth trajectories of adolescence (data from 14 to 18 years) and young adulthood (data from 19 to 25 years). As seen in Fig. 1, the mean trajectory of

drunkenness shows a steadily increasing trend up to age 18, a substantial increase in the average response between 18 and 19 (referred to here as the 'jump'), and a leveling off in drunkenness that occurs after that period. Differences in each of these parameters [slope of drunkenness across adolescence, transition point ('jump'), and slope of drunkenness across young adulthood] were tested by genotype. The model was fit in the framework of hierarchical linear modeling (Byrk & Raudenbush 1992) given varying numbers and times of assessments across individuals. Unlike conventional growth curve models using a structural equation modeling approach, hierarchical linear modeling does not have equality assumptions on the numbers and times of follow-up assessments of individuals (Mehta & West 2000). Model fitting and parameter estimation were conducted using the mixed procedure, Version 9.3 of SAS software (SAS Institute Inc., Cary, NC, USA).

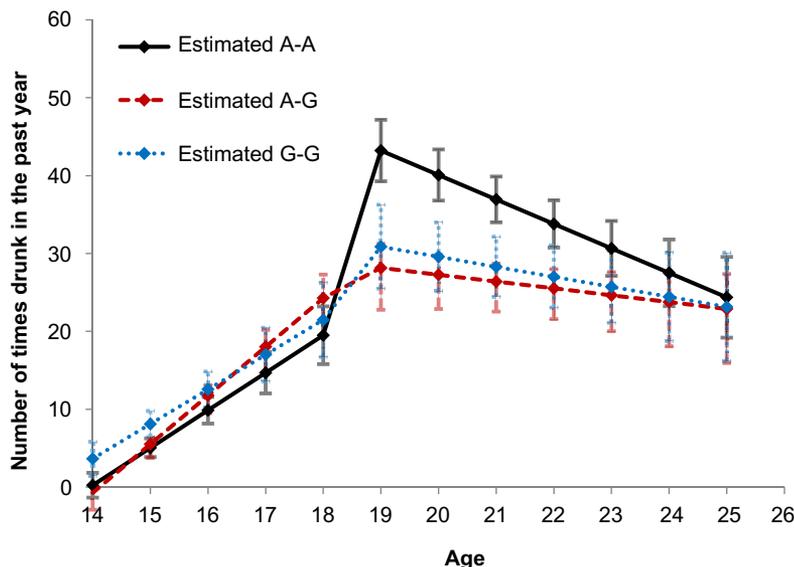
Genotype was coded as a categorical covariate with minor allele count 1 as the reference category. This is a model-free approach that does not assume a linear, recessive or dominant effect of genotype, and was chosen because different genetic models have been indicated across different samples (Dick *et al.* 2006a).

A P -value of 0.025 was used to indicate study-wide significance. This is based on a multiple testing correction performed using the web-based software SNPSpD (Nyholt 2004), which takes into account the number of SNPs genotyped and linkage disequilibrium (LD) structure between them. Based on this test, the effective number

Table 1 *P*-values of the tests of genotype effects for GABRA2 SNPs.

Parameters	Comparisons	rs497068	rs279871	rs279867	rs279858	rs279845	rs279836
Slope before 19	Minor allele count 0 versus 1	0.42	0.33	0.33	0.32	0.19	0.22
	Minor allele count 2 versus 1	0.33	0.30	0.30	0.30	0.70	0.33
Jump	Minor allele count 0 versus 1	0.0032*	0.0017*	0.0017*	0.0017*	0.0012*	0.0002*
	Minor allele count 2 versus 1	0.75	0.36	0.36	0.36	0.65	0.23
Slope after 19	Minor allele count 0 versus 1	0.13	0.11	0.12	0.14	0.28	0.07
	Minor allele count 2 versus 1	0.78	0.79	0.80	0.83	0.95	0.74

**P*-values significant after correction for multiple testing (Nyholt 2004).

**Figure 2** Observed mean trajectories of drunkenness by genotype (rs279858) for European Americans

of independent marker loci for our analyses was 2.0, resulting in the adjusted significance level of $0.05/2.0 = 0.025$.

RESULTS

Table 1 shows the *P*-values for each of the six SNPs for the different growth trajectories. After correcting for multiple testing, the only consistently significant difference across the six SNPs is the difference in the jump between minor allele count 0 and 1, with all SNPs yielding $P < 0.005$. None of the differences between minor allele count 1 and 2 were significant.

In order to test whether the jump in drunkenness was specific to the transition between ages 18 and 19, we also tested whether the genotypic effect was significant if the transition point was moved forward or backward 1 year in time. This allowed us to define the window across which the observed genotypic effect was evident. The effect was found to be specific to age 18–19; the genotypic effect associated with the homozygous major allele was not significant at age 17–18 ($P = 0.14$) or age 19–20 ($P = 0.61$).

The pattern of results was similar across all SNPs. We present in Fig. 2 results for rs279858 since it has been one of the most widely studied and replicated SNPs in GABRA2 (Covault *et al.* 2004; Edenberg *et al.* 2004; Lappalainen *et al.* 2005; Lind *et al.* 2008; Bierut *et al.* 2010). Figure 2 shows the estimated growth trajectories for each of the different genotypes. As illustrated in the figure, the trajectory of the A-A genotype (the major allele homozygote) is characterized by a significantly greater jump in drunkenness between 18 to 19 years of age. None of the slope differences for rs279858 were significant after correction for multiple testing.

Post hoc analyses were run to test for potential sex effects by fitting the models to the data for females and males separately (Tables 2 and 3). Although similar trends are observed for both females and males, the effect of genotype on the jump in drunkenness observed between adolescence and adulthood was significant only in females. Only those females who were homozygous for the major allele showed a large jump in drunkenness from 18 to 19 years (Fig. 3a). Males who were homozygous for the major allele also showed the highest levels of

Table 2 P-values of the tests of genotype effects for GABRA2 SNPs in females.

Parameters	Comparisons	rs497068	rs279871	rs279867	rs279858	rs279845	rs279836
Slope before 19	Minor allele count 0 versus 1	0.34	0.25	0.25	0.24	0.44	0.35
	Minor allele count 2 versus 1	0.13	0.22	0.22	0.22	0.91	0.35
Jump	Minor allele count 0 versus 1	0.0044*	0.0028*	0.0026*	0.0027*	0.0040*	0.0022*
	Minor allele count 2 versus 1	0.49	0.43	0.42	0.42	0.81	0.32
Slope after 19	Minor allele count 0 versus 1	0.13	0.10	0.10	0.10	0.12	0.10
	Minor allele count 2 versus 1	0.95	0.61	0.62	0.61	0.48	0.51

*P-values significant after correction for multiple testing (Nyholt 2004).

Table 3 P-values of the tests of genotype effects for GABRA2 SNPs in males.

Parameters	Comparisons	rs497068	rs279871	rs279867	rs279858	rs279845	rs279836
Slope before 19	Minor allele count 0 versus 1	0.31	0.27	0.27	0.27	0.06	0.12
	Minor allele count 2 versus 1	0.93	0.73	0.71	0.73	0.45	0.62
Jump	Minor allele count 0 versus 1	0.16	0.06	0.06	0.07	0.04	0.0124*
	Minor allele count 2 versus 1	0.66	0.66	0.64	0.68	0.78	0.53
Slope after 19	Minor allele count 0 versus 1	0.93	0.54	0.51	0.65	1.00	0.36
	Minor allele count 2 versus 1	0.57	0.85	0.97	0.77	0.47	0.85

*P-values significant after correction for multiple testing (Nyholt 2004).

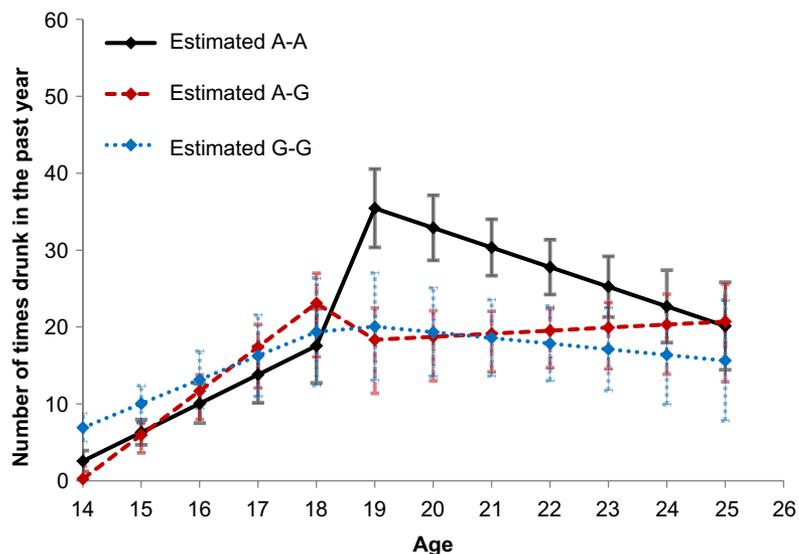


Figure 3a Observed mean trajectories of drunkenness by genotype (rs279858) for females

drunkenness (Table 3; Fig. 3b), but it was only significant with one SNP. Males overall showed higher levels of drunkenness, contributing to the lack of significant differences by genotype.

DISCUSSION

This study assesses the longitudinal, developmental trajectory of risk associated with a specific genotype in a large prospective cohort of adolescents with drinking patterns characterized from adolescence to young adulthood. Understanding risk across this period is a

particularly critical area of study in the alcohol field, as there has been a discrepancy between genetic findings for alcohol related outcomes in adolescent and adult samples (Dick *et al.* 2006a; Sakai *et al.* 2010). With longitudinal data, we demonstrate that genotype effects are associated with the transition to adulthood: genetic differences emerge as a jump in drunkenness between age 18 and 19. This is obviously a rich developmental phase that is associated with a number of milestones, such as leaving home, entering college and building new social networks (White *et al.* 2001; Borsari, Murphy & Barnett 2007). These milestones reflect enhanced independence

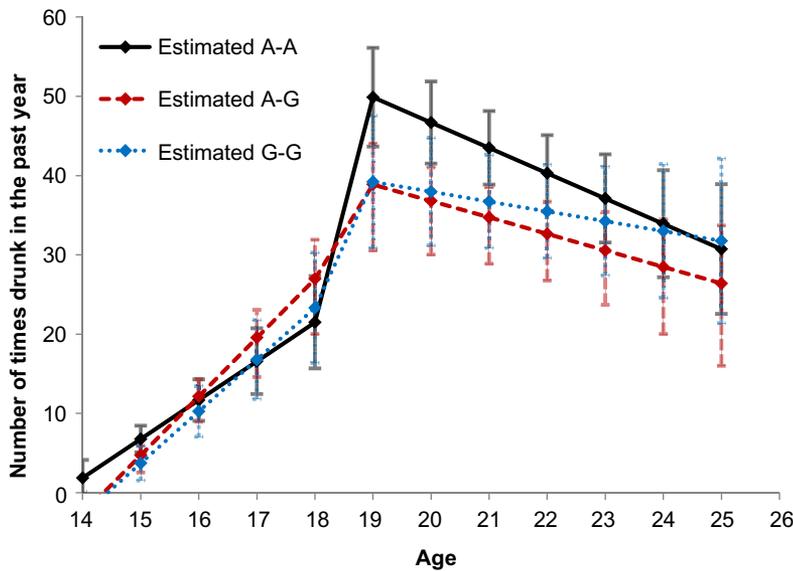


Figure 3b Observed mean trajectories of drunkenness by genotype (rs279858) for males

associated with the attainment of adult status. Twin data have indicated that environments that exert less social control and/or provide greater opportunity to engage in alcohol use allow for greater expression of genetic predispositions (Dick & Kendler 2012). This has been shown, for example, with respect to low parental monitoring (Dick *et al.* 2006d), higher peer deviance (Dick *et al.* 2007; Harden *et al.* 2008; Button *et al.* 2009) and communities with higher alcohol sales and less stability (Dick *et al.* 2001), all of which are associated with greater genetic variance. Here, we demonstrate that the effect of a specific gene on high risk alcohol use becomes evident during the transition to adulthood. Although we did not directly measure environmental characteristics in this study, this transition is generally associated with the attainment of greater autonomy and fits in the broader theoretical mechanism suggested by the gene environment interaction literature (Shanahan & Hofer 2005; Dick & Kendler 2012).

Secondary analyses reveal that the findings are more significant in females. All males, irrespective of genotype, showed a greater increase in drunkenness associated with the transition to adulthood. A number of previous studies have documented higher rates of risky drinking behaviors in males as compared to females at this age (White, Kraus & Swartzwelder 2006; White & Swartzwelder 2009), and our data support this. It is possible that this leads to a heightened culture of accepted drunken behavior in males, and that under these environmental pressures, genotypic effects are attenuated. None of the twin studies, reviewed above, showing that genetic effects are more evident in environments with less social control, have tested for sex differences. Our study illustrates the complexity of understanding how genetic predispositions interact with contextual influences and

suggests that examining environmental circumstances and pressures that may differ for males and females at the transition to adulthood is an important area to pursue.

Our findings also complement other studies that have tested for genetic effects across adolescents/young adults of different ages. In at least two independent studies examining other genes thought to be involved in alcohol related outcomes, genetic effects that were present at later ages were not evident earlier in adolescence. Using data from the Add Health study on individuals ages 13–26, genetic effects associated with five monoamine genes and patterns of alcohol consumption were only evident among individuals age 19 or older, not among younger individuals (Guo, Wilhelmsen & Hamilton 2007). Similarly, associations between *ALDH2* and alcohol consumption among Asian-Americans also showed a parallel pattern with genetic effects becoming evident late in adolescence/young adulthood (Irons *et al.* 2012). These studies together point toward a more global picture of genetic effects on alcohol related outcomes emerging in young adulthood. However, we note that the increase in drunkenness observed at the transition to adulthood in our sample is not sustained. There are no significant differences in rate of change in drunkenness after age 19, and mean levels of drunkenness do not differ significantly by genotype by the mid-20s, as illustrated by the overlapping error bars in the figures. Other analyses exploring the association between *GABRA2* and a variety of impulsivity phenotypes in this sample show that this gene is associated with subclinical levels of externalizing behavior, as measured by the Achenbach Externalizing Scale, but not with clinical-level Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV, APA 2000) symptom counts of alcohol dependence, other drug dependence or antisocial behavior, as in the

older adult COGA samples (Dick *et al.* 2013). These findings illustrate that genes can impact different outcomes across different developmental stages and underscore the importance of understanding the longitudinal pathways of risk associated with particular genes.

There has been inconsistency in the genetic model associated with *GABRA2* in different studies and in different age groups (Dick *et al.* 2006a); accordingly, we coded the genotypes so as to not assume a specific genetic model. In these analyses, we found a greater jump in drunkenness during the transition from adolescence to adulthood among individuals carrying 0 copies of the minor allele, with no difference observed among individuals heterozygous or homozygous for the minor allele. This is the same genetic model (risk associated with carrying 0 copies of the minor allele, alternately referred to as two copies of the major allele) that was associated with elevated alcohol problems in our previous cross-sectional studies of adults. In other words, the genotype originally associated with adult alcohol dependence in the parental generation of COGA is the same genotype associated with the sharper increase in drunkenness from 18 to 19 found among these prospectively followed children of COGA families.

Although we have focused these analyses on self-reports of drunkenness, as an index of high risk drinking behavior, we note that this measure correlated highly with other indices of drinking that were assessed, including frequency of drinking any alcoholic beverages ($r = 0.69$) and frequency of drinking five or more drinks in a 24-hour period ($r = 0.83$). The trajectory analyses of *GABRA2* yielded parallel results with these other indices of drinking, providing further support for the robustness of the effect, and alleviating concern that may exist about the subjective nature of what constitutes 'drunkenness'.

Our study should be interpreted in the context of several limitations. Although the age range covered encompassed 14 to 25 years, participants did not have data across this entire range. Since data collection was not age-standardized, each participant contributes to the portion of the overall trajectory for which they have data, but different timepoints have somewhat different individuals contributing. The number of individuals contributing to each age point is included in Fig. 1. In addition, although we assume that the jump in drunkenness found between ages 18 and 19 is attributable to changing environmental circumstances associated with the transition to adulthood, we have not explicitly included any measured aspects of the environment in the model. Finally, the analyses reported here are limited to the European American subset of a study that selected families rich in alcohol dependent subjects, and it is not clear if similar results would be seen in other subgroups. There are known allele frequency differences between populations

and we did not want this to lead to spurious findings; however, we note that the overall pattern of results was similar when the entire sample (of which European Americans were 62%) was analyzed jointly.

In summary, our analyses illustrate the importance of longitudinal data to characterize how genetic effects unfold across development. This allows us to go beyond simple studies of means and characterize genetic effects on patterns of use across time. By modeling patterns of high risk drinking behavior across time, our analyses suggest that the effect of this gene becomes evident during the transition to young adulthood, as evidenced by a jump in drunkenness evident between age 18 and 19 that is associated with *GABRA2* genotype. These findings fit within the broader literature suggesting that environments that exert less social control and/or allow greater opportunity to engage in alcohol use also allow for increased expression of genetic effects (Shanahan & Hofer 2005). Interestingly, our findings are more significant in females. Males overall showed larger increases in drunkenness from 18 to 19 years; accordingly, the effect of genotype were attenuated. This underscores the potential importance of studying how etiological factors may differentially impact alcohol use in males and females at this important developmental juncture. Understanding how genetic risk unfolds across development has important implications for prevention and intervention efforts.

Acknowledgements

The Collaborative Study on the Genetics of Alcoholism (COGA), Principal Investigators B.P., V.M.H., H.J.E., L. Bierut, includes 10 different centers: University of Connecticut (V.M.H.); Indiana University (H.J.E., J.I.N., T. Foroud); University of Iowa (S.K., J.R.K.); SUNY Downstate (B.P.); Washington University in St. Louis (L. Bierut, A.G., J. Rice, K.B.); University of California at San Diego (M.S.); Rutgers University (J. Tischfield); Southwest Foundation (L. Almasy), Howard University (R. Taylor) and Virginia Commonwealth University (D.M.D). Other COGA collaborators include L. Bauer (University of Connecticut); D. Koller, S. O'Connor, L. Wetherill, X. Xuei (Indiana University); G. Chan (University of Iowa); N. Manz, M. Rangaswamy (SUNY Downstate); A. Hinrichs, J. Rohrbach, J-C.W. (Washington University in St. Louis); A. Brooks (Rutgers University); and F.A. (Virginia Commonwealth University). A. Parsian and M. Reilly are the NIAAA Staff Collaborators. We continue to be inspired by our memories of Henri Begleiter and Theodore Reich, founding PI and Co-PI of COGA, and also owe a debt of gratitude to other past organizers of COGA, including Ting-Kai Li, currently a consultant with COGA, P. Michael Conneally, Raymond Crowe and Wendy Reich, for their critical contributions. This national collaborative

study is supported by NIH Grant U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA). This work is also supported by KO2AA018755 (D.M.D.). Drs. LJ Bierut, A.G., AJ Hinrichs, J Rice and J-C.W. are listed as inventors on the patent 'Markers for Addiction' (US 20070258898) covering the use of certain SNPs in determining the diagnosis, prognosis and treatment of addiction.

Conflict of Interest

No other authors have any conflicts of interest to declare.

Authors Contribution

DMD and SBC were responsible for the study concept and design. SBC, SJL and FA performed the statistical analyses. DMD and SBC drafted the manuscript. All co-authors aided with interpretation of the results and provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

References

- Almasy L, Blangero J (2001) Endophenotypes as quantitative risk factors for psychiatric disease: rationale and study design. *Am J Med Genet* 105:42–44.
- American Psychiatric Association (APA) (2000) Diagnostic and statistical manual of mental disorders (4th edn, Text Rev). Washington, DC: APA.
- Begleiter H, Reich T, Hesselbrock V, Porjesz B, Li T-K, Schuckit MA, Edenberg HJ, Rice JP (1995) The collaborative study on the genetics of alcoholism. *Alcohol Health Res World* 19:228–236.
- Bierut LJ, Agrawal A, Bucholz KK, Doheny KF, Laurie C, Pugh E, Fischer S, Fox L, Howells W, Bertelsen S, Hinrichs AL, Almasy L, Breslau N, Culverhouse RC, Dick DM, Edenberg HJ, Foroud T, Gruzza RA, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Krueger RF, Kuperman S, Lynskey M, Mann K, Neuman RJ, Nothen MM, Nurnberger JI Jr, Porjesz B, Ridinger M, Saccone NL, Saccone SF, Schuckit MA, Tischfield JA, Wang JC, Rietschel M, Goate AM, Rice JP; Gene, Environment Association Studies Consortium (2010) A genome-wide association study of alcohol dependence. *Proc Natl Acad Sci U S A* 107:5082–5087.
- Borsari B, Murphy JG, Barnett NP (2007) Predictors of alcohol use during the first year of college: implications for prevention. *Addict Behav* 32:2062–2086.
- Button TM, Stallings MC, Rhee SH, Corley RP, Boadman JD, Hewitt JK (2009) Perceived peer delinquency and the genetic predisposition for substance dependence vulnerability. *Drug Alcohol Depend* 100:1–8.
- Byrk A, Raudenbush SW (1992) Hierarchical Linear Models for Social and Behavioral Research: Applications and Data Analysis Methods. Newbury Park, CA: Sage Publications.
- Cannon TD, Keller MC (2006) Endophenotypes in the genetic analyses of mental disorders. *Annu Rev Clin Psychol* 2:267–290.
- Chahrouh MH, Yu TW, Lim ET, Ataman B, Coulter ME, Hill RS, Stevens CR, Schubert CR; ARRA Autism Sequencing Collaboration, Greenberg ME, Gabriel SB, Walsh CA (2012) Whole-exome sequencing and homozygosity analysis implicate depolarization-regulated neuronal genes in autism. *Plos Genet* 8:e1002635.
- Chen DT, Jiang X, Akula N, Shugart YY, Wendland JR, Steele CJ, Kassem L, Park JH, Chatterjee N, Jamain S, Cheng A, Leboyer M, Muglia P, Schulze TG, Cichon S, Nothen MM, Rietschel M; BiGS, McMahon FJ, Kelsoe JR, Greenwood TA, Nievergelt CM, McKinney R, Shilling PD, Schork NJ, Smith EN, Bloss CS, Nurnberger JI Jr, Edenberg HJ, Foroud T, Koller DL, Gershon ES, Liu C, Badner JA, Scheftner WA, Lawson WB, Nwulia EA, Hipolito M, Coryell W, Rice J, Byerley W, McMahon FJ, Chen DT, Berrettini WH, Potash JB, Zandi PP, Mahon PB, McInnis MG, Zollner S, Zhang P, Craig DW, Szlinger S, Barrett TB, Schulze TG (2011) Genome-wide association study meta-analysis of European and Asian-ancestry samples identifies three novel loci associated with bipolar disorder. *Mol Psychiatry* 18:195–205.
- Covault J, Gelernter J, Hesselbrock V, Nellissery M, Kranzler HR (2004) Allelic and haplotypic association of GABRA2 with alcohol dependence. *Am J Med Genet B Neuropsychiatr Genet* 129B:104–109.
- Dick DM, Aliev F, Wang JC, Gruzza RA, Schuckit M, Kuperman S, Kramer J, Hinrichs A, Bertelsen S, Budde JP, Hesselbrock V, Projesz B, Edenberg HJ, Bierut LJ, Goate A (2008) Using dimensional models of externalizing psychopathology to aid in gene identification. *Arch Gen Psychiatry* 65:310–318.
- Dick DM, Aliev F, Latendresse S, Projesz B, Schuckit M, Rangaswamy M, Hesselbrock V, Edenberg H, Nurnberger J, Agrawal A, Bierut L, Wang J, Bucholz K, Kuperman S, Kramer J (2013) How phenotype and developmental stage affect the genes we find: GABRA2 and impulsivity. *Twin Res Hum Genet* 8:1–9.
- Dick DM, Bierut L, Hinrichs A, Fox L, Bucholz KK, Kramer J, Kuperman S, Hesselbrock V, Schuckit M, Almasy L, Tischfield J, Projesz B, Begleiter H, Nurnberger JI Jr, Xuei X, Edenberg HJ, Foroud T (2006a) The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. *Behav Genet* 36:577–590.
- Dick DM, Jones K, Saccone N, Hinrichs A, Wang JC, Goate A, Bierut L, Almasy L, Schuckit M, Hesselbrock V, Tischfield J, Foroud T, Edenberg H, Projesz B, Begleiter H (2006b) Endophenotypes successfully lead to gene identification: results from the collaborative study on the genetics of alcoholism. *Behav Genet* 36:77–86.
- Dick DM, Kendler KS (2012) The impact of gene environment interaction on alcohol use disorders. *Alcohol Res Health* 34:318–324.
- Dick DM, Pagan JL, Viken R, Purcell S, Kaprio J, Pulkkinen L, Rose RJ (2007) Changing environmental influences on substance use across development. *Twin Res Hum Genet* 10:315–326.
- Dick DM, Plunkett J, Wetherill LF, Xuei X, Goate A, Hesselbrock V, Schuckit M, Crowe R, Edenberg HJ, Foroud T (2006c) Association between GABRA1 and drinking behaviors in the collaborative study on the genetics of alcoholism sample. *Alcohol Clin Exp Res* 30:1101–1110.
- Dick DM, Prescott C, McGue M (2009) The genetics of substance use and substance use disorders. In: Kim YK, ed. *Handbook of Behavior Genetics*, pp. 433–453. New York: Springer.
- Dick DM, Viken R, Purcell S, Kaprio J, Pulkkinen L, Rose RJ (2006d) Parental monitoring moderates the importance of

- genetic and environmental influences on adolescent smoking. *J Abnorm Psychol* 116:213–218.
- Dick DM, Rose RJ, Viken RJ, Kaprio J, Koskenvuo M (2001) Exploring gene-environment interactions: socioregional moderation of alcohol use. *J Abnorm Psychol* 110:625–632.
- Edenberg H, Koller DL, Xuei X, Wetherill L, McClintick JN, Almasy L, Bierut LJ, Bucholz KK, Goate A, Aliev F, Dick D, Hesselbrock V, Hinrichs A, Kramer J, Kuperman S, Nurnberger JI Jr, Rice JP, Schuckit MA, Taylor R, Webb BT, Tischfield JA, Porjesz B, Foroud T (2010) Genome-wide association study of alcohol dependence implicates a region on chromosome 11. *Alcohol Clin Exp Res* 34:840–852.
- Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO, Crowe RR, Goate A, Hesselbrock V, Jones K, Kwon J, Li TK, Nurnberger JI Jr, O'Connor SJ, Reich T, Rice J, Schuckit MA, Porjesz B, Foroud T, Begleiter H (2004) Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet* 74:705–714.
- Edenberg HJ, Xuei X, Wetherill LF, Bierut L, Bucholz K, Dick DM, Hesselbrock V, Kuperman S, Porjesz B, Schuckit MA, Tischfield JA, Almasy LA, Nurnberger JI Jr, Foroud T (2007) Association of NFKB1, which encodes a subunit of the transcription factor NF- κ B, with Alcohol Dependence. *Hum Mol Genet* 17:963–970. ddm368.
- Elia J, Glessner JT, Wang K, Takahashi N, Shtir CJ, Hadley D, Sleiman PM, Zhang H, Kim CE, Robison R, Lyon GJ, Flory JH, Bradfield JP, Imielinski M, Hou C, Frackelton EC, Chiavacci RM, Sakurai T, Rabin C, Middleton FA, Thomas KA, Garriss M, Mentch F, Freitag CM, Steinhausen HC, Todorov AA, Reif A, Rothenberger A, Franke B, Mick EO, Roeyers H, Buitelaar J, Lesch KP, Banaschewski T, Ebstein RP, Mulas F, Oades RD, Sergeant J, Sonuga-Barke E, Renner TJ, Romanos M, Romanos J, Warnke A, Walitza S, Meyer J, Palmason H, Seitz C, Loo SK, Smalley SL, Biederman J, Kent L, Asherson P, Anney RJ, Gaynor JW, Shaw P, Devoto M, White PS, Grant SF, Buxbaum JD, Rapoport JL, Williams NM, Nelson SF, Faraone SV, Hakonarson H (2012) Genome-wide copy number variation study associates metabotropic glutamate receptor gene networks with attention deficit hyperactivity disorder. *Nat Genet* 44:78–84.
- Enoch MA (2008) The role of GABA(A) receptors in the development of alcoholism. *Pharmacol Biochem Behav* 90:95–104.
- Fehr C, Sander T, Tadic A, Lenzen KP, Anghelescu I, Klawe C, Dahmen N, Schmidt LG, Szegedi A (2006) Confirmation of association of the GABRA2 gene with alcohol dependence by subtype-specific analysis. *Psychiatr Genet* 16:9–17.
- Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL, Bierut LJ, Conneally PM, Nurnberger JI, Bucholz KK, Li TK, Hesselbrock V, Crowe R, Schuckit MA, Porjesz B, Begleiter H, Reich T (2000) Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcohol Clin Exp Res* 24:933–945.
- Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 160:1–10.
- Guo G, Wilhelmsen K, Hamilton N (2007) Gene-lifecourse interaction for alcohol consumption in adolescence and young adulthood: five monoamine genes. *Am J Med Genet B Neuropsychiatr Genet* 144B:417–423.
- Harden KP, Hill JE, Turkheimer E, Emery RE (2008) Gene-environment correlation and interaction in peer effects on adolescent alcohol and tobacco use. *Behav Genet* 38:339–347.
- Hettrema JM, An SS, Neale MC, Bukszar J, van der Oord EJ, Kendler KS, Chen X (2006) Association between glutamic acid decarboxylase genes and anxiety disorders, major depression, and neuroticism. *Mol Psychiatry* 11:752–762.
- Irons D, Iacono WG, Oetting WS, McGue M (2012) Developmental trajectory and environmental moderation of the effect of ALDH2 polymorphism on alcohol use. *Alcohol Clin Exp Res* 36:1882–1891.
- Knopik V, Heath A, Bucholz KK, Madden PA, Waldron M (2009) Genetic and environmental influences on externalizing behavior and alcohol problems in adolescence: a female twin study. *Pharmacol Biochem Behav* 93:313–321.
- Lappalainen J, Krupitsky E, Remizov M, Pchelina S, Taraskina A, Zvartau E, Somberg LK, Covault J, Kranzler HR, Krystal JH, Gelernter J (2005) Association between alcoholism and Gamma-Amino Butyric Acid alpha2 receptor subtype in a Russian population. *Alcohol Clin Exp Res* 29:493–498.
- Lee SH, DeCandia TR, Ripke S, Yang J; Schizophrenia Psychiatric Genome-Wide Association Study Consortium (PGC-SCZ); International Schizophrenia Consortium (ISC); Molecular Genetics of Schizophrenia Collaboration (MGS), Sullivan PF, Goddard ME, Keller MC, Visscher PM, Wray NR (2012) Estimating the proportion of variation in susceptibility to schizophrenia captured by common SNPs. *Nat Genet* 44:247–250.
- Lind PA, Macgregor S, Agrawal A, Montgomery GW, Heath AC, Martin NG, Whitfield JB (2008) The role of GABRA2 in alcohol dependence, smoking, and illicit drug use in an Australian population sample. *Alcohol Clin Exp Res* 32:1721–1731.
- Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium (2012). A mega-analysis of genome-wide association studies for major depressive disorder. *Mol Psychiatry* 18:497–511. Epub ahead of print (Apr 3).
- Mehta PD, West SG (2000) Putting the individual back into individual growth curves. *Psychol Methods* 5:23–45.
- Nyholt DR (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 74:765–769.
- Reich W, Herjanic B, Welner Z, Gandhi PR (1982) Development of a structured psychiatric interview for children: agreement on diagnosis comparing child and parent interviews. *J Abnorm Child Psychol* 10:325–326.
- Rose RJ, Dick DM, Viken RJ, Pulkkinen L, Kaprio J (2004) Genetic and environmental effects on conduct disorder, alcohol dependence symptoms, and their covariation at age 14. *Alcohol Clin Exp Res* 28:1541–1548.
- Sakai JT, Stallings MC, Crowley TJ, Gelhorn HL, McQueen MB, Ehringer MA (2010) Test of association between GABRA2 (SNP rs279871) and adolescent conduct/alcohol use disorders utilizing a sample of clinic referred youth with serious substance and conduct problems, controls and available first degree relatives. *Drug Alcohol Depend* 106:199–203.
- Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, Ercan-Sencicek AG, DiLullo NM, Parikshak NN, Stein JL, Walker MF, Ober GT, Teran NA, Song Y, El-Fishawy P, Murtha RC, Choi M, Overton JD, Bjornson RD, Carriero NJ, Meyer KA, Bilguvar K, Mane SM, Sestan N, Lifton RP, Gunel M, Roeder K, Geschwind DH, Delvin B, State MW (2012) De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature* 485:237–241.

- Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium (2011) Genome-wide association study identifies five new schizophrenia loci. *Nat Genet* 43:969–976.
- Shanahan MJ, Hofer SM (2005) Social context in gene-environment interactions: retrospect and prospect. *J Gerontol B Psychol Sci Soc Sci* 60:65–76.
- Soyka M, Preuss UW, Hesselbrock V, Zill P, Koller G, Bondy B (2008) GABA-A2 receptor subunit gene (GABRA2) polymorphisms and risk for alcohol dependence. *J Psychiatr Res* 42:184–191.
- Stergiakouli E, Hamshere M, Holmans P, Langley K, Zaharieva I; deCODE Genetics; Psychiatric GWAS Consortium, Hawi Z, Kent L, Gill M, Williams N, Owen MJ, O'Donovan M, Thapar A (2012) Investigating the contribution of common genetic variants to the risk and pathogenesis of ADHD. *Am J Psychiatry* 169:186–194.
- White AM, Kraus CL, Swartzwelder H (2006) Many college freshmen drink at levels far beyond the binge threshold. *Alcohol Clin Exp Res* 30:1006–1010.
- White AM, Swartzwelder HS (2009) Inbound college students drink heavily during the summer before their freshman year: implications for education and prevention efforts. *Am J Health Educ* 40:90–96.
- White HR, Xie M, Thompson W, Loeber R, Stouthamer-Loeber M (2001) Psychopathology as a predictor of adolescent drug use trajectories. *Psychol Addict Behav* 15:210–218.
- Williams NM, Franke B, Mick E, Anney RJ, Freitag CM, Gill M, Thapar A, O'Donovan MC, Owen MJ, Holmans P, Kent L, Middleton F, Zhang-James Y, Liu L, Meyer J, Nguyen TT, Romanos J, Romanos M, Seitz C, Renner TJ, Walitza S, Warnke A, Palmason H, Buitelaar J, Rommelse N, Vasquez AA, Hawi Z, Langley K, Sergeant J, Steinhausen HC, Roeyers H, Biederman J, Zaharieva I, Hakonarson H, Elia J, Lionel AC, Crosbie J, Marshall CR, Schachar R, Scherer SW, Todovov A, Smalley SL, Loo S, Nelson S, Shtir C, Asherson P, Reif A, Lesch KP, Faraone SV (2012) Genome-wide analysis of copy number variants in attention deficit hyperactivity disorder: the role of rare variants and duplications at 15q13.3. *Am J Psychiatry* 169:195–204.
- Wray NR, Pergadia ML, Blackwood DH, Penninx BW, Gordon SD, Nyholt DR, Ripke S, MacIntyre DJ, McGhee KA, Maclean AW, Smit JH, Hottenga JJ, Willemsen G, Middeldorp CM, de Geus EJ, Lewis CM, McGuffin P, Hickie IB, van der Oord EJ, Liu JZ, Macgregor S, McEvoy BP, Byrne EM, Medland SE, Statham DJ, Henders AK, Heath AC, Montgomery GW, Martin NG, Boomsma DI, Madden PA, Sullivan PF (2012) Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned. *Mol Psychiatry* 17:36–48.